

Effect of Boiling on the Nutritional Value, Phytochemical Contents, and Antioxidant Activity of Commonly Consumed Potato (*Solanum tuberosum* L.) Varieties in Bangladesh

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Abstract Potatoes (*Solanum tuberosum* L.) are one of the common vegetables in Bangladesh. The present study was aimed to assess the effect of boiling on the nutritional value, phytochemical contents, and antioxidant activity of two commonly consumed potato varieties in Bangladesh; Lal Sheel and Diamant. Our results show that boiling increased moisture and crude fiber contents while ash, crude lipid, carbohydrate, and protein contents decreased in both potato varieties. Both Lal Sheel and Diamant potatoes contained considerable amounts of polyphenols (expressed as gallic acid equivalents, mg GAE per 100g sample), flavonoids (expressed as catechin equivalents, mg CE per 100g sample) and tannins (expressed as tannic acid equivalents, mg TAE per 100g sample) and all those significantly decreased after boiling. However, boiled Lal Sheel potato retained higher amounts of these phytochemicals [368.23±13.05 mg GAE/100g, 351.91±31.45 mg CE/100g, and 3946.54±298.72 mg TAE/100g, respectively] than those of boiled Diamant variety [329.12±3.72 mg GAE/100g, 312.51±12.18 mg CE/100g, and 2897.72±454.54 mg TAE/100g, respectively]. Like phytochemicals, antioxidant activity also decreased after boiling, as measured by ferric reducing antioxidant power (FRAP), phosphomolybdate reduction, and total reducing power assays, as well as, 1,1-diphenyl-2-picrylhydrazyl- (DPPH)-radical scavenging activity. Vitamin C content was good but β-Carotene, vitamin A, vitamin B1, B2 and essential minerals including iron, zinc, copper contents were relatively low, all of which also decreased after boiling. Despite the negative impacts of boiling, both Lal Sheel and Diamant boiled potatoes retained good amounts of nutrients, as well as, phytochemicals possessing antioxidant activity and thus are beneficial foodstuffs for health.

Keywords: potato, boiling effect, proximate composition, phytochemicals, antioxidant activity, vitamins

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1. Introduction

Potatoes (*Solanum tuberosum* L.) are a tuberous crop grown all over the world [1]. Potatoes, locally known as Alu, are one of the common vegetables in Bangladesh [2]. It is mainly produced in Munshiganj, Bogura, Rangpur, Dinajpur and some parts of Cumilla region of Bangladesh [3,4]. Potato production is gradually increasing and recently it has occupied an important place in the list of major food and cash crops of Bangladesh [3,4,5,6]. People who are low income group can get necessary nutrients and meet vegetable demands from potato [7]. Bangladesh produces 40 local varieties of potato. Some popular local varieties are Lal Sheel, Sheel Bilatee, Lal Pakri, and Lal Kufri [4]. Dozens of potentially high yielding varieties of potato have also been cultivated in Bangladesh. High yielding varieties are foreign varieties improved by

Bangladesh Agricultural Research Institute (BARI). Some popular high yielding varieties are Diamant, Cardinal, and Kufri Sindhury [3]. Among all, the local variety 'Lal Sheel' and the high yielding variety 'Diamant' are widely cultivated and consumed in Bangladesh. Lal Sheel potato, known as *Lal Madda* with rounded and reddish tuber, is cultivated mainly in Bogura while Diamant, with tuber oval to long shape, white to yellow skin and cream or pale yellow-colored flesh, is cultivated in all regions [3,4].

Potato tubers contain carbohydrate, protein, lipids, dietary fibers, water soluble vitamins such as C, B1, B2 and B3, carotenoids, essential minerals, and phytochemicals including polyphenols and flavonoids [1,8,9,10,11,12,13]. Environmental factors such as climate, soil, weather, fertilization, and use of pesticides affect the chemical composition of potato [13]. Phytochemicals of plants are produced for their own protection. Stress, infection, microbial attack, UV radiation, water and

nutritional deficiency can induce the production of these compounds [14,15]. Potato tubers are thought to show the antioxidant activity mainly through their antioxidant phytochemicals. Lee et al (2016) reported that colored flesh potatoes have higher antioxidant activities than the white-fleshed variety [16]. Antioxidant compounds have greatest ability to act against free radicals such as reactive oxygen species (ROS), superoxide, hydroxyl radicals etc. and thus protect cells from oxidative damages [16,17].

Potatoes are consumed all over the world after being cooked or processed [18]. Cooking improves microbiological and organoleptic qualities, increases nutritional bioavailability and digestibility and destroys toxins and antinutrients [19]. The most widely used methods of cooking are boiling, steaming, pressure cooking, baking and frying, in which destruction, release and transformations of phytochemicals take place in potato [18,19,20,21]. Potatoes are consumed also as processed products such as fries, chips, canned and mashed potatoes [1]. Available studies confirmed that retention of nutrients and phytochemicals in potatoes is affected by various cooking and processing methods [1,13,19,20,21]. When potatoes are overproduced, people store sundried potato as potato flour for long time and use it in off season in various recipes. Potato flour can be made by drying the peeled slices or whole unpeeled potato followed by grinding. Some food industries also use potato flour for manufacturing various food items such as biscuits and breads [10,22].

Impacts of cooking or processing on nutrient and phytochemical contents and antioxidant activity have been mostly reported on potatoes grown outside of Bangladesh. To date, information concerning effects of boiling on potato varieties commonly grown in Bangladesh is lacking. In the present study, we selected two most widely cultivated and consumed potato varieties in Bangladesh, Lal Sheel and Diamant; and accessed the effects of boiling on their nutritional value, phytochemical content, and antioxidant properties. Health benefits retained in both potato varieties after being boiled were also compared. Soil and ground water pollution is a threat for toxic metal contamination in potatoes which can affect human health [23]. In recent years, the farmers in Bangladesh have been using excess amount of fertilizers and doing frequent irrigation to get a very high yield. This situation may result in polluted ground water and soil. Considering this fact, it was also investigated whether our selected potato varieties contain any toxic heavy metals that could be harmful for human health.

