QUALITY ASSESSMENT OF COMMERCIAL PROCESSED APPLE JUICE AVAILABLE IN KARACHI CITY

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Abstract

Apple juice is one of the most popular fruit juices in the world. Apple fruits are the sources of electrolytes, minerals, antioxidants and vitamins, rich in fiber. Therefore, the aim of the present study was to evaluate the safety of fruit juices available in Karachi. Ten types Apple juice were collected from several areas of the city and analyzed. This study based on evaluation of physicochemical parameter (PH, Conductivity, TDS, Salinity, Dissolved oxygen, Temperature) heavy metals (Na, K, Fe, Cr, Zn, Mn, Cu, Ni, Pb Cd) and fungal contamination of HIT processed tetrapacks commonly consumed by the populace of Karachi city. The results were compared with dietary reference limit for food energy recommended by FDA.

Introduction

Crops including fruit and vegetables are processed for different reasons. Fruit can be processed into a variety of products, such as Fruit juices, fruit salads, wine etc. Fruit juices are most frequent produce products may be easily consumed by infants, children and adults to meet their nutrient requirement mainly that of micro nutrients (Nnam and Njoku, 2005).

Apple fruits and juices are broadly used as health foods for their reliable source of vitamins, minerals, antioxidant, electrolyte and fiber (Harmankaya *et al.,* 2012). Apple juice has a large concentration of natural phenols of small molecular weight including (chlorogenic acid, flavan-3-ols, and flavonols) and procyanidins (Henriette *et al* ., 2008) that may defend from diseases linked with aging due to the antioxidant effects which help reduce the possibility of developing cancer and Alzheimer's disease (Willow, 2006).

Assessment of properties and related qualities of apple fruits and juices such as antimicrobial, antimutagenic, antinflamatory, anticarcinogenic, antioxidant, antidiabetic/osteoporosis have been reported (Boyer and Liu, 2004; Gerhauser, 2008). Contamination of heavy metal in fruits, vegetables and other crops are main concern because their addition in food crops in higher concentrations might cause serious hazard to human health if the crops are consumed (Tufuor *et al.,* 2011) Ashworth and Alloway, 2004. Such accumulation has been reported (Okoronkow *et al.,* 2005). Arsenic, lead, zinc and other metals have been found in food crops a limit that top over the recommended nutritional allowance. In plant body iron, zinc are essential due to their physiological functions. The essential elements are very vital since they are concern in a variety of enzymes systems in the human body. but high concentrations are poisonous (Miller and Miller, 2000) These metals accumulate mainly in adrenal gland, liver, lungs, kidney, hair and skin, and they may cause high blood pressure, cancerous changes, damage to kidneys, liver and brain. In some cases they may also lead to mental disorders and loss of brain function (Szyczewski *et al.,* 2009). Fruits have a lot of microorganisms on their surfaces but can habitually reduce their growth until after harvest. Ripening weakens cell walls and decreases the amounts of antifungal chemicals in fruits, and physical injury through harvesting causes breaks in outer protectiving layers of fruits that spoilage organisms can develop. In acidic conditions and low water activity Mould are involved in spoilage of citrus fruits, apples, pears, and other fruits. Penicillium, Botrytis, and Rhizopus are frequently isolated from spoiled fruits (Calvo *et al.,* 2007) In bottled and canned drinks due to lack of oxygen limits mold growth. Chemical reactions that cause unpleasant sensory changes in foods are mediated by a variety of microbes that utilized food as a carbon and energy source. Spoilage microbes are often common population of soil, water, or the intestinal tracts of animals and may be dispersed through the air and water and by the activities of small animals mostly insects. They were included bacteria, yeast and mold (Doyle, 2007).

Mould troubles can be separated into two types: growth of a multiplicity of moulds due to reduced cleanliness inside the plant or field atmosphere, and growth of heat-resistant moulds within heat processed juices. The previous kind can source tainting, discolouration and other universal problems associated with unpleasant mould growth. The later type can effect in slow growth of the mould surrounded by the processed product. (Pitt. and Hocking 2009).

