University of Alberta

SUBCRITICAL WATER EXTRACTION OF FUNCTIONAL INGREDIENTS AND GLYCOALKALOIDS FROM POTATO PEEL

by

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ABSTRACT

Potato peel, a waste generated from potato processing is a disposal problem. But, it is a good source of phenolic compounds, sugars, and glycoalkaloids. This study examines the subcritical water extraction of phenolics, glycoalkaloids and sugars from potato peel and compares it to conventional solvent extraction.

Experiments were conducted in a batch stainless steel reactor at 6 MPa, 2 mL/min and 100 to 240˚C for 30-120 min. The results revealed that highest recoveries of phenolic compounds (81.23 mg/100 g; fw) and sugars (75 mg/g; fw) were obtained using subcritical water at 180°C and 30 min and at 160°C and 120 min, respectively. Low content of glycoalkaloids (1.19 mg/100 g, fw) was obtained using subcritical water. The yields of phenolics and sugars using subcritical water were 40 and 45% higher than using a conventional solvent extraction method. Therefore, subcritical water might be a good substitute to organic solvents such as methanol and ethanol to obtain functional ingredients from potato peel.

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LIST OF ABBREVIATIONS

BPR	back pressure regulator
CFA	caffeic acid
CGA	chlorogenic acid
CQA	caffeoylquinic acid
dw	dry weight
EC	extraction cell
fw	fresh weight
F-C	Folin - Ciocalteau
F-D	Folin – Denis
FRA	ferulic acid
GA	Glycoalkaloid
GAC	gallic acid
GC	gas chromatography
I.F.	inline filter
LC/MS	liquid chromatography/mass spectrometry
N.V.	needle valve
P.G	pressure gauge
PBA	*p*-hydroxybenzoic acid
PC	paper chromatography
PCA	protocatechuic acid

PCMA	*p*-coumaric acid
QNA	quinic acid
RP	reverse phase
RP-HPLC	reverse phase - high performance liquid chromatography
SCW	subcritical water
SCWE	subcritical water extraction
SPE	solid phase extraction
TGA	total glycoalkaloids
TLC	thin layer chromatography
UV	Ultraviolet
VNA	vanillic acid

1. INTRODUCTION

1.1. Rationale

Potato (*Solanum tuberosum* L.) is an important food crop worldwide. The production of potato ranks fourth in the world after wheat, rice and maize (Leo et al., 2008). After processing potatoes, a large amount of peel is generated, which represents handling and storage problem. But, potato peel is a good source of phenolic compounds (37-125 mg/100 g, dw) (Rodriguez de Sotillo et al., 1994) and carbohydrates (80%) (Augustin et al., 1979). It also contains glycoalkaloids in high amounts (48.4 - 220 mg/100 g, fw) (Kodamatani et al., 2005). Moreover, phenolic compounds and glycoalkaloids are concentrated more in the peel than in potato flesh (Weshahy and Rao, 2009; Friedman, 2006).

The main phenolic compounds present in potato peel are chlorogenic acid (CGA), caffeic acid (CFA), ferulic acid (FRA), gallic acid (GAC), protocatechuic acid (PCA), vanillic acid (VNA) and *p*-hydroxybenzoic acid (PBA) (Mader et al., 2009; Mattila and Kampulainen, 2002). These compounds possess antioxidant properties, which prevent oxidation of foods containing high fat. Phenolic compounds also exhibit medicinal properties which lower the risk of cancer, cardiovascular diseases, neurodegenerative diseases and microbial and viral infections in humans (Harborne, 2000; Nandutu et al., 2007; Hang et al., 2004).

The main glycoalkaloids present in potato are α-solanine and α-chaconine, which can be further hydrolyzed to β- and γ-solanine and β- and γ-chaconine and finally result in the aglycone solanidine (Friedman and Levin, 2009). α-Solanine

and α-chaconine comprise of 95% of total glycoalkaloids in potato. The consumption of glycoalkaloids above 20 mg/100 g results in poisoning, bitter taste and symptoms such as vomiting, nausea, diarrhea and abdominal pain (Sotelo and Serrano, 2000, Machado et al., 2007). The toxic dose of glycoalkaloids is considered to be 2-5 mg/kg of body mass (Sotelo and Serrano, 2000). On the other hand, the consumption of glycoalkaloids below 20 mg/100 g has antiallergic, antipyretic and antiflammatory effects (Friedman, 2006). It acts as a non-specific precursor and repellent against pest predators in plants (Friedman, 2006). Glycoalkaloids in the concentration of 0.1-100 µg/mL inhibit the growth of human tumour cells (Friedman, 2006).

In addition to phenolics and glycoalkaloids, potato peel also contains sugars. The total sugar content in potato is 2% of the dry weight (Lisinska and Leszczynski, 1989). The major sugars present in potato are glucose, fructose and sucrose. Potatoes with high sugar content are generally discarded before processing due to their adverse effect on the taste (Lisinska and Leszczynski, 1989). Sugars such as glucose and fructose increase the glycemic properties of foods (Kitts, 1998).

Conventional extraction methods for phenolics, glycolalkaloids and sugars from plant materials generally use organic solvents and water. These methods have some disadvantages like emissions of volatile compounds to the environment and also possess undesirable effect on food components (Ramos et al., 2002). In addition, these methods are expensive and time consuming (Ramos et al., 2002; Kubatova et al., 2001). During the last decade, consumer demands for

natural, minimally processed and safe foods has led to a desire to find alternatives to solvent extraction and to the development of new processing concepts. Recently, the application of subcritical water extraction (SCWE) has shown great potential and is gaining interest worldwide. When water exceeds its boiling point temperature (100°C) and reaches its critical point (374°C, 22.1 MPa) at a sufficient pressure to maintain the liquid state of water, the values of viscosity, density, and dielectric constant decrease (Ramos, 2002). At these conditions, water acts as a slightly polar solvent in which polar organic compounds are completely soluble. SCWE may be a feasible option and promising method for the efficient extraction of phenolics, glycoalkaloids and sugars from potato peel.

1.2. Hypothesis

Potato peel contains functional ingredients such as phenolics, sugars and glycoalkaloids that could add commercial value to the product. SCWE might be a more effective technology to extract these valuable compounds from potato peel than conventional solvent extraction.

1.3. Objectives

- To extract phenolics, glycoalkaloids, and sugars from potato peel using conventional solid-liquid extraction.
- To determine the effect of temperature and time on the extraction of phenolics, glycoalkaloids and sugars using SCWE.
- To compare SCWE with conventional solvent extraction for the removal of phenolics, glycoalkaloids and sugars.

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2. LITERATURE REVIEW

2.1. Potato

Potato is the most widely cultivated vegetable in the world (Leo et al., 2008). Potato is consumed in the cooked form. Cooking, baking, steaming, deep frying and microwaving are the predominant methods of cooking potatoes (Mader et al., 2009).

2.1.1. Potato production

Potato accounts for one third of all vegetables grown in Canada (FAO, 2008). Over 325 million tonnes of potatoes were produced worldwide in 2008, which was recognized as the international year of potato (FAO, 2008). USDA (2009) reported a 4% increase in potato production (431 million tonnes) worldwide. For the world potato production, China ranks first by producing 72 million tonnes of potato. USA and Canada rank fourth (20.4 million tonnes) and thirteenth (5 million tonnes), respectively. In Canada, potato production began in the mid 1600's by settlers in New Brunswick (FAO, 2008). Due to the increase in the demand for frozen potatoes and french fries, potato production expanded. In 2007, frozen fries (970 000 tonnes) were exported to international markets (FAO, 2008).

2.1.2. Composition of potato

Potato is a good source of carbohydrates and phytochemicals such as polyphenols and glycoalkaloids (Shahidi and Naczk, 2004). It contains low amounts of protein and lipids. It also contains ascorbic acid, riboflavin,

carotenoids and tocopherols (Augustin et al., 1979; Schieber and Saldana, 2009). It is a good source of polyphenols (4.9-46.2 mg/100 g) (Lisinska and Leszcynski, 1989). After potato processing, a large amount of waste is generated in the form of peels and trimmings, which causes a disposal problem. Potato peel contains over 80% moisture content, which are prone to microbial spoilage upon storage (Weshahy et al., 2010) but potato peel contains a number of nutritional compounds (Table 2.1) (Schieber and Saldana, 2009). Phenolic compounds and glycoalkaloids are typically concentrated in the potato peel as compared to potato flesh.

2.2. Phenolic compounds

Phenolic compounds are also reffered to as polyphenolics, which means "many phenolic groups". Phenolic compounds can be defined as compounds having an aromatic ring to which one or more hydroxyl groups are attached (Mann, 1987). Phenol is the simplest phenolic compound. There are over 9000 phenolic structures that have been identified up to 2004 (Schieber and Saldana, 2009).

2.2.1. Functional properties

2.2.1.1. Phenolic compounds as antioxidants

Recent interest in food phenolics has increased greatly because of their antioxidant properties in order to retard oxidation (Mann, 1987).

Table 2.1. Proximate composition of potato flesh and peel (adapted from Augustin et al., 1979)

Compound	Potato Flesh	Potato Peel
Dry matter, %	21.60	17.00
Ash, %	0.86	1.67
Lipid, %	n.i	0.60[a]
Protein, %	1.81	2.67
Carbohydrate (by difference), %	18.00	12.80
Phenolics, mg/100 g	9.90 [b]	41.65[c]

[a] Camire and Flint, (1991),[b] Rumbaoa et al. (2009),[c] Rodriguez de Sotillo et al. (1994), n.i: not indicated

The antioxidant activity of phenolic compounds is based on the number of hydroxyl groups and their location in the molecule. Hydroxyl groups in phenolic compounds contribute towards the formation of intermolecular hydrogen bonding, resulting in better stability and antioxidant activity than phenolic compounds that contain methoxy group. For example, gallic acid contains three hydroxyl groups, therefore it can better form hydrogen bonds and is known to be a better antioxidant when compared to protocatechuic acid and caffeic acid, which contain only two hydroxyl groups (Baum and Perun, 1962). Caffeic acid, with two hydroxyl groups, has a better ability for hydrogen bonding and is a better antioxidant than ferulic acid (that contains one methoxy group and one hydroxyl group) (Baum and Perun, 1962; Hall 2001).

2.2.1.2. Nutritional and medicinal properties of phenolic compounds

Besides their antioxidant activity, phenolic compounds exhibit antimutagenic, anticarcinogenic, antiglycemic, anticholesterol and antimicrobial properties (Friedman and Levin, 2009; Im et al., 2008). Phenolic compounds have been reported to have positive effects on cancer, cardiovascular diseases and immune disorders (Nandutu, et al., 2007; Hang et al., 2004). Etherton et al. (2002) reported the positive effect of intake of flavonoids on the reduction of coronary heart disease. High consumption of polyphenols (flavonoids) (30 mg/day) reduces 50% of the coronary heart disease mortality rate. Duthie et al. (2003) demonstrated that a diet rich in polyphenols (phenolic acids, flavonols, catechin monomers, proanthocyanidins, flavones, flavanones and anthocyanins) decreases the risk of premature mortality from major clinical conditions, like cancer and

heart disease. Cooper et al. (2004) found that red wine is a rich source of polyphenols (phenolic acids, flavonols, monomeric catechins, and polymeric anthocyanidins) that reduces the susceptibility of low density lipoprotein cholesterol to oxidation. Bazzano et al. (2003) demonstrated that an increase in the consumption of fruits and vegetables (rich in polyphenols) decreases the incidence of cardiovascular diseases and strokes.

2.2.2. Factors affecting phenolic content of potatoes

Phenolic compounds may degrade during extraction and storage conditions (Lee et al., 1990). Rodriguez de Sotillo et al. (1994) investigated the hydrolytic behavior of potato peel phenolic compounds when exposed to high temperature, light and different storage conditions. They reported degradation of phenolic compounds at high temperatures (above 100°C) and storage in the presence of light. Processing of fresh vegetables increases the risk of oxidative damage due to activation of polyphenol oxidase (Lee et al., 1990). Enzymatic discoloration has a direct relation with reduced phenolic content. First, phenols are oxidized to ortho-quinones by polyphenol oxidase. Then, ortho-quinones further oxidize and polymerize, resulting in a black pigment called melanin. This black pigment is mainly responsible for the blackening of potato that leads towards potato losses (Mondy et al., 1985). During cooking, chlorogenic acid reacts with ferrous ion, resulting in the blackening at the stem end of individual potatoes. Ewald et al. (1999) reported that boiling of vegetables leads to the reduction of flavanol content. Drying, frying and cooking also lead to the destruction of phenolic compounds (Ewald et al., 1999).

2.2.3. Phenolic compounds in potato peel

In potato, phenolic compounds are mainly distributed in between peel and adhesive tissue cortex and the concentration of phenolics decreases towards the center of the tuber (Friedman, 1997; Weshahy and Rao, 2009).

In potato peels, phenolic compounds are mostly substituted derivatives of hydroxycinnamic acid (free form phenolics) and hydroxybenzoic acid (bound form phenolics) (Shahidi and Naczk, 1995). The most common hydroxycinnamic acid derivates found in potato peel are chlorogenic acid (CGA), caffeic acid (CFA), and ferulic acid (FRA), while gallic acid (GAC), protocatechuic acid (PCA), vanillic acid (VNA), and *p*-hydroxyl benzoic acid (PBA) occurr as derivatives of hydroxybenzoic acid (Fig. 2.1) (Weshahy and Rao, 2009; Mader et al., 2009; Mattila and Kumpulainen, 2002; Nara et al., 2006).

CGA and CFA are the major phenolic compounds in potato peel (Weshahy and Rao, 2009). The replacement of acid proton of caffeic acid with quinic acid via an ester bond results in chlorogenic acid (Hall, 2001).

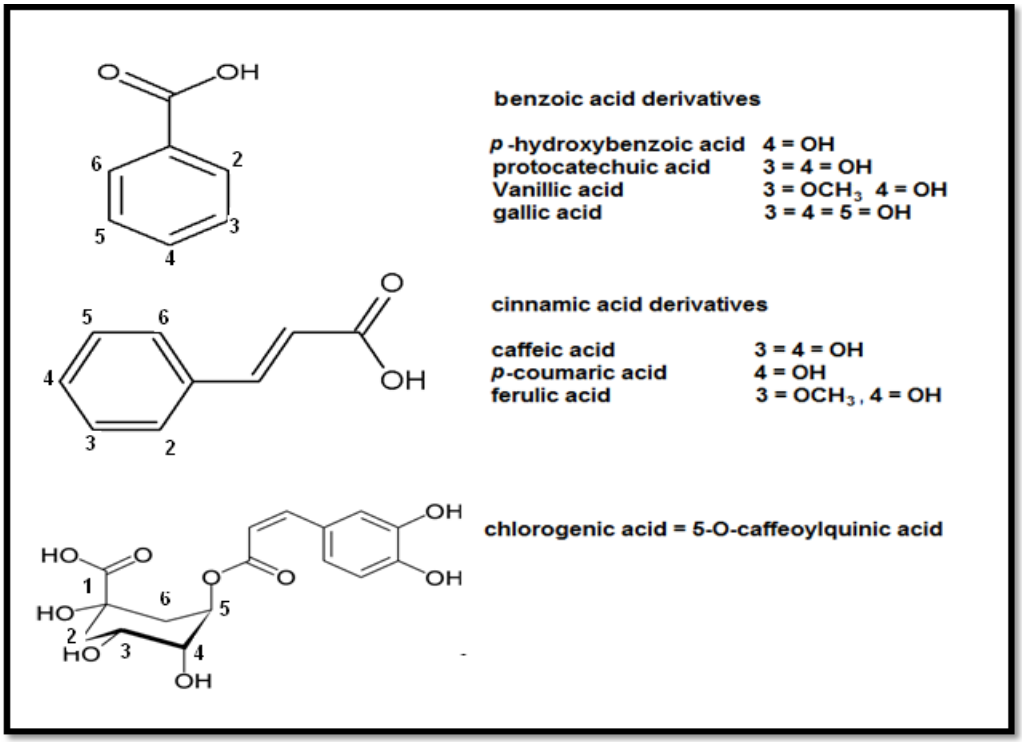

Fig. 2.1. Chemical structures of potato peel phenolic compounds (adapted from Mader et al., 2009).

2.2.4. Extraction of phenolic compounds from potato peel

For the extraction of phenolic compounds, conventional methods have been described in the literature based on solid-liquid extraction using organic solvents (Table 2.2). Other techniques such as Soxhlet or ultrasound extraction have also been applied to extract phenolic compounds. Mohadaly et al. (2009) examined extraction of phenolic compounds from potato peel with various solvents such as ethanol, methanol, acetone, water and mixtures of these solvents in water. They found the yields of phenolic compounds extracted from potato peel with different solvents were in the following order: methanol > ethanol > acetone > hexane > diethyl ether > petroleum ether.

Hertog et al. (1992) reported high solubility of flavones and flavanols in methanol. Metivier et al. (1980) extracted anthocyanins from grape pomace with methanol, ethanol and water. The highest recoveries of anthocyanins were obtained with methanol. The recoveries of anthocyanins with methanol were 15-20% more than with ethanol and 70% more than with water. Hot water has also been used as a solvent to extract phenolic compounds. The polarity of water at 25°C is quite high ($\varepsilon = 79$) but as the temperature increases, the polarity of water decreases, extracting low polar compounds such as phenolics (Ramos et al., 2002; Mohadaly et al., 2009). Rodriguez de Sotillo et al. (1994) extracted phenolic compounds from potato peel with methanol at 4°C, water at 25°C and water at 100°C. They reported higher recovery of phenolic compounds with water at 100°C (48 mg/100 g, fw) than with water at 25°C (33 mg/100 g, fw). The recovery of phenolic compounds with methanol at 4°C was 12% lower than with

water. Recovery of phenolic compounds also depends on the amount of solvent used and the extraction time. Higher amounts of phenolic compounds were obtained using two or three extractions as compared to using a single extraction. Kahkonen et al. (1999) extracted phenolic compounds from plant extracts with 80% methanol twice to recover high amounts of phenolic compounds. Extractions performed more than 5 or 6 times has negligible effect on phenolic compounds recovery (Shahidi and Naczk, 2004). Table 2.2 summarizes the extraction of phenolic compounds from potato.

Table 2.2. Extraction of phenolic compounds with organic solvents.

Potato part	Extraction Solvent	Compound determined	Reference
Peels	Ethanol	Phenolics	Weshahy and Rao (2009)
Tubers and peels	Methanol/water (70:30,v/v)	Phenolics	Mader et al. (2009)
Peels	Methanol, ethanol, acetone, hexane, diethyl ether, petroleum ether	Phenolics	Mohdaly et al. (2009)
Tuber	Methanol	Phenolics	Rumbaoa et al. (2009)
Flower, leaves, stem and tuber	Ethanol (80%)	Phenolics	Im et al. (2008)
Peels	Methanol/acetone/water (60:30:10)/ 0.1 % HCL	Phenolics, flavonoids and flavanols	Makris et al. (2007)
Tubers	Ethanol (70%)	Phenolics	Nandutu et al. (2007)

Table 2.2. Extraction of phenolic compounds with organic solvents (Continued).

Potato part	Extraction Solvent	Compound determined	Reference
Peels	Ethanol	Phenolics	Kannat et al. (2005)
Tubers	Methanol containing 2g/l of 2,3-tert-butyl-4-hydroxyanisole and 10% acetic acid (85:15)	Phenolics	Mattila and Kampulainen (2002)
Tuber and peels	Methanol (80%)	Phenolics	Kahkonen et al. (1999)
Peels	Water	Phenolics	Rodriguez de Sotillo et al. (1998)
Peels	Water (25 and 100°C) and methanol (4°C)	Phenolics	Rodriguez de Sotillo et al. (1994)
Peels	Petroleum ether/ ethanol (95%)	Fatty acids and phenolics	Onyeneho and Hettiarachchy (1993)

2.2.5. Analysis of phenolic compounds

2.2.5.1. Total phenolic content

There are numerous methods to determine total phenolic content in plant extracts, including Folin-Denis (F--D) method (Swain and Hills, 1959), the Folin-Ciocalteau (F-C) method (Singelton and Rossi, 1965) and Prussian blue method (Price and Buttler, 1977). The F-C method was developed in 1927 to analyze the reactivity of tyrosine (which contains a phenol group) with F-C reagent (Huang et al., 2005, Prior et al., 2005). Later, Singelton and Rossi (1965) developed the F-C method to quantify total phenolics from plant materials. They also optimized the amount of alkali and F-C reagent used in the reaction, the time required to reduce F-C reagent, temperature required to oxidize phenol, wavelength (765 nm) and selectivity of gallic acid as a reference phenol. In F-C method, molybdotungstate oxidizes phenol, resulting in a blue colored product with a maximum absorption at 745-750 nm. Molybdenum can be reduced easily in the reaction complex. Transferring of one or two electrons in the reactions between reductants and Mo (VI) leads to the formation of a blue color (Huang et al., 2005; Prior et al., 2005).

Phenolic compounds react with F-C reagent at pH 10, which is maintained by adding sodium carbonate. Under basic conditions, the phenolic proton dissociates and forms the phenolate anion, which finally reduces the F-C reagent to yellow color (Huang et al., 2005)

The F-C method is the simplest method for the determination of total phenolics but this method also has some disadvantages. A number of compounds

present in the sample such as sugars, aromatic amines, organic acids, ascorbic acid, Fe (II), enediols and reductones may interfere with this method, resulting in the increase in total phenolic content that leads towards inaccuracy of the method (Box, 1983). However, a uniform method can be developed by using as an standard gallic acid, following Singleton and Rossi (1965) modified method (Prior et al., 2005). The F-C method is unable to distinguish individual phenolic compounds present in the plant extract, where HPLC method is required.

2.2.5.2. HPLC analysis of phenolic compounds

Thin layer chromatography (TLC) and paper chromatography (PC) were used to isolate and quantify individual phenolic compounds but these techniques have some disadvantages such as low recoveries of phenolic compounds (Robbins, 2003). Other techniques have also been used to identify individual phenolics such as gas chromatography (GC) (Dabrowski and Sosulski, 1984), liquid chromatography/mass spectrometry (LC/MS) and high-performance liquid chromatography (HPLC) (Friedman and Levin, 2009).

In the last 20 years, reverse phase HPLC (RP-HPLC) using a C18 column is the most widely used technique for the identification and quantification of individual phenolic compounds present in food products (Friedman et al., 2009; Merken and Beecher, 2000; Robbins, 2003). HPLC columns used for the analysis of phenolic compounds are mainly reverse phase (RP) and the column length varies from 100-300 mm (Robbins, 2003). There are a number of solvents used as a mobile phase (Table 2.3). Different percentages of gradients of binary solvents

were eluted in an HPLC column for better separation of phenolic compounds. A binary solvent system consists of solvent I (a more polar solvent such as acetic acid, formic acid or phosphoric acid in water) and solvent II consists of a less polar solvent (methanol or acetonitrile) (Merken and Beecher, 2000). In general, the columns used for the phenolic analysis were kept at ambient temperature or slightly above ambient temperature (Robbins, 2003; Merken and Beecher, 2000) and sample injections generally range from 1 to 100 µL. Ultraviolet (UV) detector is mainly used in the range of 190 to 380 nm (Merken and Beecher, 2000; Robbins, 2003). Maximum absorbance wavelength for hydroxybenzoic acid derivatives ranges from 200 to 290 nm. Cinnamic acid derivatives absorption range is broader and varies from 270 to 360 nm (Robbins, 2003). RP-HPLC techniques have been commonly applied for the analysis of phenolic compounds from potato tuber and peel (Table 2.3).

Concentration of phenolic compounds in potato tuber and peel varies with the potato variety, cultivar and country of cultivation (Weshahy and Rao, 2009). Rodriguez de Sotillo et al. (1994) reported four main phenolic compounds (CGA, CFA, GAC and PCA) from industrial sample potato peel. They obtained high amounts of CGA (24 mg/100 g, fw) and GAC (12.66 mg/100 g, fw) in potato peel extracts. Oneyeneho and Hettiarachchy (1993) found eight different phenolic compounds (GAC, PCA, VNA, CFA, CGA, PBA, PCA, and FRA) from two potato peel varieties (Viking and Kennebec). CGA (753 and 821.3 mg/100 g, dw), CFA (278 and 296 mg/100 g, dw), FRA (174 and 192 mg/100 g, dw) and PCA (216 and 256 mg/100 g, dw) were the main phenolics observed in these two

potato peel varieties. In addition, Mattila and Kumpulainen (2002) identified chlorogenic acid isomers such as 3-caffeoylquinic acid (3-CQA) in potato (variety Nicola) and Mader et al. (2009) reported coumaric acid in potato peels. Weshahy et al. (2009) reported that CGA and CFA are major phenolics in Canadian potatoes (variety Siecle, Vivaladi, Yukon Gold, Purple Majesty and Dakota Pearl). They also reported *p*-coumaric acid (PCMA) and ferulic acid (FRA) in low amounts.

