LIQUEFACTION OF BARLEY HULL WITH PRESSURIZED FLUIDS

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Abstract. Canada is one of the largest barley producers, generating 10-13% of barley hull from the grain which contains valuable components, such as carbohydrates and phenolics. In this study, the extraction of carbohydrates and phenolics from the hull of a new barley variety (*BT 584*) was investigated using pressurized fluids. The effects of pressure, particle size, temperature, and static holding time on the extraction were studied. The use of pressurized ethanol aqueous solution increased the extraction of total phenolics and total carbohydrates. At 150°C and 15MPa, 21.8 and 43.25 mg phenolics/g barley hull, as well as 113.5 and 252.9 mg carbohydrates/g barley hull were extracted with pressurized water and pressurized ethanol aqueous solution (20% ethanol), respectively. To improve the extraction efficiency, the variables affecting pressurized aqueous ethanol solution were optimized using Response Surface Methodology with a central composite design. Analysis of variance showed that temperature was the most important variable, affecting the extraction of total carbohydrates and phenolics from barley hull. The pressurized aqueous ethanol extraction was the most effective method, yielding 122.4 mg total phenolics/g barley hull and 589.4 mg total carbohydrates/g barley hull at 240°C, 15MPa, with 12% ethanol concentration, and a flow rate of 5 mL/min. A kinetic model was used to fit the extraction curves with mean square error lower than 0.06. The antioxidant activity was assessed by 2,2-DiPhenyl-1-PicrylHydrazyl (89.02±0.40%) and Ferric Reducing/Antioxidant Power assay (568.47±1.7 mMol ferrous sulphate reduction/g barley hull) for the extract solutions. These extracts can be used as a good source of phenolics and carbohydrates.

Keywords: Total phenolics, total carbohydrates, barley hull, extraction, pressurized fluids.

1. Introduction

Canada is the fifth largest producer of barley in the world. In 2010-2011, the annual production of barley in Canada was 7.7 M mt [1], out of which over half of the total amount of barley was grown in the province of Alberta [2]. Barley grain contains a wide range of phenolic compounds, including benzoic and cinnamic acid derivatives, anthocyanins, proanthocyanidins, and lignans [3-5].

Barley hull, which constitutes about 10-13% of the whole grain, is a great source of phenolics as well as carbohydrates [6-8]. Höije et al. [8] reported a total carbohydrate content of 87% in the hull of Cindy or Waxy variety of barley grain. Barley hull can thus be utilized as a biomass as it has a high content of lignocellulosic (LC) material, approximately 31-34% cellulose, 24-29% hemicelluloses and 14-15% lignin content, which can be used as a source for fermentable sugars for the production of biofuel (e.g. ethanol production). Moreover, phenolic acids are also mainly concentrated in the hull portion of the barley grain [6-7]. Hao and Beta [9] determined that 1kg barley hull contains 10.71 g total phenolics as measured according to the ferulic acid equivalent. In addition, Waldron et al. [10], and Bunzel et al. [11] reported that ferulic acid and *p*-coumaric acid are associated with the cell wall constituents especially with arabinoxylans and lignin.

Phenolics contribute to the antioxidant activity of foods [12]. Further research indicated that phenolics have many beneficial activities, such as anti-allergic, anti-inflammatory, anti-microbial, cardioprotective and anti-thrombotic [13-15]. Due to these benefits, phenolics have been extracted from barley flour using aqueous solutions of methanol, ethanol and acetone separately or in combination [16-19]. Bonoli et al. [20] reported

the use of 4:1 v/v ratio of aqueous ethanol, aqueous methanol, and aqueous acetone for the extraction of free phenolics from barley flour. It was estimated that 4:1 ratio of aqueous acetone could be used to extract the highest amount of phenolics from barley flour (0.68 mg/g) after a 40 min process. Though effective, these methods involve the use of toxic petrochemical solvents and/or long extraction times up to 24 h [21], and temperatures up to 100°C, which might accelerate the oxidation of total phenolics [22] unless a reducing agent, such as cysteine is added to the solvent [23-24]. Therefore, there is a need to use a green process which could provide a high yield in short time, minimizing degradation.