Yeasts can grow up with or without oxygen and are well famous for their useful fermentations that make bread and alcoholic drinks. with a low pH of Fruit and juices yeast can grow and there are some grow on the surfaces of meat and cheese (Smits and Brul, 2005) filamentous fungi also hit a wide variety of foods Some

mould can grow in refrigerators and can tolerate harsh ecological conditions. They have a various secondary and carcinogenic mycotoxins. Patulin usually common mycotoxins linked through fruit juice, mostly apple juice (Pitt and Hocking, 1997). It frequently occurs if juice is formed as of stored apples.

There are a variety of routes through which apple fruits and juices can be contaminated. The aim of the present study was to evaluate the safety of fruit juices available in Karachi city.

Materials and Methods

Sample collection**:** Samples of apple juices were collected from different market locations and retail stores in Karachi city. Determinations of Physicochemical Perameter Immediately after opening packet of sample Physical parameters were measure by using HACH sension 105 multi parameter. First of all dissolved oxygen was measured because to avoid solubility of atmospheric oxygen.

Contamination Control**:** All the glass ware washed with acid and then deionized water dried in oven and stored in a place which was free from dust and fumes. Watman filter paper 40 was used to filter digested juice samples.

Reagent**:** HNO_3 analytical grade Merck 65% and deionized water.

Digestion of sample**:** In the present study Wet digestion methods has been used for this purposes single acid or mixture of acids with or without oxidizing agent were required(Niazi *et al.,* 1997) 50 ml of each juice sample was taken in a Acid washed 250ml of beaker and add 25ml of nitric acid then heated on the hot plate for 60 minute at 105 ^{o}C. The beaker were shaken during heating after heating mixture was cool and then, filtrate and washing were collected in a 100ml volumetric flask and then diluted up to the mark of 100 ml volumetric flask with deionized water.

Instrumentation**:** Sodium and Potassium were analyzed by Flame photometer PFP7 Jeuway while Calcium and Magnesium were analyzed by A.A.S. Heavy trace metals such as Fe, Cr, Zn, Mn, Cu, Ni, Pb were analyzed by A.A.S. Instrumental condition such as pressure of fuel, oxidant and others were adjusted according to the Atomic Absorption Spectrophotometer instrument PE-AAnalyst 700, USA model. Standard Solutions were prepared using spectrochemical grade metal solution from BHD. Three replicate per sample were used in this study. Instrument was calibrated by blank solution and finally analyzed metals content in the fruit juice sample.

Isolation of fungi**:** In direct plating technique 0.01 ml of sugarcane juice spread in hygienic Petri plate and about 10-15 ml of liquefy melt chilled SDA dextrose agar) was poured containing 200 mg/L streptomycin and then after slightly rotating the Petri plate was left for solidification. For the assessment of colony forming unit of mycobiota, two ml of Apple juice sample was suspended in sterilized test tube containing 18 ml of sterilized distilled water was shaken well which gave dilution of 1:10. Two ml of suspension from 1: 10 was transferred to second test tube which gave 1:100 dilutions. Similarly 1:1000 and 1:10000 dilutions were made. There were three replicates for 1:1000 and 1:10000 dilutions. One ml aliquot from 1:1000 and 1:10000 dilutions were transferred to the sterilized Petri plates and 10-15 ml of molten cooled agar poured containing 20,000 units/liters penicillin and 200 mg/liters streptomycin. Petri dishes were incubated at 28 ± 2 ^{o} C for 5-7 days (Nelson *et al*., 1983).

Results and Discussion

The measured physical parameters are shown in Table 1. In this study range of PH was found to range 3.5 to 4.7 were within the reference value (Faten *et al.,* 2011). Conductivity can be used to measure the total dissolved solid content. Conductivity range was found 0.97-2.7ms.cm^{-1}. Minerals, salts, metals, cations or anions dissolved in water are called Total dissolved solid. Larger the solid content indicates better dietary values of the fruit. The moisture content influences on engineering properties of fruits and vegetable simultaneously in determination of shelf life of unprocessed and processed fruit and vegetable because they may effect on physicochemical properties, microbiological spoilage and enzymatic change, the high moisture nutritional contents of the fruits and their juices make them fit for organisms and agent to produce and multiply. Moisture content has to be decreased to the limit that should make moisture unavailable for microbial growth. These can be achieved through refrigerating, freezing and drying. All sample showed highest TDS range 445 to 1338-854mg/L. In this study salinity range was 0.4 to 1.3 ppt. Dissolved oxygen measures whether the natural changes are brought about by aerobic or by anaerobic organisms (Sawyer *et al.,* 2003). The highest value of dissolved oxygen was found in SAA sample while least value was in SIP. In the intercellular spaces of fruits air is naturally present. Dissolve gas associated with pulp particle .The serums of the juices and juice blends contain cytoplasmic respiratory enzymes are present in the serum of juice and juice blend (Torres *et al.,* 2009) which are