Small amounts of phenolic compounds can be quantified after extraction but there is still a need to develop a method to determine unidentified phenolic compounds from potato peel.

Table 2.3. HPLC analysis of phenolics from potato

Product	Column	Guard Column	Detector	Mobile phase	Flow rate (mL/min)	Volume injected (µL)	Absorbance (nm)	Reference
Peels	C18 reverse phase (3.9 mm x 300 mm)	C18	Absorbance detector	Water:MeOH:Acetic acid (64:35:1, v/v/v)	1	10	313	Rodriguez de Sotillo et al. (1994)
Peels	C18 reverse phase (3.9 x 150 mm)	n.i.	Photodiode	Water:MeOH:Acetic acid (65:34:1, v/v/v)	1	n.i	n.i	Kannat et al. (2005)
Peels	ODS-3 (4.6 x 250 mm)	n.i.	Photodiode, UV visible	Elution with 10-25% acetonitrile with a 20 Mm sodium phosphate buffer (pH 3.3)	1	n.i	250-500	Nara et al. (2006)
Tubers and peels	RP-C18 Beckman Ultrasfere (4.6 x 250 mm)	n.i.	Diode array	Isocratic elution Water: MeOH: Acetic acid (65:34:1)	1	n.i	280-325	Leo et al. (2008)
Potato flower, leaves, stems	ODS-3v column (4.0 x 250 mm, 5 µm)	n.i.	UV-visible	Acetonitrile/ 0.5 % formic acid	1	20	280 and 340	Im et al., (2008)
Tubers	Inertsil ODS-3 (4.0 x 150 mm, 3 µm)	C18	Diode array	50mM H_3PO_4 : Acetonitrile	0.7	10	254, 270, 280	Mattila and Kumpulainen (2002)

MeOH: methanol; H_3PO_4: phosphoric acid; CH_3COONH_4: ammonium acetate; n.i: not indicated

Table 2.3. HPLC analysis of phenolics from potato (continued)

Product	Column	Guard Column	Detector	Mobile phase	Flow rate (mL/min)	Volume injected (µL)	Absorbance (nm)	Reference
Tubers and peels	Inertsil ODS-3 (4.0 x 150 mm, 3 µm)	C18	Diode array	50mM H_3PO_4 : Acetonitrile	0.7	10	254, 280, 329	Mattila and Hellstrom (2007)
Tubers and peels	C18 prontosil (4.6 x 150 mm, 3 µm)	n.i.	n.i	Methanol/acetic acid (2%, w/w)	1	20	285 and 325	Mader et al. (2009)
Peels	C18 (4.6 x 150 mm)	n.i.	UV-visible	n.i	n.i	n.i	n.i	Weshahy and Rao (2009)
Peels	ODS C18 (4,6 x 250 mm)	C18	Diode array	Methanol and CH_3COONH_4	1	10	280 and 354	Onyeneho and Hettiarachchy (1993)

MeOH: methanol; H_3PO_4: phosphoric acid; CH_3COONH_4: ammonium acetate; n.i: not indicated

2.3. Glycoalkaloids

Glycoalkaloids (GA) are naturally occurring secondary metabolites in potato. α-Solanine and α-chaconine are the two major GA found in potatoes, which comprise 95% of the total glycoalkaloids (TGA) (Tömösközi-Farkas et al., 2006; Eltayeb et al., 2003; Mader et al., 2009). In general, higher concentrations of TGA were reported in potato peel than in potato tuber (Kodamatani et al., 2005; Mader et al., 2009). Friedman and Dao (1992) observed the highest concentrations of TGA in sprouts and leaves. In potato tuber, GA are mostly concentrated within the first 1 mm from the outside surface (i.e. periderm, cortex and outer phloem) of the tuber (i.e. peel), decreasing towards the center of the tuber. Peeling (3-4 mm) of the outer tissue surface removes almost all of the glycoalkaloids (Friedman, 2006). α-Chaconine is the main glycoalkaloid (65-71% of the total glycoalkaloids) in potato peel that protects against pest predators. In addition, α-chaconine is also more toxic than α-solanine (Sotelo and Serrano, 2000; Machado et al., 2007).

2.3.1. Chemical structure of glycoalkaloids

The chemical structure of glycoalkaloids, α-chaconine and α-solanine, consists of a same steroidal alkaloid solanidine. But, it differs in the glycosyl moiety attached at position 3 of the aglycone, solanidine (Figure 2.2). In α-chaconine, one D-glucose and two L-rhamnose moieties are attached to the aglycone solanidine, while one D-glucose, D-galactose and L-rhamnose moieties are attached to solanidine aglycone in α-solanine (Fig. 2.2) (Schieber and Saldana,

2009; Mader et al., 2009). These alkaloids are found in plants as glycosides (solatriose and chacotriose). Glycoakaloid biosynthesis starts at the time of germination and reaches the plateau at the time of flowering (Friedman, 2006).

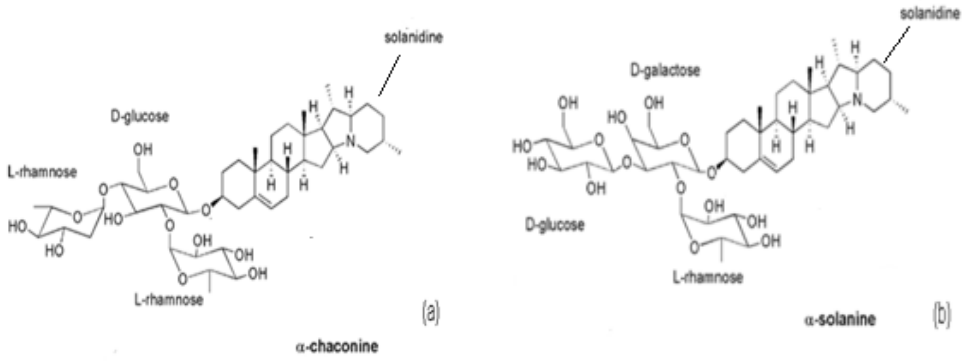

Fig. 2.2. Chemical structure of potato glycoalkaloids: (a) α-Chaconine, and (b) α-Solanine (adapted from Friedman and Levin, 2009).

The trisaccharide chain of both glycoalkaloids can be removed by acid or enzymatic hydrolysis, leading first to the formation of β- and γ-solanine and β- and γ-chaconine with the sequential removal of one or more sugar moieties and finally form solanidine, which is less toxic than α-chaconine and α-solanine (Friedman and Levin, 2009; Schieber and Saldana, 2009). Friedman (2006) observed that acid hydrolysis rate of alkaloids increases with an increment in temperature at elevated acid concentrations but decreases with an increment in the amount of water in the extraction solvent. The ratio of α-chaconine to α-solanine typically found in potato tuber is 60:40 (Slanina, 1990).

2.3.2. Glycoalkaloid adverse and beneficial effects

Glycoalkaloids are toxic if consumed in high quantities. The general safe limit of glycoalkaloids in potatoes is 200 mg/kg of fresh weight of tuber (FAO/WHO, 1999; Knuthsen et al., 2009). Consumption of TGA above recommended level leads to poisoning, bitter taste, gastro enteric symptoms such as vomiting, diarrhea, and abdominal pain (Eltayeb et al., 2003; Kodamatani et al. 2005; Machado et al., 2005). High levels of GA doses also produce symptoms such as fever, rapid pulse, low blood pressure, rapid respiration and neurological disorders (Friedman and Levin, 2009).

In plants, GA acts as non-specific-precursors and repellents against pest predators. GA also enhance potato flavor (Sotelo and Serrano, 2000).

2.3.3. Factors affecting glycoalkaloids content in potatoes

Potato tuber exposed to light can increase two to three times GA concentration (Machado et al., 2007). Percival et al. (1994) also observed the variation of glycoalkaloid levels in the presence of different light sources. They observed an increase of four to six times in glycoalkaloid level when exposed to fluorescent or sodium light as compared to mercury light. Machado et al. (2007) also observed an increase in GA content when exposed to fluorescent light (107.9 mg/kg) as compared to indirect sunlight (92.5 mg/kg), storage in darkness under refrigeration (7-8°C) (81.8 mg/kg) and storage in darkness under room temperature (19-26°C) (60.8 mg/kg). All experiments were performed between 0 to 14 days. In addition, potatoes grown in hot and dry climates contain more

glycoalkaloids than those grown in high altitude and cold climate (Dimenstien et al., 1997).

Removing the tuber skin prior to cooking can significantly reduce the glycoalkaloid content of raw potato (Bushway and Ponampallam, 1981, Knuthsen et al., 2009).

2.3.4. Glycoalkaloid extraction

The extraction of potato glycoalkaloids is challenging due to their complex chemical structure. The carbohydrate part and the alkaloid moiety make the structure slightly soluble in mixture of solvents such as ethanol, methanol or acetic acid (Coxon, 1984). The GA extraction with different solvents are reported in Table 2.4. Glycoalkaloid extraction is mainly based on wet chemical methods. In early extraction methods, Wang et al. (1972) extracted glycoalkaloids for the first time using methanol and chloroform (2:1, v/v). Then, the phase separation was done twice with sodium sulphate to obtain methanolic phase, which contains most of the glycoalkaloids. This method was later modified by Fitzpatrick and Osman (1974) by redissolving the evaporated methanolic phase in 2N sulphuric acid for hydrolysis of the glycoside moiety. Then, titration was done with 10% phenol, containing bromophenol as an indicator. However, the recoveries of TGA were found to be very low (50%). These authors only found TGA but were unable to distinguish individual glycoalkaloids.

Bushway et al. (1979) extracted and quantified α-solanine, α-chaconine and β-chaconine using HPLC analysis. They used tetrahydrofuran:water: acetonitrile (50:30:20, v/v/v; 50:25:25, v/v/v and 50:14:30, v/v/v). Bushway and Ponampallam (1981) analyzed potato glycoalkaloids from different processing conditions (baking at 218°C for 1 hr; frying at 350°C for 25 min and microwaving at 2000 W for 8 min) with methanol-chloroform (2:1, v/v). Stability of glycoalkaloids was consistent in all processing conditions at high temperatures. They also obtained 93-101% recoveries of glycoalkaloids. Sotelo and Serrano (2000) extracted glycoalkaloids from 12 different Mexican varieties of potato flesh and peel with 5 % acetic acid solution. After that, alkaline extraction with butanol was carried out twice. They obtained high contents of glycoalkaloids (25-64 mg/100 g), which is above the recommended level (20 mg/100 g). The method followed by Sotelo and Serrano (2000) was the modification of Dao and Friedman (1996) method in which they partitioned four times with saturated butanol. But, Sotelo and Serrano (2000) partitioned only twice in their extraction method to speed up the extraction.

In 2000, a standard method of extraction and analysis of potato glycoalkaloids was reported by the *Association of Official Analytical Chemists* (AOAC, 2000). In this method, extraction was carried out using a mixture of water-acetic acid-$NaHSO_3$ (100:5:0.5, v/v/w) for only 2 min (AOAC, 2000). This is a fast and reliable method of extraction. In addition, the stability of the extract is 1 week at 4°C. The same method was later adopted by Eltayeb et al. (2003/2004) and Knuthsen et al. (2009). Eltayeb et al. (2003/2004) reported α-

chaconine as the main GA (almost 60-81% of the total glycoalkaloids) in potatoes. The same amount was reported by Sotelo and Serrano (2000). Other authors (Mastuda et al., 2004 and Kodamatani et al., 2005) used only acetic acid (5% solution) for the extraction of glycoalkaloids from tubers and peels. This procedure was repeated three times obtaining 93 % of glycoalkaloids.

Table 2.4. Glycoalkaloids extraction methods

Potato Part	Extraction Solvent	Compound Identified	α-Solanine (mg/kg)	α-Chaconine (mg/kg)	Total glycoalkaloids (mg/kg)	Recovery (%)	Reference
Tuber	methanol:chloroform (2:1, v/v)	Total glycoalkaloids	n.i	n.i	68-248 (fw)	105	Wang et al. (1972)
Peel			n.i	n.i	210-630 (fw)		
Tuber	methanol:chloroform (2:1, v/v)	Total glycoalkaloids	n.i	n.i	85-94 (fw)	95	Fitzpatrick and Osman (1974)
Tuber	tetrahydrofuran-water-acetonitrile (50:30:20, v/v/v)	α-Solanine, α-Chaconine	162	284	446 (dw)	n.i	Bushway et al. (1979)
peel			150	50	200 (dw)		
sprout			1319	1578	2897 (dw)		
Tuber	methanol:chloroform (2:1, v/v)	α-Solanine, α-Chaconine	0.5-45	0.6-102	1.1-162 (fw)	93-101	Bushway and Ponampallam (1981)
peel			461-480	931-979	1390-1450 (fw)		

n.i: not indicated

Table 2.4. Glycoalkaloids extraction methods (Continued)

Potato Part	Extraction Solvent	Compound Identified	α-Solanine (mg/kg)	α-Chaconine (mg/kg)	Total glycoalkaloids (mg/kg)	Recovery (%)	Reference
Tuber	5% acetic acid	α-Solanine, α-Chaconine	4.9-31.41	1.9-40	6.3-83.7 (fw)	n.i	Sotelo and Serrano (2000)
peel			10.7-274.5	17.4-660	29-910 (fw)		
Peel	5% acetic acid	α-Solanine, α-Chaconine	3.66-166.8	8.91-362.1	12-529 (fw)	n.i	Friedman et al. (2004)
Tuber			0.45-33.9	0.56-61.95	1.01-95 (fw)		
Tuber	5% acetic acid	α-Solanine, α-Chaconine	30-321	45-497	75-818 (dw)	101-103	Kodamatani et al. (2005)
peel			218-2540	484-2200	702-4740 (dw)		
Tuber	water:acetic acid:sodium bisulphate (95:5:0.5, v/v/w)	α-Solanine, α-Chaconine	11.5-218	17.2-260	28.7-478 (fw)	n.i	AOAC (2000)

n.i: not indicated

Table 2.4. Glycoalkaloids extraction methods (Continued)

Potato Part	Extraction Solvent	Compound Identified	α-Solanine (mg/kg)	α-Chaconine (mg/kg)	Total glycoalkaloids (mg/kg)	Recovery (%)	Reference
Tuber peel	methanol: acetic acid (95:5, v/v)	α-Solanine, α-Chaconine	0.13 22.4	0.54 66.9	0.67 (fw) 89.3 (fw)	n.i	Mader et al. (2009)
Tuber	water:acetic acid:sodium bisulphate (95:5:0.5, v/v/w)	Total glycoalkaloids	n.i	n.i	8.1-84 (fw)	92-93	Knuthsen et al. (2009)
Tuber	water:acetic acid:sodium bisulphate (1:0.02:0.005, v/v/w)	α-Solanine, α-Chaconine	14-42	30-64	50-103 (fw)	n.i	Machado et al. (2007)

n.i: not indicated

2.3.5. Glycoalkaloids (GA) analysis

2.3.5.1. Conventional analysis

There are a number of analytical methods that have been developed for identification and quantification of individual GA and TGA. In the past years (1950-1980s), colorimetric and gravimetric methods were developed (Fitzpatric and Osman, 1974). Other methods such as enzyme immuno-assays, capillary isotachophoresis and thin layer chromatography have been used (Kodamatani et al., 2005) but these methods were only able to detect TGA but unable to identify and quantify individual GA.

2.3.5.2. HPLC analysis of glycoalkaloids

Table 2.5 summarizes the HPLC methods used for the GA analysis. The first HPLC method to quantify individual GA from potato was published by Bushway et al. (1979). After that, several researchers used HPLC analysis for GA quantification. GA are a challenge to analyze as they do not have a suitable UV chromophores and excessive sample clean up is needed for the removal of interfering compounds at 200 to 208 nm (Driedger and Sporns et al., 1999). Commonly, a solid phase extraction (SPE) C18 cartridge is used to clean the sample due to the presence of interfering compounds which absorb UV light, causing problems for GA quantification in HPLC analysis.

Most of the analysis performed used reverse phase columns such as C18 and NH_2 columns for the identification of individual GA. Both C18 and NH_2 columns produce excellent separation but using the amino column was observed

to have longer run times than C18 columns (Friedman and Levin, 2009). Separation of GA on a reverse phase column is based on the difference in polarity of hydrophilic sugars attached to the hydrophobic solanidine (Friedman and Levin, 2009). Glycoalkaloids were mainly detected at 200 to 202 nm but some authors reported detection at 200 to 225 nm (Table 2.5).

AOAC (2000) used a mixture of H_2O: acetic acid : $NaHSO_3$ for GA analysis. In this method, C18 sep-pak cleaning and HPLC C18 column was used for UV detection at 202 nm. The glycoalkaloids can be identified by using a single isocratic run. Bushway et al. (1979, 1986) used THF/ H_2O/ ACN, MeOH/$CHCl_3$ as HPLC buffers but other authors used different buffers. Recoveries of GA using different mobile phases varied from 88 to 101%.

Table 2.5. HPLC conditions for the determination of glycoalkaloids.

Sample	Cleaning	HPLC conditions	Detection (nm)	Recovery (%)	Reference
Tuber and peel	Ammonium precipitation	NH_2 column THF:H_2O:ACN (5:3:2, v/v/v)	208-225	n.i	Bushway et al. (1979)
Potato products	Ammonium precipitation	NH_2 column THF:H_2O:ACN (5:3:2, v/v/v)	215	93-101	Bushway et al. (1981)
Tuber	C18 Sep-pak	NH_2 column THF:H_2O:ACN (5:3:2, v/v/v)	208-225	n.i	Bushway et al. (1986)
Tuber and potato products	Ammonium precipitation	C18 column ACN:Sulfate (1:1, v/v)	200	88-90	Friedman and Dao (1992)

THF: tetrahydrofuran, ACN, acetonitrile; NH_2: amine, n.i: not indicated

Table 2.5. HPLC conditions for the determination of glycoalkaloids (Continued)

Sample	Cleaning	HPLC conditions	Detection (nm)	Recovery (%)	Reference
Leaves	n.i	C18 column ACN:Ammonium phosphate buffer (35:65, v/v)	200	88-96	Dao and Friedman (1996)
Tuber	C18 Sep-pak	C18 column Phosphate buffer:H_20	202	n.i.	AOAC (2000)
Tuber	n.i	C18 column ACN:Ammonium phosphate buffer (30:60, v/v)	200	97-99%	Sotelo and Serrano (2000)
Tuber	C18 Sep-pak	NH2 column ACN:KH_2PO_4 (75:25, v/v)	200	93	Eltayeb et al. (2003/2004)
Tuber	C18 Sep-pak	C18 column $(NH_4)_2HPO_4$:H_20 (1.2:100, w/v)	202	n.i	Tömösközi-Farkas et al. (2006)

ACN: acetonitrile, KH_2PO_4: mono potassium phosphate, $(NH_4)_2HPO_4$: ammonium phosphate, n.i: not indicated

Table 2.5. HPLC conditions for the determination of glycoalkaloids (Continued)

Sample	Cleaning	HPLC conditions	Detection (nm)	Recovery (%)	Reference
Tuber	n.i	Onyx column (10 mM formic acid with NH_4OH, 100% MeOH with 5 mM ammonium formate)	210	97.5	Shakya and Navarre (2006)
Tuber	C18 Sep-pak	C18 column Acetonitrile: phosphate buffer (50:10, v/v)	202	92-93	Knuthsen et al. (2009)

NH_4OH: ammonium hydroxide, MeOH: methanol, n.i: not indicated

2.4. Sugars

In addition to phenolics and glycoalkaloids, potato also contains sugars in the form of reducing monosaccharides such as D-glucose and D-fructose and non reducing disaccharides such as sucrose (Lisinska and Leszczynski, 1989). The sugar content of potato varies with different varieties and processing conditions. Weaver et al. (1978) reported the sugar content in three different parts of potato such as bud-end, stem-end and cortex. They observed the highest amount of reducing sugars in the cortex (7-46 mg/g, dw) relative to bud-end (5-42 mg/g, dw) and stem-end (8-33 mg/g, dw). In addition, they found large variations in glucose and fructose content in all parts of potatoes such as stem-end, cortex and bud end, while sucrose content was found to be uniform throughout. Also, sucrose and glucose contents are higher than the fructose content in potato tubers (Pritchard and Adams, 1994). Due to the high sugar content, sometimes potato tubers are discarded before processing since sugars have an adverse effect on taste of cooked food such as dehydrated and fried potatoes (Lisinska and Leszczynski, 1989).

2.4.1. Functional Properties

Sugar in food plays multifunctional roles. In general, it imparts sweetness, appearance, flavour and texture to food products (Kitts, 1998). Sugar acts as a tenderizing agent in baked products. Addition of sugar in dough batter increases the growth of yeast and enhances leavening process, which allows the dough to rise at a faster rate (Clarke, 1997). Sugar has ability to absorb water. It withdraws

the water from microorganisms and slows down their growth, which helps in preserving jams and jellies (Clarke, 1997).

Sugars exhibit antioxidant properties as they have the ability to block the reaction sites of ions such as copper, iron and cobalt, which helps in preventing the food from deterioration caused by catalytic oxidation reactions (Kitts, 1998). In pharmaceuticals, sugars are extensively used for its bodying effects in cough syrup. Sugars can be used to treat wounds and burns and retard the bacterial growth (Kitts, 1998).

2.4.2. Effect of processing and storage conditions on the sugar content of potatoes

Sucrose is transported from potato leaves to the tubers (Pritchard and Adam, 1994; Viklund et al., 2008). Hydrolysis of sucrose yields fructose and glucose. At high temperatures, swelling of intracellular starch occurs resulting in the browning reaction (Maillard Reaction) (Pedreschi et al. 2009). The reaction of a carbonyl group of reducing sugars (glucose and fructose) with an amino acid (e.g. aspargine) present in the tuber at high temperatures yields melanoidin pigments and flavor compounds (Pritchard and Adam, 1994). Viklund et al. (2008) reported that potato chips, which contain high amount of carbohydrates upon heat treatment produces acrylamide (a carcinogen and neurotoxic compound to humans). Acrylamide is a byproduct produced from the reaction of the amino acid asparagine in the presence of reducing sugars at high temperatures (Kumar et al., 2004, Takada et al., 2005). Boiling and steaming reduces the sugar content of

fresh potatoes by 0.5% (Sinoda et al., 1931). In addition, they also observed caramelization of sugars at frying temperatures of 230°C.

Storage conditions also affect sugar concentration. Low temperature storage (0-6°C) generally increases the sweetness of potatoes. Sugar loss has been observed when temperature increases from 0 to 8 °C due to starch reformation (Lisinska and Leszczynski, 1989). Tubers stored at 8-12°C have lower sugar content than those stored at 4-6°C (Kumar et al., 2004). To maintain the low level of sweetness, potato is generally stored at 8-12°C (Takada et al., 2005). Low temperature, low oxygen or physical damage of tubers during storage can also enhance sugar levels (Pritchard and Adam, 1994).

2.4.3. Extraction of sugars from potato

The three major sugars in potato tubers are glucose, fructose and sucrose (Spychalla and Desborough, 1990; Weaver et al., 1978; Picha, 1985; Liu et al., 2009). In addition, Wilson et al. (1981) identified maltose and raffinose. These sugars are highly soluble in water (Flood and Paugsa, 2000), and in mixtures of ethanol and water (Alves et al., 2007; Liu et al.,2009; Weaver et al., 1978; Picha ,1985; Wilson et al., 1981; Davies, 1988 and Pressey and Shaw, 1966).

