Original article

Chemical and Biological Food Safety Threats Associated with Fresh Juices Consumed in Capital Territory Islamabad, Pakistan

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Abstract. It is generally assumed that fruit products are safer compared to other perishable uncooked and semi cooked meat products. However, many outbreaks have been associated with the consumption of contaminated fruits and its juices. A study was conducted to evaluate the microbiological and chemical contamination status of the fresh fruit juices being served at various juice centers/markets in Islamabad, Pakistan. A total of 50 juice samples from different fruits (apple, strawberry, pineapple, orange, and carrot) were analyzed for its organoleptic microbiological (total *Escherichia coli* count), basic chemical tests and heavy metals concentration. Five homemade Juices made under hygienic conditions were taken as control and the obtained results compared with commercial juices (samples). Microbial safety assessment of the sample juices indicated that the microbial load of 31 samples were above acceptable limits. The microbial results of the sample were also supported by the physicochemical tests like pH and total solids since their higher value provide favourable conditions to microbial growth. The Heavy metals levels of the juices were evaluated using Atomic Absorption Spectrophotometer. The heavy metal concentrations in the sample juices (carrot, apple, pineapple, strawberry, orange) were found as; Cr (0.05, 0.007, 0.02, 0.06, 0.012, Mn(0.005, 0.22, 0.3, 0.26, 0.083), Co (below detection limit (BDL), BDL,0.01, 0.09, 0.002), Ni (0.008, 0.007, 0.1, 0.7, 0.008), Cu (0.045, 0.09, 0.2, 0.414, 0.14), Zn (0.07, 0.2, 0.35, 0.516, 0.21), As (BDL, BDL, BDL, not detected (ND), BDL), Cd (ND, BDL,BDL,ND,BDL), Pb (0.176, 0.041, BDL, 0.195, BDL). It indicates that the levels of some metals are within the safe limit but some of them exceed the already set limits by WHO, US-EPA and PPFR (Table 5). It was concluded that the overall quality of fresh unpasteurized juices sold in Islamabad was very poor. Furthermore, the presence of heavy metals contamination confirmed the bad quality of these juices as well as microbial contamination can cause harmful effects to human health. The occurrence of pathogenic *E. coli* is alarming enough for an immediate action by respective authorities. It is suggested that regular monitoring of the quality of fruit juices for human consumption must be introduced to avoid any future pathogen outbreaks.

Key Words: Fruit, Juice, *E.coli*, Heavy metals, microbial safety

Introduction

Fruit juices are non-fermented but likely fermentable extracts of fruits and preserved in fresh state and are consumed worldwide due to their great nutrition importance (1). Fruits are major sources of juices which comprises of vitamins, minerals and fibers that have significant effects on human health e.g. their antioxidative nature resists various diseases associated with oxidation process in the body. Juices contain carotenoid as a good source of bioavailable antioxidant and Vitamin C (Ascorbic acid) an effective dietary antioxidant agent (2). Vitamin C protect us against oxidative stress-induced cellular damages through reactive oxygen species scavenging pathway and thus reduces risks of heart disease, cancer, and other related diseases. Ascorbic acid (Vitamin C) are predominantly found in citrus fruits and their juices which enhance iron absorption (3, 4) from food. It is therefore important for children having less iron bioavailability. Juices comprises of water and are major source of phenolic compounds, glycosides, and variety of micronutrients such as potassium (K^+), calcium (Ca^{2+}), magnesium (Mg) and iron (Fe) which help in prevention from various diseases. Potassium maintains heartbeat by stimulating the blood flow, magnesium prevents from heart attack, and Calcium acts in strengthening of teeth and bones (5).

