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Microbial Growth and Chemical Analysis of Mineral Contents in Bottled Fruit Juices and Drinks in Riyadh, Saudi Arabia

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Abstract: This study aimed to determine the clinically important levels of minerals in bottled fruit juices and drinks and to determine the microbial contamination of commercially available bottled fruit juices and drinks from different supermarkets in Riyadh, Saudi Arabia. Commercially available bottled fruit juices and drinks were brought from different supermarkets in Riyadh, were examined microbiologically and mineral contents were determined by atomic absorption spectrophotometry. A total of 150 specimens (3 replicates of a total 50 samples) were examined for microbial growth on six different culture media (BAP, NA, MacConkey, CAP, Salmonella Agar and PDA). A total of 43 (28.7%) different colonies were seen on different fruit juices. Bacillus cereus was the most common isolate in all types of fruit juices. Other isolates included *Bacillus subtilis*, *Bacillus polymyxa*, *Chryseomonas luteola*, *Tatumella ptyseos*, *Streptococcus lactis* and *Candida* sp. None of the specimens taken from softdrinks and power drinks showed any microbial growth after incubation for 48 h in all six environmental plates used. Specimens from mixed juice with milk showed microbial colonies in 3 out of 10 specimens with *Lactobacillus* sp., *Streptococcus lactis* and *Lactobacillus casei*. The mineral contents of 8 specimens of fruit juices had iron content within the maximum allowed concentration. As to potassium content, 7 of 8 (87.5%) of the samples had potassium content >10 ppm. Five of 8 (62.5%) samples had sodium content >20 ppm, 7 of 8 (87.5%) had aluminum content >0.2 ppm, 4 of 8 (50%) had lithium content >0.2 ppm, 7 of 8 (87.5%) had magnesium content >30 ppm, 4 of 8 (50%) had manganese content >20 ppm, all 8 contained lead >0.2 ppm and 7 of 8 (87.5%) have zinc content >5 ppm. Commercially sold fruit juices in Riyadh, Saudi Arabia should be further investigated and regulated since they contain dangerous organisms and minerals which are toxic to the body.

Key words: Microbial contamination, toxic minerals, juices

INTRODUCTION

Fruit juices and drinks are nutritious which offer great taste and health benefits. The 2005 Dietary Guidelines for Americans (2005) recommended consumption of several cups per day of fruits and vegetables. Most fruit juices and drinks brought from grocery stores and supermarket shelves are pasteurized. This means that the liquid has been brought to a high temperature that kills harmful bacteria. However, a small percentage of fresh juices are unpasteurized. This means that there is a chance the product may contain bacteria harmful to our health. Most people can enjoy unpasteurized juice and drinks, however, for young children, the elderly and people with weakened immune systems, the effect can be severe or even deadly (Esteve and Frigola, 2007). Unpasteurized fruit and vegetable juices and drinks have posed serious public health risks in recent years. Seventy people-including a child who died-became ill in 1996 after drinking unpasteurized apple juice contaminated by a strain of *Escherichia coli* bacteria (New York Times, 1996). In 1999 and 2000, unpasteurized orange juice

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contaminated with Salmonella bacteria sickened hundreds of people in the United States and three Canadian provinces. The 1999 outbreak contributed to one death (Formanek, 2001). A 2005 study in Japan found out that up to 52% of commercial fruit juices sold in Japan were contaminated with thermostable acidophilic bacteria (Hara *et al*., 2005).

As to mineral contents of bottled juices, epidemiological studies have reported the occurrence of disease including problems with reproduction, cancer, congenital malformations of the central nervous system, cardiovascular disease and even death due to exposure to harmful trace elements and mineral contents (Amato, 1999; Eisenberg, 1992; McDowell, 1992).

Magnesium (Mg) deficiency leads to vasoconstrictions, hypertension, cardiac arrhythmia, atherosclerotic vascular disease, acute myocardial infarction, preeclampsia in pregnant women, possibly diabetes mellitus of type II and osteoporosis. The daily recommended intake for Mg is 150-500 mg (Eisenberg, 1992).

The recommended daily intake of sodium (Na) is 20 mg. A high Na^+ intake is associated with hypertension and that dietary Na^+ restriction, achieved by not adding salt and avoiding Na^+-rich foods, may effectively reduce blood pressure. Drinking certain fruit juices may unnecessarily increase Na^+ intake to a level may be detrimental for health, especially for individuals on a Na^+ restricted diet (Amato, 1999; McDowell, 1992).