A subcritical fluid (sCF) relies on the decrease of fluid polarity and dielectric constant, and an increase on the ion product with an increase in temperature. Some common examples of sCFs are water and aqueous ethanol that have been used for the extraction of phenolics from food by-products of plant origin, such as cinnamon bark [25], apple pomace [26], pomegranate seed residues [27], defatted rice bran [28], potato peel [29], and others. Most of the studies dealing with sCF extraction of phenolic acids from different matrices have used a temperature range of 100-220°C at 6-10 MPa with 2-3 mL/min of solvent flow rate [25,27-32]. Hassasroudsari et al. [32] obtained a high amount of total phenolics (17.7 mg sinnapic acid equivalent/g canola meal) using 95% ethanol at subcritical conditions. Although recent studies have demonstrated the potential of sCFs, only one study attempted the extraction of barley polysaccharides and antioxidant compounds from barley grain [33]. Our study was conducted to evaluate the ability of sCF (water and aqueous ethanol) extraction methods to obtain total phenolics and total carbohydrates from BT 584 barley hull. The extraction yields were evaluated using spectrophotometric measurements. In addition, the antioxidant activity of the extract solutions was quantified by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and Ferric Reducing/Antioxidant Power (FRAP) analysis. Furthermore, some of the Maillard reaction products of the extracts were quantitatively measured after the extraction.

2. Materials and methods

2.1 Materials

A sample of the new variety of barley, BT 584, was obtained from Alberta Agriculture (Lacombe, AB, Canada). A dehuller was used to remove the hulls from the barley. Hulls were then cleaned and milled in a cyclone sample mill equipped with a 0.5 mm screen (model 3010-030, Udy Corp., Ft. Collins, CO). Chemical reagents, such as sulphuric acid (97%, ACS reagent), ethanol (99.9%, HPLC grade), sodium hydroxide pellets (≥97%, ACS grade), Folin-Ciocalteau's phenol reagent (2M), gallic acid standard (99.9% purity) and D-(+)-glucose standard (99% purity) were purchased from Fisher Scientific Co. Ltd (Toronto, ON, Canada). Glass beads (~ 3mm) and glass wool were also purchased from Fisher Scientific Co. Ltd (Toronto, ON, Canada). The reagents used for the DPPH and FRAP antioxidant activity analysis, such as DPPH (99.99% purity), Fe_2SO_4 (98% purity), tripyridyltriazine and ferric chlorides were obtained from Sigma Aldrich (Oakville, ON, Canada).

2.2 Methods

Proximate compositional analysis. The moisture, ash and fat contents of the barley hull were analysed using the standard official method of Association of Analytical Communities (AOAC).

Subcritical fluid (sCF) extraction. sCF extraction was carried out in a semi-batch type reactor (Fig 1), earlier described in detail by Singh and Saldaña [29] and Saldaña et al. [34]. The extraction system consisted of a pump, a pre-heater, an extraction cell surrounded by heating brackets, pressure gauges, a cooling system and a back pressure regulator. The maximum pressure and temperature that can be achieved with this unit are 26 MPa and 315°C, respectively. All extractions were carried out using an extraction cell (1.3 cm internal diameter x 20.3 cm length), which was closed with inlet and outlet filters of 20 µm. The sample (1 g) was mixed with glass beads of 2.3 mm of diameter and then placed into the extraction cell. The inlet and outlet of the extraction vessel was filled with glass wool (2 mm thick) to avoid breaking the filters. The temperature of the system was monitored by a thermocouple and the pressure was maintained constant with a back pressure regulator. The extracts were stored at -18°C for further analysis.

Total phenolics. Total phenolics of barley hull extracts were determined following the methodology of Singleton and Rossi [35], with minor modifications. Briefly, 0.04mL of extract was mixed with 3.16mL of distilled water and 0.1mL of Folin-Ciocalteau's phenol reagent and the mixture was vortexed thoroughly.

