Effect of alkaline and high-pressure homogenization on the extraction of phenolic acids from potato peels

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A B S T R A C T

Phenolic acids were extracted from potato peels with NaOH treatment and a
high-pressure homogenization (HPH) process. Total phenolic content, total
flavonoid content, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity
and extraction yield were determined with different treatment conditions.

Significant improvement was observed after the alkaline and HPH treatments. HPLC analysis shows that the phenolic compounds contained gallic acid, sinapic acid, vanillic acid, syringic acid, protocatechuic acid, *p*-coumaric acid, chlorogenic acid, caffeic acid and ferulic acid. The yields of these specific phenolic acids varied with different treatments. Scanning electron microscope, nuclear magnetic resonance and particle size measurements suggest that changes in cellular structure may be the mechanisms for the improvement in extraction rendered by the HPH and NaOH treatments. In conclusion, the combined treatment of NaOH and HPH increased the extraction yield and functionality of the phenolic acids from the potato peel residues.

Keywords:

Potato peel

Phenolic acid

High-pressure homogenization

NaOH treatment

1. Introduction

Potatoes are a major crop in the world. In 2013, 376.45 million tons of potatoes were produced (FAOSTAT). One third of the production was consumed as a fresh product while the rest went into processing flow to meet the demands for convenience and fast food consumptions, which include frozen fries and wedges,

41 crisps, starch and dried potatoes. During processing, most potatoes need to be
42 peeled with various methods, resulting in a large amount of potato peel residues.

43 The potato peel residues from food processing contain starch, non-starch
44 polysaccharide, protein, and fat. Therefore, it has been used as a carbon source in
45 fermentation to produce lactic acid, biofuels ([Arapoglou, Varzakas, Vlyssides, &](#)
46 [Israilides, 2010](#)), reducing sugars ([Bhattacharyya, Chakraborty, Datta, Drioli, &](#)
47 [Bhattacharjee, 2013](#)), and cellulolytic enzymes ([dos Santos, Gomes, Bonomo, &](#)
48 [Franco, 2012](#)). Phenolic compounds and glycoalkaloids are found in potatoes,
49 especially in potato peels. Ferulic acid, gallic acid, sinapic acid, vanillic acid, caffeic
50 acid, syringic acid, protocatechuic acid, *p*-coumaric and chlorogenic acid have been
51 extracted and identified in potato peels ([Friedman, 1997](#); [Maldonado, Mudge, Ganzle,](#)
52 [& Schieber, 2014](#)). Higher quantities and stronger antioxidant activity of these acids
53 were observed in potato peels than in potato flesh ([Wu, Xu, Ma, Cao, Ma, & Ma, 2012](#)).
54 These phenolic compounds have been proven to be an source of natural antioxidants,
55 which can replace unhealthy anti-oxidant additives used in food products, such as
56 butylated hydroxyanisole, butylated hydroxytoluene, tert-butyl hydroquinone
57 ([Rehman, Habib, & Shah, 2004](#)). These natural antioxidants can retard the oxidation
58 of proteins and lipids while exhibiting antimicrobial activity. They have been used in
59 radiated lamb meat ([Kanatt, Chander, Radhakrishna, & Sharma, 2005](#)), chilled
60 minced horse mackerel ([Farvin, Grejsen, & Jacobsen, 2012](#)), soy bean oil ([Rehman,](#)

[Habib, & Shah, 2004](#)), vegetables oil ([A. A. A. Mohdaly, Sarhan, Mahmoud, Ramadan, & Smetanska, 2010](#)), among other uses.

The phenolic acids may exist in free or bound form in potato peels. Most methods can isolate almost all of the free phenolic acids, but a smaller percentage of the bound phenolic acids. Most of ferulic acid and *p*-coumaric acid found in plants are bound to the cell wall polysaccharides through ester-bonds ([Fry, 1986](#)). The bound form phenolic acids can potentially offer strong antioxidant capacity if they are released from the cellular matrix ([Nara, Miyoshi, Honma, & Koga, 2006](#)). Alkaline treatments such as dilute sodium hydroxide solution have been shown to break the ester-bonds and release the bound form phenolic acids, resulting in a higher total yield of phenolic acids ([Nara, Miyoshi, Honma, & Koga, 2006](#)).