2. Materials and Methods

2.1. Chemicals and Reagents

Gallic acid (3,4,5-trihydroxybenzoic acid), catechin, tannic acid, green vitriol ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and ascorbic acid standards; and 2,4,6-Tris(2-pyridyl)-1,3,5-triazine (TPTZ), thiourea and 2,4-dinitrophenylhydrazine reagents were purchased from Sigma-Aldrich (St. Louis, MO). Aluminium chloride (AlCl_3), sodium nitrite (NaNO_2), sodium hydroxide (NaOH), sodium acetate ($\text{C}_2\text{H}_3\text{NaO}_2$), ammonium molybdate ($(\text{NH}_4)_2\text{MoO}_4$), trichloroacetic acid

(TCA), sodium carbonate (Na_2CO_3) were purchased from Merck Co. (Darmstadt, Germany). Folin-Ciocalteu's phenol reagent, sodium phosphate monobasic and di-basic, ethanol and all other necessary chemicals were purchased from LOBA Chemie (Mumbai, India). Vitamin B1 (thiamine HCl) and B2 (riboflavin) standards were purchased from DUCHEFA (Haarlem, The Netherlands). The standard reference elements (iron, zinc, copper, lead, chromium and cadmium) were purchased from Kanto Chemical (Tokyo, Japan).

2.2. Sample Collection and Processing

Two different potato varieties, namely 'Lal Sheel' and 'Diamant', were purchased from a local store in Dhaka, Bangladesh. Both potato varieties were thoroughly washed with distilled water to remove any dirt, and then were chopped without peeling them (Figure 1). About half of each potato variety (~2.0 kg) was boiled in water for 10 minutes at 100°C and then sundried. The remaining half of each variety (~2.0 kg) was only sundried. After that the potatoes were grinded in a grinder machine (Panasonic Mixer Grinder MX AC210, Malaysia) and the sample was collected separately.

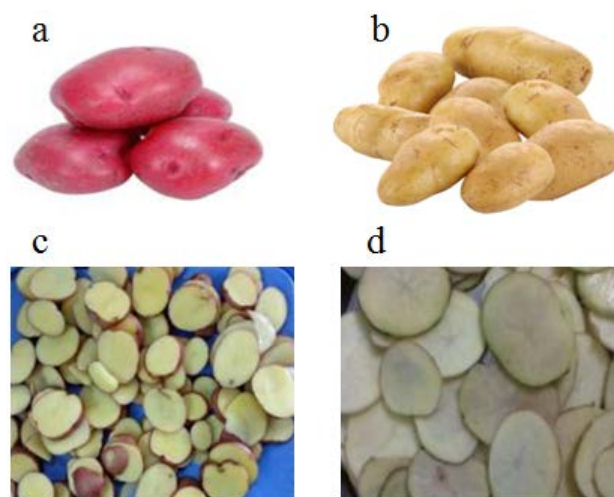


Figure 1. Lal Sheel potato, also known as *Lal Madda* and *Bograi alu*, with rounded and reddish tuber (a), and Diamant, with tuber oval to long shape, white to yellow skin and cream or pale yellow-colored flesh (b). Both Lal Sheel and Diamant potatoes were chopped at a rounded shape (c) and (d), respectively, for extract preparation

2.3. Extract Preparation

About 200g of each sample was dissolved in ethanol for 5 days while rotating at regular interval for efficient mixing of the sample with the solvent. After that, the samples were first filtered through cotton cloth and then through Whatman No. 1 filter paper which was fitted with a suction apparatus (Rocker 300). After two subsequent filtration processes through the Whatman filter paper, the solution from each sample was carefully stored for evaporation. After that, each sample was heated at 100°C using an evaporator and the residue was carefully collected in different petri plates. Then, the petri plates were kept overnight in an incubator at 42°C . Finally, each sample extract was weighed and stored for further experiments.

2.4. Estimation of Proximate Composition

Proximate composition including moisture, ash, crude lipid, crude fiber, total carbohydrate, and crude protein were analyzed. Moisture content was determined by Moisture Weighed Box (A & D company ltd. N92: P1011656, Japan). Ash content was determined according to the AOAC official method 942.05 [24]. Crude lipid was measured by Soxhlet extraction following the AOAC official method 960.39 [25]. Crude fiber was determined as described elsewhere, with slight modifications [26]. Crude protein was determined by the Kjeldahl method (nitrogen content multiplied by 6.25) [27]. Carbohydrate content was obtained by subtracting the sum of moisture, ash, crude lipid, crude fiber and crude protein contents from 100, as described by Rand et al. (1991) [28].

2.5. Estimation of Phytochemicals

Polyphenol content: Total polyphenol content was estimated using modified Folin-Ciocalteu method [29]. In brief, 0.4 mL of sample extract and different concentrations of standard gallic acid (20-100 µg/mL) were taken in different test tubes. After that, 1.6 mL of 7.5% sodium carbonate (Na₂CO₃) was added to each tube. Then, 2.0 mL of 10-fold diluted Folin-Ciocalteu's phenol reagent was added to each tube and incubated for 1-hour. Finally, the absorbance was measured at 765 nm using a PD-303S spectrophotometer (APEL Co. Ltd, Kawaguchi, Japan). Total polyphenol content was calculated as gallic acid equivalents (GAE) and expressed as mg GAE per 100g of sample.