Manganese (Mn) is necessary for protein and fat metabolism, immune support and blood sugar regulation. The United States Food and Drug Administration (US-FDA) recommended daily allowance is 2 mg. Iron (Fe) in excess can cause cancer and heart disease. The FDA recommended daily allowance for Iron is 15-18 mg. The FDA recommended daily allowance for potassium (K) is 3500 mg, needed for healthy nervous system and a regular heart rhythm. In moderate doses, zinc (Zn) enhances immunity and reproductive function. The FDA recommended daily allowance for Zinc is 15 mg. Lithium (Li) is needed for proper endocrine and brain function. The FDA recommended daily allowance for lithium is 730 mcg (www.herbshop.com/miniguide.htm.).

Other inorganic toxic substances such as aluminum (Al) and lead (Pb) are not important for human nutrition. It can have toxic effects even in small qualities (CPMC, 1996; Ponessa, 1996).

This study was designed to determine the clinically important levels of minerals in bottled fruit juices and drinks and to determine the microbiological growth and content of commercially available bottled fruit juices and drinks in Riyadh, Saudi Arabia.

MATERIALS AND METHODS

Commercially available bottled fruit juices and drinks were bought from different supermarkets in Riyadh, Saudi Arabia in April 2007 and were examined microbiologically and mineral contents were analyzed.

Samples

- 50 bottled pasteurized fruit juices.
- 20 fizzy softdrinks.
- 10 canned powerdrinks.
- 10 fruit juices fortified with milk.

All samples were bought with their security seals untampered and ensured were in their required storage temperatures with their expiry dates checked. Samples were taken to the microbiology and toxicology laboratories of Girls College of Education in Riyadh, Saudi Arabia for analysis.

Microbiological Analysis

Fifty specimens of bottled juices (pasteurized) and drinks were collected from supermarkets from different areas of Riyadh city, Saudi Arabia. Under sterilized conditions, 1 mL of each specimen were extracted and placed on Blood Agar plate, Nutrient Agar, MacConkey Agar, Chocolate Agar and Potato Dextrose Agar (PDA) for fungal growth. One milliliter of each specimen was diluted in 99 cm^3 of sterilized distilled water and 1 mL of the mixture was incubated at 30°C for 48 h. Three replica of each specimen were prepared. Identification of microorganisms was done using the API 20E system (Analytical Profile Index, Biomerieux, Durham, NC, USA).

The same method was followed for 20 specimens of fizzy softdrinks. Containers were left open in an incubated environment for 2 h to allow escape of gases. Analyses were commenced provided gases were ensured to have escaped from each specimen.

The same procedure was applied using 10 specimens taken from commercially available powerdrinks.

The same procedure was applied on 10 specimens taken from juices fortified with milk.

Chemical Analysis of Mineral Contents in Fruit Juice Specimens

Mineral contents were determined from 8 fruit juice specimens. Minerals included Fe, K, Mn, Pb, Zn, Al, Li and Mg. Samples were prepared by mixing 20 mL of juice in a 100 mL volumetric flask with 10 mL of HCl then make up to volume with water. Mixtures were shaken well, transferred to centrifuge tubes and centrifuged to remove solid particles. In cases of elements with high concentrations, further dilutions were made so that the final solution contains 5% HCl. The juice samples were hydrolyzed by a strong acid, which allows the preparation of many samples at one time, filtered and then analyzed by atomic absorption using Unicam 929 atomic absorption spectrophotometer (Unicam Atomic Absorption, Cambridge, UK).

RESULTS

Microbiological Analysis

Microbial contamination in pasteurized bottled fruit juices samples. A total of 150 specimens (3 replicates of a total 50 samples) from 10 different juice types were tested for microbial growth on six different culture media (BAP, NA, MacConkey, CAP, Salmonella agar and PDA). A total of 43 (28.7%) different colonies were seen on different fruit juices including mango juice, strawberry juice, berry juice, orange juice, apple juice, pair juice, tropical mixed fruit juice and fruit and milk juice. *Bacillus cereus* was the most common isolate in all types of fruit juices except from apple juice and mixed orange and carrot juice. Other isolates included *Bacillus subtilis*, *Bacillus polymyxa*, *Chryseomonas luteola*, *Tatumella ptyseos*, *Streptococcus lactis* and *Candida* sp. (Table 1, 2).

Microbial contaminants in fizzy softdrinks and powerdrinks samples. None of the specimens taken from softdrinks and power drinks showed any microbial growth after incubation for 48 h in all six environmental plates used.

Microbial contaminants in milk fortified fruit juices. Specimens taken from mixed juice with milk showed microbial colonies in 3 out of 10 specimens. The isolated colonies showed *Lactobacillus* sp., *Streptococcus lactis* and *Lactobacillus casei* (Table 1, 2).