There have been many studies on the conventional methods for extraction of phenolic compounds from potato peels, using organic solvents such as methanol, acetone, ethanol, and ethyl acetate at high temperatures. It has been shown that methanol and ethanol can extract more phenolic acids from potato peels compared to acetone, hexane, diethyl ether and petroleum ether([Adel A. A. Mohdaly, Sarhan, Smetanska, & Mahmoud, 2010](#)). In addition, a few new methods have been developed to extract the active components in potato peels and other crop residues. These include ultrasonic assisted extraction ([Chen, Zhao, & Yu, 2015](#)), subcritical water or pressurized liquid extraction at above 100 °C under high pressure ([Alvarez,](#)

[Cahyadi, Xu, & Saldana, 2014](#); [P. P. Singh & Saldana, 2011](#)), acidified water- and ethanol-based solvents ([Maldonado, Mudge, Ganzle, & Schieber, 2014](#)), and microwave-assisted extraction ([A. Singh, Sabally, Kubow, Donnelly, Gariepy, Orsat, et al., 2011](#)).

High-pressure homogenization (HPH) has been reported to reduce particle size dramatically ([Grau, Kayser, & Müller, 2000](#); [Li, Ruan, Chen, Liu, Pan, Lin, et al., 2004](#)), and is used for pharmaceuticals, food and other materials manufacturing industries. Homogenization is mainly a mechanical process that disrupts cellular structures; for example, it breaks cell walls and compartments, resulting in the direct release of cellular products and increased access of solvent to cellular materials. Microfluidization, the HPH technique used in this study, can generate 160 Mpa, whereas conventional homogenization techniques usually generate 30 Mpa. In the microfluidization technique, high velocity micro-streams are created as a fluid accelerates into chambers so that the high impact shear can damage the cell walls and reduce the particle size more significantly ([McCrae, 1994](#)).

However, there is no report on the use of a method combining HPH and alkaline treatment to extract phenolic acids from potato peels. This research seeks to compare a combined method to alkaline treatment and HPH methods used separately; to study the effect of alkaline treatment time and concentration on the yield of total phenolic acid, total flavonoids and radical scavenging capacity; and to

determine the effect of the combined treatment process on the yield of individual major phenolic acids.

2. Materials and Methods

2.1 Materials and chemicals

The potato peel residues were provided by Old Dutch Food Inc. (Roseville, MN, USA). HPLC-grade acetonitrile and water were purchased from Sigma-Aldrich (St. Louis, MO, USA). Phenolic standards of highest purity available were used. Ferulic acid (FER), gallic acid (GAR), sinapic acid (SIN), vanillic acid (VAN), caffeic acid (CAF), syringic acid (SYR), protocatechuic acid (PRO), *p*-coumaric acid (*p*-COU) and chlorogenic acid (CHL) were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were analytical grade ordered from Sigma-Aldrich (St. Louis, MO, USA).

2.2 Sample preparation, chemical analysis and experiment procedure

Potato peels were lyophilized and milled with a laboratory bench mill, and then screened by sieves. The samples with particle size between 125µm and 355µm were used for the extraction experiments. The chemical composition of potato peels was analyzed by the Minnesota Valley Testing Laboratories, Inc (New Ulm, MN, USA). The moisture (AOAC 930.15), carbohydrate (calculated), fat (AOAC 2003.05), crude fiber

(AOCS BA6A-05), protein (AOAC 990.03), starch (MVTL R&D) and ash (AOAC 942.05) were determined before the process. The extraction procedure is as follows: 4 g potato peel residue powder was pretreated in ethanol with 100 ml NaOH of varying concentration (0-0.4 mol/L) at 40 °C for varying amounts of time (0-24 h). Table 2 shows the experimental conditions used for each sample. Sample Number 1 is the control sample that did not go through the NaOH pretreatment and HPH process. After that, the samples were neutralized with HCL in ethanol to pH 7.0 and passed through the High Pressure Homogenizer twice at 158.58 Mpa. All samples were diluted with ethanol to 200 mL and incubated at 40 °C for 1 h for extraction. In succession, the samples were centrifuged at 8000rpm for 10 min and filtrated through a 0.45 µm membrane. The upper layer liquid (supernatant) was collected and stored in -20 °C for further testing.