Flavonoid content: Total flavonoid content was estimated by colorimetric assay [30]. At first, 1.0 mL of the extract and different concentrations of standard catechin (7.8-250 µg/mL) were taken in different test tubes and then 4.0 mL of distilled water was added. After that, 0.3 mL of 5% sodium nitrite (NaNO₂) was added to each tube. After 5 minutes, 0.3 mL of 10% aluminium chloride (AlCl₃) was added to each tube. Then, 6 minutes later 2.0 mL of 1.0M sodium hydroxide (NaOH) was added to each tube. After that, 2.4 mL of distilled water was added to each tube and made the volume 10.0 mL. Finally, the solutions were mixed well and the absorbance was measured at 510 nm using spectrophotometer. Total flavonoid content was calculated as catechin equivalents (CE) and expressed as mg CE per 100g of sample.

Tannin content: Total tannin content was estimated by Folin-Ciocalteu method as described elsewhere [31]. At first, 0.1 mL of sample extract and different concentrations of standard tannic acid (31.25-1000 µg/mL) were taken in different test tubes. Then, 7.5 mL of distilled water was added. After that, 0.5 mL of folin-ciocalteu's phenol reagent and 1.0 mL of 35% sodium carbonate were added. Then, 0.9 mL of distilled water was added and incubated for 30 minutes. Finally, the absorbance was measured at 725 nm. Tannin content was calculated as tannic acid equivalents (TAE) and expressed as mg TAE per 100g of sample.

Determination of β-carotene: β-carotene content was determined as described previously [32]. Briefly, 100 mg of sample extracts were vigorously shaken in a 10.0 mL of acetone-hexane mixture (4:6) and then filtered through

Whatman No. 1 filter paper. The absorbance of each filtrate was measured at 453, 505, and 663 nm. Finally, the content of β-carotene was calculated according to the following equation:

$$\begin{aligned} \beta\text{-Carotene (mg/100 mL)} \\ = 0.216 \times A_{663} - 0.304 \times A_{505} + 0.452 \times A_{453}. \end{aligned}$$

The assay was carried out in triplicate and the result was expressed as mg carotenoid/100g of sample.

2.6. Vitamin A and C contents

Determination of vitamin A: Vitamin A content was determined according to the Retinol Equivalent (RE) concept of FAO/WHO, 1967 as described elsewhere [32]. Following relationships were applied to determine vitamin A content:

$$1 \mu\text{g retinol} = 1 \text{ RE}$$

$$1 \mu\text{g } \beta\text{-carotene} = 0.167 \mu\text{g RE}$$

$$1 \mu\text{g other pro-vitamin A carotenoids} = 0.084 \mu\text{g RE}.$$

The conversion factors were only average estimates for a mixed diet. Result was calculated as RE/100g of sample and finally converted into International Unit (IU) by multiply with 0.3.

Estimation of vitamin C: Vitamin C content was colorimetrically estimated as previous [30]. In brief, 0.5 mL of sample extract and different concentrations of standard ascorbic acid (0.312-10 µg/mL) were taken in different test tubes which were diluted by 5% TCA. Then, 0.1 mL of DTC solution (2,4-dinitrophenylhydrazine-thiourea-copper sulfate) was added to each tube, mixed well and incubated for 3 hours at 37°C. After that, 750 µL of 65% ice cold sulfuric acid was added, mixed well and kept at room temperature for 30 minutes. Finally, the absorbance was measured at 520 nm. Vitamin C content was calculated as ascorbic acid equivalents (AAE) and expressed as mg AAE per 100g of sample.

2.7. Determination of Antioxidant Activity

Ferric reducing antioxidant power (FRAP) assay: Total antioxidant activity of the sample was determined by Ferric Reducing Antioxidant Power (FRAP) assay as described elsewhere [33]. FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10.0 mM TPTZ solution in 40 mM HCl, and 20.0 mM FeCl₃·6H₂O in the ratio of 10:1:1. For the assay, 0.2 mL of sample extracts at various concentrations (0.0625-1.0 mg/mL) were taken in different test tubes. Then, 1.5 mL of FRAP reagent was added to each tube and incubated at 37°C for 4 minutes. Finally, the absorbance was measured at 593 nm against the blank. Green vitriol was used as reference standard.

Phosphomolybdate assay: Total antioxidant activity of the sample was also determined by phosphomolybdate assay method with slight modifications [34]. In brief, 0.3 mL of sample extracts at different concentrations (0.0625-1.0 mg/mL) were mixed with 3.0 mL of the reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4.0 mM ammonium molybdate) and incubated in a boiling water bath at 95°C for 90 minutes. After cooling to

room temperature, the absorbance was measured at 695 nm against the blank. Catechin was used as reference standard. The phosphomolybdate reduction power of the tested samples was compared with that of the standard.

Total reducing power assay: Total reducing power of the sample was assayed as described elsewhere [35]. Briefly, 1.0 mL of sample extracts at different concentrations (0.0625-1.0 mg/mL) was mixed with 2.5 mL of phosphate buffer and 2.5 mL of potassium ferricyanide. Then, the mixtures were kept in a water bath at 50°C for 30 minutes. After cooling, 2.5 mL of 10% TCA was added and centrifuged at 3000 rpm for 10 minutes. Then, the upper layer of each solution (2.5 mL) was mixed with distilled water (2.5 mL) and freshly prepared 0.1% ferric chloride (0.5 mL). Finally, the absorbance was measured at 700 nm against blank that contained distilled water and phosphate buffer. Increase in absorbance indicates increased reducing power of the sample. Ascorbic acid was used as reference standard. The reducing power of the sample extracts was compared with that of the standard.