The selection of solvent or mixture of solvents plays an important role in the recovery of sugars from plant extracts. For example, the solubility of sugars decreases when concentration of ethanol increases in water. Alves et al. (2007) reported the solubility of glucose in different water-ethanol mixtures

(50, 60, 70, 80%). They obtained a higher amount of glucose at 60°C in 50% ethanol (62.6 mg/100 g) than in 60% ethanol (54 mg/100 g), 70% ethanol (42.6mg/100 g) or 80% ethanol (36.2 mg/100 g). An increase in the concentration of ethanol in water from 40 to 80% reduces the solubility of glucose (35%). Flood and Paugsa (2000) also reported the solubility of glucose and fructose in different ethanol concentrations (40, 60 and 80%) and found higher solubilities of glucose and fructose in ethanol 40% than at high ethanol concentrations (Table 2.6).

Extractability of sugars from potato varies with the use of solvents, temperature and time as reported in Table 2.7.

Table 2.6: Solubility of sugars in different concentrations of ethanol in water

Sugars	Ethanol concentration (%, v/v)	Sugar content (%, w/w)	Reference
Fructose and glucose	40	0.30	
	60	0.15	Flood and
	80	0.004	Paugsa (2000)
Glucose	50	0.70	Alves et al.
	60	0.50	(2007)
	70	0.30	
	80	n.i	

Table 2.7. Extraction of sugars from potato tuber

Compound analyzed	Extraction solvent	T (°C)	Time (min)	Total sugars (mg/g)	Reference
Glucose, fructose, sucrose	Water	80	60	n.i	Liu et al. (2009)
Glucose, fructose, sucrose	Water	60, 75, 90	120	10-55 (fw)	Pedrechi et al. (2009)
Glucose, fructose, sucrose	Acetonitrile: water (70:30, v/v)	60	30	8-34 (fw)	Spychalla and Desborough (1990)
Glucose, fructose, sucrose	Ethanol (70%)	b.t	60	17-51 (dw)	Weaver et al. (1978)

b.t: boiling temperature, n.i: not indicated

Table 2.7. Extraction of sugars from potato tuber (Continued)

Compound analyzed	Extraction solvent	T (°C)	Time (min)	Total sugars (mg/g)	References
Glucose, fructose, sucrose, maltose	Ethanol (80%)	b.t	16	1.6-4.6 (fw)	Picha (1985)
Glucose, fructose, sucrose, maltose, raffinose	Ethanol (70%), water	b.t	30	4.22-535 (dw)	Wilson et al. (1981)
Reducing sugars	Ethanol (80%)	70	90	n.i	Davies (1988)
Total, reducing sugars	Ethanol (95%)	b.t.	40	n.i	Pressey and Shaw (1966)

b.t: boiling temperature, n.i: not indicated

2.4.4. Analysis of sugars

2.4.4.1.Conventional methods of analysis

Various analytical methods have been developed to identify and quantify total sugars from plant materials. Colorimetric, gravimetric and titration methods have been used in the past. McCready (1950) developed the anthrone method for the determination of total sugars, which was later modified by Jermyn (1975). In this method, sugars react with the anthrone reagent under acidic conditions, resulting in a blue-green color and absorbance is measured at 620 nm. Because of the presence of strong oxidizing sulfuric acid, this method measures both reducing and non reducing sugars. But, this method is not accurate and generates more than 10% error. Besides, this method does not measure sugar content in alcoholic concentration and consumes more time to evaporate the ethanol (Buysse and Merckx, 1993). Moreover, absorbance values were stable only for 12 min after extraction. Buysse and Merckx (1993) developed a phenol-sulphuric acid method in which sugars were quantified by varying the phenol concentration in the reagent. In this method, the phenol solution was added to the sugar solution followed by the addition of sulfuric acid. After 15 min, absorbance was measured at 490 nm and the values were stable for 1 hr. This method converts all non reducing sugars to reducing sugars and determines the total sugar present.

There are a number of other colorimetric methods using reagent 3, 5-dinitro salicylic acid, picric acid, alkaline ferricyanide and copper based formulations (Davies, 1988, Browne and Zerban, 1912). Some physical methods such as

polarimetry and refractive index were also developed to measure sugar content. But, these methods only measure total sugar content and are unable to distinguish individual sugars in the plant extract.

2.4.4.2. HPLC analysis of sugars

To identify individual sugars, HPLC (Picha, 1985, Wilson et al., 1981, AOAC, 1977), GC (Davies et al., 1988) and thin layer chromatography (Picha, 1985) have been used. GC has some disadvantages in the separation of sugars. GC requires a time consuming derivatization step, while results obtained from thin layer chromatography technique is semi-quantitative (Picha, 1985).

HPLC analysis is commonly used to identify and quantify sugars from food sources and plant materials as shown in Table 2.8. HPLC methods were used to analyze sugars from honey (AOAC, 1977), boiled sweets and jellies, milk and ice cream, milk chocolate and confectionary products (Wilson et al., 1981). It was also used to analyze sugars from potato tubers (Table 2.7) (Picha, 1985; Wilson et al, 1981; Den et al., 1986). To observe sugars below 200 nm with a UV detector requires extensive purification (Binder, 1979, Ball, 1990). Refractive index or electrochemical detection can be used. For the selection of the stationary phase, different types of columns have been used as shown in Table 2.8. Guard columns were also selectively used by different authors in sugar analysis (Table. 2.8). The guard column may be packed with similar or different material to that of the analytical column (Ball, 1990).

The mobile phase for the analysis of sugars generally consists of water or water/acetonitrile mixtures (Table 2.8). Temperature of the column was generally in the range of 60-80 °C (Palmer and Brandes, 1974; Picha, 1985). Flow rates were in the range of 0.75-2.2 mL/min (Table 2.8).

Table 2.8. HPLC specifications for sugar analysis from different food and plant materials

Product	Column	Guard column	Detector	Mobile phase	Flow rate (mL/min)	Volume injected (µL)	Reference
Potato tuber	Carbohydrate packing (300 x 3.9 mm)	C18	Differential refractometer	acetonitrile/water (75:25, v/v)	1.8	n.i	Wilson et al. (1981)
Potato tuber	Amino 5S (250 x 4 mm)	n.i.	Refractive index	acetonitrile/water (60:40, v/v)	1	10	Den et al. (1986)
Potato tuber	Aminex resin (300 x 7.8 mm)	Amino	Refractive index	distilled water	1.2	20	Picha et al. (1985)
Honey	µ Bondapak/ Carbohydrate (300 x 4 mm)	n.i.	Refractive index	water/acetonitrile (87:13, v/v)	1	10	AOAC (1977)
Berry juice	Amine (150 x 4.6 mm)	n.i.	Refractive index	0.75 % acetonitrile in water	1	10	Xie et al. (2009)

n.i: not indicated

Table 2.8. HPLC specifications for sugar analysis from different food and plant materials (Continued)

Product	Column	Guard Column	Detector	Mobile phase	Flow rate (mL/min)	Volume injected (µL)	References
Deionized juices	Aminex cation exchange resin (600 x 9.5 mm)	n.i.	Refractive index	distilled water	0.75	10	Palmer and Brandes (1974)
Milk and ice cream	Carbohydrate packing (300 x 3.9 mm)	Corasil cation and anion exchange	Refractive index	acetonitrile/ water (75:25, v/v)	2.2	n.i	Warthesen and Kramer (1979)
Dough and baked products	Amino propyl-bonded[a]	n.i.	Refractive index	acetonitrile/ water (75:25, v/v)	n.i.	n.i	Langemeier and Rogers (1995)
Pineapple	Carbohydrate column[a]	n.i.	Refractive index	acetonitrile/ water (75:25, v/v)	0.9	n.i	Camara et al. (1996)

n.i: not indicated, [a] column specification not mentioned

2.5. Subcritical water extraction (SCWE) technology

Water is termed as subcritical when it is brought between the boiling point temperature (100°C) and the critical point temperature (374°C) and sufficient pressure is applied to prevent its transition to the gaseous state. It will exist in the subcritical state up to its critical temperature (374°C) and pressure (22 MPa) (King, 2003; Herrero et al., 2006). Water above its critical point is known as supercritical water (Fig. 2.3). Subcritical water extraction (SCWE) is also known as pressurized low polarity water extraction, high temperature water extraction, superheated water extraction or hot liquid water extraction.

Under SCW conditions, the dielectric constant of water (polarity) decreases by increasing the temperature. Akerlof and Oshry (1950) calculated the dielectric constant of water at temperatures up to 240°C (Table 2.9). The dielectric constant of ethanol, methanol and of pure water at ambient temperature and pressure are 27, 32.5 and 79.9, respectively. As temperature increases to 250°C and pressure increases to 5 MPa, dielectric constant of water decreases from 79.9 to 32.5 and 27, which is similar to the dielectric constant of methanol and ethanol, respectively (Fig. 2.4) (Amashukeli et al., 2007, Ramos et al., 2002). SCW exhibits unique properties such as high reactivity and intermediate diffusivity (Baek et al., 2008). SCW can solubilize organic compounds of low molecular weight and also has ability to hydrolyse ester and ether (Baek et al., 2008). At temperatures of 100-374°C, viscosity and density of water also decreases.

The most common advantages of SCW extraction over conventional methods are the high quality of extract, environment friendly technique, its short extraction time and low cost (Herrero et al., 2006). Another important factor is cost and energy consumed during the process. Basile et al. (1998) reported a low energy consumption of SCWE (30-150 °C, 1.5 MPa) relative to steam processing (100 °C). Energy consumed to heat the water from 30 to 150°C at 1.5 MPa was 505 kJ/kg, which is four times less than steam processing (2550 kJ/kg) at 100 °C.

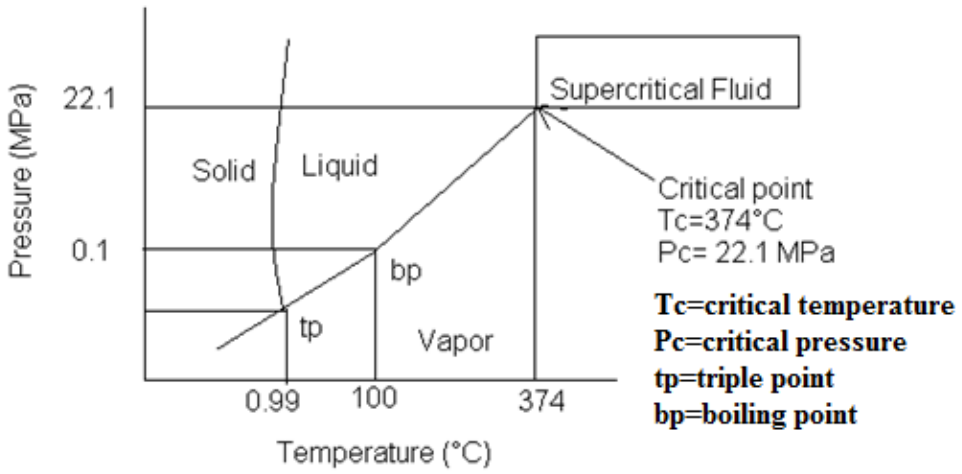

Fig. 2.3. Phase diagram of water

Table 2.9. Dielectric constant of water at various temperatures (Adapted from Akerlof and Oshry (1950))

Temperature (°C)	Pressure (bar)	Dielectric constant
100	1.1	55.39
110	1.4	52.89
120	2.0	50.48
130	2.7	48.19
140	3.6	46.00
150	4.8	43.89
160	6.2	41.87
170	7.9	39.96
180	10.0	38.10
190	12.6	36.32
200	15.6	34.59
210	19.1	32.93
220	23.2	31.32
230	28.0	29.75
240	33.5	28.24

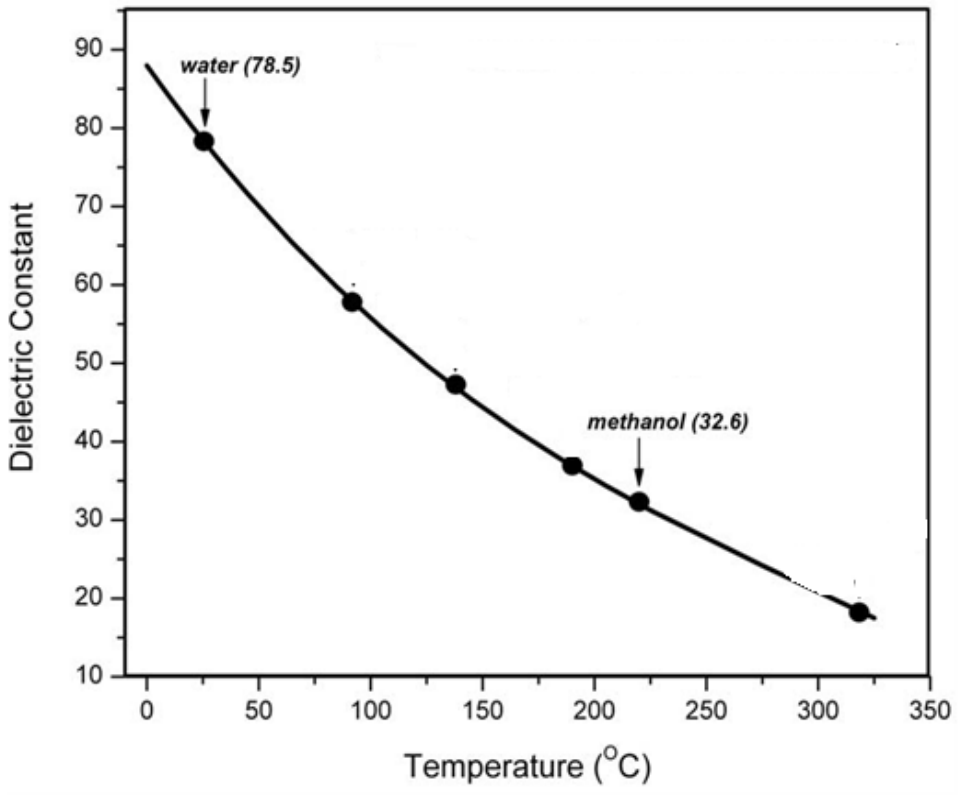

Fig. 2.4. Behavior of water's dielectric constant at 20 MPa (adapted from Amashukeli et al. (2007))

2.5.1. Subcritical water extraction of phenolic compounds

Studies using SCW to extract phenolic compounds from some plants are shown in Table 2.10. The temperature range varies from 100°C to 170°C and product degradation was reported in some cases above 170°C. Kim and Mazza (2006) investigated SCWE of phenolic compounds, including *p*-hydroxybenzaldehyde, vanillic acid, vanillin, acetovanillone and ferulic acid from flaxshive. They found high recovery of total phenolics (5.8 mg/kg, dw) at 230.05°C, flow rate of 0.7 mL/min and high NaOH (0.65 M) concentration. Kim and Mazza (2007) found that there was an increase in internal and external diffusion as flow rate increases from 0.7 to 2 mL/min and obtained maximum concentrations of free form phenolics (5.7 g/kg, dw) and total carbohydrates (26 g/kg, dw) at 230°C and 2 mL/min.

Total phenolics, total flavanols and anthocyanins from the winery residues were extracted with SCW and ethanol (Rodriguez et al., 2007). They obtained three times more anthocyanins (17510 µg/g), seven times more phenolics (126 mg/g) and eleven times more flavanols (35 mg/g) with SCW as compared with SCW + ethanol. Superheated acidified water was also used as an extracting medium but the recovery was very low.

Marino et al. (2006) extracted phenolic compounds from winery by-products with SCWE and compared with MeOH/H_2O (75:25, v/v) extraction. High amounts of total phenolic content (582 mg/100 g) was obtained with subcritical water extraction at 150°C and 10.34 MPa as compared to conventional

MeOH/H_2O extraction (292.7 mg/100 g). King (2003) extracted anthocyanins from dried elderberry seeds and stems by SCWE and compared the results with conventional extraction method. They found higher concentration of anthocyanins (272 µg/g) with SCWE at 160 °C than with ethanol extraction (228 µg/g). Li-Hsun et al. (2004) reported the extraction and fractionation of five isoflavones from defatted soybean by SCW at 110°C, 1-1.5 mL/min and 2-6 MPa. They found the highest yield of total isoflavones (90%) at 110°C, 1.5 mL/min and 4.25 MPa. Xu et al. (2008) demonstrated the use of hot water (30-100°C) for 30-90 min to extract phenolic compounds from citrus peel and reported 90% recovery of phenolics at 100 °C for 90 min. Overall, SCWE is a more effective method to extract high amounts of phenolics than conventional methods.

Table 2.10. SCWE of phenolic compounds

Raw material	Feed (g)	Sample preparation		Bioactive compound	Extraction conditions					Recovery (%)	Reference
		Particle size (mm)	Moisture (%)		T(°C)	P(MPa)	Flow rate (mL/min)	Time (min)	Solid/Solvent Ratio		
Flaxshives	2.5	1-2	n.i	Phenolics	230	5.2	0.5-5	50-200	n.i	100	Kim and Mazza (2007)
Citrous peel	5	Powder	n.i	Phenolics	30-100	n.i	n.i	30-90	n.i	90	Xu et al. (2008)
Wine making residues	n.i	n.i	n.i	Phenolics, flavonols, anthocyanins	120	8	1.2	30	n.i	n.i	Rodriguez et al. (2007)
Flaxshives	0.85	1-2	n.i	Phenolics	180	5.2	0.3-4	10-120	1:180, 1:60	100	Kim and Mazza (2006)
Soybean flakes	180	n.i	n.i	Isoflavons	110	4.3	1-1.5	150	1:10	90-120	Li-Hsun et al. (2004)
Fruit berry	n.i	n.i	7.4-9.3	Anthocyanins	110-160	4	n.i	40	n.i	90	King (2003)

n.i: not indicated

2.5.2. Other SCWE applications

SCWE technique is not limited to phenolic compounds extraction and can be applied to the extraction of a variety of compounds from plant material. A summary of studies investigating SCWE for other bioactive compounds from plant material is provided in Table 2.11. For example, Ueno et al. (2008) extracted pectin by SCW without using chelating agents or acid, as required by the conventional method. They recovered high amounts of pectin from citrus fruits (80%) at 160°C, 20 MPa and a flow rate of 7 mL/min.

Natural antioxidants from boldo leaves were extracted by SC-CO_2 + methanol and hot pressurized water (Del Valle et al., 2005). They found high yield of recovery of boldine from 36.9 % at 100 °C to 53.2 % at 125°C with SCW. But, the amount recovered decreases when temperature increases to 175 °C, while recovery of 50% was obtained with SC-CO_2 at 45 MPa and 50 °C and boldine degraded at 60 MPa at the same temperature. Basile et al. (1998) extracted flavor compounds such as α-pinene, camphene, limonene, camphor, borneal and oxygenates from rosemary leaves. The data indicated high selectivity of this method at 150°C using SCW. Ibanez et al. (2003) found similar yield of antioxidants (carnosol, rosmanol, carnosic acid, methyl carnosate) and flavonoids (cirismaritin and genkwanin) with SCWE at 100, 150 and 200°C, 4-6 MPa and 1 mL/min. They did not report any effect of temperature on the extraction. Shalmashi et al. (2007) reported high yield of extraction of caffeine from 100 to 175 °C. They further investigated that small particle size (0.5 mm) and high flow

rate (4 mL/min) accelerated the process. Ho et al. (2007) extracted lignans, carbohydrates and proteins from flaxseed meal using SCW. The maximum yield of lignans (21 mg/g, dw) was obtained at pH 9 and 170°C. Maximum recovery of carbohydrates (215 mg/g, dw) was found at pH 4 and 150°C. Protein degradation and carbohydrate hydrolysis were relatively low at 160°C than at 190°C. While Kim and Mazza (2006) recovered maximum amounts of lignans and other flaxseed bioactives, including proteins at 160°C. However, they reported that on a dry weight basis, the most concentrated extracts in terms of lignans and other phenolic compounds were extracted at 140°C. Sereewatthanawut et al. (2008) obtained high yield of proteins (219 mg/g) and amino acids (8 mg/g) from deoiled rice bran with SCW extraction at 200°C for 30 min. High antioxidant activity of deoiled rice bran was obtained at 200 °C for 30 min.

Pongnaravane et al. (2006) compared the effectiveness of using SCWE of anthraquinones from *Morinda citrifolia* with other conventional extraction methods, such as ethanol extraction in a stirred vessel, soxhlet extraction and ultrasound-assisted extraction. The results revealed that 96% of anthraquinones were recovered at 200°C in 120 min as compared to soxhlet extraction (93%) at 78 °C for 1 hr and solvent extraction (79-81%) at 25-60 °C for 2-3 hrs. Furthermore, SCW extracts had higher antioxidant activity than ethanol extracts and ultrasound-assisted extracts. Anekpankul et al. (2007) extracted anticancer demnacanthal from *Morinda citrifolia* roots. They also proved that the recovery of demnacanthal was high (0.72 mg/g) at 170°C. However, degradation of demnacanthal (0.227 and 0.197 mg/g, respectively) was observed at 200 and

220 °C. Kubatova et al. (2001) isolated kava lactones from kava root by SCWE. They compared the extraction efficiency with organic solvents. They also demonstrated degradation of lactones at high temperatures. They found high concentration of lactones (104 mg/g) at 170°C which was 40-60 % higher than quantities obtained by soxhlet extraction (48 mg/g) for 6 hr and boiling (57 mg/g) for 2 hrs. Baek et al. (2008) investigated the extraction of glycyrrhetic acid, glycyrrhizin and liquiritin from licorice roots by using SCWE. They also found high content of glycyrrhetic acid and glycyrrhizin at 100°C for 30 and 60 min, while the highest liquiritin content was obtained at 300°C for 60 min. Observations from different authors show that subcritical water can be used to extract bioactive compounds from plants. Recoveries vary depending on the processing conditions as described below.

2.5.3. Effect of process parameters on SCWE

Process parameters for SCWE include, but are not limited to, those presented in Table 2.11 i.e. temperature, pressure and flow rate. These parameters are discussed in the following sections.

2.5.3.1. Effect of temperature

Temperature is the most important factor contributing to increase extraction efficiency in SCWE (King 2002; Shalmashi et al., 2007). An increase in the extraction temperature can promote high solubility and mass transfer rate of bioactive compounds. High temperature also decreases the viscosity and increases the surface tension of the solvent and improves extraction efficiency (Mendiola et al., 2007). Temperature might also cause degradation of compounds (Shotipruk et

al., 2004; Anekpankul et al., 2007; Kubatova et al., 2001). The effect of temperature varies from product to product and depends upon the concentration of the bioactive compound in the related product (Anekpankul et al., 2007). Increasing temperature from 100 to 180°C results in high extraction recovery of compounds such as demnacanthal from *Morinda citrifolia* (Anekpankul et al., 2007), oxygenates from savory and peppermint (Kubatova et al., 2001), anthraquinones (Shotipruk et al., 2004), essential oils from coriander leaves (Ekani et al., 2007) and isoflavones (Li-Hsun et al., 2007). But, further increases in temperature above 180°C might cause destruction of anthocyanins (King, 2003), antioxidant compounds from rosemary (Ibanez et al., 2003) and lactones from kava roots (Kubatova et al., 2001).

At high temperatures above 170°C, pyrolysis of main constituents in plants has also been observed. Ekani et al. (2007) reported the degradation of linalool (essential oil) at 175°C and reported a burning smell at this temperature. However, high linalool content was observed at 125 °C. Temperatures between 100-180°C are optimal temperatures for high extraction recovery of bioactive compounds such as kava lactones from lactones (Kubatova et al., 2001), and other compounds.

2.5.3.2. Effect of pressure

Pressure has a very limited effect on the extraction yield during SCWE (Shalmashi et al., 2007). High pressure prevents the transition of liquid to vapor at temperatures above its boiling point. Therefore, it is necessary to increase the

pressure to avoid the formation of steam that is corrosive and might degrade the extract (Ramos et al. 2002). High pressure also increases the yield of extraction and provides faster extraction (Mendiola et al., 2007).

2.5.3.3. Effect of flow rate

An increase in flow rate results in an increase in superficial velocity. Li-Hsun et al. (2004) extracted high amounts of isoflavones from defatted soy bean oil when the flow rate of water increases from 5 to 10 mL/min. The same effect was observed by Shalmashi et al. (2007) while extracting caffeine from black tea leaves. High recoveries of caffeine were obtained when flow rate increased from 1 to 4 mL/min (Table 2.11). The extraction yield of anthraquinones was high at flow rate of 2 mL/min (Table 2.11) (Shotipurk et al., 2004). Therefore, a high flow rate does not always accelerate the mass transfer and it depends upon internal structure of food matrix.