Although Fruit juices are greatly recommended due to their effective nutritional values however worries about their quality and safety aspects are increasing day by day. In light of the global concern about food safety in last decades greatly intended researchers to carry out studies concerning the risks linked with utilization of juices/food stuff contaminated by pathogens, trace metals and other toxins (6). Contamination in fruits can occur before harvesting by various sources including human handling, fertilizers, pesticides, and waste/polluted water (7). Mainly in developing countries, food-borne diseases are caused by pathogenic bacterial strains e.g. *S. spp, E. coli,* and others which enormously influence the health care systems (8). Additionally, Juices undergo contamination through pathogens from raw materials and equipment used for extracting juice. Processing atmosphere of juice preparation and unhygienic practices by vendors may also cause contamination (9). These kinds of spoiled juices caused potential food related diseases in public who frequently utilize them. In developing countries *E. coli* (which usually present in contaminated fruit juices) accounts for 25% of the infant diarrhea (1).

Fruit juices have substantial expose to heavy metals contamination. Metals like lead (Pb), cadmium (Cd), mercury (Hg) and arsenic (As) exert harmful effects even in low concentration of 10 to 50 ppm. Pd and Cd in exceeding than permissible limits add serious health complications including nervous, cardiovascular, bone and kidney diseases (10, 11). Heavy metals like Fe, Cu, and Zn apart from their nutritional significance, can also cause certain health problems when ingested in high concentration. For example, too much iron intake has been reported and linked to overall increase in colorectal cancer risks while excessive Zn intake results in electrolytes imbalance and anaemia (12). Food consumption is one of the main pathways of heavy metal intake by humans since these food stuffs/human diets have high chances of contamination with the heavy metals and other contaminants (13). As discussed, the fruit juices comparatively have high exposure to contamination to microbes and toxic elements which make it necessary to physicochemical and biological assessment of the fruit juices consumed by common public and suggest adopting safe and standard procedures.

In this connection, the present paper describe the physicochemical parameters, *E.coli* count and heavy metal analysis of the fruit juices commonly sold at local juice centres/marketsin Islamabad, Pakistan, and comparing microbial quality of the commercially available juices with homemade juices between January to June 2017.

Materials and Methods

Samples collection and preparation

Glass bottles were used for the study. All the glass bottles were washed primarily with distilled water and autoclaved for fifteen minutes at a temperature of 121ºC while keeping the pressure as 15 Psi. After then the glass bottles were dried at 105˚C in an oven. Fresh unpasteurized juices of Apple (*Malus pumila*), Orange (*Citrus sinensis*), Strawberry (*Fragaira ananassa*), Pineapple (*Ananas comosus*), and Carrot (*Daucus carota*) were obtained from different restaurants/cafeterias of Islamabad city (Pakistan) in 250 mL of the pre cleaned glass bottles. It was then stored in ice cubes containing cool box. Then for analysis purpose, the sample juices were shifted to the microbiology laboratory located in IESE-NUST. Additionally, five samples of fresh juices from the same fruits were also prepared at home in a hygienic environment. Each sample was distributed into three parts for the three different kind of analysis i.e. physicochemical, microbial and heavy metals analysis.

Physicochemical Analysis

Determination of pH, density, moisture and total solid (TS) contents. pH of all the juice samples were determined by using pH meter made by SCHOTT company#A053106011 while density of all the samples were measured by employing densitometry method. Accordingly, 10 mL of the sample juice was drawn by a pipette, weighted and subsequently measured its density by employing the formula;

$$Dencity = \frac{mass}{volume}$$

Similar method was adopted for all samples.

Moisture content and total solids (TS) of the juice samples were determined by adding 30 mL of the sample to a china dish and weighted. The dish was then kept at 70°C in an oven and next day it was taken to desiccator for cooling. After then the dish was weighted and then the moisture content and Total Solid data was obtained by employing the following formula.

$$Moisture\% = I - \frac{F}{I} \times 100$$

$$I = inial\ weight$$

$$F = Final\ weight$$

$$Total\ solids = 100 - \%\ Moisture$$

For all the samples, same procedure was adopted.