DPPH-radical scavenging activity: DPPH-radical scavenging activity was assessed by determining the decrease in absorbance of DPPH at 517nm [36]. DPPH is a deep purple colored free radical which is decolorized when picks up an electron from an antioxidant. Briefly, 1.0 mL of sample extracts at various concentrations (0.0312-0.50 mg/mL) was taken in different test tubes. Then, 1.0 mL of 0.1 mM DPPH in methanol was added, mixed, and finally allowed to incubate in the dark at room temperature for 35 minutes. The discoloration of DPPH was measured against methanol blank. Ascorbic acid was used as reference standard. The inhibition percentage for scavenging DPPH radical was calculated according to the following equation:

$$\% \text{ inhibition} = \left[\frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \right] \times 100$$

Where, Abs control is the absorbance of DPPH radical in methanol, Abs sample is the absorbance of DPPH radical in the presence of either the sample extract or the standard. Control was prepared by adding 1.0 mL of methanol with 1.0 mL of DPPH reagent.

Then, the curves were constructed by plotting the percentage of DPPH inhibition against the concentration of the sample extracts. The DPPH scavenging activity was expressed as IC₅₀ value, which was calculated from the equation of the curve corresponding to the sample concentration that reduced the initial DPPH absorbance by 50%. The IC₅₀ values of the sample extracts obtained were compared to that of the standard ascorbic acid. A smaller IC₅₀ value corresponds to a higher free radical scavenging activity.

2.8. Estimation of B complex Vitamins

Vitamin B1 and B2 contents were estimated by high performance liquid chromatography (HPLC) as described by Aslam *et al.* (2008), with some modifications [37]. A high performance liquid chromatographic system (Shimadzu-UFLC Prominence), equipped with an auto sampler (Model-SIL 20AC HT) and an UV-visible detector (Model-SPD 20A), was used for the analysis. The data were recorded using LC-solutions software. An

analytical reversed phase C18 column (250×4.6 mm, 5 μm) was used (Ultremex C18, Phenomenex Inc). Buffer was prepared using 1.08g of hexane sulphonic acid sodium salt, 1.36g of potassium dihydrogen phosphate, 995 mL of double distilled HPLC water, and 5.0 mL of triethylamine, and its pH (3.0) was adjusted with orthophosphoric acid. Mobile phase, consisted of prepared buffer and HPLC grade methanol in a ratio of 50:50 (v/v), was delivered at a flow rate of 1.0 mL/min with UV detection at 254 nm. Standard curves were plotted based on concentration and peak area. The data of peak area versus concentration of vitamin standards were treated by linear least-square regression, from which the regression equations were obtained and vitamin contents were estimated.

2.9. Determination of Mineral and Heavy Metal Contents

Essential minerals and heavy metals were determined by atomic absorption spectroscopy (AAS) as described by Anwar *et al.* (2004), with some modifications [38]. At first, 2.0g of each sample was taken into 100 mL glass beaker. Then, 25.0 mL of 65% HNO₃ was added to each beaker, gently shaken and kept aside for few minutes. After that, the beakers were placed on the hot plate and set the temperature to 120°C. After 1-hour of acid digestion the beakers were put away from the hot plate and cooled down. Then, 10.0 mL of perchloric acid was added to each beaker which turned the sample solution into orange color. Then, the digestion process was continued on the hot plate at temperature between 100-135°C and stopped when the color of the sample solution changed from orange to yellowish. After cooling, 2.0 mL of HCl was added and made the volume 60.0 mL with deionized water which turned the color of the sample solution into light green. The solution was filtered in a conical flask through Whatman No. 1 filter paper and 40.0 mL of deionized water was added to make the volume 100.0 mL. Finally, the contents were transferred into plastic bottles for assessment using Atomic absorption spectrophotometer (Shimadzu AA-7000).

2.10. Statistical Analyses

All the analyses were performed in triplicate and the data are expressed as mean ± standard deviation (SD). Two tailed t-test was performed to assess statistical significance ($P < 0.05$). All statistical calculations were performed using MS Excel 2013 of windows 10.

3. Results

3.1. Proximate Composition

Proximate composition of two different potato varieties is shown in Table 1. Moisture content was almost the same whereas crude fiber content was significantly higher in unboiled Diamant potato than that of unboiled Lal Sheel variety. When boiled, both moisture and crude fiber contents increased in both potato varieties. In contrast, unboiled Lal Sheel potato had relatively higher ash and

crude lipid contents than those of Diamant potato and due to boiling both ash and crude lipid contents significantly decreased. Results also show that total carbohydrate and crude protein contents were almost the same in Lal Sheel and Diamant potatoes in unboiled condition, both of which slightly decreased after boiling.

Table 1. Proximate composition (% per 100g) of two different potato varieties in unboiled and boiled conditions

Parameters	Lal Sheel		Diamant	
	Unboiled	Boiled	Unboiled	Boiled
Moisture	71.98 ± 0.73 ^a	73.12 ± 0.55 ^a	71.81 ± 0.58 ^a	73.07 ± 0.80 ^a
Ash	1.92 ± 0.11 ^a	1.52 ± 0.05 ^b	1.78 ± 0.17 ^a	1.49 ± 0.14 ^b
Crude lipid	0.75 ± 0.08 ^a	0.49 ± 0.07 ^b	0.70 ± 0.09 ^a	0.54 ± 0.10 ^b
Crude fiber	1.39 ± 0.11 ^a	1.96 ± 0.05 ^b	1.84 ± 0.12 ^b	2.40 ± 0.15 ^c
Carbohydrate	22.14 ± 0.72 ^a	21.15 ± 0.56 ^a	22.07 ± 0.52 ^a	20.78 ± 0.80 ^a
Protein	1.82 ± 0.24 ^a	1.76 ± 0.49 ^a	1.80 ± 0.37 ^a	1.72 ± 0.42 ^a

Data are expressed as mean ± standard deviation. $P < 0.05$ was considered statistically significant. Values that do not share a common superscript letter within the same row are significantly different from each other.