After 6 min of reaction, 0.3 mL of sodium carbonate solution was added to the mixture. The mixture was then incubated at room temperature for 2 h. After incubation in a dark place, the absorbance was read at 765 nm and the measurements were compared with the calibration curve of gallic acid solutions. Total phenolics were expressed as milligrams of gallic acid equivalents per gram of barley hull.

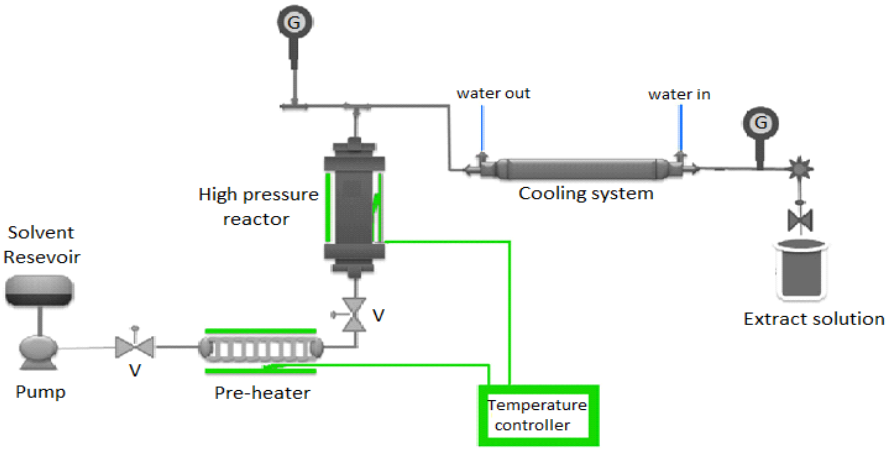

Figure 1. Subcritical fluid extraction system (G: Gauge, and V: Valve)
(Adapted from Saldaña et al., 2012)

Total carbohydrates. Total carbohydrate content was determined using the methodology of Dubois et al. [36] for all the extracts obtained. Dilutions of the extracts were performed depending on the concentration of each extract sample. Then, 0.5mL of phenol and 2.5mL of sulfuric acid (96%) was added to an aliquot of 1mL of the diluted extract. Good mixing was attained by vortexing each sample for approximately 1 min. The reaction was then stopped using a cold water bath at 20°C for 20 min. The calibration curve for total carbohydrates was prepared using glucose solutions, ranging from 0.5 to 10 mg/g solution. The absorbance was measured at 490 nm using a spectrophotometer (Genova MK3, New Malden, Surrey, UK). The final results were expressed as milligrams of glucose equivalents per gram of barley hull.

DPPH assay. The methodology of Blois [37] previously described by Gülçin [38] was used with slight modifications to assess the DPPH free radical scavenging capacity of the extracts. Briefly, 19.71 g DPPH was dissolved in 500 mL of ethanol to prepare 0.1 mM solution of DPPH. Then, 1.5 mL of this DPPH solution was added to 0.5 mL of barley hull extract. These solutions were vortexed thoroughly and incubated in a dark environment at room temperature for 1 h. The blank samples (Milli-Q water or aqueous ethanolic solutions) were also prepared using the same method. Then, the absorbance of these mixtures was measured at 517 nm using a Genova spectrophotometer (Genova MK3, New Malden, Surrey, UK). The decrease in absorbance of a sample was calculated in comparison with the DPPH solution. Measurements were performed in duplicate. The antioxidant activity, defined as the percentage of the DPPH free radical, was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = \frac{\mathbf{A - B}}{\mathbf{A}} \times \mathbf{100} \qquad (1)$$

where: A = absorbance of the DPPH control solution (0.1 mM DPPH solution), and
B = absorbance of the DPPH control solution + absorbance of the extract.