2.3 Determination of total phenolic content

A modified Folin-Ciocalteu assay ([Singleton, 1999](#)) was employed to determine the total phenolic content of potato peel extracts. Gallic acid was used as a standard and a series of gallic acid solutions (0-100 mg/L) was prepared to establish the standard curve, which was a plot of gallic acid concentration vs. absorbance at 760 nm. For the analysis, 100 µL Folin-Ciocalteu Reagent was added to 1 mL sample or blank. After 5 min, 1 mL Na₂CO₃ solution (12%) was added, followed by addition of 5

mL distilled water. After incubation for 1 h at room temperature, the absorbance of samples was measured on a DR5000 Spectrophotometer (Hach Company, Germany) at 760nm in a 1 mL cuvette. The results were expressed as mg gallic acid equivalent/g dry weight (mg GAE/g DW).

2.4 Determination of total flavonoid content (TFC)

The total flavonoid content was determined based on the method reported by ([Zhishen, Mengcheng, & Jianming, 1999](#)) with slight modification. A series of catechin solutions (0-100 mg/L) was prepared to establish the standard curve. Briefly, 1 mL sample or blank was diluted with 250 μ L of distilled water, and then 75 μ L NaNO_2 (5%) was added. After 5 min, 150 μ L AlCl_3 (10%) was added. After another 5 min, 500 μ L of 1M NaOH was added and finally the solution was diluted with 2 mL distilled water. After incubation for 30 min at room temperature, the absorbance was read at 510 nm on the DR5000 Spectrophotometer (Hach Company, Germany). The total flavonoid content was calculated by comparing the readings against the standard curve and expressed as mg catechin equivalent/g dry weight (mg catechin/g DW).

2.5 Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging capacity

The DPPH assay described by ([Brand-Williams, Cuvelier, & Berset, 1995](#)) was modified and used to measure the radical scavenging capacity of potato peel extracts. 300 µL sample or blank was mixed with 1 mL of 1 mM solution of DPPH in ethanol. The absorbance was read on the DR5000 Spectrophotometer (Hach Company, Germany), at 515 nm every 5 min until the reaction reached a plateau (around 70 min). The radical-scavenging activity (RSA) was calculated using the equation below as a percentage of DPPH discoloration:

$$RSA = \frac{(A_{control})_{t=60 \text{ min}} - (A_{sample})_{t=60 \text{ min}}}{(A_{control})_{t=60 \text{ min}}} \times 100$$

where A_{sample} is the absorbance of the sample at 515 nm, and A_{control} is the absorbance of a blank at 515 nm.

2.6 Extraction yield measurement

The yield measurement was carried out through freeze drying. Briefly, 10 mL of potato peel extract was lyophilized and the residue was collected and weighed. The extraction yield was calculated and displayed as mg (bioactive component)/g (potato peels). Every sample was measured in triplicate.

2.7 Particle size distribution

The particle size distribution was measured using a laser diffraction particle size analyzer (LS-13-320, Beckman Coulter Inc., Brea, CA) with the universal liquid module. The mean and median of the particle size was calculated from the triplicates.