Chemical Analysis of Mineral Contents of Fruit Juice Specimens

Table 3 shows the mineral contents of 8 specimens of fruit juices sold in different supermarkets in Riyadh, Saudi Arabia. All 8 samples had iron content within the maximum allowed concentration. As to potassium content, 7 of 8 (87.5%) of the samples had potassium content >10 ppm. Five of 8 (62.5%) samples had sodium content >20 ppm, 7 of 8 (87.5%) had aluminum content >0.2 ppm, 4 of

Table 1: Isolated microorganisms and number of colonies in tested fruit juices in Riyadh, Saudi Arabia

Juice types	Brand	No. of colonies	Microorganisms (No. of colonies)
Mango juice	A	4	*Bacillus cereus* (2) *Bacillus subtilis* (2)
	B	2	*Bacillus cereus* (1) *Bacillus polymyxa* (1)
	C	2	*Bacillus cereus* (1) *Chryseomonas luteola* (1)
	D	2	*Tatumella ptyseos* (2)
Strawberry juice		4	*Bacillus cereus* (1), *Candida* sp. (2) *Chryseomonas luteola* (1)
Berry juice		1	*Bacillus cereus*
Orange juice	A	3	*Bacillus cereus* (2) *Candida* sp. (1)
	B	2	*Bacillus cereus* (2)
Apple juice		2	*Candida* sp. (2)
Pair juice		2	*Bacillus cereus* and *Candida* sp.
Tropical fruits	A	2	*Bacillus cereus* (1) *Streptococcus lactis* (1)
	B	2	*Bacillus cereus* and *Candida* sp.
Mixed fruits	A	3	*Bacillus cereus* (1) *Tatumella ptyseos* (2)
	B	3	*Bacillus cereus* (1) *Streptococcus lactis* (2)
	C	3	*Bacillus cereus* (2) *Candida* sp. (1)
	D	3	*Bacillus cereus* (2) *Tatumella ptyseos* (1)
Orange and Carrot		3	*Candida* sp. (2) *Tatumella ptyseos*
Mixed milk w/juice		3	*Lactobacillus* sp. (1), *Lactobacillus casei* (1) *Strep. lactis* (1)

Table 2: Bacterial and fungal growth on different culture media for tested fruit juices in Riyadh, Saudi Arabia

Juice types	Brands	BAP	NA	MacConkey	CAP	*Salmonella*	PDA
Mango juice	A	1	1	1	1	-	-
	B	-	1	-	1	-	-
	C	-	1	1	-	-	-
	D	1	-	-	-	1	-
Strawberry juice		1	2	-	1	-	-
Berry juice		-	-	1	-	-	-
Orange juice	A	1	-	1	-	1	-
	B	1	-	1	-	-	-
Apple juice		-	2	-	-	-	-
Pair juice		-	1	-	-	1	-
Tropical fruits	A	-	-	1	1	-	-
	B	1	1	-	-	-	-
Mixed fruits	A	2	-	1	-	-	-
	B	1	1	1	-	-	-
	C	1	-	2	-	-	-
	D	-	1	-	1	1	-
Orange and Carrot		1	-	1	1	-	-
mixed milk w/ juice			2	1			

Note: Entries signify the No. of colonies in specified culture media

Table 3: Mineral contents of tested fruit juices (ppm) in Riyadh, Saudi Arabia

	Maximum allowed (ppm)*								
	15	10	20	0.2	0.2	30	20	0.3	5
Samples	Fe	K	Na	Al	Li	Mg	Mn	Pb	Zn
1	0.0417	84.850	25.070	51.623	0.1820	8498.50	20.460	3.909	21.810
2	-0.0160	-0.081	-1.213	51.260	0.0870	6016.70	11.730	5.220	23.080
3	0.0350	63.050	24.780	-0.878	0.0570	-291.91	-0.1268	0.778	-6.350
4	0.0330	44.550	29.020	55.290	0.1550	6507.30	33.620	1.589	26.600
5	0.0411	80.670	10.020	43.668	0.2540	6336.40	14.970	3.892	14.130
6	0.0422	265.430	23.220	217.690	0.2049	14105.20	17.470	2.097	23.120
7	0.0440	77.600	21.150	47.450	0.3812	7627.40	34.483	3.850	16.020
8	0.0350	92.880	10.480	87.061	0.2020	10044.50	24.920	2.763	26.784

*: Dietary Guidelines for Americans (2005) and US-FDA (2006)

8 (50%) had lithium content >0.2 ppm, 7 of 8 (87.5%) samples had magnesium content >30 ppm, 4 of 8 (50%) had manganese content >20 ppm, all 8 samples contained lead >0.3 ppm and 7 of 8 (87.5%) have zinc content >5 ppm.

DISCUSSION

Present study showed the presence of different species of bacteria namely, *Bacillus cereus*, *Bacillus subtilus*, *Bacillus polymyxa*, *Chryseomonas luteola*, *Tatumella ptyseos*, *Streptococcus lactis* and *Candida* sp. in supposedly bacteria-free commercially available fruit juices is of a concern. Their presence may pose risks to consumers' health and should not be taken for granted.