FRAP assay. FRAP analysis was performed according to the methodology reported by Benzie and Strain [39] with some minor modifications. This method considers the ferric to ferrous ion reduction at low pH, which results in a blue colored ferrous-tripyridyltriazine complex. The FRAP solution was prepared by mixing 47.5 mL of buffer acetate of pH 3.6, 4.75 mL of 10 mM TPTZ solution and 4.75 mL of 20 mM ferric chloride solution. The FRAP solution (3mL) reacted with 0.1 mL of the extract solution and 0.3 mL of water. The solution was then incubated at 37°C in a water bath for 30 min. Absorbance of the colored product was then measured at 593nm. The calibration curve was prepared using ferrous sulphate solutions and the results

were expressed in moles of ferrous sulphate per gram of sample. Additional dilutions were used when the reading of the FRAP solution was higher than the linear range of the calibration curve.

Statistical analysis. The software Design Expert (version 6) was used for the statistical analysis of the different parameters for the subcritical fluid extraction methods. All the experiments were performed in a randomized order. The experimental design consisted of 6 replicates at the center point. The validation of the model was done using the analysis of variance (ANOVA).

3. Results and Discussion

3.1 Proximate Compositional Analysis of Barley Hull

The proximate compositional analysis of BT 584 variety of barley hull is reported here for the first time (Table 1). The moisture content of BT 584 hull was low (7.54% on weight basis). Krawczyk et al. [40] reported that the total solid content of barley hulls (variety not specified) was 92%, therefore the amount of moisture in barley hull accounted for 8% of the total weight. The ash content of the barley hull in this study was 8.18%, which was similar to the result obtained by Krawczyk et al. [40]. However, an average ash content of 15.49% was reported by Garrote et al. [41], with increasing ash content for samples with smaller particle size. Total fat content of BT 584 hulls during this study was 1.45%, while a value of 3% fat content was reported for barley hulls from a variety named Cindy or waxy barley [8]. The protein content analyzed in this study was 5.53%, which is consistent with 4.45-10.65% protein content reported in the hull of other barley varieties [8,41,42]. The total phenolics obtained in this study (13.102±0.169 mg/g) were not similar to the 33-36 mg/g barley hull obtained by Garrote et al. [41], possibly due to the high temperature of autohydrolysis (190-229°C) used by Garrote et al. [41]. The total carbohydrate was the most abundant component of barley hull, accounting to approximately 75% on a weight basis. Barley hull in this study was composed of a high percentage of cellulose (32.6%), followed by hemicellulose (26.08%) and lignin (14.26%). The differences in the values obtained in this study with the values reported in the literature are probably due to differences in barley varieties used in the studies and/or different methodologies used for those analyses.

Table 1. Proximate compositional analysis of BT 584 hull

Component	This study	Literature	Reference
Moisture (%)	7.537±0.006	8-10	[40,43]
Ash (%)	8.187±0.001	9.1	[40,43]
Fat (%)	1.445±0.012	1-2	[8,43]
Protein (%)	5.533±0.006	7-10	[8,41,42]
Total phenolics (mg/g hull)	13.102±0.169	33-36	[41]
Total carbohydrates (%)	75.148±0.034	85-90	[43]
Cellulose (%)	32.66±0.116	26.5-28.8	[8,43,44]
Hemicellulose (%)	26.08±0.004	33.5-33.7	[43]
Lignin (%)	14.26±0.248	21.4-22.8	[8,43,44]

3.2 sCF extraction

The sCF extraction was performed in two different stages and further analysis was conducted on the basis on these two stages. The first stage was carried out to screen experiments to study the influence of some of the most important parameters (temperature, pressure, static holding time, and particle size). After obtaining the significant parameters, a second set of experiments was conducted to obtain the optimal values of the significant parameters using the RSM. For the preliminary study, temperature, pressure, particle size, and

static holding time were considered as the main factors that influenced the extraction. All these factors were analyzed on the basis of total carbohydrates and phenolics extraction.