2.8 Isolation and quantification of individual phenolic acids

The HPLC analysis was performed on a Varian Pro Star HPLC system, equipped with Polaris solvent delivery modules (Palo Alto, CA), employing a Acclaim C30 column (Thermo Fisher Scientific Inc. Waltham, MA), 3 μ m, 4.6 x 150mm. A gradient profile was developed for this test. In detail, the elution was carried out using a binary gradient consisting of 2% acetic acid (A) and 100% acetonitrile (B). The column temperature was set at 30 °C and the flow rate was 0.5 mL/min. The solvent gradient program was set as follows: 5% B for the first 5 min, then increased to 100% over 25 min. Then B was kept 100% for 13 min and decreased to 5% over 5 min. At the end B was kept at 5% until 60 min. The injection volume was 10 μ L and all the samples were filtered through a 0.45 μ m membrane. Gallic acid, sinapic acid, vanillic acid, syringic acid, protocatechuic acid, p-coumaric acid and chlorogenic acid were monitored at a wavelength of 280 nm, while caffeic acid and ferulic acid were detected at 320 nm using a Varian Pro Star 325 UV detector (Palo Alto, CA). The

concentrations were calculated from standard curves prepared under the same conditions.

2.9 Scanning electron microscopy

Scanning electron microscopy (SEM) was carried out to provide a visual confirmation of modification to the ultrastructure of potato peels. Samples were mounted on aluminum stubs with double-sided carbon adhesive tabs, and sputter coated with gold-palladium (60%-40%). Specimens were observed in a Hitachi S3500N scanning electron microscope at an accelerating voltage of 5 kV.

2.10 Statistical analysis

All experiments were carried out in triplicate and the results were expressed as mean $\pm$ standard deviation. All the data were subjected to analysis of variance ($p < 0.05$), and the results were processed by SPSS Version 23.0 (SPSS Inc., Chicago, IL, USA).

3 Results and discussion

3.1 Proximate composition of potato peels residue

The chemical composition of potato peels expressed as a percentage of weight is shown in Table 1. Carbohydrates were found to be the main component accounting

for 72.21-72.57 % of the potato peels. Among the carbohydrates, 45.60-49.45% was starch and 6.58-9.09% was crude fiber. Lower protein and higher moisture were observed compared with the data for samples obtained through abrasion and steam peeling procedures ([Camire, Violette, Dougherty, & McLaughlin, 1997](#)) . Similar ash, moisture, fat, protein and total carbohydrate can be found in potato peel powders of different particle size. However, more starch and lower crude fiber can be found in lower particle size samples.

3.2 Effect of NaOH and homogenization pretreatment on the extract yield

As shown in Table 2, the extraction yield (EY) increased from 24.83 mg/g to 102.33 mg/g with 0.1 mol/L NaOH treatment for 18 h, and improved a large amount when treated by HPH. The combined treatment yielded the highest EY. With the increase of NaOH treatment time from 6 h up to 24 h, the EY increased slightly. The homogenization process and concentration of NaOH have a significant impact on the extraction yield, while the pretreatment time did not affect it significantly ($p < 0.05$). Similar trends of total phenolic content (TPC) and total flavonoid content (TFC) can be observed from this table. The bound bioactive components, especially the phenolic acid, were released with alkaline treatment and HPH process. The TPC and TFC rose from 2.88 mg/g and 2.61 mg/g to 3.87 mg/g and 3.63 mg/g respectively with 0.1 mol/L NaOH treatment for 18 h. The HPH process augmented them to 3.67

mg/g and 3.11 mg/g. Furthermore, the combined treatment can push the TPC up to 4.16 mg/g with 0.1 mol/L NaOH treatment for 18 h, and the TFC up to 3.95 mg/g with 0.1 mol/L NaOH treatment for 12 h. Higher TPC and TFC can be obtained with 0.1 and 0.2 mol/L NaOH compared with 0.3 and 0.4 mol/L NaOH, whether or not they were treated by HPH. Consequently, the lower concentration alkaline treatment can contribute more to the active component release process. Similarly, with the rise in NaOH treatment time, no significant improvements of TPC or TFC were obtained. This could be due to the process finishing in 18 h and some antioxidant component being gradually oxidized after that. ANOVA ($p < 0.05$) also showed that both TPC and TFC were affected significantly by homogenization and NaOH concentration. However, pretreatment time of NaOH only have significant effect on TFC. From Table 2, it can be seen that the combined extraction method did not improve the TPC and TFC as much as the summation of two individual methods. One reason may be the starch pastes formed during the direct alkaline extraction, because the major component of the potato residue from the factory is starch. The starch pastes with a high viscosity affected the extraction efficiency and inhibited the diffusion of the extract ([Zhou, Robards, Helliwell, & Blanchard, 2004](#)). This effect reduced the velocity of the sample in the chamber, which in turn reduced the release of the active component. This can also be supported by the finding that the particle size of the control sample is lower than that of the samples treated by NaOH. The other reason