Determination of titratable acidity

Titrimetric method recommended by AOAC was applied to determine total titratable acidity (TTA) of all the samples (14). According to the method, 0.1M NaOH solution in 500 mL of distilled H_2O was prepared initially. It was then filled in a burette up to its zero mark. About 10 mL of the sample juice was taken in a beaker (250 mL). It was then added 50 mL of the distilled water. As an indicator, Phenolphthalein (3 drops)was added to the beaker having a diluted juice sample. The base from the burette was then added slowly into the beaker containing solution of the sample juice and continuous swirling was maintained till appearance of the indicator colour (distinct pink colour) which was persisted for thirty seconds. It was considered as an end point i.e. pH 8.2. At this point the titre (NaOH) volume was recorded. The recorded reading was used in calculating acidity of the juice sample, by using the formula;

$$\%\ Acid = titre \times acid\ factor \times 100/(10\ ml\ of\ juice)$$

Acid Factor: i) citric acid = 0.0064

ii) Malic acid = 0.0067

Same method was employed for all the samples.

Measurement of the RS (reducing sugar) values

The reducing sugar in the juices were measured through Benedict's test. For this test, initially benedict's solution was prepared by taking 173g of sodium citrate. Anhydrous Na_2CO_3 of about 100g dissolved in 800 mL distilled H_2O and diluted further by adding 850 mL of the distilled H_2O. About 17.3g of $CuSO_4$. $5H_2O$ dissolved in a 100 mL of distilled H_2O was taken in another beaker to prepare carbonate-citrate solution. The mixture was further added 1.0 L of the distilled water. Then separately a stock solution (100 g/L) of glucose standard was prepared by dissolving 100 g of glucose in distilled water there by diluting it up to 1L. From this standard stock solution, diluted standard of different concentrations (10g/L, 8g/L, 6g/L, 4g/L and 2g/L) were prepared through dilution method. The benedict's solution was then used for estimation of reducing sugars. For this purpose, eleven test tubes were taken and to each one was poured 1 mL of the Benedict's solution. Five milliliters of the distilled H_2O were poured to one test tube and 5 mL of each glucose standard solution to the other five tubes. To the rest of the five tubes 5 mL of each juice sample was added. The tubes were sealed with aluminum foil. These were then kept at 60 °C for 5 minutes in a water bath which were then removed and allowed to cool. The material from each tube was then transferred to centrifuge tube of 15 mL and centrifugation was performed for 2 to 4 minutes until we observe a clear solution. The filtrates were then transferred to other tubes and subjected to spectrophotometric analysis. The spectrophotometer was set at 735 nm. For the distilled H_2O, it was set at 100% transmittance (T). Graphs obtained for the standard and concentration for the unknown samples were measured through a linear equation as below.

$$y = 0.0539x + 0.0157$$

$$x = \text{absorbance (A)}$$

Stock solution was kept in refrigerator for further analysis and stayed stabilized at 25°C for 2 hours before preparation of its dilute standard. Additionally, each of the glucose standard was prepared just before its analysis.

Bacterial identification (Escherichia coli count)

Eosin Methylene Blue (EMB) agar plates were prepared according to a reported standard method (15). *E. coli* count of the fruit juices samples were performed by serial dilution agar plate technique. Also, the serial dilution technique was followed for dilution and experiment was carried out under Laminar flow. After dilution, spread plate technique was employed to plate the samples. Accordingly, one mili litter of the sample was spread aseptically onto Eosin Methylene Blue (EMB)agar plates by using sterilized glass spreader. These plates were then incubated at 37°C for twenty-four hours. Only those plates which showed countable colonies of 30 to 300 were considered for counting. Total *Escherichia coli* count/ 0.1 mL were calculated as;

Colonies/plate = N
Dilution volume added to a plate = 1 mL
Colonies number/1 mL = Number of colonies × dilution factor

Morphological characterization

Single colonies were studied for their color, shape, size, margin, elevation, texture gram reaction etc. The gram positive and negative bacterial cells were distinguished through a standard Gram staining method (15).