Table 2.11: SCWE of other bioactive compounds

Raw material	Feed (g)	Sample preparation		Bioactive compound	Extraction conditions					Recovery (%)	Reference
		Particle size (mm)	Moisture (%)		T(°C)	P(MPa)	Flow rate (mL/min)	Time (min)	Solid/Solvent Ratio		
Boldo leaves	1	n.i	n.i	Boldine (oils)	100-175	n.i	2-4	180	1:10	n.i	Del Valle et al. (2005)
Rosemary	1	n.i	n.i	Antioxidants	25-200	6-7	1	30	n.i	48.6	Ibanez et al. (2003)
Tea leaves	5	0.5-2	n.i	Caffeine	100-175	2	1-4	120 ,180	1:48	3.7 % (dw)	Shalmashi et al. (2007)
Flaxseed meal	1	n.i	n.i	Lignans	130-190	n.i	0.6 -7.4	n.i	1:77-150	n.i	Kim and Mazza (2007)

n.i: not indicated

Table 2.11: SCWE of bioactive compounds (Continued)

Raw material	Feed (g)	Sample preparation		Bioactive compound	Extraction conditions					Recovery (%)	Reference
		Particle size (mm)	Moisture (%)		T(°C)	P(MPa)	Flow rate (mL/min)	Time (min)	Solid/Solvent Ratio		
De-oiled[a] rice bran	1	n.i	10	Proteins, Aminoacids	100-220	0.1-3.97	n.i	5-30	1:5	n.i	Sereewatthanawut et al. (2008)
Morinda citrifolia	0.5	n.i	n.i	Anthraquinones	150-200	4	2-6	n.i	0.5	n.i	Pongnaravane et al. (2006)
Morinda citrifolia	1	n.i	n.i	Demnacanthal	150-220	4	4	200	n.i	90	Anekpankul et al. (2007)
Kawa root	0.5	2-4	n.i	Lactones	100-175	6-7	1	10-120	n.i	100	Kubatova et al. (2001)
Coriander seeds	4	0.25-1	n.i	Essential oils	100-175	2	1-4	20	n.i	n.i	Eikani et al. (2007)
Morinda citrifolia		5	20	Anthraquinones	110 – 220	7	2 -6	180	n.i	n.i	Shortipruk *et* al. (2004)

[a]hydrolysis, n.i. not indicated

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3. EXTRACTION OF PHENOLICS FROM POTATO PEEL

3.1. Introduction

Potato is one of the major staple foods of human diet that grows in more than 100 countries (Leo et al., 2008)**.** After potato processing, a large amount of waste is generated in the form of peels and trimmings, causing handling and storage problems. But potato peel is rich in carbohydrates (12 g/100g, fw), protein (2.56 g/100g, fw), ash (1.6 g/100g, fw) and lipid (0.1g/100g, fw) (USDA, 2008). In addition, potato peel contains phenolic compounds (37-125 mg/100 g, dw), which exhibit antimutagenic, anticarcinogenic, antiglycemic, anticholesterol and antimicrobial properties (Friedman and Levin, 2009; Im et al., 2008) that avoid neurodegenerative and cardiovascular diseases (Nandutu et al., 2007; Hang et al., 2004). Phenolic compounds also possess antioxidant properties, which prevent oxidation of food containing high amounts of lipids (Andrich et al., 1999). Phenolics are observed more in the peel and adhesive tissues than in the tuber (Weshahy and Rao, 2009).

Phenolic compounds are present in both free and bound forms. GAC, CGA, CFA and PCA are the major phenolic compounds reported in potato peel (Rodriguez de Sotillo et al., 1994).

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For the extraction of phenolic compounds, conventional methods have been described in the literature based on solid-liquid extraction using organic solvents (Rodriguez de Sotillo et al., 1994; Kannat et al., 2005; Friedman and Levin, 2009; Im et al., 2008; Weshahy and Rao, 2009; Mattila and Kumpulainen, 2002 and Nara et al., 2006). Mohdaly et al. (2009) reported high yield of phenolic compounds from potato peel and with methanol extraction, which is a toxic solvent. Moreover, these techniques require a long extraction time and result in low yields of extraction.

To avoid the use of chemical solvents, Andrich et al. (1999) extracted phenolic compounds from potatoes with supercritical CO_2 using a co-solvent of 50% ethanol or pure ethanol at 30 and 50 MPa and 50 and 80°C. The authors recovered high amounts of phenolics with pure ethanol (10.93 mg/g) at 50 MPa and 80°C as compared to a mixture of supercritical CO_2 and ethanol (50%) at the same conditions (1.75 mg/g). Moreover, pure CO_2 due to its non polar characteristics is a poor solvent for the extraction of phenolics even in the supercritical state (Andrich et al., 1999). Okuno et al. (2002) extracted antioxidants such as β-carotene and α-tocopherol from sweet potato waste powder with supercritical CO_2 at 10-35 MPa and 40-80°C for 2 hrs. These authors reported low recoveries of antioxidants (5%) at high processing conditions (35 MPa and 80°C) compared to 69% at low processing conditions (10 MPa and 40°C). Furthermore, they reported that a temperature of 40°C or 60°C has the same effect on the recoveries of antioxidants.

Another attractive alternative to obtain phenolic compounds is the use of subcritical water (SCW) extraction. SCW technology uses water at temperatures between 100-374°C and enough pressure to maintain water in the liquid state. The critical temperature and pressure of water are 374°C and 22.1 MPa, respectively (Ramos et al., 2002; King, 2003).

Under subcritical conditions, the intermolecular hydrogen bonds of water break down. The dielectric constant, viscosity and density of water decrease and SCW behaves like solvents such as ethanol, methanol or acetonitrile. SCW acts as a low polar solvent in which low polar compounds become completely soluble (He et al., 2008).

Recently, SCW has been gaining worldwide attention as a promising technique for the selective and efficient extraction of bioactive compounds from plant materials. SCW has been used for the extraction of anticancer damnacanthal from *Morinda citrifolia* (Anekpankul et al., 2007). It has also been used to extract flavonoids, phenolic acids and anthocyanins from red grape skins (Rodriguez et al., 2007), nutraceuticals such as glycyrrhetic acid, glycyrrhizin and liquiritin from licorice roots (Baek et al., 2008), antioxidants from rosemary oil (Basile et al., 1998) and isoflavones from soybean flakes (Li-Hsun et al., 2002). Ho et al. (2007) extracted lignans, proteins and carbohydrates from flaxseed meal, while Kim and Mazza (2006) extracted phenolic compounds from flax shives using SCW. But, there are no reports on the use of subcritical water for the extraction of phenolics from potato peels. Therefore, the main objective of this study was to

extract phenolic acids from potato peel using SCWE and compared it to conventional solvent extraction.

3.2. Material and Methods

3.2.1. Materials

Three different varieties of potato peel were used in this study. Potato, variety *Red* was purchased from a local supermarket (Edmonton, AB, Canada). *Red Norland* potato peel was obtained from a company in Lacombe, AB, Canada and *Russet Burbank* potato peel was obtained from a company in Calgary, AB, Canada. All phenolic compound standards (with purity ≥ 96%) were obtained from Sigma Aldrich (St. Louis, MO). Organic solvents such as methanol and ethanol were obtained from Fisher Scientific (Fair Lawn, NJ).

3.2.2. Sample preparation

Potato variety *Red* was washed and peeled manually with a knife. Other varieties such as *Red Norland* and *Russet Burbank* were directly obtained from the companies in peeled form. The peel of each potato variety was freeze dried for 3 days using a Vertis freeze drier (Gardiner, NY). The dried sample was then ground in a Fritsch cutting mill, Model #14-4050 (Idar-Oberstein, Rhineland, Germany) using a 0.5 μm sieve size. The ground potato peel was packed and sealed in Uline Ziploc bags (Edmonton, AB, Canada) and stored in a freezer at -18°C.

3.2.3. Proximate composition analysis of potato peel samples

3.2.3.1. Moisture content

Moisture content of the potato peel was determined gravimetrically following the AOAC methodology (AOAC, 2000). Aluminum dishes were pre-dried in an oven at 100°C for 6 hours and cooled to ambient temperature in a dessicator (Fisher Scientific, Ontario, Canada). Then, the aluminum dishes and lids were weighed to 4 decimal places. Two grams of potato peel sample of varieties *Russet Burbank, Red Norland* and *Red* were accurately weighed in the dish using an analytical balance and spread evenly throughout the dish using a spatula. The total mass was recorded. Samples were dried in a pre-heated oven at 105°C for 4 hours. Then, samples were cooled in a dessicator for 1 hour and weighed. Moisture content was determined using the following equation:

$$\% \text{ (w/w) Moisture} = \frac{WSDL1 - WSDL2}{WSDL1 - d} \times 100 \quad \ldots\ldots\ldots\ldots\ldots (3.1)$$

where:

WSDL1 = weight of sample + dish + lid before drying, in g

WSDL2 = weight if sample + dish + lid after drying, in g

d = weight of dry dish + lid, in g

All samples were analyzed in duplicate.

3.2.3.2. Ash content

The ash content of the potato peel was determined according to AOAC Method 923.03 (AOAC, 2000). One gram of potato peel sample from each variety was weighed into pre-weighed porcelain crucibles using an analytical balance. Then, porcelain crucibles were transferred into the muffle furnace Model F-A1730 (Thermolyne Corporation, Dubuque, IA) set at 550°C for 15 hours. Following ashing, crucibles were cooled in the dessicator for 1 hr. Total ash content of the potato peel was determined using the following equation:

$$\% \text{ (w/w) ash} = \frac{WCS - WC}{WS} \times 100 \quad \text{..... (3.2)}$$

where:

WCS = weight of crucible + sample, in g

WC = weight of crucible, in g

S = weight of sample, in g

3.2.3.3. Lipid content

Approximately two grams of dry ground sample was transferred onto a 150 mm filter paper (Whatman No. 4) and placed inside the extraction thimble (Whatman International Ltd., Maidstone, Kent, UK) and covered with glass wool (Supelco Inc., Bellegonte, PA). The extraction beakers were weighed and 40 mL of petroleum ether was placed into it. A blank sample was also prepared and run by using 40 mL of petroleum ether throughout the entire extraction process. All samples were then placed in the sample holders and attached to clamps in the condenser units. An extraction beaker containing ether as an extraction solvent

was attached by tightening the beaker ring clamp. Water condenser was turned on. The extraction was performed by applying heat to the extraction beakers for 4-6 hours. Once the extraction was completed, the extraction beaker was dried in an oven (Despatch Oven Co., Minneapolis, MN) at 110-120°C for 30 min and cooled at room temperature. The extraction beaker was weighed. The total lipid content was calculated as follows:

$$\% \text{ (w/w) Lipid} = \frac{(WB+WE-WBR) - (WB)}{WS} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.3)$$

where:

WB= weight of beaker, in g

WE= weight of extract, in g

WBR= weight of blank residue, in g

WS= weight of sample, in g

3.2.3.4. Protein content

The estimation of the protein content of the potato peel was carried out using a Leco nitrogen analyzer Model FP-428 (Leco instruments Ltd., Mississauga, ON, Canada). Freeze dried potato peel sample (0.16 g) was transferred into an aluminum foil cone and inserted into the Leco analyzer sample port. The Leco analyzer was calibrated with caffeine standards, each weighing 0.16 g. The protein was calculated using the following equation:

$$\% \text{ (w/w) protein} = (N/S \times 100) \times 6.25 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.4)0$$

where:

N = weight of nitrogen in the sample, in g

S = weight of the dry sample, in g

3.2.3.5. Carbohydrate content

The carbohydrate content was calculated by difference based on 100% minus the sum of moisture, ash, protein and lipid contents.

3.2.3.6. Total phenolics

The total phenolic content was measured by the Folin-Ciocalteau (F-C) method proposed by Singleton and Rossi (1965). For the extraction of phenolic compounds, see section 3.2.4 and 3.2.5.6. A sample of 0.1 mL was mixed with 7.9 mL of water. Then, F-C reagent (0.5 mL) was added and allowed to stand for 5 min. Sodium carbonate (20% w/v; 1.5 mL) was then added to the mixture. After shaking, the mixture was incubated for 90 min. The total phenolic content was determined using a gallic acid standard calibration curve. A stock solution of 5g/L of gallic acid was prepared by dissolving 0.5 g of gallic acid in 100 mL of distilled water. Different concentrations of standards in the range of 50-750 mg/L were prepared by diluting the stock solution in distilled water. The absorbance of all standards was measured at 765 nm using a spectrophotometer (Genova MK3, New Malden, Surrey, UK). The calibration curve was constructed by plotting the absorbance of different standards versus the standard concentration. The concentration of phenolics in the samples were calculated using the calibration curve equation, which had a correlation coefficient greater than 0.995. All

samples were analyzed in duplicate and final results were expressed as milligrams of gallic acid equivalents per 100 g of potato peel.

3.2.4. Conventional solvent extraction of phenolics

Freeze dried potato peel (50 g) was extracted with 100 mL of methanol (≥ 98% purity) and 50% ethanol (≥ 98% purity) at 65°C for 1 hr. The slurry was filtered under light vacuum through filter paper Whatman No. 4, and the solid residue was re-extracted twice under the same conditions. The extracts were pooled and the volume made up to 300 mL with the same solvent. The extracts were centrifuged at 6000 rpm for 15 min and then concentrated in a rotary evaporator at 40°C. The dried samples were redissolved with 10 mL of methanol or 10 mL of 50% ethanol, respectively. The solution was then centrifuged at 10000 rpm for 10 min and passed through a 0.45 µm nylon filter prior to HPLC analysis. Ethanol and methanol extractions were performed in duplicates.

3.2.5. Subcritical water extraction of phenolics

First, the SCWE system was set up to carry out extractions using subcritical water. Earlier, King et al. (2003) set up a subcritical water system in which water was pumped by an HPLC pump to the extraction cell containing raw material by an intermediate step, where water was preheated at 100°C. Then, it was transferred to the extraction cell, which was mounted in a heated oven and subcritical water conditions were applied. The extract then passed through a water bath to cool. After the cooling section, a micrometering valve was installed to

control the amount of sample collected in a sample vial. A similar set up was used in this study, which is discussed below.

3.2.5.1. Design of the extraction cell

The extraction cell (Fig. 3.1) is one of the main components of a SCWE system. The extraction cell, designed for a maximum pressure of 34.5 MPa and a temperature of 500°C, was purchased from Swagelok (Edmonton, AB, Canada). The volume of the extraction cell is 90 mL and it can be loaded with 10 g of sample. Glass wool was used at both ends of the extraction cell to prevent clogging of the outlet tubing. At both openings of the cell, frits (5 μm) were also installed to prevent blockage of the inlet and outlet tubing with sample. To connect the extraction cell with the tubing, an adapter was used (Fig. 3.1). A heating bracket surrounds the extraction cell and can attain maximum temperature up to 500°C. A type K thermocouple was placed between the extraction cell and the heating brackets. The temperature of the extraction cell was measured and controlled by a temperature controller.

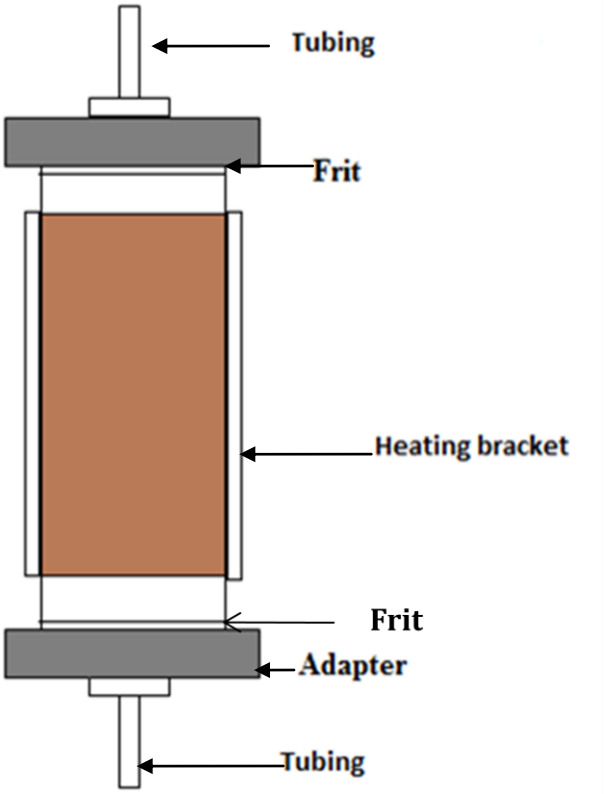

Fig. 3.1. Design of the extraction cell

3.2.5.2. Design of the pre-heater

In the SCWE system, water was preheated (120°C) to reduce the time to achieve the subcritical condition in the extraction cell. The pre-heater mainly consists of a stainless steel rod, tubing (1/16”) and a heating bracket. A long stainless steel rod (8” L x 1.5” W) was obtained from Steel market (Edmonton, AB, Canada). Tubing (1/8”) was coiled around the stainless steel rod (8” L x 1.5”o.d.). Heating brackets (8"L x 1.75" W) were clamped on stainless steel rod. In between the heating bracket and tubing, a type K thermocouple was placed and connected to the temperature controller.

3.2.5.3. Set up of the SCWE system

The SCWE system (Fig. 3.2) consists of an HPLC pump (Gilson 305, Villiers-le-Bel, France) connected to a pre-heater and an extraction cell. Both extraction cell and preheater were surrounded by heating brackets (Valax, Burnaby, BC, Canada). The temperature of the preheater and the extraction cell was controlled by the temperature controller (Omega, Montreal, QC, Canada). Pressure of the extraction cell was measured by a pressure gauge (Swagelok, Edmonton, AB, Canada) connected on the top of the extraction cell. After the pressure gauge, a safety head was installed to avoid the risk of high pressure attained by the extraction cell above pressure limits. The pressure range of the safety head was set to 34.5 MPa by adjusting the rings on the safety head assembly. The outlet of the extraction cell was connected to a cooling system (Swagelok, Edmonton, AB, Canada), which lowers the temperature and avoids

degradation of the extract. The cooling system mainly consists of a hollow cylinder in which cold water was circulated. The tubing containing the extract after the extraction cell was passed through the cooler. After the cooling system, a needle valve (Swagelok, Edmonton, AB, Canada) and a micrometering valve (Autoclave, Erie, PA) were connected to control the amount of sample flow to the collection vial. The micrometering valve was later changed with a back pressure regulator (Tescom, Elk River, MN). All parts used in the SCW system are described in Table 3.1.

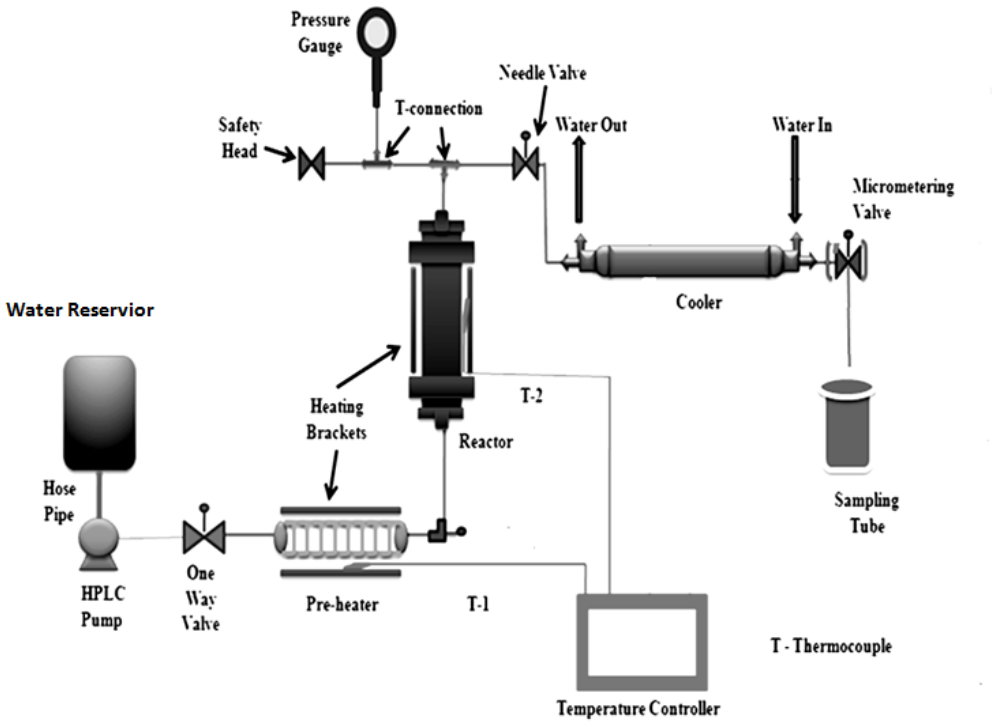

Fig. 3.2. SCWE system (T-1: pre-heater thermocouple; T-2: extractor thermocouple).

Table 3.1. Specifications of different parts used in the SCWE system

Part	Specification	Manufacturer
HPLC pump	Flow rate (0.5 - 10 mL/min)	Gilson, Roissy, Villiers-le-Bel, France
Extraction cell	0.75” i.d. x 1”o.d. x 9.5” L	Swagelok, Edmonton, AB, Canada
Preheater	Temperature up to 350°C, 8" L x 1.75" W, 120 VAC, 700 W, 6A	Valax, Burnaby, BC, Canada
Extraction heater	Temperature up to 500°C, 6" L x 1" W, 120 VAC, 450 W, 3A	Valax, Burnaby, BC, Canada
Thermocouple	Type K, 1/16", 316 SS, 0-1260°C	Wika, Edmonton, AB, Canada
Temperature controller	6 zone, RS-232 digitial communication	Omega, Montreal, QC, Canada
Cooling system	Volume (250 mL)	Swagelok, Edmonton, AB, Canada
Pressure gauge	0-69 MPa, 200°C, 0-69 MPa, 100°C	Swagelok, Edmonton, AB, Canada
Back pressure regulator	1.4 - 69 MPa	Tescom, Elk-River, MN

Table 3.1. Specifications of different parts used in the SCWE system (Continued)

Part	Specification	Manufacturer
Safety head assembly	34.5 MPa	Autoclave, Erie, PA
Inline filter	5 µm pore size	Swagelok, AB, Edmonton, Canada
Needle valve	69 MPa	Swagelok, Edmonton, AB, Canada
Micrometering valve	69 MPa	Autoclave, Erie, PA
Preheater rod	8" L x 1.5" W	Stainless steel market, Edmonton, AB, Canada
Tubing, T connections and reducers	1/8", 1/4" to 1/8"	Swagelok, Edmonton, AB, Canada

3.2.5.4. System specifications

The SCWE system was set up at Agri Food Discovery Place (Edmonton, Canada). Several trial runs with ethanol, water and sample were conducted at different conditions of temperature and pressure. A trial run with a maximum pressure of 10 MPa and a temperature of 374°C was successfully attempted at flow rates of 2, 4 and 6 mL/min. This system can operate up to 34.5 MPa, 500°C, and 0.5-10 mL/min (Table 3.2). In most of the literature of SCWE, temperatures of 100-240°C, pressures of 2-7 MPa, flow rates of 1-6 mL/min and time of 5-200 min were used to extract bioactive compounds from plant materials (see Table 2.11).

Some problems were faced during the trial runs. There were some fluctuations observed in the pressure during the extraction process that could not be controlled by the micrometering valve. The other challenge was that potato peel contains high amount of carbohydrate content and the carbohydrate content from the sample was plugged in the 1/8" tubing at the outlet of the extraction cell. Therefore, the system was further modified.

Table 3.2. SCWE system specifications

Conditions	Maximum operating conditions	Operating conditions (Literature)[a]	Operating conditions (This study)
Temperature (°C)	500	100-240	100-240
Pressure (MPa)	34.5	2-7	6
Flow rate (mL/min)	0.5-10	2-8	2
Time (min)	n.a	5-200	10-120

[a] refer to Table 2.11, n.a: not applicable.

3.2.5.5. System modifications

To maintain a contant pressure, King et al (2003) used a micrometering valve, which was unsuccessful in this study. Basile et al. (1998) also used only a needle valve at the outlet of the extraction cell. But, instead of using a micrometering valve, other researchers used a back pressure regulator (Shalmashi et al., 2007; Anekpankul et al., 2007; Ong et al., 2006; Shotipruk et al., 2004). Therefore to avoid pressure fluctuations, the micrometering valve was replaced with a back pressure regulator (Fig. 3.3).

