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Total phenolic, chromium contents and antioxidant activity of raw and processed sugars

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A B S T R A C T

Processing improves the visual quality of food; however, the ingredient and nutritional values may alter. In present study, the sugar samples (refined, raw, gur (Jaggery/jaggeree) and molasses) were collected from 20 different agro-climatic regions of Pakistan and analyzed for the chromium concentration, total phenolic and antioxidant activity, in order to evaluate the processing effect. The concentration of Cr was determined by digestion method using atomic absorption spectrophotometer, while antioxidant activity was determined by DPPH (1,1-diphenyl-2-picrylhydrazyl) and reducing power. It was observed that chromium content in Jaggery was 74% higher than raw sugar, while molasses showed 21.27% higher concentration versus jaggery. The total phenolic contents were found considerably higher in molasses (3751 μg GAE/g) followed by Jaggery (3285 μg GAE/g), raw sugar (27.75 μg GAE/g) and refined sugar (23.81 μg GAE/g). The DPPH scavenging activity and reducing power was also found dependent to sugar type. Form results, it can be concluded that processing significantly affected the chromium contents, total phenolics and antioxidant activity.

1. Introduction

Chromium is present in all living individual and essential for the metabolism of carbohydrates and lipids [1,2] and deficiency can leads to the disorder in metabolic activities and causes diseases such as diabetes mellitus which is a global disease, increasing rapidly and has been doubled since last two decades. At present, more than 240 million people are pretentious to this disease [3]. Diabetes mellitus is a disease of metabolism abnormality due to chronic chromium deficiency and is implicated in several cardiovascular and metabolic pathologies such as non insulin dependent

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diabetes mellitus (NIDDM) [2]. Reports revealed that the chromium supply had significant beneficial effect on Glycated hemoglobin, glucose, insulin and cholesterol regarding type II diabetes [4]. Therefore, the chromium is a nutritionally essential element with a requirement in human from 10 to 60 μg/day for healthy person [5–8]. Attempts have been made to identify the chromium deficiency in diabetes patients [9,10]. Therapeutic trials with trivalent chromium gave good results and lead to significant improvement in glucose tolerance with lowering fasting glucose, plasma total cholesterol, LDL cholesterol [11] and reduction in plasma triglyceride in type 2 diabetes patients [12]. For diabetes patients, the World Health Organization [8] suggested the utilization of chromium rich sources foods, approved by National Bureau of Standards such as Brewer's yeast, Bovive liver, orchard leaves, spinach, pine needles and tomato leaves to mitigate the chromium deficiency [4,10,11]. Refined sugar is used as sweetener in beverages (juices, syrup and tea coffee) and in making desserts such as jams and cakes. According to Bratakos et al. [13], the concentration of chromium vary based on food processing methods and source which might affect the nutritional value of food. Sugars are obtained from sugarcane and sugar beet crops. In sugar industry, initially, raw sugar (brown sugar) is produced, which is a sucrose sugar with brown color due to the presence of molasses, which is an unrefined or partially refined sugar consisting of sugar crystals with some residual molasses content (natural brown sugar). Refined sugar is made from raw sugar by further processing, which removes molasses and resultantly, white crystals are obtained. Molasses also called black treacle, is a viscous by-product of refining sugarcane or sugar beets into sugar. Jaggery is a traditional non-centrifugal cane sugar, which is commonly consumed in Asia, Africa and America. The major constituents of Jaggery are 50% sucrose, 20% invert sugars, 20% moisture and remaining part contains insoluble matter (wood ash, proteins, and bagasse fibers etc.) [14–17].