Heavy metals Analysis

Analysis for the heavy metals (Chromium, Manganese, Cobalt, Nickel, Copper, Zinc, Arsenic, Cadmium, and Lead) was performed by using Varian 6 flame atomic absorption spectrophotometer (FAAS) at Quaid-e-Azam University (QAU), Islamabad as per APHA methods 1995.Prior to analysis the juice samples were digested as per described method of Unaegbu and his co-workers (16). Calibration plot method (as described in the British Pharmacopoeia, was adopted during analysis on FAAS (17). A 1000 ppm stock standard solution of the selected metal ion was prepared initially where weight obtained was equal to 1.0 g of the metal ion. Volume of the sample fruit solution (equal to 1.0 g of metal) was dissolved in 1000 mL to obtain 1000 ppm. After then a 100 ppm working solution was make ready from the sample fruit stock solution. Their absorbance was recorded on FAAS and the experiment was repeated in triplicate and standard deviation (SD) from the reading was calculated. The detection limit for the metals under observation was derived from the SD and calculations were based on reference/blank readings. Deionized H_2O was used as a reagent blank. Calibration curve (noted at 5 points ranging from 0.5 to 10 mg/dL) was plotted from reading of three standard solutions while the metals concentrations were derived from regression equation.

Results and Discussion

Our work begins with random collections of the fresh juices of Apple, Orange, Strawberry, Pineapple, and Carrot, from different restaurants and cafeterias of Islamabad, Pakistan. Homemade juice samples were also prepared, and all the samples were processed as described in material and method. The prepared juice samples were subjected for assessment of their Physicochemical, microbial properties and heavy metal contents.

Physicochemical Properties of Juices

Physicochemical characteristics such as pH, density, moisture contents, titratable acidity, total solids and reducing sugars for all the juice samples were measured. The results obtained about pH, titratable acidity, and density of the juices are given in table 1.

The results about moisture contents, total solids and reducing sugars of the juices samples are shown in table 2.

Table 1. Physio-chemical Analysis of the selected Fresh Juices*

	Physico-Chemical parameter					
Juice samples	**pH**	**Mean±SD**	**TA (g/100 mL)**	**Mean±SD**	**Density (g/cm³)**	**Mean±SD**
Carrot	5.80-8.99	6.3±0.91	0.12-0.39	0.27 ±0.09	0.96-1.05	1.002± 0.03
Apple	3.82-4.92	4.1±0.29	0.22-0.40	0.30 ±0.06	0.98-1.017	0.995 ±0.03
Pineapple	3.40-3.79	3.7±0.11	0.75-0.89	0.83± 0.06	0.967-1.023	0.98 ±0.03
Strawberry	3.44-3.55	4.1±0.29	0.44-0.79	0.64±0.1	0.94-1.02	0.99 ±0.02
Orange	3.50-4.09	6.3±0.9	0.57-0.88	0.8± 0.1	0.98-1.02	1.01 ±0.01
WHO standards (Apple juice)	3.20-3.55		0.36-0.80		1.05	
WHO standards (Carrot juice)	5.88-6.40		0.3-2.0		1.02	
WHO standards (Pineapple juice)	3.20-4.00		0.7-1.6		1.06	
WHO standards (Strawberry juice)	3.00-3.90		0.6-1.1		1	
WHO standards (Carrot juice)	5.88-6.40		0.3-2.0		1.02	

**NG: Not given, FAO, 2005(CODEX STAN 247:2005)*

Table 2. Physio-chemical Analysis of the selected Fresh Juices*

	Physico-Chemical parameter					
Juice samples	**Moisture %**	**Mean±SD**	**TS (g)**	**Mean±SD**	**RS (g/L)**	**Mean±SD**
Carrot	88.5-94.1	90.62± 1.95	5.9-12.02	9.39 ±1.95	5.002-7.88	6.14± 0.9
Apple	82.1-88.6	84.86 ±1.98	11.4-17.9	15.14 ±1.98	7.77-9.04	8.32 ±0.50
Pineapple	75.9-82.5	79.51 ±2.04	17.5-24.1	20.49 ±2.04	8.25-9.75	9.1 ±0.5
Strawberry	83.8-88.4	86.23 ±1.94	11.7-17.3	3.5 ±0.1	6.9-9.2	7.64 ±0.7
Orange	86.9-92.4	89.13 ±1.52	7.6-13.1	10.87 ±1.52	4.23-4.99	4.7 ±0.30
WHO standards (Apple juice)	NG		13.3		NG	
WHO standards (Carrot juice)	NG		7.0		NG	
WHO standards (Pineapple juice)	NG		14.3		NG	
WHO standards (Strawberry juice)	NG		8.0		NG	
WHO standards (Carrot juice)	NG		7.0		NG	