After the cooling section, temperature decreases. This might lead to change in pressure or pressure fluctuation in the system. To observe the effect of pressure upon cooling, another pressure gauge was installed (Fig. 3.3). It was observed that there was no change in pressure reading before and after the cooling system. Another problem faced was clogging of solute in the outlet 1/8" tubing. Therefore, this tubing was substituted with ¼" tubing and a filter (5 μm) was also installed (Fig 3.3).

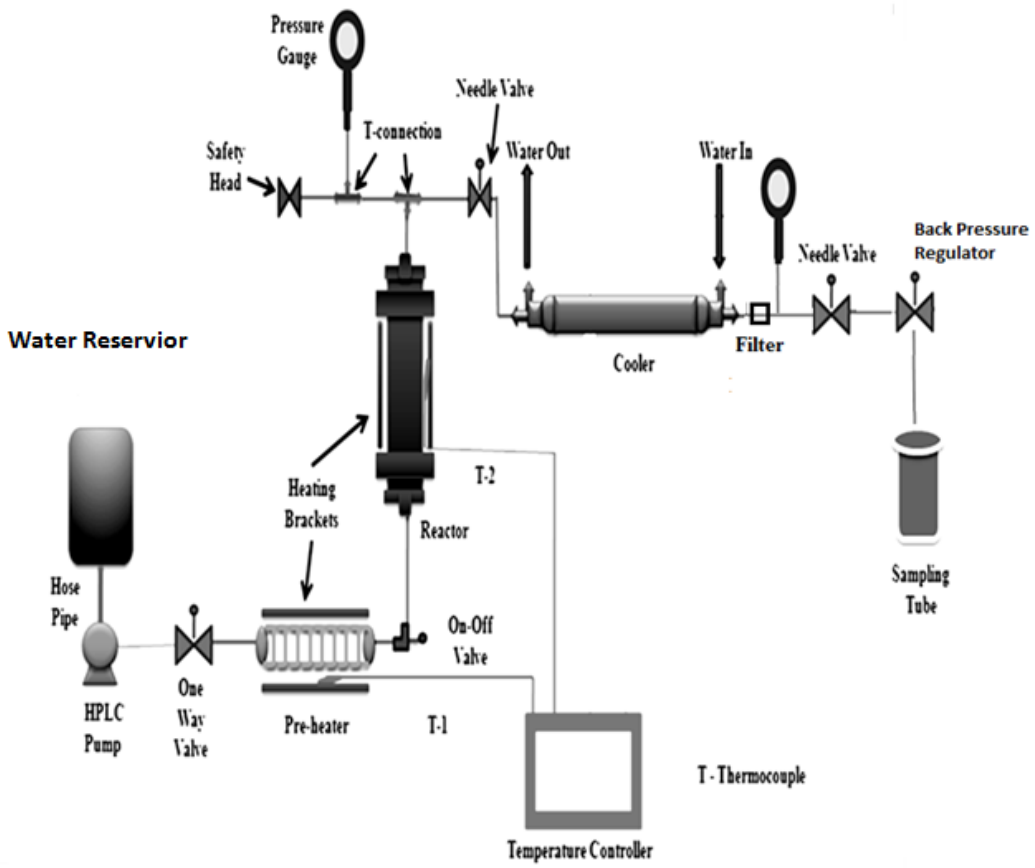

Fig. 3.3. Modifications in the SCWE system (T-1: pre-heater thermocouple; T-2: extractor thermocouple)

3.2.5.6. SCWE of phenolics from potato peel

Freeze dried potato peel (10 g) dissolved in 45 mL of water was loaded into the reactor. To prevent plugging, glass wool (10 mm thick) was placed at both ends of the extraction vessel.

In a typical experiment, distilled water was first degassed and then delivered with the HPLC pump at a constant flow rate (2 mL/min) to the preheating section. Then, it was passed through the extraction vessel preloaded with the potato peel. The pressure of the system was adjusted to 6 MPa by using the back-pressure regulator. The temperature of the system was monitored by a temperature controller (Omega Engineering, Stamford, CT). After the extraction cell, the extract passed through a cooling system using cold water to prevent degradation. The extraction was carried out for 120 min and the samples were collected in vials every 30 min. Extractions were carried out at temperatures of 100-240°C, at a constant pressure of 6 MPa and using a constant flow rate of 2 mL/min. The residue left after each extraction was re-extracted with 10 mL of methanol and extracted for 1 hr. All extractions were performed in duplicates. The extracts were stored at 4°C for further analysis of total phenolics by using the F-C method and of individual phenolic composition by using HPLC. At the end of each experiment, ethyl alcohol was used to clean the SCWE unit by pumping it throughout the system.

3.2.6. Phenolic compound analysis

HPLC analysis was performed using a Varian Prostar HPLC system (Varian, Palo Alto, CA) equipped with a 401 model autosampler, pumps, and a UV model 1305 detector. The column used was Luna RP-18 (150 mm x 4.6 mm i.d. x 5 μm) with a Phenomenex security guard column C18 (4 mm x 3 mm) (Phenomenex, Irvine, CA).

The HPLC methodology adapted from Pellati et al. (2005) was modified for the quantification of phenolic compounds from potato peel. The mobile phase consisted of: (A) Formic acid (0.5%; v/v) in water, and (B) Formic acid (0.5%, v/v) in methanol. The elution profile consisted of an eight step linear gradient using formic acid 0.5% in water and methanol, rising from 16 to 19% of (B) in 15 min, 19 to 27% of (B) in 25 min, 27 to 41% of (B) in 26 min, followed by further increase to 65% of (B) in 36 min to 100% of (B) in 44 min and then back to the initial concentration in 45 min. The total run time was 50 min and the volume injected was 10 μl. The flow rate was 1 mL/min. The phenolic compounds were detected at 280 nm.

Phenolic compounds including chlorogenic acid (CGA), caffeic acid (CFA), gallic acid (GAC), protocatechuic acid (PCA), coumaric acid (CMA), ferulic acid (FRA), syringic acid (SGA), vanillic acid (VNA), *p*-hydroxy benzoic acid (PBA) were identified and quantified.

3.3 Results and discussion

3.3.1. Compositional analysis of potato peel

The compositional analysis of three varieties of potato peel is reported in Table 3.3. The potato peel contains high moisture content (85-90%) and carbohydrate content (8-11%). The protein content of potato peel varies from 0.6-2.2 %, while fat content was found to be very low (0.02-0.4%).

According to USDA (2008), potato tuber and peel typically contain approximately 83% moisture content, 2.6% protein, 0.1% lipid, 1.6% ash and 12% carbohydrate by difference. These contents are quite similar to the results obtained in this study and are in agreement with results reported by Augustin et al. (1979).

A big variation was found in the phenolic content of the three varieties (Table 3.3). High content of total phenolics was found in potato peel variety *Red* (55.7 mg/100 g, fw) than *Russet* (2.2 mg/100 g, fw) and *Red Norland* (2.7 mg/100 g, fw). On the basis of these results (i.e. the low amounts of phenolics recovered from *Red Norland* and *Russet Burbank* potato peel), further studies on phenolic compounds with SCWE was only performed with *Red* variety.

Table 3.3. Compositional analysis of potato peel

	This work			Augustin et al. (1979)	USDA (2008)
	Red	***Red Norland***	***Russet Burbank***		
Moisture, %	87.7 ± 0.29	90.1 ± 0.79	84.7 ± 0.37	79.0	83.29
Ash, %	1.7 ± 0.01	1.2 ± 0.05	1.9 ± 0.02	2.5	1.61
Protein, %	2.2 ± 0.06	0.6 ± 0.01	1.7 ± 0.02	1.8	2.57
Fat , %	0.01 ± 0.00	0.03 ± 0.00	0.4 ± 0.09	n.i	0.10
Carbohydrate (by difference), %	8.4 ± 0.36	8.0 ± 0.85	11.0 ± 0.50	12.7	12.44
Total phenolics[a]	55.7 ± 0.67	2.7 ± 0.12	2.2 ± 0.25	n.i	n.i

[a] mg gallic acid equivalents per 100 gram of potato peel, n.i: not indicated

3.3.2. Conventional solvent extraction of phenolics

Phenolic compounds were extracted from *Red* potato peel with methanol and with 50% ethanol. Higher amounts of phenolics were obtained with methanol (46.59 mg/100 g, fw) than with ethanol 50% (29.52 mg/100 g, fw). These results are in agreement with data reported by Rodriguez de Sotillo et al. (1994) and Im et al. (2008) (See Table 3.5). Mohdaly et al. (2010) investigated solubility of phenolic compounds in different organic solvents and observed a high solubility of low polar phenolic compounds in methanol (2.91 mg/g, dw) than in ethanol (2.74 mg/g, dw). They also reported the high extraction yield of phenolics from potato peel with methanol as compared to ethanol, acetone, hexane, diethyl ether and petroleum ether.

Previous studies reported in Table 3.4 showed that methanol was the best extraction solvent to obtain phenolic compounds from potato peel. For example, Sotilo et al. (1994) obtained high amount of total phenolics (41.56 mg/100 g, fw) with methanolic extraction compared to the study of Im et al. (2008) that used ethanolic extraction (7-10 mg/100 g, fw). However, as observed in Table 3.4, the phenolic content in potato peel might vary with color and potato variety. Weshahy and Rao (2009) studied six potato peel varieties, finding high phenolic contents in *red Siècle* variety (333 mg/100 g, dw) and *Purple majesty* variety (2.96 mg/100 g, dw) compared to *Yukon gold* variety (1.51 mg/ 100 g, dw). Im et al. (2008) also observed high amounts of phenolic compounds in red and purple colored peel compared to yellow and brown potato peel. Overall, the

results in Table 3.4 showed different phenolic compounds obtained from potato peel and different amounts of phenolics extracted.

In this study, HPLC analysis was performed to identify different phenolic compounds from potato peel. Two major phenolic compounds (CGA: 28.55 mg/100 g and CFA: 12.22 mg/100 g) from potato peel were obtained using pure methanol. Other phenolics (PCA, GAC, SGA, CMA and FRA) reported in Table 3.5 were also obtained in low quantities. Furthermore, recovery of gallic acid was low with either methanol extraction (0.46 mg/100 g, dw) or ethanol extraction (0.60 mg/100 g, dw). This is in agreement with findings of Weshahy and Rao (2009) that only reported CGA (86-279 mg/100 g, dw) and CFA (26-72 mg/100 g, dw) from six Canadian varieties of potato peel. But, other studies (Sotilo et al., 1994; Oneyeneho & Hettiarachchy, 1993) found a considerable amount of gallic acid (58-63 mg/100 g, dw) in potato peel using water and methanol as extraction solvents. This might be explained as the presence of individual phenolic compounds depends on the variety, the cultivar and might vary from country to country. In general, conventional extraction methods are expensive as they require solvents, which are expensive. They also require long time of extraction and high energy consumption to recover pure phenolic compounds. Moreover, organic solvents such as methanol are toxic and less acceptable by consumers.

Table 3.4. Total phenolic content obtained from various potato peel varieties.

Potato variety	Extraction solvent	Phenolic compound	Total phenolics (mg/100 g)	Reference
Siecle, vivaladi, Yukon, Purple, Majesty and Dakota pearl	Methanol	CGA and CFA	151-333 (dw)	Weshahy and Rao (2009)
Kennebec, Norchip, Russet Burbank, Red Norland, Red Pontiac and Viking	Methanol (95%)	GAC, PCA, VNA, CFA, CGA, PBA, CMA and FRA	48-821 (dw)	Onyeneho et al. (1993)
Sieglinde Frances, Nicole, ISC1 4052 and ISCI 67	Methanol (80 %)	CGA, CFA, CMA and FRA	221-427 (dw)	Leo et al. (2008)
Sumi	Ethanol (80%)	CGA and CFA	7-10 (fw)	Im et al. (2008)
Kufri chandermukhi	Ethanol	CGA	70 (fw)	Kannat et al. (2005)
Industry sample (n.i.)	Methanol	GAC, PCA, CGA and CFA	42 (fw)	Rodriguez de Sotillo et al. (1994)
Red	Methanol	CGA, CFA, PCA, GAC, SGA, CMA and FRA	47 (fw)	This study
Red	Ethanol (50 %)	CGA, CFA, PCA, GAC, SGA, CMA and FRA	34 (fw)	This study

fw: fresh weight; dw: dry weight; n.i. not indicated; CGA: Chlorogenic acid; CFA: Caffeic acid; GAC: Gallic acid; PCA: Protocatechuic acid; CMA: Coumaric acid; FRA: Ferulic acid; SGA: Syringic acid; VNA: vanillic acid; PBA: *p*-hydroxy benzoic acid.

Table 3.5. Phenolic compounds extracted with organic solvents.

Phenolic compound	Yield (mg/100 g, fw)[a]	
	Methanol	**Ethanol (50%)**
Chlorogenic acid (CGA)	28.55 ± 2.47	8.67 ± 0.62
Caffeic acid (CFA)	12.22 ± 0.16	5.18 ± 0.39
Protocatechuic acid (PCA)	1.90 ± 0.01	1.97 ± 0.04
Gallic acid (GAC)	0.46 ± 0.03	0.60 ± 0.01
Syringic acid (SGA)	0.85 ± 0.01	1.70 ± 0.02
Coumaric acid (CMA)	0.83 ± 0.01	6.50 ± 0.02
Ferulic acid (FRA)	1.50 ± 0.02	4.90 ± 0.01
Total (expressed as mg/100 g, fw)	46.59 ± 2.71	29.52 ± 1.11

[a]Values are means of at least two determinations ± standard deviation

3.3.3 Subcritical water extraction of phenolics

3.3.3.1 Effect of temperature

Temperature is a critical parameter in the subcritical water extraction process (Shalmashi et al., 2007). In this study, the release of phenolic compounds from potato peel can be observed in the color of the extracts as a function of temperature. Figs. 3.4a and 3.4b show the color of the extracts and residues obtained from potato peel extraction with subcritical water at a pressure of 6 MPa and temperatures of 100-240°C. At 100 °C, a very light brown color of the extract was seen. As temperature increases from 100 to 120°C, the color of the extract becomes darker (Fig. 3.4 a), while the color of the residue becomes lighter (Fig. 3.4 b). At high temperatures (140-180°C), high concentration of phenolic compounds were recovered and color of the extract was found to be darker (Fig. 3.4 a) than at lower extraction temperatures. But, at temperatures of 180-240°C, the color of the extract becomes quite dark.

Sample pyrolysis above 180°C results in degradation of phenolic compounds. Also, as stated earlier by Pitchard and Adam (1994), reactions of reducing sugars with amino acids (Maillard reaction) at high temperatures occur. Thomas (1981) observed that the color of cooked potato water was darker due to the high phenolic compound present than in pre-peeled cooked potato water. During cooking of potatoes in boiling water, phenolic compounds percolate to the cooking water and contribute to the dark color.

100°C 120°C 140°C 160°C 180°C 200°C 220°C 240°C

Fig. 3.4a. Color of extracts obtained at 6MPa, 100-240°C and 30 min

100°C 120°C 140°C 160°C 180°C 200°C 220°C 240°C

Fig. 3.4b. Color of residues obtained at 6MPa, 100-240°C and 30 min

Table 3.6 shows data for the extraction of phenolic compounds using subcritical water at 6 MPa and 100-240°C with a constant flow rate of 2 mL/min for 30-120 min. The use of subcritical water extraction resulted in higher amounts of phenolic (81.23 mg/100 g, fw) than the amounts obtained with methanol extraction (49.59 mg/100 g, fw) or with ethanol extraction (33.6 mg/100 g, fw). Furthermore, using subcritical water, six different phenolic compounds were identified by HPLC (Table 3.6 and Fig. 3.8 b). Phenolic compounds such as GAC (29.56 mg/100 g, fw) was extracted using subcritical water at 180°C but it was not detected using the conventional solvent extraction method at 65°C for 3 hr (Fig. 3.8 a). Rodriguez de Sotillo et al. (1994) obtained higher amounts of PCA from potato peel using water at 100°C (3.77 mg/100 g, fw) relative to water at 25°C (0.78 mg/100 g, fw). In this study, at temperatures of 100-180°C, the bound form PCA (13.58 mg/100 g, fw) and PBA (11.18 mg/100 g, fw) were also obtained. Above 180°C, degradation of these bound form phenolics was observed.

As the extraction temperature increases from 100°C to 180°C (Table 3.6), the yield of phenolic compound also increases. At 180°C, the highest recovery of phenolic compound (81.23 mg/100 g; fw) was obtained. Overall, the results show that extraction yield increases as the temperature increases as a result of increased solubility of phenolics in water. Above 180°C, degradation of phenolic compounds was observed.

Table 3.6. Phenolic compounds extracted with subcritical water at 6 MPa, 2 mL/min and different temperatures.

Phenolic compound	Yield (mg/100 g)[a]							
	100°C	120°C	140°C	160°C	180°C	200°C	220°C	240°C
CGA	6.11 ± 0.26	12.11 ± 0.97	15.00 ± 0.75	15.75 ± 0.93	14.59 ± 0.98	11.21 ± 0.33	6.09 ± 0.1	6.15 ± 0.6
CFA	6.15 ± 0.49	8.98 ± 0.33	8.63 ± 0.63	8.73 ± 0.45	9.23 ± 0.73	5.83 ± 0.12	2.13 ± 0.06	2.1 ± 0.01
GAC	7.23 ± 0.97	11.17 ± 0.65	18.30 ± 0.97	20.80 ± 1.13	29.56 ± 0.79	25.11 ± 1.59	19.87 ± 1.15	17.27 ± 1.19
PCA	1.16 ± 0.07	3.31 ± 0.03	9.32 ± 0.10	9.41 ± 0.46	13.58 ± 0.13	n.d	n.d	n.d
SGA	1.91 ± 0.20	2.23 ± 0.09	3.63 ± 0.06	3.61 ± 0.63	3.69 ± 0.06	2.23 ± 0.21	3.63 ± 0.11	3.61 ± 0.01
PBA	n.d	0.69 ± 0.08	2.17 ± 0.06	4.12 ± 0.09	11.18 ± 0.16	n.d	n.d	n.d
Total	22.56 ± 1.99	38.49 ± 2.15	59.20 ± 3.51	62.42 ± 3.69	81.83 ± 2.85	44.38 ± 2.25	31.72 ± 1.42	29.13 ± 1.81

n.d: not detected; [a]Values are means of at least two determinations ± standard deviation and are expressed as fresh weight; CGA: Chlorogenic acid; CFA: Caffeic acid; GAC: Gallic acid; PCA: Protocatechuic acid; CMA: Coumaric acid; FRA: Ferulic acid; SGA: Syringic acid; VNA: vanillic acid; PBA: *p*-hydroxy benzoic acid.

Sotilo et al. (1994) reported the formation of CFA (0.64 µmol/g) when CGA degraded (0 µmol/g) upon storage at 25°C. They observed that light, storage (more than 9 days) and extraction temperature (above 100°C) are the main parameters causing degradation of CGA and CFA. In this study, extraction of phenolic compounds were carried out at eight different temperatures (100-240°C) to observe the degradation of phenolic compounds mainly chlorogenic acid. The highest amount of CGA (16.1 mg/100 g, fw) and CFA (9.23 mg/100 g, fw) were obtained at 160 and 180°C, respectively, using subcritical water (Fig. 3.5). At 100-160°C, chlorogenic acid content increases, while there is almost no variation in the amount of caffeic acid. In Fig. 3.5, further increase in temperature (160-180°C) promotes the degradation of CGA and formation of caffeic acid. The amount of CGA was reduced to 1.5 mg/100 g, while the amount of CFA was increased to 1.27 mg/100 g. Further increase in temperature (above 180°C) resulted in burning and degradation of both phenolic compounds.

After the subcritical water extraction, the potato peel residue left in the reactor was re-extracted with methanol for 1 hr to determine if any phenolic compounds were left (Fig. 3.6). At 100°C, the amount of phenolic compounds concentrated in the residue was high (10.1 mg/100 g, fw). At 180°C, most bound form phenolics such as PBA, PCA and GCA were extracted (Table 3.6). Phenolic compounds in the residue were found to be low (0.41 mg/100 g, fw) at 180°C. Approximately 96% of phenolic compounds were recovered (this is based on spiking of the extract with 2 mg/mL of all phenolic standard).

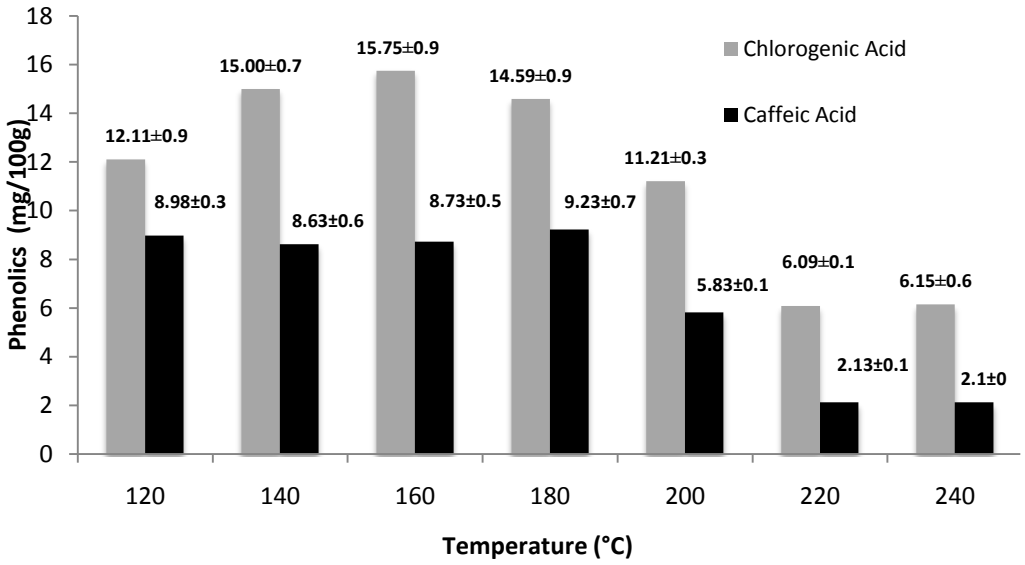

Fig. 3.5. Chlorogenic and caffeic acid profiles at various temperatures

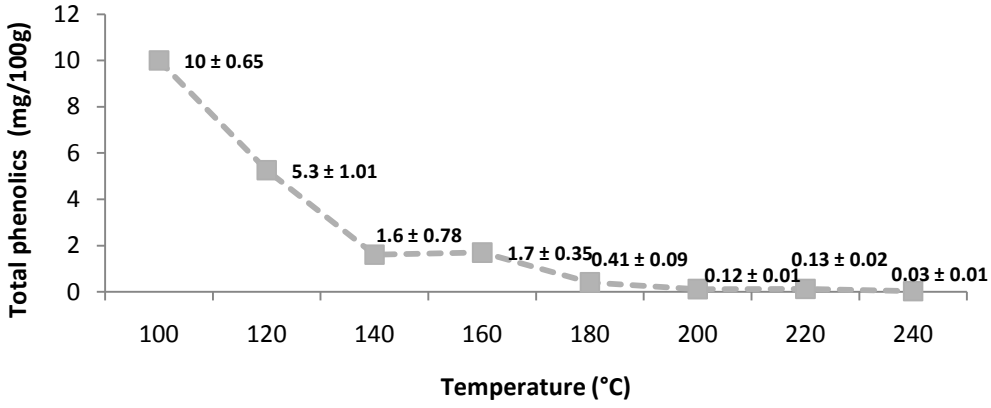

Fig. 3.6. Phenolic content in residues after extractions at various temperatures

3.3.3.2. Effect of Time

In Fig. 3.7, three different temperatures (120, 180 and 240°C) were selected to optimize the time to extract phenolic compounds from potato peel. All extractions were carried out for 120 min. High yield of phenolic compounds were observed at all selected temperatures at 60 min of extraction. After that, the content of phenolic compounds was almost constant (Fig. 3.7). Low content of phenolic compounds were found at 240°C and 60 min due to sample degradation. The best condition for the extraction of phenolics from potato peel was 180°C for 60 min.