The plant and plant products have been the main focus in the search for nutraceuticals to combat oxidative stress induced diseases [18]. Free radicals are generated during normal cellular metabolism and their effect is neutralized by antioxidants. However, this balance between the oxidants and antioxidants molecules is disturbed by free radicals derived from exogenous sources like ozone, exposure to UV radiations and cigarette smoke. The free radical production in cells can be significantly increased by certain toxic redox cycling compounds such as drugs and carbon tetrachloride. Importantly, the main biomolecules like DNA, lipids and proteins are vulnerable to free radical damage resulting in cell destruction. Damaged cells lead to abnormal functioning and results in oxidative stress induced diseases. A potent scavenger or quencher of free radicals may serve as a possible preventive measure for free radical mediated diseases and naturally occurring plant product are the potent source of scavenger. However, the processing of the natural product may affect the quality of food products [19,20].

Sugarcane extract has been reported to be excellent source of organic compounds i.e., phenolic compounds and minerals [21] with significant antioxidant activity [22]. However, it has been reported that processing/refining of food product can alter the food quality [23] i.e., blanching results in losses of vitamins and minerals. Also, milling and extrusion can cause the physical removal of minerals during processing. The nutritional quality of minerals in food depends on their quantity as well as their bioavailability. Moreover, the bioavailability also reported to be significantly different in processed and raw foods. The contents of food components are altered by various processing methods including milling, fermentation, extraction and thermal processing. Bioactive components are highly sensitive to the processing methods as well as the processing conditions [24].

In view of processing effect on food quality, present study was aimed to appraise the processing effect on chromium content, antioxidant activity and phenolic contents of sugars. Different products i.e., refined, raw sugar, jaggery (Gur) and molasses were collected from 20 different agro-climatic region of Pakistan and were analyzed for chromium concentration, antioxidant activity and total phenolic compounds in order to evaluate the processing effect on sugar quality.

2. Materials and methods

2.1. *Chemicals and reagents*

The chemical and reagents used were of analytical grade i.e., HNO_3 (70%), HCl (⩾99%), chromium standard (TraceCERT®, 1000 mg L^{-1} Cr in nitric acid), Folin–Ciocalteu reagent (F 9252), DPPH (CS0790-1KT), sodium carbonate (⩾99%), Tris–HCl buffer (99%), ethanol (99.5%), disodium hydrogen

Table 1 – The station selected for sampling of jaggery, molasses, raw and refined sugar.

Code	Station	Location	Code	Station	Location
1,21	Tandilnwala	Kanjwani/Faisalabad	11,31	Noon	Bhalwal/Sargodha
2,22	Chaudhry	Gojra/Toba Tek Singh	12,32	Pahriawali	Lalian/Jhang
3,23	Chistia	Sillanwali Chistia/Sargoda	13,33	Pattoki	Pattoki/Kasur
4,24	Crescent	Nishtabad/Faisalabad	14,34	Ramzan	Chainot/Jhang
5,25	Csk(phalia)	Phalia/Mandi Bahauddin	15,35	Shahtaj	Mandi Bahauddin
6,26	Gojra Samundri	Gojra/Faisalabad	16,36	Shakarganj Jhang	Jhang
7,27	Haseeb waqas	Nawabshah	17,37	United	Rahim Yar Khan
8,28	Hussain	Jaranwala/Faisalabad	18,38	Yousaf	Sargoda
9,29	Kamalia	Kamalia	19,39	Adam	Chistian/Jhang
10,30	National	Bhalwal/Sargodha	20,40	Shakarganj Bhone	Jhang

Table 2 – The chromium contents, range, mean and SD form 20 different Agro-ecological Zones.