is that some kinds of phenolic acids were degraded during the extraction process which was supported by the result of HPLC.

3.3 Effect of NaOH and homogenization pretreatment on the antioxidant of extracts

Table 2 also shows that the radical-scavenging capacity expressed by DPPH was noticeably improved by NaOH and HPH treatment. The highest DPPH was 58.62 when the samples were processed by combined HPH and 0.2 mol/L NaOH treatment for 2 pass cycles and 18 h respectively. HPH changes the physical structure of the sample to obtain more active component, resulting in the increase of antioxidant capacity. The NaOH treatment softens the cell wall to release the bound active component. In conclusion, the NaOH and HPH treatment can be combined to improve the efficiency of extraction except for improvement of anaerobic digestion efficiency ([Fang, Zhang, Zhang, Jin, Li, Zhang, et al., 2014](#); [Zhang, Zhang, Zhang, Ma, Wu, & Ma, 2012](#)). The radical-scavenging capacity of extract from plants can be improved by HPH, which was also reported by([Hu, Nie, & Xie, 2013](#)). Varying concentration did not significantly affect DPPH, which also agrees with the findings of ([Hu, Nie, & Xie, 2013](#)). Additionally, with the prolonging of treatment time, the DPPH declined slightly because of the decomposition and oxidation of the active components in the extraction. The homogenization and concentration of NaOH significantly affected the DPPH, while the NaoH pretreatment time did not.

3.4 Effect of NaOH and homogenization pretreatment on the microstructure of potato peels

SEM micrographs of NaOH and HPH treatment potato peels are shown in Fig. 1 (500x). A through C show the samples pretreated with 0, 0.1 and 0.4 mol/L NaOH without HPH treatment while D through F express the samples pretreated with 0, 0.1 and 0.4 mol/L NaOH with HPH treatment. The structure of potato peels was damaged by HPH. The elliptical shape granules in Fig. 1 D-F are starch; half of the component in potato peels was starch (see Table 1), and starch tends to retain granules after HPH ([Guo, Zeng, Lu, Zhou, Zheng, & Zheng, 2015](#); [Le Thanh-Blicharz, Lewandowicz, Baszczak, & Prochaska, 2012](#)). The crude fiber was broken and the protein structure was changed; these segments adhered to the starch granules or separated, as reported by ([Sugumaran, Vimal, Kapur, & Narula, 2015](#)). During this process, the phenolic acids and flavonoids, which had been bound to the fiber structure, were released; this resulted in a dramatic increase of total phenolic acids and total flavonoids. In Fig. 1, more small segments are observed with increasing concentration of NaOH treatment. The reason could be that the alkaline treatment may solubilize extracellular polymeric substance ([Fang, et al., 2014](#)). The ester link was broken and the bound-form phenolic acids such as ferulic acid and p-coumaric were released. We can conclude that the combined treatment of NaOH and HPH produced many smaller segments found in the micrographs. The cellular structure

was altered by the treatment and more bioactive components were released during this process. Additionally, some crystals can be seen after the NaOH pretreatment and a large amount is observable when the concentration is 0.5 mol/L. These are probably the NaCl crystals from the neutralized reaction of NaOH and HCl, so the amount increased with increased concentration. Similar structures were found by ([Eswaran, Stoops, & Abtahi, 1980](#)).