**NG: Not given, FAO, 2005(CODEX STAN 247:2005)*

pH and total titratable acidity (TA). The pH analysis of the juice shows significant differences depending on the fruit used. pH values of the juice samples were ranging from 3.7 to 6.3. Carrot juice showed highest pH while lowest pH value was shown by Pineapple juice. About 81.8% (for n=55) of the sample juices showed pH values within the permissible limits. Low pH values indicated that the juices are rich in organic acids. Additionally, the juices which showed pH greater than 4.3 have higher chances for rapid pathogenic growth. The titratable acidities showed consistency like the pH values. Acidity range showed by the sample juices were 0.27 to 0.83. The pineapple juice samples, orange sample juices and strawberry juices indicated high TTA values compared to the apple juices and carrot juice samples. Considering titratable acidity, 87.3% (where n=55) of the sample fruit juices showed titratable acidity values within the permissible limits. Higher values of TA (titratable acidity) shows that citric acid (a weak organic acid) is present which occurs naturally in many fruits and their juices (18). Its high quantity reduces pathogens growth, increases the flavor of the juices and enhances shelf life of the beverages. Additionally, some juices also have malic acids, particularly in Apple juices (19).

Density. The density values obtained for all juice samples were found in a range of 0.98-1.01 g/cm^3. Among the tested juice samples, orange juice possesses higher density while Pineapple juice samples showed the lowest density value. It was inferred that 90.9% juice samples had their densities within permissible limits. Density is considered as one of a qualitative parameter for the juices (20). Juice having higher density value are considered to have high amount of pulp, total solids (TS) and sugars, compared to those having low density values. For the selected sample, since orange juice had higher density thus it was suggested that orange juice comprises of more pulp, total solids (TS) and sugars while rest of the juices have comparatively lower values of pulp, total solids (TS) and sugars.

Moisture Content of the sample juices. Moisture content is an amount of H_2O present in a substance. Considering moisture contents in the sample juices it was found that all the juices have high percentage of moisture which is enough to quench the thirst. The values obtained for the selected juices falls within a range of 84.86 - 90.62 % while it is recommended to be in range of 79 - 81% (21). Determining moisture content/percentage is an important parameter to measure taste and texture of the sample juices. The higher percentage of moisture content indicated that the juices are weak, watery, and have less shelf-stability.

Total Solids (TS). All the minerals, cations, anions, salts, metals, or any of these dissolved in water are referred as total solids. In juices fruit pulp, sugars and fruit acids are usually considered as total solids. They are major contributors of pectin, glycosidic substances, metallic salts, Na, K, and Ca etc. Total Solids are considerably associated with storage, maturity, and ripening conditions of the sample juices. These conditions are important with respect to the quality for majority of the fruit juice samples (22). For the tested juices, the TS (Total solid) ranged from 9.39 to 20.49. Majority of the fruit juices had TS values greater than the reported standards (CODEX STAN 247:2005) for fruit juices and nectars described in FAO.,2005 (23). The enhanced values show that high pulp level is present in the juices. The RS (reducing sugars) values ranged from 4.7 to 9.1 where the RS of Pineapple juice was highest while that of the orange juice showed least amount.

Microbial Status of the Sample Juice

All the juice samples were investigated for their microbial (*E. coli*) contamination and the results obtained are presented in table 3.

The *E. coli* count for all the samples ranged from 3.0×10^3 to 2.10×10^8 (Cfu/mL) where strawberry juice and carrot juice sample were more contaminated than the apple juice, orange juice and pineapple juices. According to WHO standards the *E. coli* should be zero in fresh juices/food stuff. Among the 55 analysed samples, 31 (56.66%) were positive for *Escherichia coli*.

The morphological characterization results are given in table 4.