able to convert sugars to pyruvic acid, in the presence of oxygen pyruvic acid in the mitochondria decompose to carbon dioxide and water through respiratory enzymes., adenosine triphosphate (ATP), a high energy biocompound, is formed in the aerobic respiration pathway. ATP is essential for the protection of the structures of organelle membranes, aerobic respiration process and the synthesis of pigments and flavour compounds in the fresh juice. If oxygen is lacking after that anaerobic respiration may proceed. Pyruvic acid is decomposed to off-flavour compounds such as ethanol, lactic acid and acetaldehyde in the anaerobic respiration hence, only small amounts of ATP are formed and thus membrane structures may decay with probable value fall of the juice in storage (Caldron and Boli, 1990).

The quantitative average distribution of Na, K, Ca and Mg in the Apple juice is shown in Figure 1. (a) and (c). The highest concentration of Na was seen with a concentration of 158.317 ppm which was less than the average (Noss and Rolfes, 2002) while Ca was determined at the near to the ground level was seen with the concentration 0.111 ppm. This was far away from the required averaged. Sodium, Potassium, Calcium and Magnesium are electrolytes. They are macroelement found naturally in the body they are required to maintain the muscle contraction, and brain function. The concentrations of heavy metals, Cr, Fe, Mn, Co, Ni, Cu, Zn, , Cd, and Pb, in analyzed commercial Apple fruit juice presented in F ig 1. (b) and (c). The highest concentration of Fe was seen with a concentration of 9.286 ppm, which is low within the reference. (Noss and Rolfes, 2002). Defficiency can lead to anemia. Whereas Cu was determined at the low level was seen with the concentration 0.053 ppm. Copper is a component of various enzymes. Cu content was found below the reference value. (Noss and Rolfes, 2002). Copper is involve in oxidative process and electron mechanism, deficiency of Cu can lead deactivation of the above processes (Bhatta charya, 2005). The highest concentration of Pb was seen with a concentration of 0.77 ppm. Lead replaces Ca in the bone (Mills, 1971). Its effect is growing and long time contact has been noted to cause severe health risk (Essien, 1992) which contain inhibition of the synthesis of hemoglobin and also negatively affect the central and peripheral nervous system as well as the kidney (Bhata, 2002).

The fungal species isolated are presented in Table 3. Following species were obtained *A. flavus, A. niger Rhizopus sp, Mucor, Candida. SIP*, INF, PY and JUCY showed fungal contamination The Genus *Aspergillus* was the most frequent. They are heat resistant and their spore can survive in soil and decaying fruits, therefore they become the main cause of pollution for fruit and utensils used for transportation and handling out. *Rhizopus sp* were found in SIP, INF, JUCY samples and *Mucor was* only found in SIP sample. *Candida albicans* was found in one sample these fungi are involved in spoilage of fruits, some vegetables and dairy products (Casey and Dobson, 2003).

Table 1. Physico chemical parameters of commercial apple juices.

Sample code	Packing material	pH	Conductivity	TDS	Salinity	DO	Temp
			Sm^{-1}	mg/L	Ppt	mg/L	^{O}C
N	Tetra pack	3.92	$1.135x10^{-5}$	539	0.5	0.95	27.6
TOP	Tetra pack	3.63	$1.322x10^{-5}$	617	0.6	0.82	27.6
SIP	Tetra pack	4.72	$1.667x10^{-5}$	854	0.8	0.44	24
SHE	Tetra pack	3.84	$1.875x10^{-5}$	865	0.8	0.59	30.4
INF	Tetra pack	3.5	$1.559x10^{-5}$	700	0.7	0.54	30.5
OLF	Tetra pack	3.87	$1.074x10^{-5}$	539	0.5	0.46	24.1
JUCY	Tetra pack	3.98	$1.721x10^{-5}$	719	0.7	0.62	31.1
PY	Tetra pack	3.86	$1.339x10^{-5}$	599	0.6	1.54	29.5
F-O	Tetrapack	3.62	$9.7x10^{-6}$	445	0.4	1.52	28.7
SAA	Tetra pack	4.1	$2.79x10^{-5}$	1338	1.3	1.56	28.6
Reference value		3.9[a]				2-9[b]	