Based on the results obtained in the screening study, the RSM was used to evaluate the effect of temperature (120-180°C), static holding time (2-20 min), flow rate (2-6 mL/min), and ethanol concentration (0-20%) to optimize the extraction of total carbohydrates and phenolics (Table 2). Changes in temperature had significant effects on the extraction, and also on the scavenging effect. The rise in temperature from 120 to 180°C at 15 MPa after approximately 30 min resulted in a 60% increase of the antioxidant activity. In general, phenolic compounds have high antioxidant activity [12]. A study of Pourali et al. [48] reported that the antioxidant activity using DPPH analysis of rice bran extract collected from the subcritical water treatment had the same profile as the total phenolics content of the extract.The increase in temperature also had a positive effect on the FRAP antioxidant activity. Treatment of barley hull during this study at 180°C for 25 min resulted in a maximum extraction of phenolics and thus the highest FRAP value (426 mMol ferrous sulphate reduction/g barley hull), while treatment at 120°C for 25 min resulted in a low antioxidant activity (54 mMol ferrous sulphate reduction/g barley hull) (Table 2). As observed using the ANOVA analysis, the temperature was the most significant factor for the maximum extraction of total carbohydrates (p = 0.01) and phenolics (p = 0.04), producing also a positive influence on the antioxidant activity.

Table 2. Response values for the sCF (aqueous ethanol) extraction

Run	Experimental				Total		Antioxidant activity	
	T** (°C)	Ethanol (%)	SHT** (min)	FR** (mL/min)	CHO** (mg/g)	Phe** (mg/g)	DPPH (%)	FRAP (mMol FS/g)
1	150	10	2	4	209.8	28.00	20.7	37.2
2	135	15	16	3	213.1	24.34	44.4	161.1
3	165	15	7	3	451.6	46.01	75.8	315.6
4	165	15	16	3	352.3	45.74	80.4	412.9
5	150	20	11	4	252.9	43.25	43.9	104.3
6	135	15	7	3	194.9	22.59	64.6	293.3
7	165	5	7	3	373.4	31.91	53.1	196.1
8	165	15	16	5	398.6	44.74	78.6	342.6
9	150	0	11	4	238.6	37.48	60.3	254.7
10	135	5	7	3	194.7	12.87	36.9	83.3
11	135	15	16	5	224.2	35.72	66.2	209
12	165	15	7	5	334.8	43.3	80.1	371.8
13	165	5	16	3	376.77	32.14	81.9	326.2
14	135	15	7	5	86.16	13.63	40.0	91.5
15	165	5	16	5	397.41	27.39	78.6	333
16	150	10	20	4	384.19	45.42	77.5	286
18	135	5	7	5	207.84	11.74	58.5	188.9
20	165	5	7	5	334.28	28.37	73.9	269.5
21	120	10	11	4	110.57	10.81	25.9	53.8
22	150	10	11	6	398.43	48.27	75.8	283.9
23	180	10	11	4	489.53	74.84	46.2	425.5
24	135	5	16	3	180.1	13.26	66.1	202.8
26	150	10	11	2	412.97	39.56	77.8	306.4
27	135	5	16	5	194.83	14.51	31.5	68.1
30*	150	10	11	4	424.7±8	45.30±1	74.1±7	270.7±1

**T: temperature, SHT: Static holding time, FR: Flow rate, CHO: carbohydrates, Phe: phenolics, DPPH: 2,2-DiPhenyl-1-PicrylHydrazyl, FRAP: Ferric Reducing/Antioxidant Power, FS: Fe_2SO_4 .
*Center point (based on 6 replicates): mean ± uncertainty.

Data obtained for total carbohydrates using Design Expert™ fitted well (R^2 = 0.945) into the quadratic polynomial equation (Fig 2a) while data for total phenolics fitted well (R^2 = 0.618) to the linear polynomial equation (Fig 2b).

The RSM optimized the different parameters (temperature, ethanol concentration, static holding time and flow rate) for the extraction, and generated the combination of the parameters which predicted the maximum extraction of total carbohydrates and phenolics. The optimal point for the extraction of total carbohydrates and phenolics was 180°C, 15 MPa using 12% ethanol at a flow rate of 5 mL/min. Therefore, extractions in duplicates were conducted at this optimal point and the average of the total carbohydrates and phenolics obtained after 20 min extraction were 450.3±7.83 mg/g and 70.3±1.33 mg/g, respectively. The predicted values for the total carbohydrates (460.0 mg/g) and the total phenolics (61.6 mg/g) were in agreement with the experimental values obtained during this study. The 1% difference in the predicted and the experimental values of total carbohydrates and phenolics may be due to the experimental errors. Since the optimal temperature for the maximum extraction for total carbohydrates and phenolics was 180°C (Table 2), further studies were performed to evaluate the effect of temperature on the extraction efficiency.