The microbial status results showed that the fruit juices were prepared in an unhygienic environment. The

Table 3. *E. coli* counts (cfu/mL) of Fresh Unpasteurized Juice Samples

Samples	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**%age frequency**
Apple	5.5×10^7	2.86×10^2	-:	1.02×10^6	-:	-:	1.40×10^4	2.80×10^4	1.70×10^7	2.70×10^7	70%
Carrot	5.0×10^3	-:	6.0×10^6	2.50×10^4	1.60×10^8	2.70×10^6	-:	2.20×10^4	1.30×10^5	4.0×10^5	80%
Pineapple	1.20×10^4	1.30×10^6	1.00×10^7	1.10×10^4	-:	-:	3.0×10^3	5.0×10^4	-:	-:	60%
Strawberry	-:	2.10×10^8	1.70×10^5	-:	-:	1.10×10^4	3.0×10^3	5.0×10^4	-:	-:	50%
Orange	1.67×10^7	2.17×10^6	-:	-:	5.60×10^4	-:	-:	-:	-:	-:	30%
Homemade	-:	-:	-:	-:	-:	-:	-:	-:	-:	-:	0%

Note: -: either absent or below countable range

Table 4. Morphological characterization

S.NO	Morphological Attributes	Measuring Techniques
1	Form	Circular
2	Elevation	Raised
3	Size	Small
4	Margin	Entire
5	Surface	Shiny
6	Pigmentation	Intracellular
7	Opacity	Opaque
8	Gram reaction	Gram negative

findings propose that consumers (particularly middle-income group people) of these unpasteurized juices have high chance of risk for contaminated food-related diseases. Findings of the present study is also compatible to the reported results (2, 18). Additionally, the highest bacterial (*E. coli*) contamination was observed in Carrot juice, Apple juice and Strawberry juice sample respectively. The high contamination observed could be associated to greater pH values, high moisture contents as well as high TS values of the juices as these conditions support microbial growth. In some other studies (24) it was inferred that greater pH and temperatures greater than 28 °C favor bacteria growth that ultimately reduces shelf life of the juices. Similar results have also been revealed in literature (25-27) reported that *Escherichia coli* was the predominant strain in the street vended juices. It suggests that careful handling and strict processing in fruit juice preparation are necessary to maintain the quality. It has also been reported that transformation of microbes to the fruit juices linked with the environment microflora harboring over the fruits (use for juices) and on the surfaces of utensils, in dust form generated by traffic (28). Moreover, unhygienic washing of fruits and the utensils before fruit juice extraction led to greater microbial load. Codex standard (CX/NEA 03/16:2002) revealed that *Escherichia coli* in fruit juices/drinks should be at zero level. *Escherichia coli* which was found as55% in the present study, indicate that the sample juices were of low quality which cause food-borne diseases in the consumers. Compared to the results (99% *E. coli* contamination) revealed in a report (29) for juices from Bangladesh, the juices studied in the present work showed low contamination.

Heavy Metals Concentration in Fresh Juices

Fresh Fruit Juices obtained from local markets of Islamabad, Pakistan were pre-subjected to sample preparation (as described in material and method section) and were then analyzed for their heavy metals contents by using flame atomic absorption spectrophotometer (FAAS). The results obtained are shown in table 5.

The results shown in above table reveal that not all samples juice have contents of all the ten investigated heavy metals. It was also found that in some juices the heavy metal concentration was below the detection limits. It may be due to the very small detection limit of FAAS. In our study, the results were compared with WHO, US-EPA and Punjab Pure Food Rules 2011. Maximum permissible limits (MPL) of WHO, US-EPA and PPFR for theses heavy metals in fruit juices are given in the table 5. Cadmium (Cd) and arsenic (As) in all fruit juice samples were absent or below the detection limit. As the acceptable limit for Cd and As is (WHO0.003, US-EPA 0.005, PPFR 0.1), (PPFR0.1) respectively so all the juice samples were not harmful in case of Cd and As because their concentration did not exceed the maximum allowed limits. The Cr concentration in 36% of the sample fruit juices were exceeding the MPL. It may be due to presence of this metal in fruits from which these juices have been prepared. Chromium is one of the toxic essential heavy metals. It is highly harmful to humans when its concentration exceeds tolerable limits. It aids in the biosynthesis of glucose tolerance factor (30), utilization of sugar protein and fats (31), catabolism of fat and carbohydrates, and the maintenance of blood glucose. In case of Mn all the samples show the concentration within the WHO limits but only orange juice exceeded the US-EPA limit which is 0.05 and the mean concentration of Mn in orange juice is 0.083. Manganese plays an essential role in living things, including humans, such as oxidative phosphorylation, fatty acid and cholesterol metabolism, mucopolysaccharide metabolism, and activation of some enzymes (32). By the oral route, manganese is often regarded as one of the least toxic elements, however if consumed in large amount can cause neurological impairment (33). Mean concentration of Co in all the samples was within the MPL.