a .Faten *et al* ., 2011, b. Calderon and Bolin, 1990.

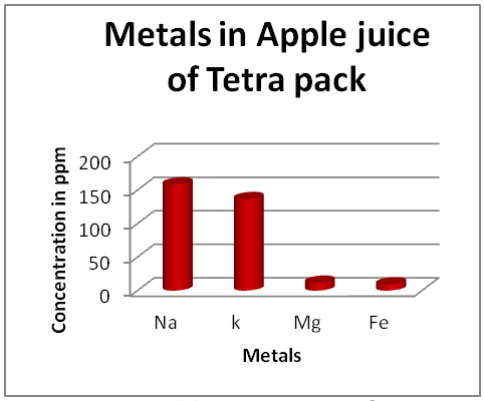

Fig 1. (a). Na, K, Mg and Fe.

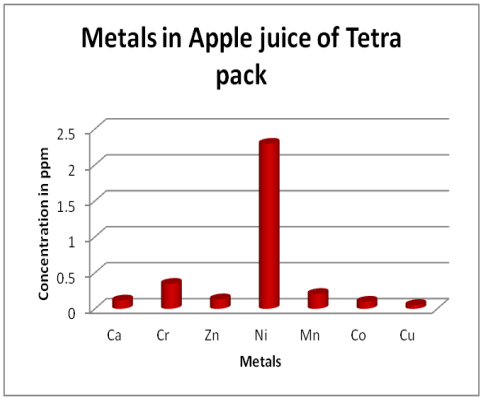

Fig 1. (b). Ca, Cr, Zn, Ni, Mn, Co and Cu.

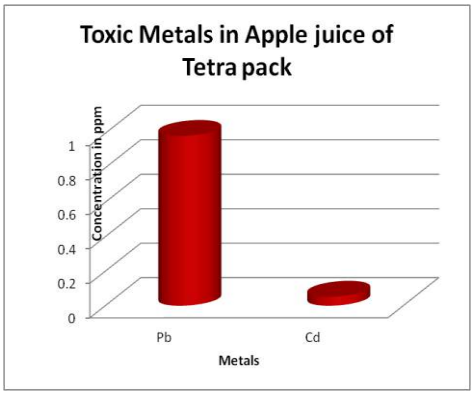

Fig 1. (c). Pb and Cd.

Fig. 1. Metals concentration in apple juice of tutra pack.

Table 2. Reference values developed by FDA specifically used for Food.

Metals	**Amount (mg/day)**
Sodium	2400
Calcium	1000
Iron	18
Copper	2
Reference	
Eleanor Whitney Noss and Sharon Rady Rolfes (2002).	

Table 3. Fungal species isolated from apple juices.

Code	**Identified fungal species**	**Cfu/ml**
N	-	-
TOP	-	-
SIP	*Rhizopus, A. niger, A. flavus, Mucor*	0.4
SHE		-
INF	*Rhizopus, A. niger, Candida albicans*	o.-5
OLF	-	
JUCY	*A. flavus, Rhizopus*	0.75
PY	*A. flavus, A. niger*	2.25
F-O	-	-
SAA	-	-

References

Ashworth, D.J. and Alloway, B.J. (2004). Soil mobility of sewage sludge-derived dissolved organic matter,copper, nickel and zinc. *Envirmental pollution* 127: 137-144.

Bhata, S.C. (2002). *Environmental Chemistry*. CBS Publishes and Distributors, New Delhi pp 442.

Bhattacharya, P.K. (2005). Metal ion Biochemistry. Narosa Publishing House, New Delhi. (P. 191-193,195).