S. No. Sugar mills	N	Chromium contents											
		Refined sugar			Raw sugar			Jaggery (gur)			Molasses		
		Range (ppm)	Mean	SD	Range (ppm)	Mean	SD	Range (ppm)	Mean	SD	Range (ppm)	Mean	SD
1. Tandilnwala	6	0.019–0.022	0.021	0.002^{d}	0.04–0.05	0.51^{c}	0.03	0.901–0.908	0.91^{b}	0.005	1.12–1.20	1.16^{a}	0.05
2. Chaudhry	6	0.028–0.031	0.030	0.002^{d}	0.04–0.05	0.52^{c}	0.04	0.89–0.92	0.91^{b}	0.023	1.11–1.19	1.16^{a}	0.06
3. Chistia	6	0.028–0.031	0.030	0.002^{d}	0.52–0.53	0.53^{c}	0.01	0.932–0.93	0.94^{b}	0.005	1.05–1.14	1.10^{a}	0.07
4. Crescent	6	0.025–0.028	0.027	0.002^{d}	0.51–0.52	0.52^{c}	0.01	0.913–0.91	0.92^{b}	0.004	1.12–1.15	1.14^{a}	0.02
5. Csk(phalia)	6	0.011–0.016	0.014	0.004^{d}	0.55–0.59	0.58^{c}	0.03	0.908–0.93	0.92^{b}	0.020	1.11–1.24	1.18^{a}	0.09
6. Gojra	6	0.013–0.014	0.014	0.001^{d}	0.57–0.059	0.58^{c}	0.02	0.94–0.97	0.96^{b}	0.020	1.02–1.17	1.15^{a}	0.18
7. Haseeb	6	0.024–0.027	0.026	0.002^{d}	0.52–0.60	0.57^{c}	0.06	0.91–0.96	0.94^{b}	0.033	1.16–1.18	1.17^{a}	0.02
8. Hussain	6	0.017–0.018	0.018	0.001^{d}	0.56–0.58	0.58^{c}	0.01	0.92–0.95	0.94^{b}	0.024	1.14–1.18	1.17^{a}	0.03
9. Kamalia	6	0.028–0.031	0.030	0.002^{d}	0.49–0.53	0.51^{c}	0.03	0.98–1.08	1.03^{b}	0.073	0.97–1.10	1.04^{a}	0.10
10. National	6	0.017–0.019	0.018	0.001^{d}	0.57–0.58	0.58^{c}	0.01	0.93–0.95	0.94^{b}	0.013	0.98–0.99	0.99^{a}	0.01
11. Noon	6	0.025–0.027	0.026	0.001^{d}	0.55–0.57	0.56^{c}	0.01	0.90–0.93	0.92^{b}	0.018	1.18–1.21	1.20^{a}	0.02
12. Pahriawali	6	0.024–0.025	0.025	0.001^{d}	0.55–0.56	0.55^{c}	0.00	0.94–0.98	0.97^{b}	0.023	1.06–1.04	1.08^{a}	0.02
13. Pattoki	6	0.018–0.041	0.030	0.016^{d}	0.52–0.53	0.53^{c}	0.01	0.88–0.98	0.94^{b}	0.069	1.21–1.33	1.27^{a}	0.08
14. Ramzan	6	0.011–0.038	0.025	0.019^{d}	0.41–0.52	0.49^{c}	0.10	0.95–0.98	0.96^{b}	0.012	1.11–1.17	1.14^{a}	0.04
15. Shahtaj	6	0.016–0.041	0.029	0.018^{d}	0.37–0.53	0.46^{c}	0.12	0.91–1.02	0.97^{b}	0.072	1.15–1.22	1.19^{a}	0.05
16. Shakarganj	6	0.01–0.04	0.028	0.018^{d}	0.43–0.54	0.49^{c}	0.07	0.91–0.99	0.96^{b}	0.051	1.18–1.22	1.20^{a}	0.03
17. United	6	0.008–0.021	0.015	0.009^{d}	0.58–0.59	0.59^{c}	0.01	0.92–0.97	0.95^{b}	0.037	1.11–1.33	1.22^{a}	0.16
18. Yousaf	6	0.013–0.014	0.014	0.001^{d}	0.49–0.62	0.56^{c}	0.09	0.92–0.98	0.96^{b}	0.042	0.99–1.18	1.06^{a}	0.09
19. Adam	6	0.012–0.019	0.016	0.005^{d}	0.61–0.62	0.62^{c}	0.01	0.92–0.97	0.95^{b}	0.031	0.97–1.29	1.14^{a}	0.23
20. Bhone	6	0.011–0.018	0.015	0.005^{d}	0.56–0.61	0.59^{c}	0.03	0.94–0.95	0.95^{b}	0.008	1.02–1.25	1.14^{a}	0.16

*Different letters in column indicates Cr contents difference among sugar brands at 95% confidence interval of mean, N = number of sample analyzed for each sugar mill phosphate (99%), sodium chloride (⩾99.5%) potassium ferricyanide (99%), trichloroacetic acid (99%) were purchased from Sigma–Aldrich (USA).