For a more specific analysis, the particle size of potato peels with different treatment is shown in Fig. 2. Evidently, the HPH process reduced the mean particle sizes from 206.40-266.80 μm to 44.66-60.32 μm , with similar changes occurring in the median value as well. This result is supported by the SEM measurement result and those found by ([Hu, Nie, & Xie, 2013](#); [Valencia-Flores, Hernandez-Herrero, Guamis, & Ferragut, 2013](#)). It also can be concluded that the HPH process can reduce the particle size of potato peels dramatically due to the strong shear and high impact force broke the cell walls to generate the small cellulose fragment and smaller sphere. On the other side, the effect of NaOH treatment on the particle size is not significantly. It is supported by the SEM measurement result, we can see the NaOH treatment gained only small segments so that the mean and median of particle size declined slightly. The reason may be during the treatment produced small segments and swelled the other segments simultaneously. However, when the treatment time was over 12 h the particle size became smaller compared to the short time

treatment. Almost the same particle size can be obtained when they are carried out with HPH and NaOH combined treatment.

3.5 Effect of NaOH and homogenization pretreatment on the specific phenolic acid

Fig. 3. shows the results of HPLC analysis for extracts with different treatment conditions and revealed the major phenolic acid changes under different treatments. All treatments increased the phenolic yields to different degrees. The gallic acid yield was the major portion in the extract, while the syringic acid was the lowest. More chlorogenic acid and sinapic acid were produced with NaOH treatment while the protocatechuic acid, caffeic acid and vanillic acid yields declined slightly. The reason could be that NaOH can hydrolyze the bound acid and speed up the degradation of caffeic acid significantly, which has been reported by([Nardini, Cirillo, Natella, Mencarelli, Comisso, & Scaccini, 2002](#)) The NaOH treatment increased the bound-form phenolic acid yield, which was mainly contributed by the increased yields of ferulic acid, *p*-coumaric and syringic acid. This has been observed in the report of ([Nara, Miyoshi, Honma, & Koga, 2006](#)), in which the samples were treated with 4 mol/L NaOH for 1 hour at room temperature. The yield of protocatechuic acid, vanillic acid and caffeic acid, most of which are non-bound, can apparently be improved by the HPH.

4 conclusions

This study is the first time that HPH treatment was used to improve the extraction of phenolic acid from potato peels residues. The total phenolic acid extraction yield can be improved by the combined NaOH and HPH treatment. However, the NaOH concentration and treatment time were not the main factors affecting the extraction. From the results of particle size and SEM measurements, it is clear that the HPH process produced the smaller segments that significantly helped the release of the active components. The HPLC measurement also demonstrated that the NaOH and HPH treatment improved the extraction yields of most of the individual phenolic acids. In conclusion, we have shown that the combined NaOH and HPH treatment is an efficient method to promote the release and extraction of phenolic acids from potato peel residues.

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Fig. 1. Scanning electron micrographs of potato peels of control (A), treatment with 0.1 N NaOH (B), 0.5 N NaOH (C), HPH (D), 0.1 N NaOH+HPH (E) and 0.5 N NaOH (F) and all the NaOH treatment were processed for 18 h..

Fig. 2. the effect of NaOH concentration (a) and NaOH treatment time (b) combined with HPH treatment on the mean and median of potato peels particle size.

Fig. 3. (a) HPLC chromatogram of the standard samples mix, peak 1, gallic acid; 2, protocatechuic acid; peak 3, chlorogenic acid ; peak 4, vanillic acid; peak 5, syring acid; peak 6, *p*-coumaric acid; peak 7 sinapic acid; peak 8, caffeic acid; peak 9 ferulic acid. (b) the effect of NaOH and HPH treatment on different phenolic compounds, the NaOH treatment was processed with 0.1 N NaOH for 18 h

Fig. 1. Scanning electron micrographs of potato peels of control (A), treatment with 0.1 N NaOH (B), 0.4 N NaOH (C), HPH (D), 0.1N NaOH+HPH (E) and 0.4N+HPH NaOH (F), and all the NaOH treatment were processed for 18 h.

(a)

(b)

Fig. 2. the effect of NaOH concentration (a) and NaOH treatment time (b) combined with HPH treatment on the mean and median of potato peels particle size.