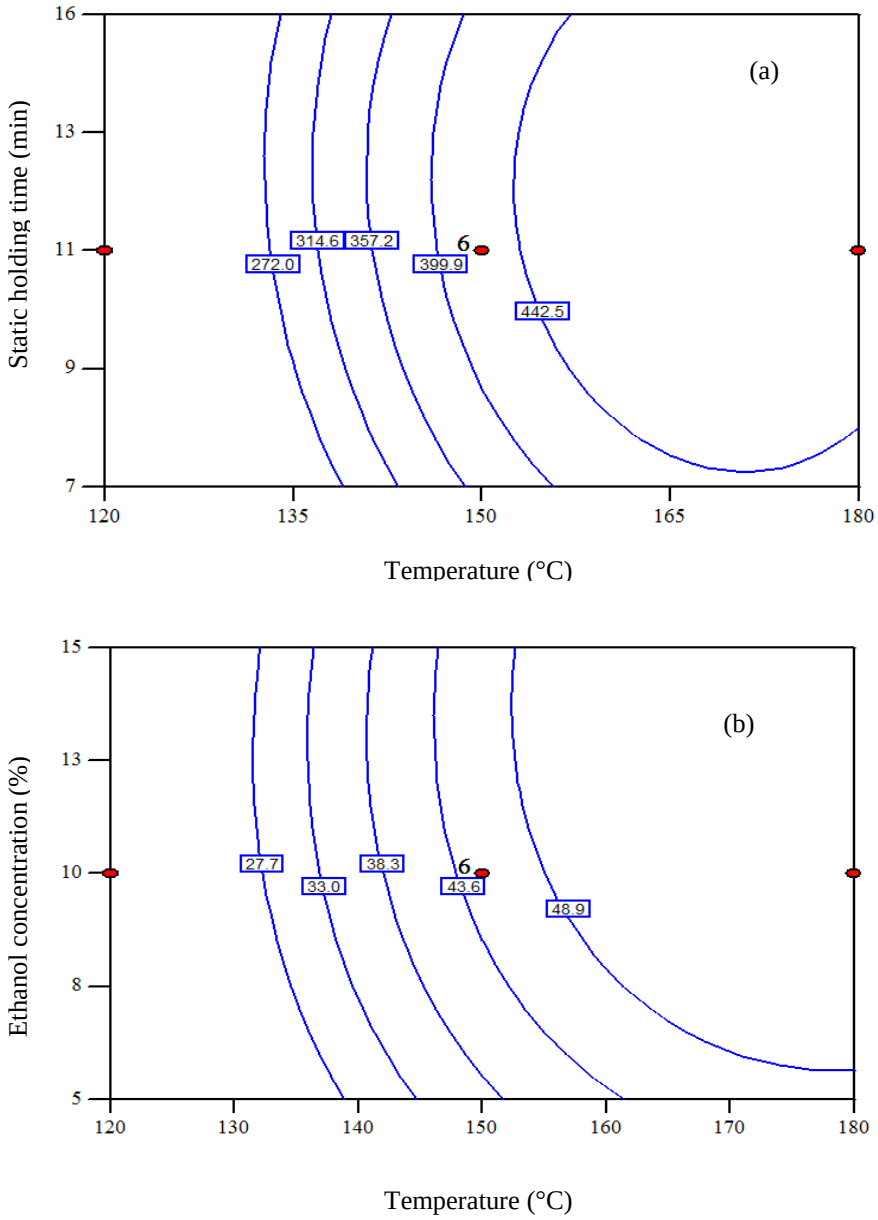

Figure 2. (a) Yield of total carbohydrates (mg/g) as a function of temperature and static holding time, and (b) yield of total phenolics (mg/g) as a function of temperature and ethanol concentration, for the sCF extraction of barley hull.