2.2. Samples collection and analysis

Refined, raw, and molasses samples were collected from 20 different sugars mills from Punjab and Sindh, Pakistan (Table 1) and jiggery sample was collected from same area. The samples were collected twice in a year in triplicate (total six samples from each point). The collected samples were dried at 105 °C to constant weight and preserved in glass jars [11] and analyzed for chromium, total phenolic contents and antioxidant activity.

2.3. Chromium content measurement

For chromium contents measurement, digestion method was adopted as precisely reported elsewhere [25]. Briefly, homogenized sample (1 g) was weighed into a Kjeldahl flask and 12 mL of aqua-regia digestion mixture was added and placed on hot plate for 2 h. Then, the mixture was cooled to room temperature, filtered through a Whatman filter paper (Grade 42) and diluted to 50 mL with de-ionized distilled water. The prepared samples were analyzed by atomic absorption spectrophotometer (Perkin Elmer A Analyst 300), using air acetylene flame in combination with single element hollow cathode lamp (wavelength 357.9 nm, Band pass 1.3 nm, Lump Current 7.5 mA, Fuel pressure 0.40 kg cm^{-2}, Burner height 7.5 mm, Calibration range 0.02–0.50 mg L^{-1}, Detection limit 0.01 mg L^{-1}, Oxidant pressure 1.60 mg L^{-1}). The samples and standard (chromium Standard for AAS, TraceCERT®, 1000 mg L^{-1} chromium in nitric acid obtained from Sigma–Aldrich) were run under same conditions and chromium concentration was measured with the help of standard curve [26].

2.4. Total phenolic contents and antioxidant activity evaluation

The total phenol content of refined sugar, raw sugar, jaggery and molasses were determined calorimetrically using the Folin–Ciocalteu method [27]. A sample aliquot of 100 μL was mixed with 900 μL of water, 5 mL of 0.2 N Folin–Ciocalteu reagent and 4 mL of saturated sodium carbonate solution (100 g/L). The mixture was after incubation for 2 h at room temperature and finally, absorbance was measured at 765 nm (CE Cecil, 7200, UK). The total phenolic content was expressed as micrograms of gallic acid equivalent (GAE)/g sample. The effect of different sugar varieties on DPPH radical was estimated according to the reported method by Lai et al. [28]. Sugar samples (50 μg/mL) in 200 μL aliquot was mixed with 100 mM Tris–HCl buffer (800 μL, pH 7.4) and then, 1 mL of 500 l M DPPH in ethanol was added. The mixture was shaken vigorously and kept in dark at room temperature for 20 min. after stipulated period of time; the absorbance was measured at 517 nm (CE Cecil, 7200, UK). The capability to