3.2. Phytochemical Contents

Phytochemical contents of two different potato varieties are shown in Table 2. Among the phytochemicals, total flavonoid and β-carotene contents were higher but total polyphenol and tannin contents were lower in unboiled Lal Sheel potato than those of unboiled Diamant potato. Except β-carotene in Diamant variety, contents of all the phytochemicals significantly decreased in both varieties due to boiling. However, boiled Lal Sheel potato retained significantly higher amount of these phytochemicals than those retained by boiled Diamant variety.

Table 2. Phytochemicals, vitamin A and vitamin C contents of two different potato varieties in unboiled and boiled conditions

Parameters	Lal Sheel		Diamant	
	Unboiled	Boiled	Unboiled	Boiled
Total polyphenol (mg GAE/100g)	398.62 ± 1.34 ^a	368.23 ± 13.05 ^b	500.28 ± 5.11 ^c	329.12 ± 3.72 ^d
Total flavonoid (mg CE/100g)	1013.41 ± 5.00 ^a	351.91 ± 31.45 ^b	868.35 ± 12.01 ^c	312.51 ± 12.18 ^d
Total tannin (mg TAE/100g)	4359.24 ± 94.53 ^a	3946.54 ± 298.72 ^b	5082.94 ± 272.51 ^c	2897.72 ± 454.54 ^d
β-carotene (mg carotenoid/100g)	0.109 ± 0.014 ^a	0.053 ± 0.003 ^b	0.047 ± 0.005 ^{bc}	0.039 ± 0.007 ^c
Vitamin A (IU)	5.46 ± 0.70 ^a	2.66 ± 0.15 ^b	2.35 ± 0.25 ^{bc}	1.95 ± 0.35 ^c
Vitamin C (mg AAE/100g)	90.44 ± 2.30 ^a	61.17 ± 1.96 ^b	145.33 ± 8.15 ^c	115.19 ± 3.99 ^d

Data are expressed as mean ± standard deviation. $P < 0.05$ was considered statistically significant. Values that do not share a common superscript letter within the same row are significantly different from each other. GAE: gallic acid equivalents, CE: catechin equivalents, TAE: tannic acid equivalents, IU: international unit, and AAE: ascorbic acid equivalents.

3.3. Vitamin A and C contents

Vitamin A content was relatively higher while vitamin C was lower in unboiled Lal Sheel potato than those of unboiled Diamant potato (Table 2). Like phytochemicals, both vitamin A and C contents also decreased after boiling. Our results suggest that Lal Sheel and Diamant potatoes were good sources of vitamin C but poor sources of vitamin A as of β-carotene.

3.4. Antioxidant Activity

Due to the presence of phytochemicals, we investigated whether Lal Sheel and Diamant potatoes possessed antioxidant activity or not. The total antioxidant activity as per FRAP assay is shown in Figure 2a. According to this assay, antioxidant activity of both varieties of unboiled potatoes increased in a dose dependent manner as like as the standard green vitriol. However, unboiled Diamant potato exhibited relatively higher extent of antioxidant activity than that of unboiled Lal Sheel variety. In contrast, phosphomolybdate reduction assay shows that unboiled Lal Sheel potato possessed higher extent of antioxidant activity than that of unboiled Diamant potato (Figure 2b). As per both FRAP and phosphomolybdate assays, antioxidant activity of Lal Sheel and Diamant potatoes reduced due to boiling. However, both potato varieties possessed similar extent of antioxidant activity after boiling.

Antioxidant activity was also quantified by total reducing power assay. Our finding shows that unboiled Lal Sheel and Diamant potatoes had similar extent of total reducing power (Figure 2c). Both showed the reducing power ability in a dose dependent manner which was comparable to that of the standard. Due to boiling, total reducing power decreased and almost similar activity was observed among the two varieties after boiling. Both Lal Sheel and Diamant potatoes were able to reduce the purple color of DPPH, indicating their DPPH scavenging ability (Figure 2d). Both possessed considerable scavenging activity when compared their IC₅₀ with that of the standard ascorbic acid. However, among the two varieties, unboiled Lal Sheel potato showed higher DPPH scavenging activity than that of unboiled Diamant potato (IC₅₀: 0.128 vs. 0.215 mg/mL). Results also show that boiling reduced the DPPH-radical scavenging activity of both potato varieties. But, even after boiling, Lal Sheel potato exhibited relatively higher scavenging activity than that of Diamant variety (IC₅₀: 0.418 vs. 0.819 mg/mL). All the results indicate that both Lal Sheel and Diamant potatoes possessed considerable antioxidative activity before and after boiling.

3.5. Vitamin B Contents

Vitamin B1 and B2 contents were determined by HPLC where the retention time for both vitamins was observed when the eluents were monitored by an UV detector of HPLC system (Figure 3a, Figure 3b). Calibration curves for vitamin B1 and B2 were prepared by plotting average peak areas against concentrations. The curves were linear for the concentration ranges shown in Figure 3c and Figure 3d. From the calibration curves, slopes (m) and intercepts (c) were obtained and vitamin contents in both potato varieties were calculated.