3.3.3.3. Comparison of subcritical water extraction with solvent extraction

SCWE (180°C, 6 MPa and 2 mL/min) removed higher amounts of phenolic compounds from potato peel (81.83 mg/100 g, fw) than methanol extraction (46.50 mg/100 g, fw) or ethanol extraction (33.6 mg/100 g, fw) at 65°C and at atmospheric pressure. Overall, the amount of phenolic compounds obtained by SCWE was 40% higher than those obtained by the conventional solvent extraction. In addition, the time of extraction used for SCWE was shorter (60 min) than that used by conventional solvent extraction (3 hrs). The volume of solvent used by SCWE was lower (150 mL) than that used by conventional extraction (300 mL). However, more work is needed with the same feed:solvent ratio for a better comparison of the two extraction methods.

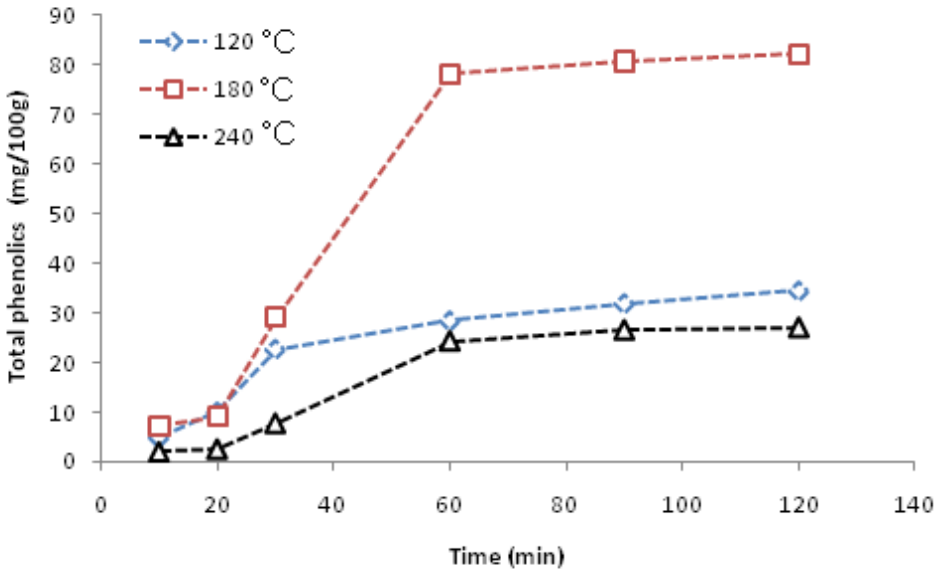

Fig. 3.7. Effect of time on the extraction of phenolic compounds using SCW

The chromatogram of Fig. 3.8a shows that a very low amount of bound form gallic acid was obtained with either methanol extraction (0.45 mg/100 g, fw) or ethanol extraction (0.6 mg/100 g, fw). However, other studies (Sotilo et al., 1994; Oneyeneho et al., 1993) reported considerable amounts of gallic acid (58-63 mg/100 g, dw) in their extracts. This might be the effect of climate and soil conditions and the solvent used for extraction. Using subcritical water, a high amount of gallic acid was observed in the chromatogram of Fig. 3.8b. In Fig. 3.9, the content of gallic acid was low (7.23 mg/100 g, fw) at 100°C. As temperature increases, more bound form phenolics were removed from potato peel in subcritical water. Therefore, the gallic acid content increases to 29.56 mg/100 g at 180°C but decreases above 180°C as observed in Fig. 3.9.

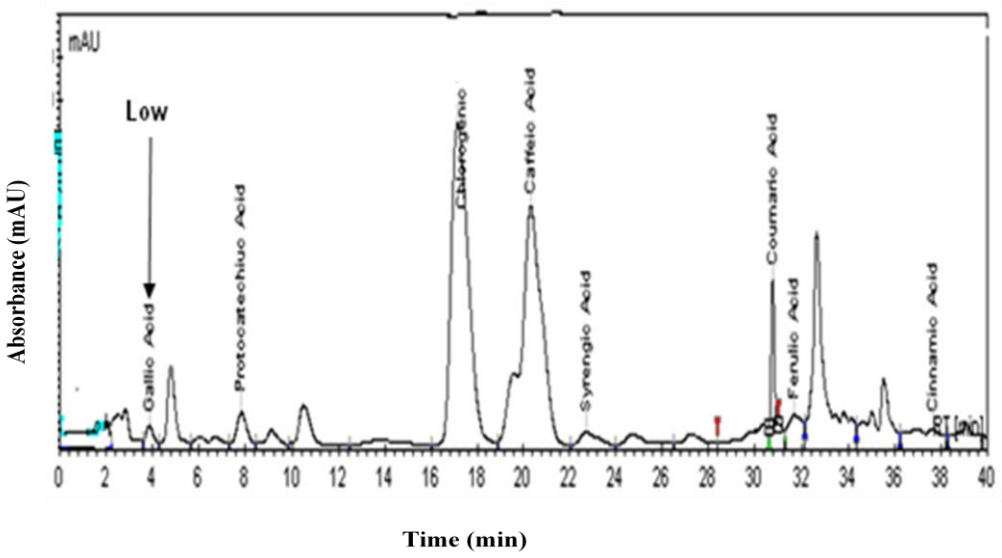

Fig. 3.8 a. HPLC chromatogram of phenolic compounds with methanol extraction

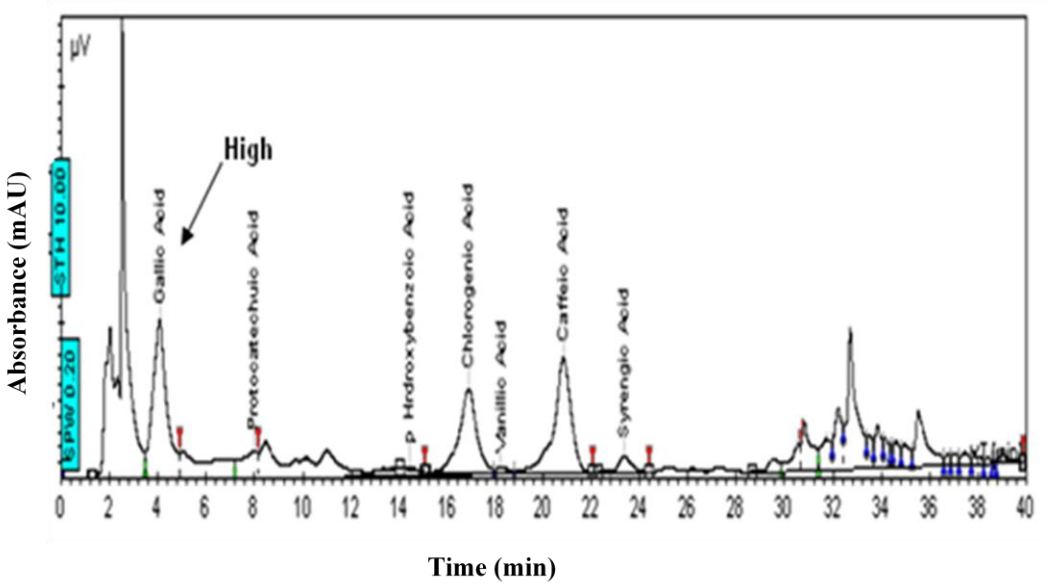

Fig. 3.8 b. HPLC chromatogram of phenolic compounds with SCWE

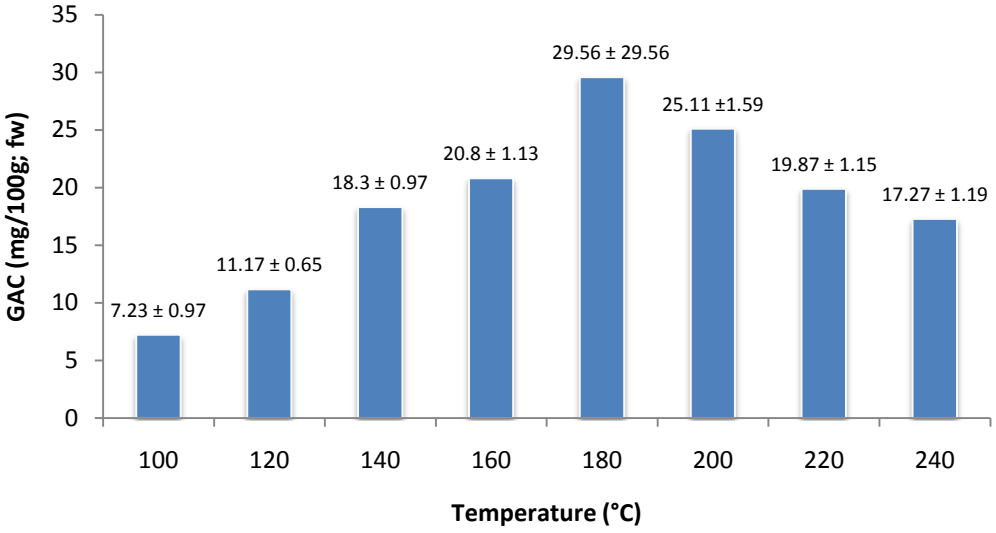

Fig. 3.9. Extraction of gallic acid using SCWE

3.4. Conclusions

- Phenolic compounds were successfully extracted from potato peel using subcritical water. Moreover, the yield of phenolic compounds with SCWE was also higher than with methanol or ethanol extraction.
- The results also demonstrated that extraction yield increases as the temperature increases, resulting from an increased solubility of phenolics in water. In addition, a higher content of gallic acid was obtained using subcritical water than using methanol or ethanol.
- The subcritical water extraction of phenolic compounds were maximized at 180°C and 60 min. Above 180°C, degradation of phenolic compounds occurred.
- Subcritical water removed high amounts of phenolic compounds from potato peel in a shorter time and required 50% less solvent (150 mL) for the extraction than methanol or ethanol extraction (300 mL).
- Using subcritical water, it is possible to extract different types of free and bound form phenolics from potato peel. These compounds could be used in food and nutraceutical industries.

3.5. Recommendation

- The extraction time can be reduced by increasing the flow rate and reducing the amount of solute. More work is needed to optimize the extraction time.

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4. EXTRACTION OF GLYCOALKALOIDS FROM POTATO PEEL

4.1.Introduction

Glycoalkaloids are naturally occurring compounds present in potato. The concentration of glycoalkaloids is higher in potato peel than in the tuber (Kodamatani et al., 2005 and Mader et al., 2009). Peeling of potato removes more than 90% of the glycoalkaloids (Friedman, 2006).

The major glycoalkaloids present in potato peel are α-solanine and α-chaconine, accounting 95% of the TGA. (Mader et al., 2009). α-Solanine and α-chaconine consist of the same alkaloid solanidine but different sugar moieties attached to it. Concentration of α-chaconine was found to be higher (65-71%) than that of α-solanine (Sotelo and Serrano, 2000). Further acid or enzymatic hydrolysis of α-solanine and α-chaconine results in solanidine. (Friedman and Levin, 2009).

Consumption of potato containing high levels of glycoalkaloids (above 20 mg/100 g) may be toxic to humans (Kodamatani et al., 2005). FAO/WHO (1999) reported that the general safe limit of potato glycoalkaloids should not exceed 20 mg/100 g. But, Nordiac view of health risks reported that the concentration of TGA in new varieties of potato peel should not exceed above 10 mg/100 g (Knuthsen et al., 2009).

Presence of glycoalkaloids below recommended level enhances the potato flavor (Sotelo and Serrano, 2000). Consumption of glycoalkaloids has antiallergenic, antipyretic and antiflammatory effects (Friedman, 2006). But, consumption of potatoes with GA above recommended levels leads to poisoning, and bitter taste (Friedman and Levin, 2009; Eltayeb et al., 2003; Kodamatani et al., 2005 and Machado et al., 2005). Several investigations were conducted with volunteers to found out the symptoms caused by excessive consumption of glycoalkaloids. Most of the volunteers after consuming glycoalkaloids (0.95-2.6 mg/kg of the body weight) suffered from nausea and vomiting (McMillan and Thompson, 1979; Mensinga et al., 2005; Kubo et al., 1996 and Friedman , 2002).

Glycoalkaloid content in potatoes depends upon factors such as poor growing conditions, adverse climate, fungal attack, mechanical injury, light and storage temperature. Cooking, baking, boiling and microwaving have no effect on glycoalkaloid content (Tömösközi-Farkas et al., 2006). In addition, Friedman (2006) reported that glycoalkaloids are stable and do not degrade at 180°C during frying.

Due to the presence of carbohydrates and alkaloids in the glycoalkaloid structure, glycoalkaloids are slightly soluble in solvents such as methanol, ethanol and acetic acid. Friedman and McDonald (1997) reported that methanol-chloroform (2:1, v/v) and tetrahydrofuran-water-acetonitrile-acetic acid (5:3:2:0.1, v/v/v/v) were the best solvents for the extraction of glycoalkaloids. Other extraction method for the removal of glycoalkaloids from potato used methanol-chloroform (2:1, v/v) (Wang et al., 1972; Bushway and Ponampallam, 1981),

water-acetic acid-$NaHSO_3$ (95:5:0.5; v/v/w) (AOAC, 2000) and acetic acid-water (5:95, v/v) (Mastuda et al., 2004; Friedman et al., 2004 and Kodamatani et al., (2005). Recoveries of glycoalkaloids with all these extraction solvents were above 90%.

HPLC method is the preferred analytical method to determine individual glycoalkaloids from potato (Bushway et al., 1979; Bushway et al., 1986; AOAC, 2000; Knuthsen et al., 2009). But, for their detection, sample clean up is needed to remove interfering compounds, which absorbs UV light. Glycoalkaloids are visible at 200-208 nm with a UV detector (Driedger and Sporns, 1999). Most of the glycoalkaloid HPLC determinations were performed with C18 and NH_2 columns. Both columns were observed to have more than 90% of GA recovery (Friedman and Levin, 2009). The glycoalkaloids can be identified by using a single isocratic run with buffers such as acetonitrile/phosphate buffer (Knuthsen et al., 2009), acetonitrile/KH_2PO_4 (Eltayeb et al., 2003/2004) and H_20/HOAc/$NaHSO_3$ (AOAC, 2000).

The methods of extraction of GA are based on the use of chemicals and solvents. Moreover, these methods require cleaning of samples which is a time consuming step. Nowadays, there is a need to find alternative methods to reduce organic solvent consumption. SCWE technology has shown to be a feasible option to solve the problem of using conventional extraction methods. Therefore, the main objective of this study was to extract glycoalkaloids from potato peel with SCWE.

4.2. Material and Methods

4.2.1. Materials

Three varieties of potato peel, reported in section 3.2.1, were used in this study. Chemicals and solvents such as α-solanine and α-chaconine, acetic acid, sodium bisulphate, acetonitrile, potassium monohydrogen phosphate (0.1 M), potassium dihydrogen phosphate (0.1 M), potassium phosphate buffer (0.1 M) were purchased from Sigma-Aldrich (St. Louis, MO) and provided by the Crop Diversification Centre (Brooks, AB, Canada)

4.2.2. Sample preparation

The same potato peel samples used in the extraction of phenolic compounds (for details see section 3.2.2.) were used here.

4.2.3. Glycoalkaloid extraction

4.2.3.1 Conventional solvent extraction of glycoalkaloids

Conventional extraction of glycoalkaloids was performed in collaboration with Dr. Driedger at the Crop Diversification Centre.

4.2.3.1.1. Reagents preparation

Extraction Solution: One liter of distilled water was mixed with 50 mL of glacial acetic acid. Then, $NaHSO_3$ (5 g) was added to the solution and mixed it until complete dissolution.

Glycoalkaloids Standard Stock Solution: 0.1 mg of α-solanine and α-chaconine were dissolved in liquid chromatography (LC) mobile phase solution and transferred into a 1 L volumetric flask. Volume of 1 L was completed with LC mobile phase solution.

Solid Phase Extraction (SPE) Wash: A 150 mL of acetonitrile was mixed with 850 ml of distilled water for sep-pak cleaning.

Potassium Monohydrogen Phosphate (0.1 M): Anhydrous K_2HPO_4 (17.4 g) was dissolved in distilled water and transferred into 1 L flask. Volume of 1 L was completed with distilled water.

Potassium Dihydrogen Phosphate (0.1 M): Anhydrous KH_2PO_4 (13.6 g) was dissolved in distilled water and transferred into 1 L flask. Volume make up was done with distilled water.

Potassium Phosphate Buffer (0.1 M, pH 7.6): A 100 mL of K_2HPO_4 solution was placed in a beaker with a magnetic stirrer and KH_2PO_4 solution wad added until pH of 7.6 was reached. Then, the phosphate buffer was passed through a 0.45 µm filter.

LC Mobile Phase: For HPLC analysis, a buffer was prepared by adding 100 mL of phosphate buffer with 300 mL of distilled water. The solution was then mixed with 600 mL of acetonitrile and passed through a 0.45µm filter.

LC Flush Solution: After each HPLC run, the column was flushed with a mixture of acetonitrile-water (3:2, v/v).

4.2.3.1.2 Method of extraction

Freeze dried peel (4 g) of each variety (*Red, Red Norland and Russet*) and fresh peel (*Red*) were weighed and dissolved in 80 mL of extraction solution (water: acetic acid: $NaHSO_3$, 100:5:0.5, v/v/w). The mixture was homogenized using a Polytron homogenizer for 2 minutes at high speed. Then, the solution was centrifuged at 7220 rpm for 30 min. The supernatant was collected and passed through whatman no. 4 filter paper and stored at -4°C in dark vials. The extracts were cleaned using Sep-Pak C18 cartridges (Water Sep-Pak C18, 500 mg, Milford, MA). Sep-Pak cartridges were first conditioned with 5 mL of LC grade acetonitrile. Then, a 10 mL of extraction solution was passed through the cartridge. Afterward, the sample solution (10 mL) was passed through the cartridge. The cartridge was washed by passing 4 mL of SPE wash solution. After that, 4 mL of LC mobile phase was eluted in a catridge and recovered it in a 5 mL volumetric cylinder. The final volume was adjusted to 5 mL with LC mobile phase. Eluted sample was then transferred into a dark amber vial and stored at -4°C for further HPLC analysis.

During the extraction process, sample spiking was also performed to verify the accuracy of the method. Glycoalkaloid solution standard (2Ml , 100 µg/mL) was eluted in the Sep-Pak cartridge before loading 10 mL of sample on to Sep-Pak cartridge following above extraction procedure. Spiking was performed to increase in 10 µg/mL in the TGA concentration.

4.2.3.2. Subcritical water extraction of glycoalkaloids

Similar procedure and conditions were followed as in the subcritical water extraction of phenolic compounds (see section 3.2.5.7.). Same extract (obtained from SCWE of phenolic compounds) was analyzed for glycoalkaloid content. However, after extraction, samples were cleaned using Sep-Pak column following detailed procedure in section 4.2.3.1.2.

4.2.3.3. HPLC analysis

The method of HPLC analysis was adapted from AOAC (AOAC, 2000). This analysis was performed using a HPLC model # 1100 (Agilent, Palo Alto, CA) equipped with a Phenomenex Hydro-RP C18 column (Palo Alto, CA) (250 x 4.6 mm id, 5 µm particle size), Phenomenex Hydro-RP guard column with UV Detector (Palo Alto, CA). A 20 µL glycoalkaloid sample was loaded into an HPLC vial and placed in the autosampler. Isocratic elution with 60% acetonitrile in 0.01M phosphate buffer, where pH 7.6 was used. Flow rate of 1.5 mL was maintained constant. Each sample was analyzed for 20 min. The column temperature was maintained constant at 30°C. Quantification was carried out with UV-detection at 202 nm and compared using external standards of α-solanine and α-chaconine. The retention times of α-solanine and α-chaconine were 7.5 min and 9 min, respectively. All samples were analyzed in duplicate. The standard curve using 5, 10, 20, 30, 40, 50 and 100 µg/mL of α-solanine and α-chaconine solutions were prepared in LC mobile phase. Glycoalkaloid content was

quantified using Agilent Chemstation software. The glycoalkaloids contents were expressed as mg per 100g of fresh weight of sample.

Calculations

- Dry weight (dw) concentration of α-solanine and α-chaconine in the sample

$$GA = \frac{4 \times \text{glycoalkaloids concentration in the sample}}{\text{Sample weight (g)}} \quad \text{... (4.1)}$$

- Fresh weight (fw) concentration of α-solanine and α-chaconine in the sample

$$GA = \frac{\text{Dry basis concentration (mg/100 g)} \times (100 - \text{moisture \%})}{100} \quad \text{… (4.2)}$$

- Spike Recovery

$$\text{Recovery (\%)} = \frac{(\text{C in spiked sample} - \text{C in unspiked sample}) \times 100}{10\ \mu g/mL^{a}} \quad \text{….. (4.3)}$$

C = Concentration, [a] increase in concentration due to spike

4.3. Results and Discussion

4.3.1. Conventional solvent extraction of glycoalkaloids

Freeze dried samples of *Red*, *Red Norland* and *Russet* varieties were extracted following AOAC methodology (AOAC, 2000). Fresh sample of *Red* variety obtained from a local market was also analyzed to evaluate the variation of GAs after frozen, freeze dried, and extraction. Glycoalkaloids were identified and quantified by HPLC. Retention time for the standards α-solanine and α-chaconine were 7.0 and 8.3 min, respectively (Fig. 4.1 a). Figs. 4.1b-d and Table 4.1 show that all three potato peel varieties contain α-solanine and α-chaconine. The recoveries of glycoalkaloids obtained from potato peel (*Red* variety) were 98.9% of α-solanine and 99.6 % of α-chaconine.

TGA content of *Red* potato peel was higher (31.74 mg/100 g) than the recommended safe level (20 mg/100 g, fw) (FAO/WHO, 1999), while the GA contents in *Red Norland* (2.02 mg/100 g, fw) and *Russet* (11.36 mg/100 g, fw) were lower than the recommended safe level (Table 4.1). Data was obtained in duplicate. The variation in glycoalkaloid level in potatoes depends on the variety of potato. Sotelo and Serrano (2000) analyzed 12 different Mexican potato varieties. They found high level of glycoalkaloids above recommended level in 5 varieties: Alpha (24.52 mg/100 g, fw), Michoacan (42.46 mg/100 g, fw), Juanita

3.48 mg/100 g, fw), Nortena (91.63 mg/100 g, fw) and Rosita (50.75 mg/100 g).

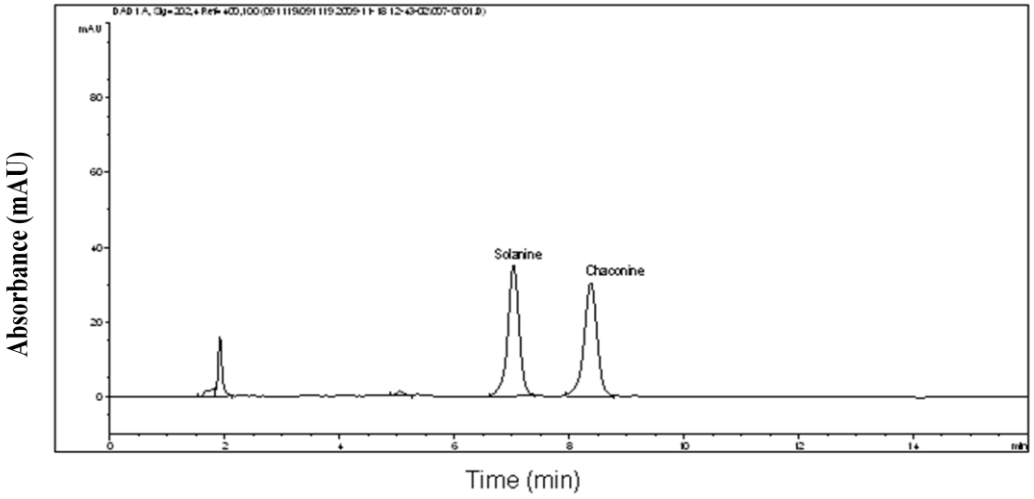

Fig. 4.1 a. HPLC chromatogram of glycoalkaloid standards

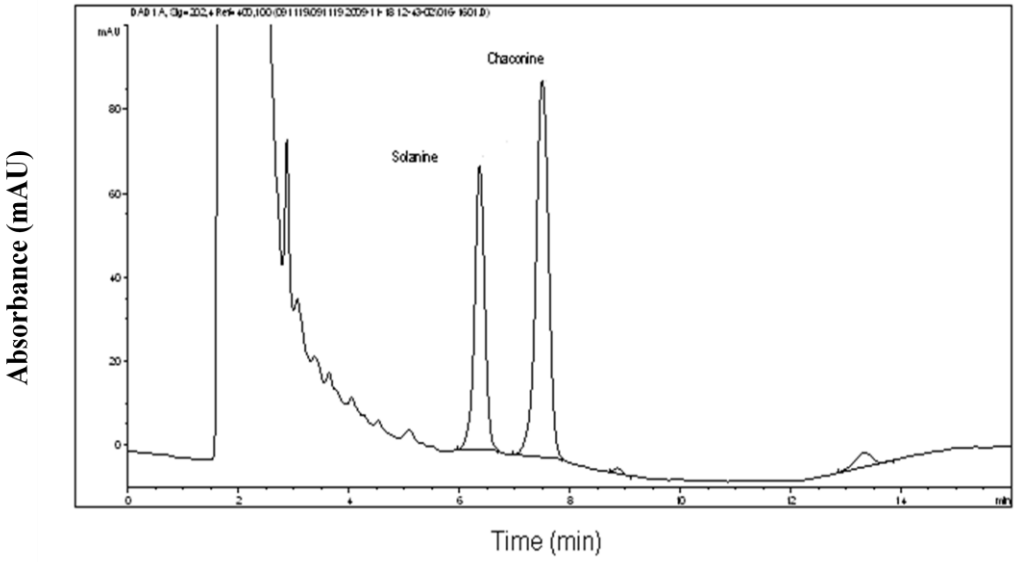

Fig. 4.1 b. HPLC chromatogram of freeze dried potato peel variety *Red*.