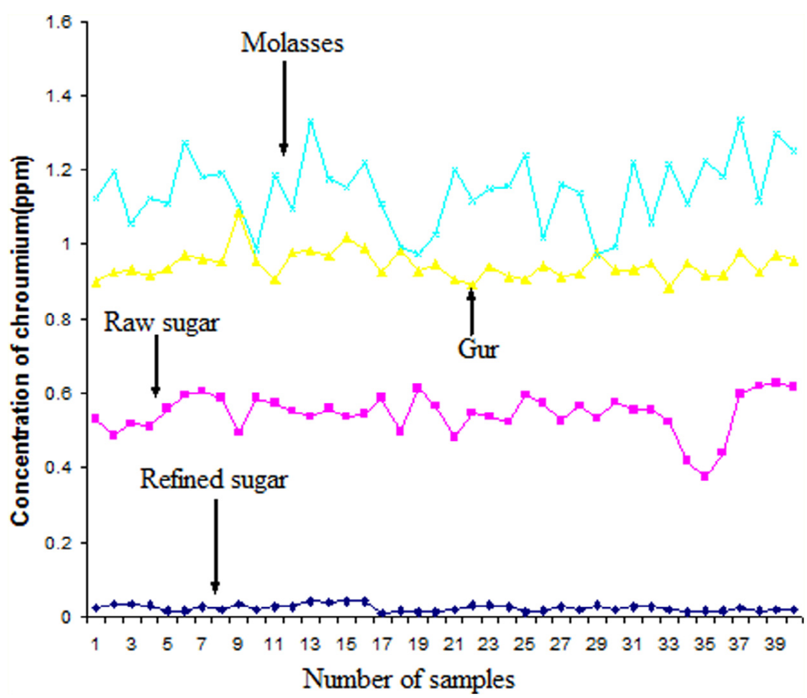

Fig. 1 – The chromium comparison of molasses, jaggery (gur), raw and refined sugar from different agro-climatic region. The sugars, jaggery and molasses sample were collected from Punjab districts, Pakistan as; 1, 21 (Kanjwani/Faisalabad), 2, 22 (Gojra/Toba Tek Singh), 3, 23 (Sillanwali Chistia/Sargoda), 4, 24 (Nishtabad/Faisalabad), 5, 25 (Phalia/Mandi Bahauddin), 6, 26 (Gojra/Faisalabad), 7, 27 (Nawabshah), 8, 28 (Jaranwala/Faisalabad), 9, 29 (Kamalia/Toba Tek Singh), 10, 30 (Bhalwal/Sargodha), 11, 31 (Bhalwal/Sargodha), 12, 32 (Lalian/Jhang), 13, 33 (Pattoki/Kasur), 14, 34 (Chainot/Jhang), 15, 35 (Mandi Bahauddin), 16, 36 (Jhang), 17, 37 (Rahim Yar Khan), 18, 38 (Sargoda), 19, 39 (Chistian/Jhang) and 20, 40 (Jhang).

scavenge DPPH radical was calculated using relation shown in Eq. (1). The EC_{50} (effective concentration for 50% DPPH radical scavenging activity) was also measured.

$$\text{Scavenging effect } (\%) = [(A_c - A_s)/A_c] \times 100 \quad (1)$$

Where A_c and A_s are the absorbance values of control and sample, respectively.

For reducing power measurement, sugar sample (10 mg/mL) was mixed with an equal volume of 0.2 M phosphate buffer, pH 6.6 and 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min following addition of an equal volume of 10% trichloroacetic acid and centrifuged at 5000×*g* for 10 min. The supernatant was mixed with distilled water and 0.1% ferric chloride (1:1:2) and the absorbance was measured at 700 nm (CE Cecil, 7200, UK). The change in absorbance was considered as reducing power of the sugar samples [29].