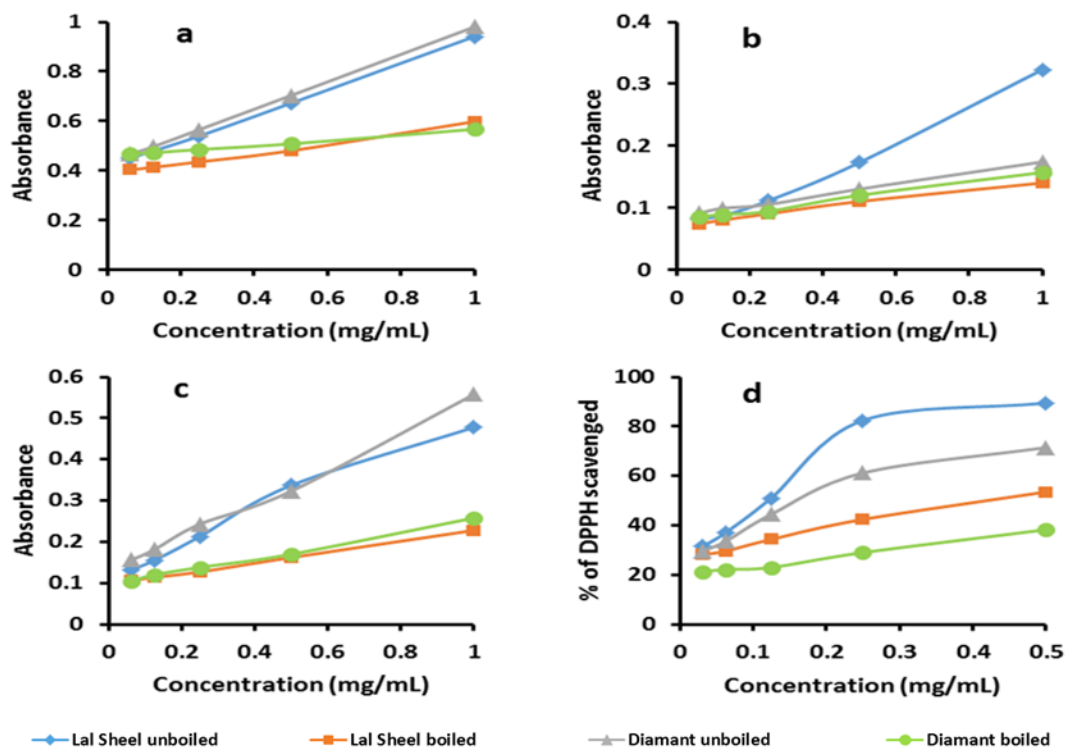


Figure 2. Antioxidant activity of Lal Sheel and Diamant potatoes in unboiled and boiled conditions as per FRAP assay (a), phosphomolybdate assay (b), total reducing power assay (c) and DPPH-radical scavenging activity (d). The antioxidant activity in each case was compared with that of the respective standard. The IC_{50} for DPPH scavenging potential of potato varieties was calculated from the equation of the curve corresponding to the sample concentration that reduced the initial DPPH absorbance by 50%.

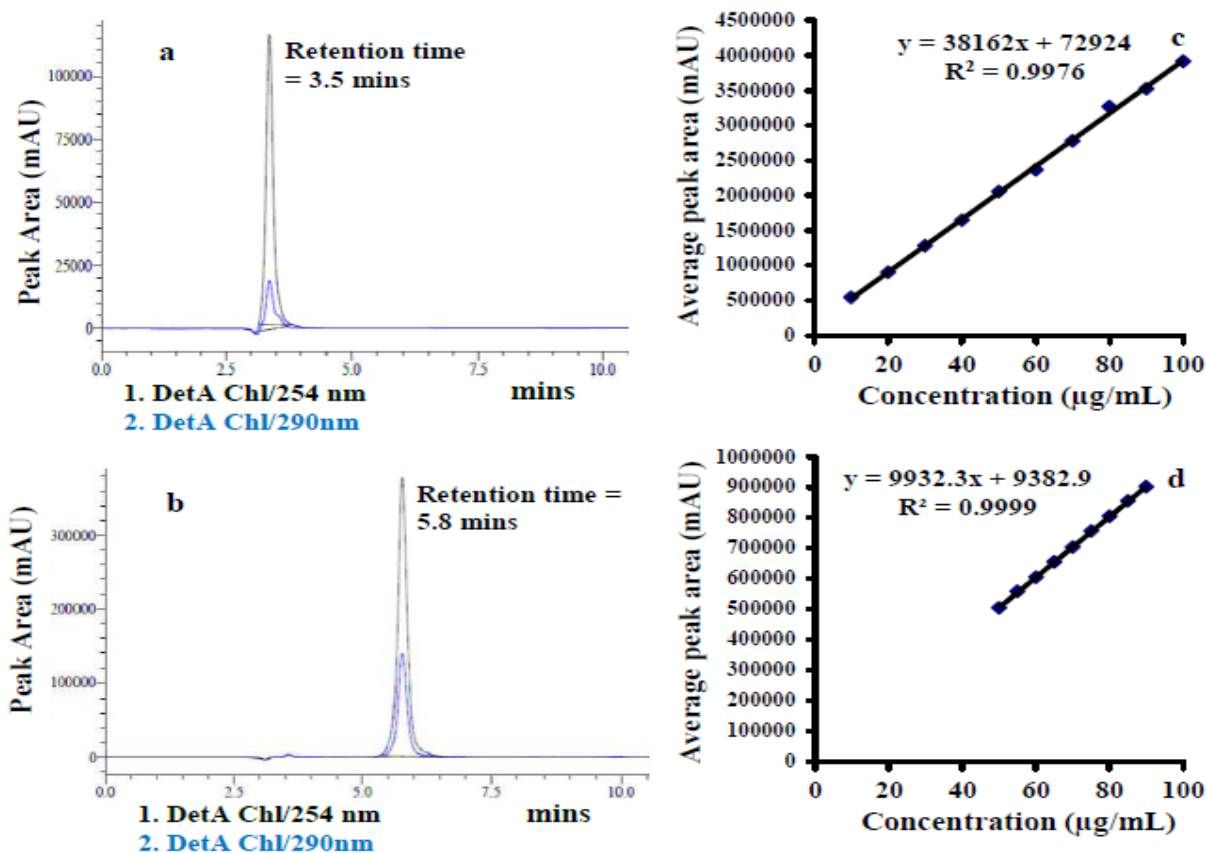


Figure 3. Chromatogram of (a) vitamin B1 (thiamine-HCl) and (b) vitamin B2 (riboflavin) standard obtained from an UV detector of HPLC system. Retention time was 3.5 minutes for vitamin B1 and 5.8 minutes for vitamin B2 at 254 nm. Calibration curves for vitamin B1 (c) and vitamin B2 (d), both of which were linear for the concentration ranges 10-100 $\mu\text{g/mL}$ and 50-90 $\mu\text{g/mL}$, respectively. Here, m = slope, c = intercept, and r = correlation coefficient

Vitamin B1 was detected only in unboiled Lal Sheel potato but not in either unboiled or boiled Diamant potato (Table 3). In contrast, vitamin B2 was detected in both potato varieties before and after boiling. After boiling, vitamin B2 content significantly decreased in both potato varieties; however, boiled Lal Sheel potato retained relatively higher level of it as compared to that of boiled Diamant potato.