Table 5. Heavy metals concentration (Mean ± SD) in fresh juices*

Sample juices &various organization limits	**Cr** (mg/L)	**Mn** (mg/L)	**Co** (mg/L)	**Ni** (mg/L)	**Cu** (mg/L)	**Zn** (mg/L)	**As** (mg/L)	**Cd** (mg/L)	**Pb** (mg/L)
Carrot	0.05±0.04	0.005±0.004	BDL	0.08±0.09	0.045±0.039	0.07±0.065	BDL	ND	0.176±0.165
Apple	0.007±0.003	0.22±0.09	BDL	0.07±0.06	0.09±0.05	0.2±0.1	BDL	BDL	0.041±0.03
Pineapple	0.02±0.001	0.3±0.1	0.01±0.003	0.1±0.08	0.2±0.083	0.35±0.082	BDL	BDL	BDL
Strawberry	0.06±0.046	0.26±0.4	0.09±0.03	0.7±0.65	0.414±0.502	0.516±0.57	ND	ND	0.195±0.26
Orange	0.012±0.009	0.083±0.009	0.002±0.001	0.08±0.07	0.14±0.07	0.21±0.09	BDL	BDL	BDL
Detection Limits	0.003	0.0015	0.009	0.006	0.0015	0.0015	0.15	0.0008	0.015
WHO limits	0.05	0.4	NM	0.07	2	NGL	NM	0.003	0.01
US-EPA Limits	0.1	0.05	0.1	0.1	1.3	5	NM	0.005	1.5
PPFR	0.02	NM	NM	0.025	5	5.0	0.1	1.0	0.2

**NGL: no guideline, because it occurs in drinking water at concentrations well below those at which toxic effects may occur. NM: not mentioned, BDL: below detection limit, ND: not detected. WHO, (2017), US-EPA, 2008, Punjab pure Food Rules 2011*

In case of Ni considering WHO limits carrot (0.08), Strawberry (0.7), and Orange (0.08) and considering PPFR all the samples are contaminated. If we consider WHO and US-EPA limits 48% of the juice samples were contaminated. Nickel (Ni) contaminated soil probably transfer Ni into the fruits which ultimately transfer it into its juices. Although Ni intake in small amount is necessary but its higher intake may cause bed effects on human health. These adverse effects may be lung cancer, larynx cancer, prostate cancer, nose cancer, lung embolism, asthma, chronic bronchitis, and some heart related diseases (34). Lead in carrot, apple and pineapple juices exceeded the MPL set by WHO where it was BDL in orange and strawberry juices. Mean concentration of Cobalt, Copper and Zinc in all the juices samples were within the permissible limits.

Conclusion

Fruit juice bought from the markets in Islamabad posed a health risk based on the presence of *E. coli* and the concentration of heavy metals analyzed in the present work. Chromium, Manganese and Nickel posed the greatest risk as their level exceeded the safe limits set be WHO, US-EPA and PPFR. Copper, Cobalt and Zinc levels were within the limit set by WHO, US-EPA and PPFR and therefore pose no threat to public health. Arsenic and Cd were either not detected or were below the detection limit. The study inferred that the overall quality of fresh unpasteurized juices was poor due to the presence of heavy metal concentration, and occurrence of pathogenic *E. coli* that imposes serious threats to the consumers life and is enough for an immediate action and proper monitoring by respective authorities.

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