3. Results and discussion

3.1. Chromium contents

The results of chromium concentration in different sugar types (refined, raw, jaggery and molasses) from 20 sugar mills, located in different agro-climatic regions are shown in Table 2 (range, mean and SD). The difference among regions was insignificant regarding chromium content, while sugars types showed significant difference in chromium contents. A marginal higher chromium concentration were found in Pattoki and Shahtaj sugar mill (refined sugar), Yousaf and Adam (raw sugar) and Adam and Bhone (molasses), while the concentration of chromium in jaggery was found to be high from Kamalia region. According to [30], the geographic location, soil type, oxidation–reduction potential, cation exchange capacity, clay content, nature of water used for irrigation can affect the absorption of mineral and marginal difference between regions might be due to variable uptake of chromium from soil. However, anthropogenic input associated with agricultural practices, mineral exploration, industrial process and solid waste management are considered the important contributors [30,31,12,32]. Results showed that the concentration of chromium varies from region to region, but overall variation was non-significant ($P < 0.05$). However, variation among sugar types was highly significant ($P < 0.05$), which is the pure effect of processing on chromium contents. The concentration of chromium varies from 0.011 to 0.041 ppm in refined sugar, raw sugar (0.419–0.628 ppm), jaggery (0.887–1.084 ppm) and molasses (0.97–1.33 ppm) (Table 2). A comparison of chromium concentration among all sugar types is shown in Fig. 1. The concentration of chromium in jaggery (gur) was found to be 74% higher versus raw sugar, while 21.27% higher in comparison to jiggery, which indicates that refined sugar has very low concentration of chromium versus other sugar types (raw sugar, jiggery and molasses). The results are in accordance with the previous finding reported by Awadallah et al. [33] from Egypt regarding processing effect on chromium contents. Author reported that the chromium contents in processed products are affected by the possessing methods such as evaporation, filtration and purification. So far, it is noted that during the production of refined sugar, the chromium content decreased from 1.33 ppm to 0.01 ppm (refined sugar). The jaggery and raw sugar showed 43 and 25 folds higher Cr(III) versus refined sugar and the higher chromium content in molasses may be due to chelating tendency of chromium [13]. So far, the body chromium content may reduce by eating refined sugar, because chromium continuously excreted in the urine naturally [34]. Also, under infection, acute exercise, pregnancy, lactation and stress conditions, the chromium contents affected negatively in body [35] and chromium supply is necessary to maintain proper homeostasis of the body [36]. Therefore, based on present findings, it can be concluded that the chromium contents were significantly low in refined sugar versus raw sugar and ingestion of refined sugar might have negative impact on normal body functioning, especially in diabetes patients i.e., Jeejeebhoy et al. [37], Freund et al. [38] and Brown et al. [39] investigated the effect of Cr(III) supplementation in diabetes patients and it have been revealed that patients fed intravenously with Cr(III) showed better health, neuropathy and glucose tolerance. Similarly, Althuis et al. [40] also revealed that chromium supply is helpful in controlling

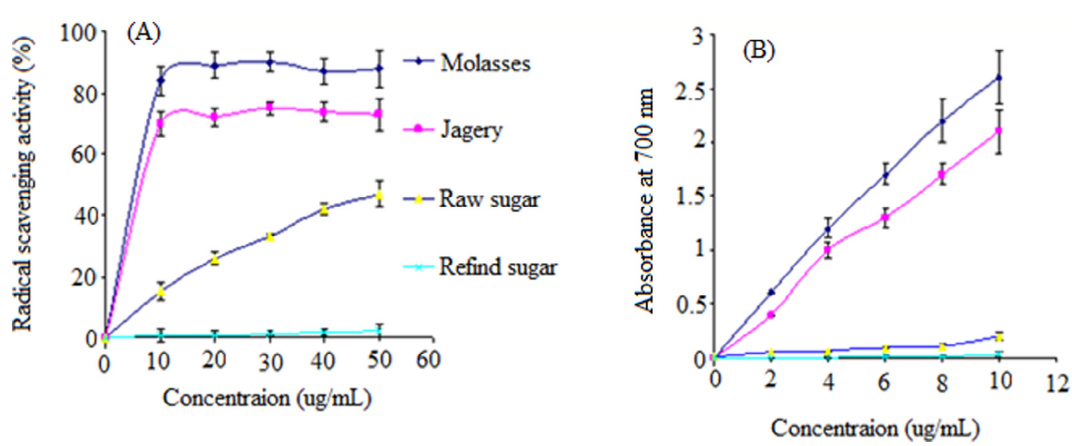

Fig. 2 – (A) DPPH radical scavenging activity of jaggery, molasses, raw and refined sugar samples. (B) The reducing power of jaggery, molasses, raw and refined sugar samples. Values are mean ± SD (*n* = 6).