Bacilus cereus is an emerging pathogen that causes invasive disease in immunocompromised patients (El-Saleeby *et al*., 2004). These bacteria can survive, grow and sporulate despite changes in water activity, pH and temperature (Jaquette and Beuchat, 1998; Collado *et al*., 2003). Whether the food or juice is improperly cooled or prepared, *Bacillus cereus* could grow because their spores survive cooking and can germinate (US-FDA, 2006). *Bacillus cereus* can cause diarrhea and emetic illness causing dehydration and possibly death.

Bacillus subtilis has been used in animal feed, baking, cleaning and wastewater, food and beverage because of its capability to produce alpha amylase, crackerase and neutral protease that works at higher pH and temperature range that relaxes dough to get uniform crackers and improve cracker flavors (www.bio-cat.com). However, in the preparation of this enzyme needs inactivation of the sporulating capability of *B. subtilis* using high pressurized conditions (Vasantha and Freese, 1979).

Tatumella ptyseos is a new member identified with Enterobacteriacea found in clinical specimens from the respiratory tract (Hollis *et al*., 1981). *Chryseomonas luteola*, a gram-negative bacillus is associated with nosocomial infections and *Candida albicans* can cause serious disease in humans (Chihab *et al*., 2004). *Lactobacillus* sp. is a probiotic bacteria which can provide opportunities for the prevention or treatment of inflammatory bowel disease (Pena *et al*., 2005). *Lactobacillus casei* is present in fermented dairy products and has beneficial properties for human health. In the human digestive tract, it prevents the establishment of ingested lactic acid bacteria (Oozeer *et al*., 2006). *Streptococcus lactus* is said to be a non-toxic microorganism that can be transformed by a plasmid used as an ice nucleating agent or a functionally equivalent protein capable of inducing ice nucleation, lowering the temperature of the mixture, thereby preserving products such as fruit juices to below -5°C (US Patent, 1993). This organism also produces E234 Nisin, a polypeptide antibiotic used in cheese and milk preservatives (www.elixirmre.com).

Minerals are important parts of drinking water and even fruit juices and are of both direct and indirect health significance. Sufficient evidence is now available to confirm that a certain minimum amount of minerals is desirable, since their deficiency have many negative health effects: diseases and possible aggression from toxic elements and bacteria. In present study results, our samples were supposedly within the required mineral contents as stated in their company's levels. However, the variability of minerals and elemental contents is great from bottle to bottle. In fact, no two brands of bottled water were identical in their mineral content (www. opsi.gov.uk/legislation).

Minerals such as magnesium and zinc are just as critical to maintaining optimal health, or that, taken in excess, these minerals can be toxic. Iron is essential for blood cells; potassium is needed for a healthy nervous system, lithium for controlling emotions and anger, magnesium for effective muscle function, manganese necessary for protein and fat metabolism and zinc to enhance immunity and for reproductive function. However, when taken in amounts over the recommended maximum allowable range, they can be toxic to health. These effects occur in nervous system. Present study showed 7 out of 8 samples had aluminum levels above the maximum allowable value of 0.2 ppm. Another is the presence of lead in all of our tested samples is alarming. Aluminum, as a metal when present in our food, water supply and soil can induce individuals to suffer from aluminum toxicity. After years of accumulated exposure and storage in our body, it can result to brain degeneration and skeletal deformities (www.drpepi.com). It is believed that Alzheimer's disease is related to aluminum toxicity (Derouesne, 2004). On the other hand, exposure to even minute amounts of lead should be avoided.

Lead is highly toxic to humans especially in young children who love to drink fruit juices. It has no known physiologic value to humans. In children, even very low levels of exposure to lead can result to reduce IQ, learning difficulties, attention deficit disorders, behavioral problems, impaired hearing and kidney damage. In adults, it can increase BP and can cause fertility problems, nerve disorders, muscle and joint problems, irritability and memory or concentration problems (www.nsc.org/library/facts). These 2 identified metals are toxic to consumers and thus require further examination, verification and further testing of samples.

The results of our study have several implications for the consumption of these commercial fruit juices in Saudi Arabia. The differences in the mineral content for toxic substances should be further investigated since the issue of potability and safety may advertently harm consumers. It is therefore recommended that corresponding authorities should be vigilant in the issuance of permits and licenses in the manufacture and production of these products.

CONCLUSION

Further analysis of commercially sold fruit drinks should be done. Regulation in the issuance of permits to produce and sell these products should be under strict quality control to reduce and mitigate exposure to harmful microbes and toxic chemicals deleterious to consumers' health.

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