3.3 Effect of temperature during the sCF extraction

As temperature was the most significant parameter observed during the sCF extraction (Table 2), further studies at higher temperatures were evaluated. Fig 3 showed that temperatures up to 240°C produced a positive effect on the extraction of total phenolics (122.4±1.304 mg/g barley hull) and total carbohydrates (589.4±3.902 mg/g barley hull) after 20 min of extraction. The total carbohydrates (350.1±21.54mg/g barley hull) and total phenolics (95.2±3.79mg/g barley hull) at 260°C showed a substantial decrease, possibly due to thermal degradation [45-47]. The phenol-sulfuric acid method used for the analysis of total carbohydrates accounts for low molecular weight carbohydrates, such as monosaccharides and disaccharides [47]. Thus, the decrease of total carbohydrates at high temperatures (260°C, Fig 3) can be related to the degradation of carbohydrates into acids due to the acidity (pH~2) of the extracts [48]. In addition, the experiment at 260°C had a detrimental effect on the extraction of phenolics (97.9 mg/g barley hull) in Fig 3.

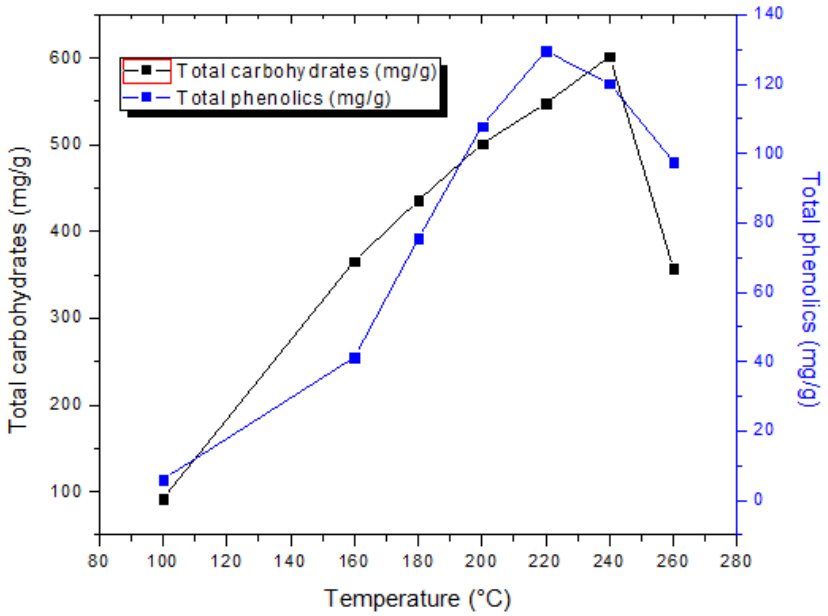

Figure 3. Effect of temperature on the extraction of total carbohydrates (mg/g) and total phenolics (mg/g) at 15 MPa using 12% ethanol at 5 mL/min, and 15 min of static holding time.

In addition to the total carbohydrates and phenolics, the antioxidant activity was also evaluated in the extracts. The change in the antioxidant activity (FRAP analysis) and scavenging activity (DPPH) with the change in temperature from 100 to 260°C is shown in Fig 4. The increase of temperature had a positive impact on the antioxidant activity. Hata et al. [49] observed that the increase in temperature from 50 to 250°C using subcritical water significantly increased the solubility of antioxidants, proteins and carbohydrates after a 5 min extraction. The same trend was observed for the antioxidant activity of the extracts in this study. The extract obtained at 100°C and 15 MPa using 12% aqueous ethanol at 5 mL/min had a very low DPPH value (36.97±6.44%) compared to the DPPH of the extract collected at 240°C (89.02±0.40%). The antioxidant activity determined by the FRAP assay for the extract solution collected at 240°C also showed a maximum antioxidant activity in the range of 568.47±1.7 mMol ferrous sulphate reduction/g barley hull.