(a)

(b)

Fig. 3. (a) HPLC chromatogram of the standard samples mix, peak 1, gallic acid; 2, protocatechuic acid; peak 3, chlorogenic acid ; peak 4, vanillic acid; peak 5, syring acid; peak 6, *p*-coumaric acid; peak 7 sinapic acid; peak 8, caffeic acid; peak 9 ferulic acid. (b) the effect of NaOH and HPH treatment on different phenolic compounds, the NaOH treatment was processed with 0.1 N NaOH for 18 h

Particle size	125-212µm (%)	212-355µm (%)
Moisture	6.00	5.55
Carbohydrate	72.57	72.21
Starch	49.40	45.60
Fiber, Crude	6.58	9.09
Fat, ethyl ether	1.04	1.33
Protein N×6.25	12.90	13.60
Ash	7.49	7.31

Table 1 Proximate composition of potato peels residue

Number	Homogenization	NaOH Concentration (mol/L)	NaOH Preatment Time (h)	EY (mg/g)	TPC (mg/g)	TFC (mg/g)	DPPH (%)
1	0	0	18	24.83±1.76 e	2.88±0.07 c	3.07±0.11 c	36.38±2.26 c
2	0	0.1	18	102.33±1.04 cd	3.87±0.04 ab	3.63±0.17 bc	46.14±1.25 b
3	0	0.2	18	94.50±3.50 d	3.82±0.10 ab	3.40±0.04 c	45.67±0.50 b
4	0	0.3	18	97.17±3.61 d	3.59±0.05 b	3.12±0.24 c	44.91±2.36 b
5	0	0.4	18	103.50±3.00 cd	3.56±0.15 b	3.12±0.06 c	44.25±3.06 b
6	2	0	18	61.50±5.68 d	3.67±0.06 b	3.69±0.13 bc	45.51±3.32 b
7	2	0.1	18	104.50±4.36 cd	4.16±0.22 a	3.90±0.09 bc	55.24±1.25 a
8	2	0.2	18	101.33±1.61 cd	4.09±0.02 ab	4.40±0.22 a	58.62±2.48 a
9	2	0.3	18	136.17±3.32 a	3.93±0.15 ab	3.50±0.07 c	51.39±3.76 ab
10	2	0.4	18	124.17±2.57 bc	3.78±0.11 ab	3.40±0.04 c	51.82±4.10 ab
11	0	0.1	0	17.83±4.01 e	2.73±0.11 c	2.61±0.10 d	29.59±2.31 c
12	0	0.1	6	101.50±2.00 cd	3.75±0.09 b	3.32±0.21 c	45.35±2.44 b
13	0	0.1	12	103.67±5.80 cd	3.83±0.18 ab	3.55±0.21 bc	47.38±2.44 b
14(2)	0	0.1	18	102.33±1.04 cd	3.87±0.04 ab	3.63±0.17 bc	46.14±1.56 b
15	0	0.1	24	110.33±4.16 c	3.85±0.10 ab	3.43±0.08 c	42.64±2.13 bc
16	2	0.1	0	60.50±2.66 d	3.60±0.26 b	3.11±0.12 c	34.62±2.91 c
17	2	0.1	6	100.00±1.82 cd	3.83±0.04 ab	3.86±0.05 bc	56.38±2.43 a
18	2	0.1	12	104.83±7.26 cd	4.09±0.19 ab	3.95±0.23 b	54.84±1.51 ab
19(7)	2	0.1	18	104.50±4.36 cd	4.16±0.22 a	3.90±0.09 bc	55.24±1.25 a
20	2	0.1	24	118.17±5.25 bc	4.02±0.08 ab	3.68±0.15 bc	55.84±3.63 a

*Mean ± standard deviations within columns for each experiment are significantly different (p<0.05, Tukey HSD test) when followed by different letters.

Table 2 The results of extraction yield (EY), total phenolic content (TPC), total flavonoid content (TFC) and DPPH

radical-scavenging capacity (DPPH) under different treatment condition