diabetes (type 2) since chromium is an essential element that have beneficial role in the regulation of insulin and regulates carbohydrate, protein and lipid metabolism. Studies also showed that people with type 2 diabetes have deficiency of chromium versus normal persons. Present study indicates that the jaggery has significantly higher chromium concentration and in case of diabetes disorder, the jaggery use may be helpful in managing chromium deficiency. The molasses also revealed high chromium contents, however, it can not be utilized directly and to make it useable, it need further processing. Therefore, the jaggery intake will be more viable to avoid chromium deficiency and other health issues.

3.2. *Total phenolic content and antioxidant activity*

The variation in total phenolic content was found significant ($P < 0.05$) among sugar types (refined, raw, jaggery and molasses). The higher phenolic contents were observed in molasses followed by jaggery, raw and refined sugar. The phenolic contents in molasses, jaggery, raw and refined sugar were 3751 μg GAE/g, 3285 μg GAE/g, 27.75 μg GAE/g and 23.81 μg GAE/g, respectively. The antioxidant activity of molasses, jaggery, raw and refined sugar was evaluated by DPPH radical scavenging and reducing power. The percentage radical scavenging activity of molasses, jaggery, raw and refined sugar is shown in Fig. 2. The free radical scavenging ability of different sugar types was found higher in molasses followed by jaggery, raw and refined sugar, which indicates that DPPH scavenging activity of molasses, jaggery and raw was higher, while refined sugar showed marginal activity verses other sugar types. The EC_{50} values were found out to be 6.72 and 51.08 μg/mL, respectively for jaggery and raw sugar, which indicates the potency of sugar types to donate electron for reduction of oxidative stress. The reducing power of molasses, jaggery, raw and refined sugar was evaluated by measuring their abilities to reduce ferric chloride and potassium ferricyanide complex (Fig. 2). The reducing power of molasses and jaggery was found considerably higher as compared to refined sugar. Furthermore, the reducing power was found to be dose dependent and the antioxidant activity as evaluated by DPPH radical scavenging ability ($R^2 = 0.901$) and reducing power ($R^2 = 0.975$) showed the dominant antioxidant potential of the molasses and jaggery. The considerable antioxidant activity indicates the presence of bioactive compound in molasses and jiggery versus other sugar types.

The low chromium concentration, total phenolic and antioxidant activity of refined sugar indicates that the processing affects the sugar nutritional value. Now a day the terms "low fat" and "no fat" is completely backwards. The real culprits are refined and processed products (responsible for number of diseases). The death rate was uncommon when refined products were not common and cardiovascular disease was almost unknown in world before 1920 [41]. The micronutrient deficiency in foods (processed products) is important to consider i.e., deficiency of chromium, absence of phenolic and lack of antioxidant activity in refined sugar in present investigation proved that consumption of refined sugar might be harmful and in case of diabetes patients, its value is likely to be white poison because chromium is positively correlated with glucose metabolism in body [5]. Therefore, the use of natural raw food/moderately processed should be adopted to avoid deficiency of biologically important bioactive compounds [42–52].

4. Conclusions

Different sugar types (refined, raw, gur (Jaggery/jaggeree), molasses) from 20 different agro-climatic regions of Pakistan were collected and subjected to chromium contents, total phenolic and antioxidant activity analysis. Refined sugars showed very low chromium contents as well as low reducing power, DPPH radical scavenging activity and total phenolic contents. The chromium contents found out to be marginally different with respect to locality; however, the sugar types (Raw, Refined, Jaggery and Molasses) showed significantly different chromium contents, total phenolic contents and antioxidant activities. Therefore, the quality of sugar may change significantly as a result of processing. The refined products give color, aroma and better sensation to the products, but nutrient (ingredient) as well as caloric values may change. So far, the processing may alter the nutritional value and quality of food and there is need to utilize raw/moderately processed products to avoid negative health impacts.

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