Table 3. Vitamin B1 and B2 contents of two different potato varieties in unboiled and boiled conditions

Potato variety	Condition	Contents (mg/100g)	
		Vitamin B1	Vitamin B2
Lal Sheel	Unboiled	1.02 ± 0.03	0.68 ± 0.13 ^a
	Boiled	ND	0.46 ± 0.11 ^b
Diamant	Unboiled	ND	0.98 ± 0.15 ^a
	Boiled	ND	0.31 ± 0.09 ^b

Data are expressed as mean ± standard deviation. $P < 0.05$ was considered statistically significant. Values that do not share a common superscript letter within the same column are significantly different from each other. ND = Not detected.

3.6. Mineral and Heavy Metal Contents

Mineral contents of two different potato varieties are shown in Table 4. Unboiled Diamant potato had relatively higher iron and copper contents than those of unboiled Lal Sheel potato. Boiling significantly decreased the contents of iron and copper in both potato varieties. Zinc was present in very small amount which didn't have any notable boiling effect. Among the toxic metals, lead was detected but chromium and cadmium were not (Table 4). However, lead content was not significantly affected by boiling in both potato varieties.

Table 4. Mineral and heavy metal contents (mg/100g) of two different potato varieties in unboiled and boiled conditions

Parameters	Lal Sheel		Diamant	
	Unboiled	Boiled	Unboiled	Boiled
Essential minerals:				
Iron	1.04 ± 0.04 ^a	0.88 ± 0.09 ^b	1.52 ± 0.11 ^c	1.01 ± 0.08 ^a
Zinc	0.07 ± 0.01 ^a	0.06 ± 0.02 ^a	0.09 ± 0.02 ^a	0.07 ± 0.01 ^a
Copper	0.12 ± 0.03 ^a	0.04 ± 0.01 ^b	0.21 ± 0.07 ^c	0.09 ± 0.02 ^{ab}
Heavy metals:				
Lead	0.059 ± 0.009 ^a	0.056 ± 0.020 ^a	0.067 ± 0.014 ^a	0.063 ± 0.010 ^a
Chromium	ND	ND	ND	ND
Cadmium	ND	ND	ND	ND

Data are expressed as mean ± standard deviation. $P < 0.05$ was considered statistically significant. Values that do not share a common superscript letter within the same row are significantly different from each other. ND = Not detected.

4. Discussion

Boiling of potatoes with or without their skin is one of the common cooking methods to make them edible. In the

present study, effects of boiling on Lal Sheel and Diamant, which are commonly consumed potato varieties in Bangladesh, were investigated. When potatoes were boiled, moisture content increased in both potato varieties and this fairly agrees with the finding obtained by Elfaki and Abbsher (2010) [21]. The softening of potato tissues due to boiling enhances water absorption and retention capacity which might cause the increase in moisture content [39]. In contrast, ash content in Lal Sheel and Diamant potatoes significantly decreased after boiling. Ash is the total mineral content of a food. Thus, the reduction in ash content could be attributed to the solubilization and leaching of minerals into the water during boiling [39]. Lipid content in the tested potato varieties was relatively low, which also significantly decreased after boiling. Boiling causes rupturing of membrane and changes in tissue structure of potato which permit diffusion of lipids into the water and hence the reduction in lipid content [8]. Like moisture, crude fiber content significantly increased due to boiling. A study by Elfaki and Abbsher (2010) showed that boiling slightly increased fiber content in 'Alpha' variety of potato [21]. Gelatinization and retrogradation of starch during and after boiling can convert some part of potato starch into non-degradable polysaccharides which could increase the crude fiber content [39].

Carbohydrate provides energy for all form of body activities. Lal Sheel and Diamant potatoes had considerable amount of carbohydrate, which remained almost unaffected due to boiling, suggesting that both potato varieties possess high calorific value before and after boiling. This result is consistent with the finding obtained by Lewu et al. (2010) [39], but does not agree with the result obtained by Elfaki and Abbsher (2010) who reported that boiling significantly decreased carbohydrate content [21]. Protein content in tuber crops is usually low. Protein availability in potato is dependent on genotype and growing condition [39]. In our study, both Lal Sheel and Diamant potatoes had the similar amount of protein which was slightly decreased by boiling. This result is consistent with the finding obtained by Elfaki and Abbsher (2010) that boiling decreased protein content of potato from 1.60% to 1.40% [21].