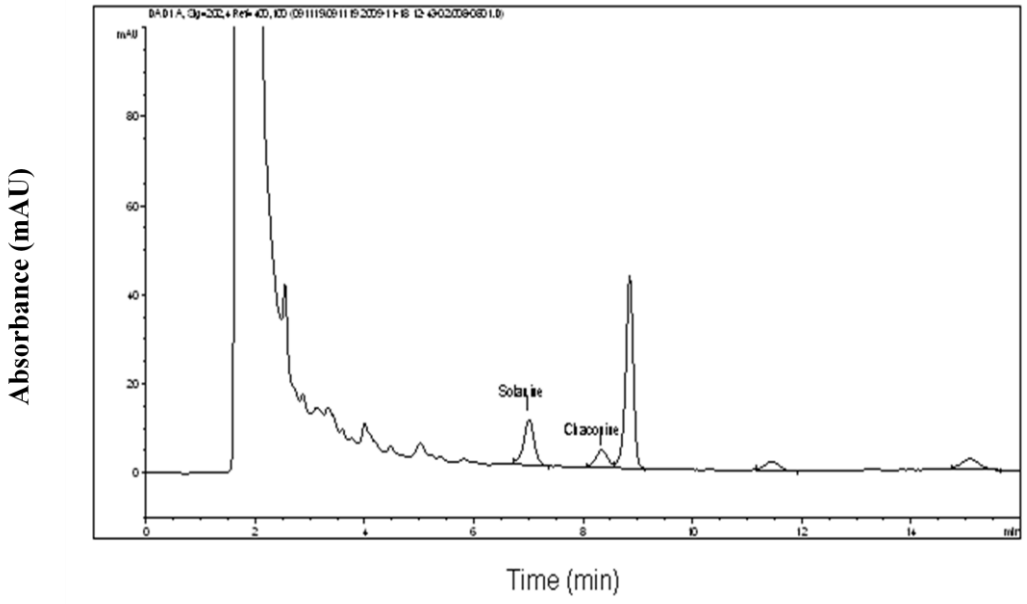

Fig. 4.1 c. HPLC chromatogram of freeze dried potato peel variety *Red Norland*

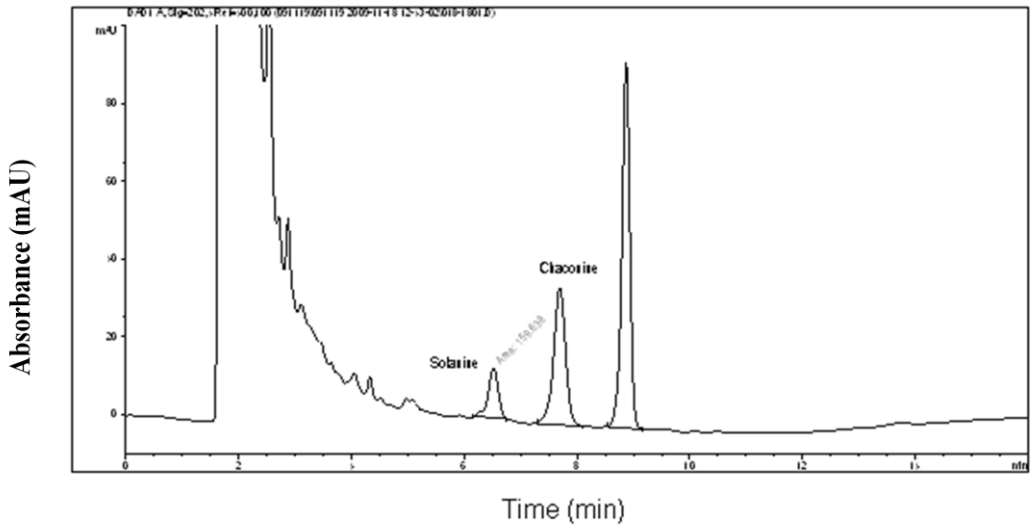

Fig. 4.1 d. HPLC chromatogram of freeze dried potato peel variety *Russet*

Table 4.1. Glycoalkaloid content[a] observed in different varieties of potato peel

Potato variety	α–Solanine	α–Chaconine	TGA
Red (freeze dried)[b]	11.89 ± 0.67	19.85 ± 1.08	31.74 ± 1.75
Red (frozen)	11.41± 0.79	19.09 ± 0.95	30.50 ± 1.74
Red Norland (freeze dried)	1.45 ± 0.03	0.57±0.05	2.02 ± 0.08
Russet (freeze dried)	2.86 ± 0.09	8.50 ± 0.5	11.36 ± 0.59

TGA: Total glycoalkaloids, [a]Values are means of at least two determinations ± standard deviation and are expressed as fresh weight (fw) concentration, [b] converted to fresh weight using equation 4.2

Eltayeb et al. (2003/2004) found above recommended level of glycoalkaloids in thirteen of the eighteen varieties of potatoes grown in Oman (*Latona, Estima, Vivaldi, Cyclon, Lady Rosseta, Turbo, Spunta, No. 3, Diamont, Aida, Atlas, Durbas, Caeser, Lady Clair, No. 10 and Cantate*). They found that the glycoalkaloid content in all varieties was in the range of 87-543 mg/100 g of fresh weight. Only five varieties of potatoes (*Estima, Vivaladi, Ceaser, Lady Christl and Cantate*) among 18 varieties were found below recommended level (20 mg/100 g, fw). Knuthsen et al. (2009) analyzed 386 potato samples of the Danish market and found high glycoalkaloid content (123-223 mg/ kg, fw) in 3 potato varieties (*Grenailles, Pompadour, and Sieglinde*). The authors also

reported the variation in the glycoalkaloids content of identical varieties obtained in six different years.

Bushway and Ponnampalam (1981) analyzed glycoalkaloid content in various commercial products such as chips, frozen steak fries, frozen French fries, frozen fried potato balls, frozen dried potato, dehydrated potato flour, canned sliced white potato, canned peeled whole potato, canned home fries and fried peels. Glycoalkaloids content of all the processed potato samples were under recommended level (0.09-16.2 mg/100 g, fw) except for fried peels (139-145 mg/100 g, fw). Mader et al. (2009) analyzed the effect of steam peeling, cooking, blanching, mashing and drying on the glycoalkaloid content. They observed degradation of glycoalkaloid content during steam peeling (from 29.7 to 6.7 mg/100 g, dw) and blanching (29.7 to 3.4 mg/100 g, dw). Overall, higher concentration of glycoalkaloids was found in peels than in the flesh (Sotelo and Serrano, 2000). Whole potato contains 10% of potato peel, which is very low in terms of contribution of GAs in the peel to the flesh. Hence, potato peel above recommended level is safe for human consumption if consumed with potato flesh (Sotelo and Serrano, 2000).

The main glycoalkaloid found in this study was α-chaconine, comprising 60% of the total glycoalkaloids (Table 4.1). This result is in agreement with results reported earlier by Eltayeb et al. (2003/2004) and Sotelo and Serrano (2000). Eltayeb et al. (2003/2004) analyzed 18 different potato varieties grown in Oman and found high ratios of α-chaconine relative to α-solanine (60:40 to 79:21, α-chaconine:α-solanine). On the other hand, Sotelo and

Serrano (2000) analyzed 12 different varieties of Mexican potatoes and found high quantities of α-chaconine (41-220 mg/100 g, fw) as compared to α-solanine (6.70-161.69 mg/100 g, fw) with an average ratio of α-chaconine:α-solanine (60:40). The high percentage of α-chaconine may be due to the greater activity of α-chaconine against pathogens and predators (Friedman and McDonald, 1997).

The results show that potato peel (*Red*) contains higher amount of α-chaconine (19.95 mg/100 g, fw) than α-solanine (11.89/100 g, fw). The same trend was found in potato peel variety Russet (2.86 mg/100 g of α-solanine and 8.5 mg/100 g of α-chaconine). Bushway and Ponannampalam (1981) also found higher content of α-chaconine (93.1-97.9 mg/100 g, fw) than α-solanine (46.1-48.0 mg/100 g, fw) in fried peels. AOAC (AOAC, 2000) interlaboratory results also reported high content of α-chaconine (17.2 -26.08 mg/100 g, fw) than α-solanine (1.5-21.8 mg/100 g, dw).

Mader et al. (2009) reported higher content of α-chaconine (669 mg/100 g, dw) than α-solanine (224 mg/100 g, dw) in potato peel. However, a different trend was observed in *Red Norland* variety, in which higher content of α-solanine (1.45 mg/100 g, fw) than α-chaconine (0.57 mg/100 g, fw) was found (Table 4.1). For the varieties, *Red Norland* and *Russet*, this study found low quantities of α-chaconine. In addition, an unidentified peak was observed after α-chaconine in the HPLC chromatograms of Figs. 4.1 c-d. This unknown peak might be a hydrolysis product of mainly α-chaconine and α-solanine or the aglycone (solanidine). Due to unavailability of standards in the market, this unknown peak was not identified. In addition, this unknown peak was only found in the two potato peel samples

provided by the industry, that were stored for a week at -18°C and then freeze dried and stored at -18°C for 3 months. But, this peak was not found in potato peel variety *Red* as shown in the HPLC chromatogram of Fig 4.1 b. This potato was obtained from a local market and the peels were freeze dried and stored at -18 °C for 2 months.

For the GA extraction method, solid phase extraction (SPE) was used with a sep-pak catridge to remove interfering compounds. Fig. 4.2 shows that no GAs peaks were detected without a previous sample cleaning.

Literature reviewed earlier shows that the extraction of glycoalkaloids is usually carried out with freeze dried samples (Friedman et al., 2004; Kodamatani et al., 2005; AOAC, 2000 and Eltayeb et al., 2003/2004). In this study, a freeze dried sample and a frozen sample at -18°C were used for the extraction of glycoalkaloids. There was a slightly difference in the content of total glycoalkaloids in the frozen sample (30.50 mg/100 g, fw) and the freeze dried sample (31.74 mg/100 g, fw). Extraction and quantification of glycoalkaloids from fresh or frozen sample is reliable and consume less time. Fresh or frozen potato peels can be useful in the potato processing industries for quick glycoalkaloid determination as it avoids the freeze drying step. In addition, overall time for extraction and quantification would not exceed 45 min by removing the freeze drying step. Besides, the peaks obtained from HPLC analysis of fresh potato peel were clear and can be easily quantified (Fig. 4.3).

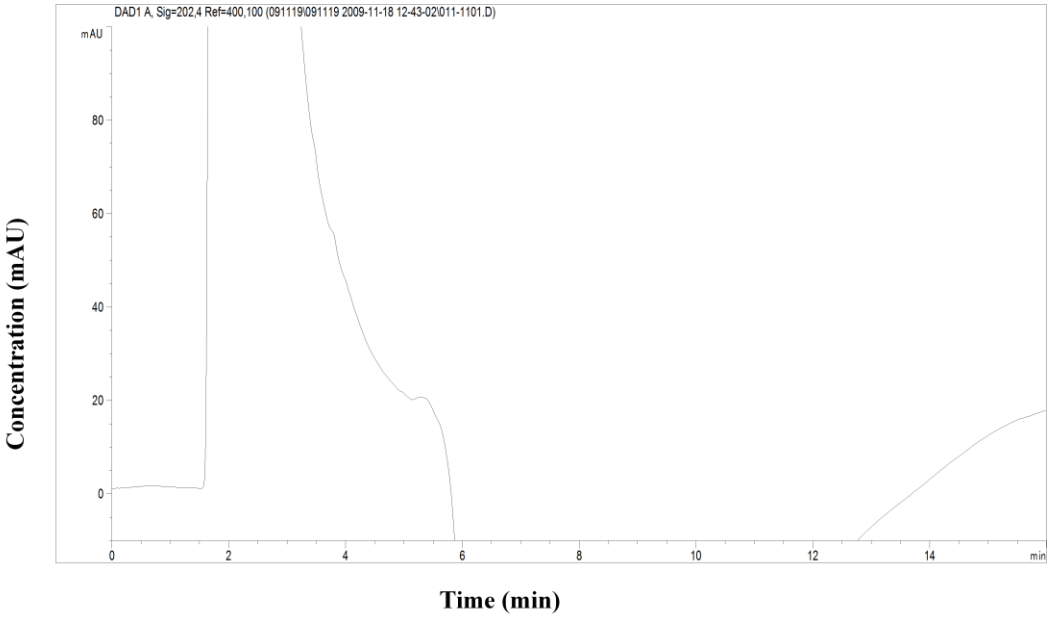

Fig. 4.2. HPLC chromatogram of glycoalkaloids without sep-pak cleaning

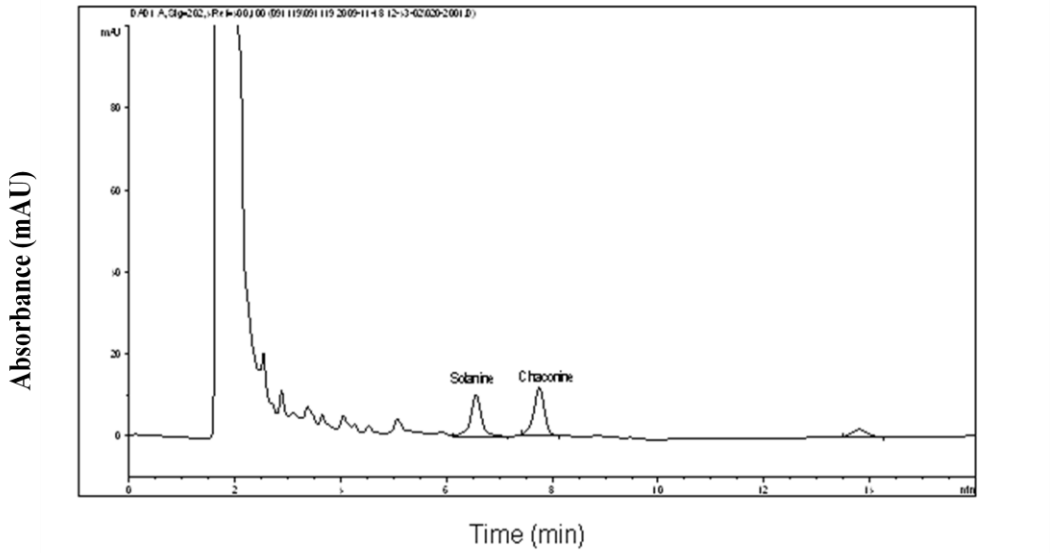

Fig 4.3. HPLC chromatogram of fresh potato peel variety *Red*

4.3.2. Subcritical water extraction of glycoalkaloids

Due to the high yield of glycoalkaloids observed in potato peel variety *Red* than in varieties *Russet* and *Red Norland*, SCW extraction of glycoalkaloids was only performed with potato peel variety *Red* (Table 4.1). After SCW extraction, α-solanine was identified but not quantified due to overlapping of peaks in the HPLC chromatograms. Other hydrophilic compounds were observed in the HPLC chromatograms. The solid phase extraction (SPE) with Sep-Pak was only able to remove some of the interfering compounds, but some of these compounds eluted together with glycoalkaloids.

In conventional extraction of glycoalkaloids, the solid/solvent ratio used was 1:20 (AOAC, 2000). But, in this study, solid to solvent ratio used in SCWE was 1:6. High amount of solid (potato peel) and low amount of solvent (water) used in SCWE might influence the concentration of interfering compounds in the extract, resulting in the overlapping of peaks during HPLC analysis. Moreover, high amount of solid requires more purification to remove other compounds from the glycoalkaloids. α-Chaconine was detected in SCW extracts but its content was lower (0.35-1.19 mg/100 g, fw) than in conventional extraction (19.85 mg/100 g, fw). Eltayeb et al. (2003/2004) extracted potato peel with water-acetic acid-sodium bisulphate (95:5:0.5) at 1:5 solid to solvent ratio, which is similar to the solid to solvent ratio used in this study for the SCW extraction process. Eltayeb et al. (2003/2004) observed the presence of both α-solanine and α-chaconine in potato peel and found high content of total glycoalkaloids (8-56.9 mg/100 g, fw) in potato peel. This indicated that the

presence of slightly acidic conditions (water-acetic acid-sodium bisulphate) influences the glycoalkaloid extraction from potato peel. In this study, water was the only solvent used at subcritical conditions.

4.3.2.1. Effect of Temperature

α-Solanine content was identified in the preliminary studies but not quantified at 100-240°C (Table 4.2). At the same temperature range, α-chaconine was not identified but a very low amount (0.94 mg/100 g, fw) of α-chaconine was observed at 160°C and a little increase in the α-chaconine content (1.19 mg/100 g, fw) was observed at 240°C. Due to low amount of α-chaconine observed in SCW extracts, the effect of temperature is not clear.

4.3.2.2. Effect of Time

Due to low amounts of glycoalkaloids found in SCW extracts of potato peel, α-chaconine content was not observed after 30 min, while α-solanine was not detected at any time in these preliminary studies.

Table 4.2. Effect of temperature on the removal of α-chaconine* (mg/100 g) using SCWE of *Red* potato peel at 6 MPa and 30 min.

Temperature (°C)	α-Chaconine[a]
100	n.d
120	n.d
140	n.d
160	0.94 ± 0.07
180	0.35 ± 0.01
200	0.91 ± 0.12
220	n.d
240	1.19 ± 0.14

[a]Values are means of at least two determinations ± standard deviation, n.d: not detected,*α-Solanine was detected but unable to be quantified

4.4. Conclusions

- This study quantified glycoalkaloid content in three Canadian potato peel varieties (*Red, Red Norland and Russet*). Two industrial varieties, Red Norland and Russet, contain low glycoalkaloid content (2.02 and 11.36 mg/100 g, fw) as compared to the variety *Red* (31.74 mg/100 g, fw).
- The glycoalkaloid level in variety *Red* was higher than the recommended level (> 200 mg/kg, fw). But, due to low contribution of the peel to whole potatoes, peel with high concentration of glycoalkaloid is safe when consumed with potato.
- The concentration of α-chaconine was higher than α-solanine for the two varieties (*Red and Russet*) investigated in this study. Higher content of α-solanine (1.45 mg/100 g, fw) than α-chaconine (0.57 mg/100 g, fw) was observed in variety *Red Norland*.
- Similar recoveries of glycoalkaloids were obtained after extraction of freeze dried and frozen samples. Using a frozen sample speeds up the extraction procedure by eliminating the freeze drying step.
- Based on preliminary results, conventional solvent extraction was found to be more efficient than SCWE for glycoalkaloids from potato peel. Subcritical water did extract a very low amount of α-chaconine.

4.5. Recommendations

- Solid to solvent ratio in SCWE needs to be optimized to obtain high yield of glycoalkaloids from potato peel. Sep-Pak cleaning of subcritical water samples is required to quantify glycoalkaloids.

- Further investigation is needed to identify the unknown peak obtained in the variety *Red Norland* and *Russet*.

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5. EXTRACTION OF SUGARS FROM POTATO PEEL

5.1. Introduction

Carbohydrates are the most predominant component in potato, representing 80% of the potato composition (Lister and Munro, 2000). Carbohydrates are present in the form of starch, sugars and non starch polysaccharides (Lisinska and Leszczynski, 1989). Potato tuber contains 76% starch, 2.10% sugars and 2.32% crude fiber (Lisinska and Leszczynski, 1989). The potato peel obtained after processing of potatoes contains about 40% starch that varies with the peeling process used (Schieber and Saldana, 2009). Camire et al. (1997) reported high starch content obtained during abrasion peeling (51%, w/w) than with steam peeling (28%, w/w).

The sugar content in potato is an important factor, affecting the quality of potato (color). It is responsible for browning (Maillard reaction) during processing of potatoes at high temperatures (Kumar et al., 2004). The main sugars present in potato are glucose, fructose and sucrose (Picha et al., 1985) and low concentrations of raffinose and maltose (Den et al., 1986). The sugar content in potato varies with the variety, size and storage temperature. Spychella and Desborough (1990) reported 35 mg/g and 8 mg/g of fresh weight of sugar content in potatoes stored at 3°C and 9°C, respectively. Generally, tubers are stored at 8-12°C to maintain low level of sugar content. Processing of potatoes containing high sugar content has adverse effect on the taste of dehydrated and fried potatoes. Sometimes, high sugar content potatoes are discarded before processing

(Liainska and Leszczynski, 1989). In addition, potatoes with high sugar content have more chances of browning during processing, which lead to the formation of a carcinogen and neurotoxin compound, acrylamide (Takada et al., 2005). A blanching process is commonly used by potato processing industries to avoid browning during frying. This process improves color and texture of potatoes (Pedreschi et al., 2009).

Sugars are mostly soluble in water and mixtures of ethanol and water (Alves et al., 2007 and Flood and Paugsa, 2000). Den at al. (1986) reported higher yields of total sugars (533 mg/g, dw) with 70% ethanol than with water (4.33 mg/g, dw). Alves et al. (2007) extracted sugars with 50-80% ethanol-water mixtures and found higher yield of sugars (0.7%, w/w) with 50% ethanol-water mixtures than (0.3%, w/w) with 70% ethanol-water mixture. The same effect was observed by Flood and Paugsa (2000). They found high sugar content (0.3%, w/w) with 40% ethanol than with 80% ethanol (0.003% w/w).

Temperature is also a critical parameter, which enhances the sugar extraction from the plant material. Macedo et al. (2005) reported high amount of pure sugars (glucose, fructose and sucrose) recovery with water at 60°C than at 40 or 25°C. Alves et al. (2007) recovered high amount of standard glucose using different concentrations of ethanol at 60°C (36-62 mg/100 g) than at 10°C (3.3-8.2 mg/100 g).

There are various methods developed to analyze sugars from food and plant materials. Earlier, gravimetric methods (Browne and Zerban, 1912),

colorimetric and chemical methods (McCready, 1950, Buysse and Merckx, 1993) were used to quantify total sugars but these methods are unable to identify and quantify individual sugars.

HPLC is the most suitable technique to determine individual sugars (Wilson et al., 1981). The solvent extraction method of sugars from plant material is mostly based on chemical solvents. However, water can be used to extract sugars. No data was found on the extraction of sugars from potato peel using SCW. This study mainly focussed on the extraction of sugars from potato peel using SCW.

5.2. Material and Methods

5.2.1. Materials

All sugar standards such as glucose, fructose and sucrose were obtained from Sigma Aldrich (St. Louis, MO). Pure ethanol and HPLC grade water were obtained from Fisher Scientific (Fair Lawn, NJ).

5.2.2. Sample preperation

Same potato peel samples were used as in extraction of phenolic compounds (see section 3.2.2). Only *Red* potato peels were used to determine the sugar content.