The trend for the FRAP assay (Fig 4) is similar to the trend of total phenolics as observed in Fig 3. Pourali et al. [48] reported that phenolics extracted from rice bran possessed antioxidant activity as determined using the DPPH method. Thus, the change in the antioxidant activity in our study can be related to the phenolics in the extracts. Moreover, it has been reported that besides phenolics other non-phenolic compounds, such as carbohydrates and protein derived compounds possess antioxidant activity [49]. In this study, it was observed that the total carbohydrates and phenolics extracted at different temperatures (Fig 3) had the same shape profile as the antioxidant activity analysed by the FRAP assay (Fig 4).

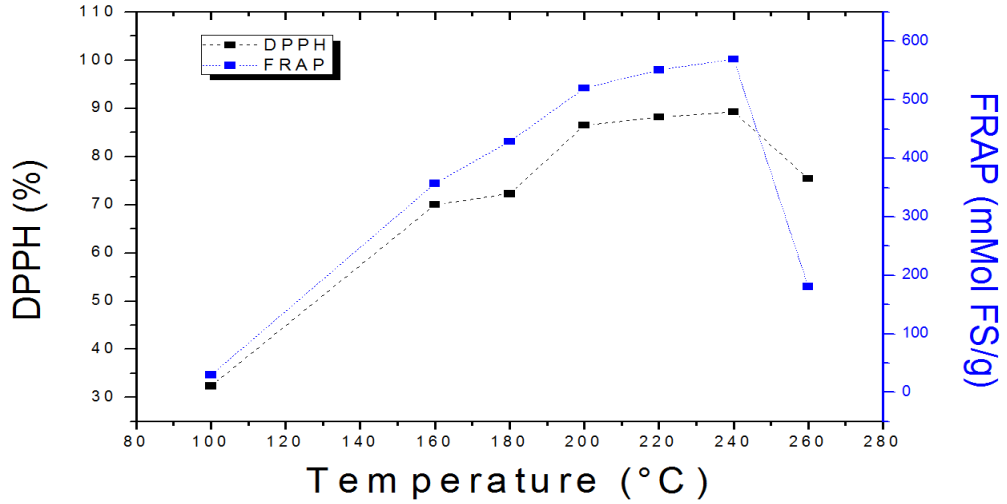

Figure 4. Effect of temperature on the FRAP and DPPH values at 15 MPa using 12% ethanol concentration at 5 mL/min, and 15 min of static holding time.

After determining the optimal extraction parameters (240°C, 15 MPa using 12% ethanol aqueous solutions at a flow rate of 5 mL/min) to maximize the amount of total carbohydrates and total phenolics extracted, the kinetics of the extraction was obtained. The extract solutions were collected every 5 min. After 30 min of extraction, there was no significant change in the extraction of total carbohydrates and phenolics. This indicated that the extraction time can be reduced to 30 min to obtain the maximum extraction of total carbohydrates (627.3 mg/g barley hull), and less than 20 min can be used for the highest extraction of total phenolics (129.7 mg/g barley hull).

4. Conclusions

Total carbohydrates and total phenolics were successfully extracted using the sCF extraction method. Temperature and static holding time ($p \leq 0.05$) were the most important variables for the sCF extraction. The addition of a polar solvent, such as ethanol to water solutions increased the extraction of total phenolics and total carbohydrates. At 150°C and 15MPa, subcritical water was able to extract 21.8 mg phenolics/g hull while 43.25 mg phenolics/g barley hull was extracted with the addition of 20% ethanol. In addition, the use of 20% ethanol increased the total carbohydrates extraction (252.9 mg carbohydrates/g barley hull) compared to subcritical water alone at 150°C and 15MPa (113.5 mg carbohydrates/g barley hull). The maximum extraction of total carbohydrates and phenolics was obtained at 240°C, 15MPa, 15 min static time, and 12% ethanol flowing with 5 mL/min using subcritical aqueous ethanol (589.4 and 122.38 mg/g, respectively). The sCF extraction can thus be scaled up to produce extracts rich in phenolics and carbohydrates.

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