Polyphenols constitute the largest group of phytochemicals which are known to act as antioxidants [40,41]. Lee et al. (2016) reported that purple colored potatoes had higher polyphenol content than white and yellow colored varieties [16]. Our result is somewhat different from their finding. In our study, Diamant potato had higher polyphenol content than that of reddish Lal Sheel variety. Due to genetic variation and climate condition the amount of polyphenols might be different. When boiled, polyphenol losses were 7.62% and 34.21% in Lal Sheel and Diamant potatoes, respectively. Similar observations of a reduction in total polyphenol in potato tubers after boiling have also been reported [20,42]. The difference in the reduction is likely due to diffusion of polyphenols into the water at different extent during boiling [43]. Flavonoids possess radical scavenging activity and their presence influences the flavor and color of fruits and vegetables [12,41]. Lal Sheel potato had higher amount of flavonoids than that of Diamant variety. This result is in good agreement with the previous studies

that colored-flesh potatoes contained more flavonoid than white-fleshed [16,20]. Like polyphenols, the amount of flavonoids also decreased in both potato varieties after boiling, which is consistent with the finding obtained by Perla et al (2012) [20]. However, good amounts of polyphenols and flavonoids were retained in both potato varieties after boiling. Thus, boiled Lal Sheel and Diamant potatoes could be a good source of antioxidants which can protect from harmful effects of radicals. Tannins are phenolic compounds and potential antioxidants [44]. Like polyphenols and flavonoids, tannin content was higher in unboiled potatoes, but reduced by ~9.5% in Lal Sheel and 43% in Diamant potato after boiling. However, after being boiled both potato varieties retained significant amount of tannin, which was higher than the amount reported previously for an African potato variety [39]. Tannins affect the nutritive value of food products by forming a complex with protein. Thus, the reduction in tannin content indicates that boiling could increase digestion and absorption of potatoes.

β -carotene, one of the pro-vitamin A carotenes, is a potential antioxidant but is found in trace level in most potato varieties [45,46]. As expected, Lal Sheel and Diamant potatoes also contained small amounts of β -carotene as well as vitamin A; however, both of these were relatively higher in Lal Sheel than in Diamant potato before and after boiling. Potato contains significant amount of vitamin C, which may account for up to 13% of its total antioxidant capacity [45]. In the present study, our tested potato varieties contained higher amount of vitamin C than that reported for Irish potato [19] and an Indian variety [47]. Boiling resulted in significant loss of vitamin C in both Lal Sheel and Diamant potatoes, which could be due to the leaching of a portion of vitamin C into the water and oxidative destruction during boiling [19,48]. Despite the loss, both potato varieties retained good amount of vitamin C after boiling.

Several methods can be applied to assess antioxidant activity. In the present study, we first adopted ferric reducing antioxidant power (FRAP) assay which is based on the reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) by the antioxidants. As per FRAP assay, unboiled Diamant potato had higher antioxidant activity than that of unboiled Lal Sheel potato. In contrast, when examined by phosphomolybdate assay method, unboiled Lal Sheel potato exhibited higher antioxidant activity than that of unboiled Diamant potato. Exact reason(s) for this difference remained unclear. Both FRAP and phosphomolybdate assays confirm that boiling decreased antioxidant activity of the tested potatoes. However, the extent of antioxidant activity was found similar for both varieties after boiling. The loss of antioxidant activity could be related with the loss of antioxidant phytochemicals during boiling [20]. Reducing power may serve as a reflection of antioxidant activity [35]. Thus, to evaluate the antioxidant activity we also adopted total reducing power assay, which is based on the reduction of ferric ferricyanide complex (Fe^{3+}) to ferrous ion (Fe^{2+}) by the substances having reduction potential [35]. As per this assay, antioxidant activity of Lal Sheel and Diamant potatoes also decreased after boiling and the trend was fairly similar with that observed when adopted FRAP

assay. Both Lal Sheel and Diamant potatoes also exhibited DPPH-radical scavenging activity in a dose dependent manner. IC_{50} , the concentration required to scavenge 50% of DPPH radical, of unboiled Lal Sheel potato was 0.128 mg/mL and that of unboiled Diamant potato was 0.215 mg/mL, suggesting that unboiled Lal Sheel potato possesses relatively greater scavenging potential than that of Diamant variety. Boiling decreased the scavenging activity of Lal Sheel and Diamant potatoes, but yet both the boiled potatoes possessed good antioxidant activity to scavenge the free radicals (IC_{50} : 0.418 mg/mL and 0.819 mg/mL, respectively). Antioxidant phytochemicals retained in potato might play this scavenging role after boiling [20].

Vitamin B1, B2 and essential minerals including iron, zinc and copper contents were relatively low and all these nutrients decreased after boiling in Lal Sheel and Diamant potatoes. Vitamin and mineral contents in potato usually depend on genotype, growing condition, soil property, and the use of herbicides [49,50]. Furthermore, the reduction in water soluble vitamins B1, B2 and minerals in potato could be related with their leaching into the water during boiling [13,19]. Heavy metals such as lead, mercury, cadmium, chromium etc. cause environmental hazards and are known to be toxic. Hence, the trace heavy metal content in potato is an important consideration [51]. Several studies detected lead, chromium, and cadmium at different levels in the potatoes grown in different parts of Turkey and Slovakia [23,51,52], where the levels obtained for these metals were above the FAO/WHO highest acceptable limits [53]. In the present study, when these heavy metals were examined, only lead was detected, which slightly reduced in both Lal Sheel and Diamant potatoes after boiling. The lead levels detected in both varieties before and after boiling were slightly higher than the FAO/WHO acceptable limit [53]. Genotype, potato cultivation close to the polluted area, use of fertilizer, and heavy metal concentration in the soil could be the cause for the difference in heavy metal contamination in the potatoes of different areas [23,51,52]. Absence of toxic metals or presence below the permissible level is good for the quality of a food. Thus, attention should be given and effective measures need to be taken on to avoid lead accumulation in both Lal Sheel and Diamant potatoes grown in Bangladesh.

5. Conclusions

The study revealed that Lal Sheel and Diamant potatoes grown in Bangladesh are good sources of nutrients and antioxidant phytochemicals including polyphenols, flavonoids and tannins. Boiling significantly affected their nutritional value and phytochemical contents and hence the antioxidant activity. However, after being boiled both Lal Sheel and Diamant potatoes retained good amounts of nutrients and phytochemicals and only small differences of these were observed between the two varieties. Thus, both Lal Sheel and Diamant boiled potatoes are beneficial foodstuffs for health, at least, from the perspective of phytochemical contents and antioxidant potential that could prevent free-radical damages.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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