5.2.3. Conventional solvent extraction

Freeze dried potato peel (10 g) was first homogenized with 100 mL of 80% ethanol for 2 min. The slurry obtained after homogenization was boiled for 15 min. Then, the extract was cooled and centrifuged at 6000 rpm for 10 min. The supernatant obtained was filtered through Whatman No. 4 filter paper and the residue left was re-extracted at the same conditions. Supernatant of the extraction was collected and filtered through Whatman No. 4 and the volume of extract was adjusted to 200 mL with 80% ethanol. The supernatant was then concentrated in a rotary evaporator at 40°C and the final concentrated volume adjusted to 100 mL. The final concentrated extract was then passed through 0.45 µm nylon filter prior to HPLC analysis.

5.2.4. Subcritical water extraction

The methodology used for the SCW extraction of sugars was similar to SCW extraction of phenolics. Sugars from potato peel samples were extracted at temperatures of 100-240°C, at a constant pressure of 6 MPa and using a constant flow rate of 2 mL/min. The residue left after each extraction was diluted with 10 mL of methanol and extracted for 60 min. All extractions were performed in duplicates and extracts were stored at 4°C. At the end of each experiment, ethyl alcohol was used to clean the subcritical water extraction unit, pumping it throughout the system.

5.2.5. HPLC analysis of sugars

HPLC analysis was performed using a Dionex ultimate 3000 HPLC system (Bannockburn, IL) equipped with a 401 model autosampler, ultimate RS 3000 pump, and a Shodex refractive index (RI) 101 detector. The column used was Aminex HPX-87P (300 mm x 7.8 mm), serial #425473 with an Aminex guard column (40 mm x 4.6 mm).

The mobile phase mainly consisted of HPLC grade water. The elution profile consisted of an isocratic run with water and the total run time was 35 min. The volume of injection was 10 µL. The flow rate was 0.6 mL/min. Different concentration of standards (0.001, 0.1, 1, 5, 30, 60 mg/mL) were prepared. Three different sugars standards including glucose, fructose and sucrose were analyzed by HPLC (Fig. 5.1).

5.3. Results and Discussion

5.3.1. Identification of sugars

HPLC analysis was carried out to identify and quantify sugars from potato peel using the methodology adopted from Sluiter et al. (2007). Fig. 5.1 shows three main peaks from the standards injected. Sucrose was first observed in the chromatogram at 10.23 min of retention time, while the retention times for glucose and fructose were 12.42 min and 16.68 min, respectively.

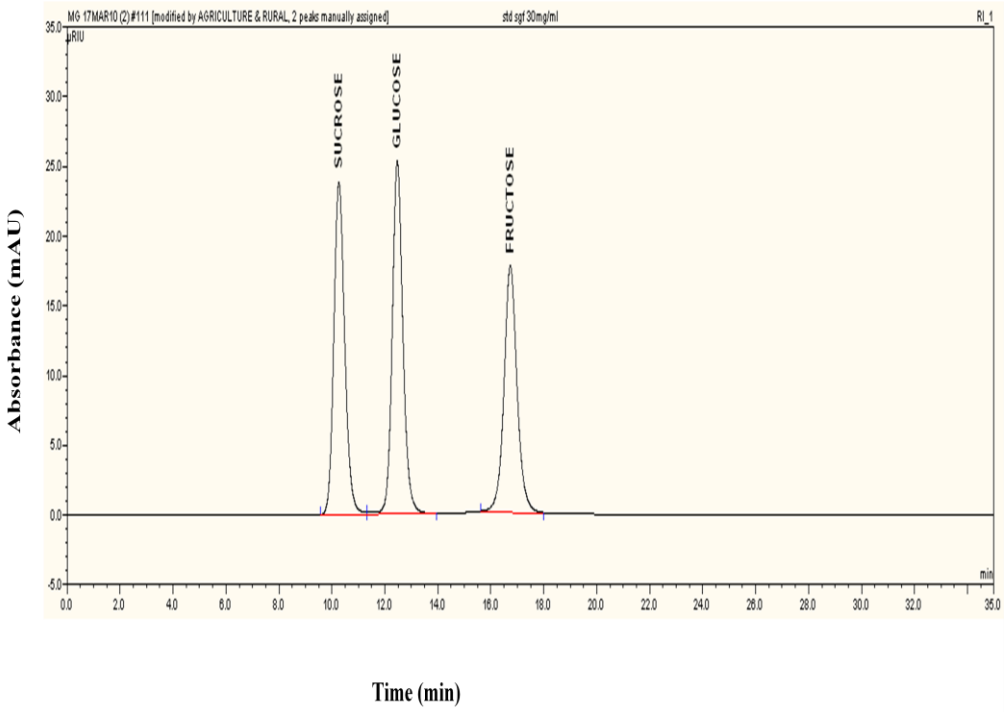

Fig 5.1. HPLC chromatogram of standard sugars

5.3.2. Conventional solvent extraction of sugars

In earlier studies, water and mixtures of water/ethanol were used to extract sugars from potato. Den et al. (1986) compared the extraction recoveries of ethanol (70%, v/v) and boiling water to extract sugars from raw and cooked potato. They found high recoveries of saccharides with ethanol (70%, v/v) (182 mg/g, dw) as compared to boiling water (135 mg/g, dw). In this study, sugars from potato peel were extracted with 80% ethanol. The method of extraction was adapted and slightly modified from Picha (1985). The total sugar content of potato peel was found to be 45.41 mg/100 g of fresh weight (Table 5.1). The main sugars identified in the peel were glucose, sucrose and fructose (Fig. 5.2). Fructose was the main sugar, accounting for 84% (w/w) of the total sugars while glucose and sucrose recoveries were only 8% (w/w). The results obtained were comparable with earlier results of Den et al. (1986). They extracted sugars from new varieties of potato (VSP-1, VSP-2, VSP-3 and BNAS-51) with ethanol/water (70/30, v/v) and obtained a higher amount of sucrose (76-93%, w/w). On the other hand, Wilson et al. (1981) extracted sugars from potato (Kennebec and Russet). They recovered higher amount of fructose (8 mg/g, fw) than sucrose (2.51 mg/g, fw). In this study, fructose was found to be the most abundant sugar in potato peel (Table 5.1).

Table 5.1. Extraction of sugars from *Red* potato peel

Sugars	Yield[a] (mg/g, fw)	
	Conventional extraction	SCWE
Glucose	3.65 ± 0.77	2.96 ± 0.22
Fructose	38.2 ± 3.68	74.45 ± 1.52
Sucrose	3.56 ± 0.47	5.64 ± 1.01
Total	45.41 ± 4.92	83.05 ± 2.75

[a]Values are means of at least two determinations ± standard deviations

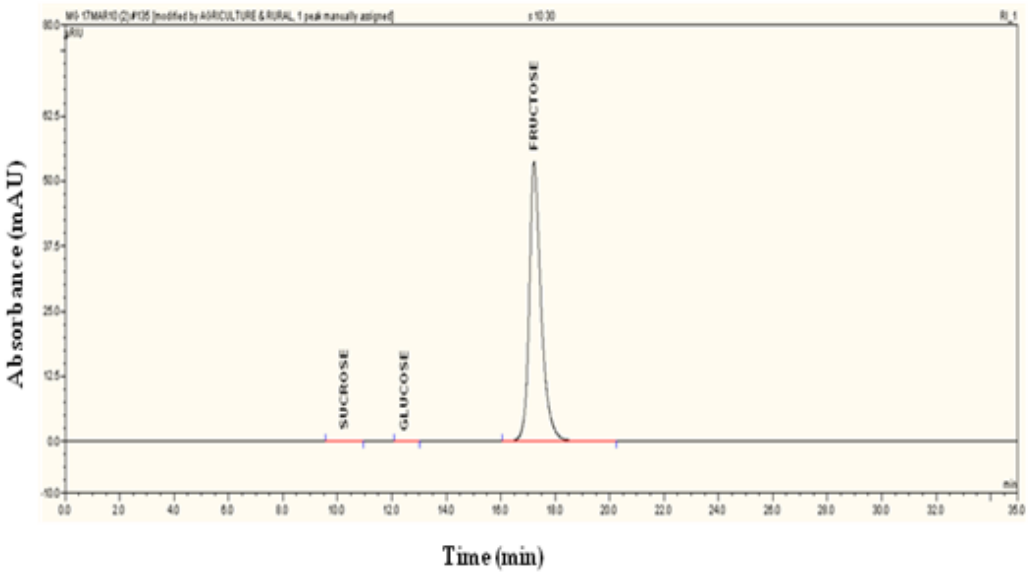

Fig. 5.2. HPLC chromatogram of sugars in potato peel extracted with 80% ethanol.

5.3.3. Subcritical water extraction of sugars

No data was found in the open literature that documented the extraction of sugars from potato peel using subcritical water. High yield of total sugars (83 mg/g, fw) were obtained at 160°C, 120 min, 2 mL/min and 6 MPa compared to the conventional extraction method (45.56 mg/g, fw). Fructose was found in higher quantities in potato peel than sucrose and glucose after SCW extraction (Table 5.1). At these conditions (160°C and 2 mL/min), water's dielectric constant changes, resulting in changes in the water properties and water behaves like an organic solvent (Ramos et al., 2002).

5.3.3.1. Effect of temperature

Similar to phenolics extraction, temperature was the critical parameter, which influenced sugar extraction. The effect of temperature in subcritical water extraction was studied at 100-240°C, 6 MPa, 30-120 min and 2 mL/min. Pressure was kept constant because it has little effect on the solvent properties and is only required to maintain the liquid state of water (Ramos et al., 2002). Flow rate was kept low (2 mL/min) to avoid excessive sample recovery as it is time consuming to evaporate extracts containing water in the rotary evaporator.

Fig. 5.3 shows the effect of temperature on the recovery of sugars from *Red* potato peel at 6 MPa and 2 mL/min. This figure shows that the concentration of sugar extracted increased as temperature increased from 100 to 160°C. At high temperature, water polarity decreases (Ghoreishi et al., 2008), resulting in the decrease of the viscosity and surface tension but increase of diffusion rates, which

results in increased solubility of the solute in the solvent (Ramos et al., 2002, Ghoreishi et al., 2008). The highest amount of sugar was obtained at 160°C (83.05 mg/100 g, fw) as shown in Table 5.2. As temperature increases above 160°C, degradation of sugars was observed (Table 5.2 and Figure 5.3). In addition, at temperatures above 200°C, burning of sample was noticed during the extraction process. A char was found at the side walls of the reactor and color of the extract was black. This might be the reason for decreasing yield of sugar content at temperatures above 160°C. Ghoreishi et al. (2008) also observed burning of product at temperatures of 120-150°C. They extracted mannitol from olive leaves at temperatures of 100-150°C. They observed 37% increment in the yield of mannitol, when temperature increased from 60 to 100°C. But, mannitol content decreased by 16% at temperatures above 100°C.

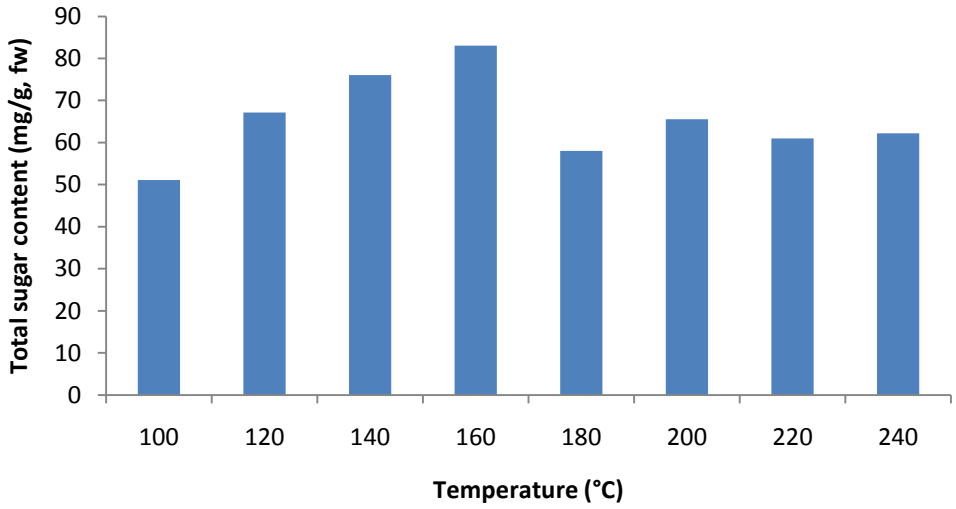

Fig. 5.3. Effect of temperature on sugar content of potato peel.

Table 5.2. SCWE of sugars at various temperatures.

Temperature (°C)	Yield (mg/g, fw)			
	Glucose[a]	Fructose[a]	Sucrose[a]	Total sugars[a]
100	1.27 ± 0.25	47.55 ± 3.16	2.09 ± 0.17	51.12 ± 3.58
120	2.68 ± 0.16	60.32 ± 4.43	4.13 ± 0.21	67.12 ± 4.80
140	2.62 ± 0.42	69.14 ± 3.01	4.26 ± 0.62	76.02 ± 4.05
160	2.96 ± 0.23	74.45 ± 1.50	5.64 ± 1.00	83.05 ± 2.73
180	0.98 ± 0.33	53.93 ± 0.82	3.07 ± 0.12	57.98 ± 1.27
200	1.64 ± 0.01	60.77 ± 2.20	3.17 ± 0.27	65.57 ± 2.42
220	1.74 ± 0.18	57.25 ± 2.40	2.01 ± 0.14	61.00 ± 2.72
240	1.72 ± 0.38	59.09 ± 1.40	1.38 ± 0.16	62.19 ± 1.94

[a] Values are means of at least two determinations ± standard deviation

Khajavi et al. (2005) reported complete degradation of pure sucrose at 190-200°C. At high temperatures of 160-200°C, hydrolysis of sucrose occurs, resulting in increase in the concentration of glucose and fructose. Saito et al. (2009) reported degradation of glucose in SCW at 240°C. They also reported the conversion of glucose into aldehydes, organic acids and furans at 240°C. To avoid degradation of sugars, Giannoccaro et al. (2006) extracted soluble sugars (glucose, fructose and sucrose) from ground soybean seeds at low temperatures (25, 50 and 80°C) with water. They observed an increment in total sugars at 25 and 50°C (12.54%, w/w) than at 80°C (11.50%, w/w). Sugar degradation and

extent of thermal degradation of sugars varies with the type of plant material, its composition and type of sugar present in the plant material.

5.3.3.2. Effect of time

Extraction time is an important parameter. Nowadays, industries are still modifying extraction techniques to avoid a time consuming extraction process. SCWE of sugars from *Red* potato peel was carried out for 120 min. Every 30 min, a sample was collected for further HPLC analysis. Different sugar concentrations were obtained at 30-120 min (Table 5.3). High yield of fructose was observed at a selected temperature and 120 min of extraction. There was a cumulative (32%, w/w) increase in the fructose content when extraction time increases from 30 to 60 min (Table 5.3 and Fig. 5.4). Further increase in time from 60 to 120 min, increases fructose content (44%, w/w). Same effect was seen in the sucrose content (Table 5.3 and Fig. 5.5). Sucrose recovered in 120 min was (74%, w/w) more than sucrose recovered after 30 min (Fig. 5.5). Due to the low glucose content found in potato peel, the maximum recovery of glucose was obtained at 90 min of extraction. There was no significant change observed after 90 min up to 120 min (Fig. 5.5). Ho et al. (2007) extracted complex carbohydrates such as lignins from flaxseed meal at 130-190°C for 130-190 min. They found high recoveries of carbohydrates (65%) in the extract at 160°C and 120 min. Kubaova et al. (2001) found high recoveries of lactones (100%) from kawa roots in 120 min. Generally, extractions in SCW were carried out for 10-200 min (Table 2.22).

Due to high concentration of fructose in *Red* potato peel sample, more than 120 min of extraction time is needed (Fig. 5.4). In conventional extraction of sugars, extractions were carried out for 30-90 min as in other studies (Den et al., 1986, Camara et al., 1996). In some of these studies, re-extractions were carried out to obtain higher yields of sugars, which is also a time consuming step (Picha et al., 1985, Den et al., 1986).

To shorten the extraction time and obtain a maximum yield, two parameters, weight of solute and flow rate can be modified. The initial weight of solute (10 g) used in all experiments can be reduced. Due to the high content of sugars in the raw material, saturation of sugars in the low flow rate of water (2 mL/min) can be assumed. In addition, solid to solvent ratio was not sufficient for the extraction of sugars from the potato peel. Therefore, studies using high flow rates and different solid to solvent ratios are recommended to recover high yield of sugars in short times.

Red potato peel residue left in the reactor after SCWE was extracted following conventional extraction methodology described in section 4.2.3 and analyzed for remaining sugar content. Fructose was the only sugar found in the residual (Table 5.4). Fructose content in potato peel residues at 100°C and 160°C were 3.9 mg/g and 8.15 mg/g, respectively. At 160°C, the highest recovery of sugar content in potato peel was observed in 120 min. However, over 120 min of extraction was required to remove all fructose from potato peel. This might be the reason that at 160°C, the highest amount of fructose has been retained in the potato peel residue (Table 5.4). Above 200°C, fructose was not detected because

of sample burning (Table 5.2). Due to the low content of glucose and sucrose observed in potato peel sample, both sugars were not detected in the residual analysis (Table 5.4). Sugar content obtained with SCWE was 45% higher than conventional solid-liquid extraction. Due to high content of sugar obtained with SCWE, this study unble to explain the mass balance between the extract and residue obtained after the process.

Table 5.3. Extraction of sugars using SCW at different time intervals and at 160°C, 6 MPa and 2 mL/min

	Yield (mg/g, fw)		
Time (min)	**Fructose[a]**	**Glucose[a]**	**Sucrose[a]**
30	27.79 ± 2.41	1.17 ± 0.12	1.49 ± 0.16
60	13.37 ± 0.43	0.98 ± 0.02	1.48 ± 0.32
90	14.82 ± 2.39	0.82 ± 0.14	1.05 ± 0.14
120	18.47 ± 1.92	nd	1.62 ± 0.45

[a] Values are means of at least two determinations ± standard deviation, nd: not detected

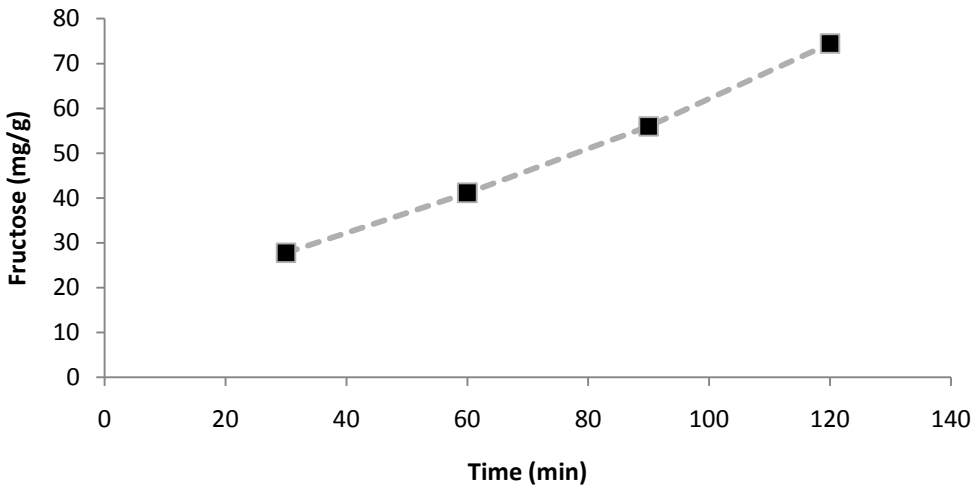

Fig. 5.4. Effect of extraction time on fructose content at 160°C, 6 MPa and 2 mL/min

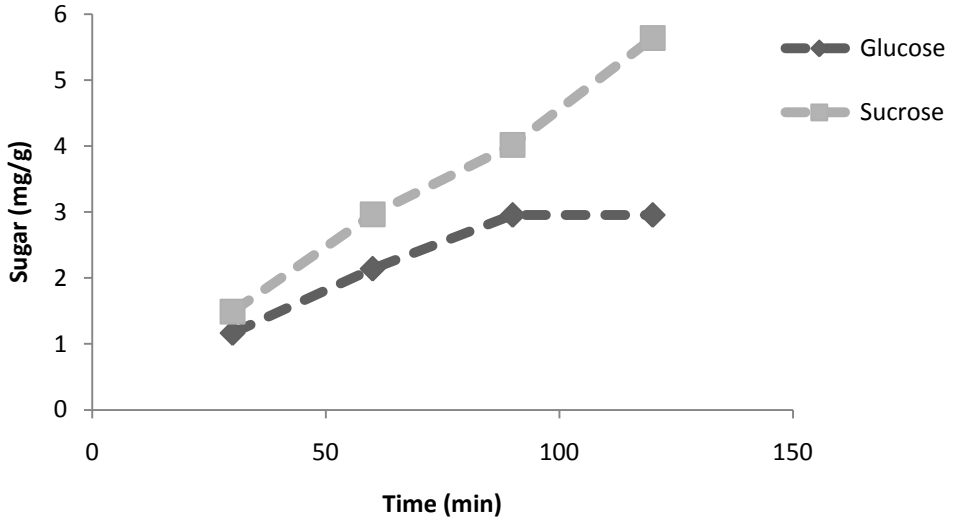

Fig. 5.5. Effect of extraction time on glucose and sucrose content at 160°C, 6 MPa and 2 mL/min

Table 5.4. Sugar content* in potato peel residue after SCWE

Temperature (°C)	Fructose (mg/g, fw)
100	3.90 ± 0.78
120	4.30 ± 0.85
140	3.30 ± 0.56
160	8.15 ± 2.19
180	2.35 ± 0.21
200	6.90 ± 0.14
220	n.d
240	n.d

[a] Values are means of at least two determinations ± standard deviation, n.d: not detected, *only fructose was found in the residual. No glucose and sucrose were detected.

5.4. Conclusions

- Removal of sugars from potato peel using SCWE depends on the temperature and time. High recoveries of sugars (83.05 mg/g, fw) were obtained using SCW at 160°C, 2 mL/min, 6 MPa and 120 min. Temperatures above 160°C degraded sugar and temperatures above 200°C resulted in burning of potato peel.
- Extraction recoveries of total sugars using subcritical water (83.05 mg/g, fw) were 45% higher than using 80% ethanol extraction (45.41 mg/100 g, fw).
- SCWE is a feasible method for the extraction of glucose, fructose and sucrose from potato peel. It can be possible to implement this technology on a large scale industrial extraction system.

5.5. Recommendation

Using SCWE, the effect of time needs to be studied. Fructose in potato peel residue after SCWE was still high in 120 min. To overcome the long extraction time and to recover high sugar content, either the weight of solute can be reduced or the flow rate can be increased. Therefore, the solid to solvent ratio still needs to be optimized to reduce the extraction time.

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6. CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

- Higher amounts of phenolics and sugars from potato peel were obtained using SCWE than conventional solvent extraction method. But, low amounts of glycoalkaloids were extracted with SCWE than with solvent extraction in preliminary experiments.
- Temperature and time were the main parameters investigated. High yields of phenolics at 180°C and 60 min, high yields of sugars at 160°C and 120 min and low yields of glycoalkaloids at 240°C and 30 min were obtained using SCWE at a constant pressure of 6 MPa and flow rate of 2 mL/min.
- Chlorogenic acid (CGA), gallic acid (GAC), caffeic acid (CFA) and protocatechuic acid (PCA) were the main phenolic compounds obtained from potato peel. Using SCWE, recoveries of total phenolic compounds were 40% higher than ethanol (50%) and methanol extraction. A small amount of chlorogenic acid degradation (7%) was observed at 180°C. Gallic acid, which was not extracted with ethanol or methanol, was recovered in high quantities (30 mg/100 g) using SCW.
- Using subcritical water, recoveries of sugars were 45% higher than ethanol (80%) extraction. The glycoalkaloids extraction using SCW was lower than conventional solvent extraction. α-Solanine was not detected in SCWE due to interfering compounds observed in HPLC chromatograms.
- Overall, the results indicate that SCWE may be an effective and alternative way of extracting phenolics and sugars from potato peel. The results indicated

that SCWE requires only water at high temperature and does not require high pressure, making it a cost effective process. Therefore, it can be easily implemented on a large scale industrial extraction system.

6.2. Recommendations

- Time of extraction can be minimized by increasing the flow rates or using low amount of potato peel.
- More cleaning of samples are required to identify α-solanine. Moreover, solid to solvent ratio also needs to be optimized to extract high recoveries of glycoalkaloids from potato peel.