Boyer, J. and Liu, R. (2004). Apple Phytochemicals and their benefit. *Nutr.* J. 3:5.

Calderon, M. and Bolin, B. (1990). Physiological and Biochemical Effects of Controlled Atmosphere on Fruits and Vegetables. *Food Preservation by Modified Atmosphere,* CRC Press, Boca Raton, Fla., 197-207.

Calvo, J., Calvente, V., De Orellano, M.E., Benuzzi, D. and De Tosetti MIS. (2007). Biological control of postharvest spoilage caused by *Penicillium expansum* and *Botrytis cinerea* in apple by using the bacterium *Rahnella aquatilis. Int. J. Food Microbiol*. 113: 251-257.

Casey, G.D. and Dobson, A.D.W. (2003). Moleculardetection of Candida krusei contamination in fruit juice using the citrate synthase gene cs1 and a potential role for this gene in the adaptive response to acetic acid. *J. Appl Microbiol.* 95:13-22.

Doyle, E.M. (2007). Microbial Food Spoilage - Losses and Control Strategies P: 3.

Essian, E.U. (1992). Differential accumulation of lead on selected edible vegetables associated with roadside gardening in Nig. *Trop. J. Applied Sciences* 1(2): 83-86.

Faten, S.N., Khaled, M.S., Amal, T. A. and Askar, K.A. (2011). Factors affecting the fungal contamination of some fruit juices packaged in tetra pack, *African Journal of Biotechnology* 10: 12957-12918.

Gerhauser, C. (2008). Cancer chemopreventive potential of apples, apple juice and apple components. *Planta Med.,* 74: 1608-1624.

Harmankaya M., Sait G. and Mehmet .O. (2012). Comparative evaluation of some macro-and micro element and heavy metal content in commercial fruit juices. *J. Environ Monit Assess*: 5415-5420.

Henriette Z., Lydia, P., Frank, W., Karin, K., Jutta, K., Regina, N., Wolfgang, H., Robert, O., Elke, R., Norbert, F., Peter, S., Hans, B. and Clarissa, G. (2008). *Molecular Nutrition & Food Research, Supplement: Natural Products and Dietary Prevention of Cancer*. Volume 52, Issue Supplement 1, pages: S28–S44.

Miller, D.M. and Miller, W.P. (2000). Land application of waste. Summer M.E(ed) Handbook of soil science CRC Books New York.

Mills, A. (1971). Lead in the environment. *Chem. in Britain* 7(4): 160-162.

Nelson, P.E., Toussoun, T.A. and Marasas, W.F.U. (1983). Fusarium species: *An Illustrated Manual for Identification*. The State Univ. Press pp :193.

Niazi S.B., Mahmood, F. and Asghar, M.Z. (1997). Levels of some major and Trace element in Commercial Mango Fruit juices determined by Flame Atomic Absortion Spectrometry. *J. Chem. Soc. Pak.* 19: 2.

Noss, E.W. and Rolfes, S.R. (2002). Understanding Nutrition 9th Edition Thomson wadsworth corporation ,p: 52, y

Nnam, N.M. and Njoku, I.E. (2005). Production and evaluation of nutrient and sensory properties of juices made from citrus fruits. *Nigerian Journal of Nutrition Science* 26(2): 62-66.

Okoronkow, N.E., Igwe, J.C. and Nwuchekwa, E.C. (2005). Risk and health implications of polluted soils for crop production. *African J. of Biotechnology* 4(13): 1521 1524.

Pitt, J.I. and Hocking, A.D. (2009). Primary keys and miscellaneous fungi. New York, Springer 1(9): 122-124.

Smits, G.J. and Brul, S. (2005). Stress tolerance in fungi to kill a spoilage yeast. *Curr Opin Biotechnol.* 16:225-230.

Sawyer, C.N., Perry, L., Gene, M. and Parkin, F. (2003). *Chemistry for Environmental Engineering and science*. 5th Edition Tata hill Publishing Company Limited. P: 595.

Szyczewski, P., Siepak, J. and Niedzielsk, P. (2009). Research on Heavy Metals in Poland Polish. *J. of Environ. Stud*. Vol. 18, No. 5: 755-76.

Torres, R., Garc´ıa, Ponagandla, N.R., Rouseff, R.L., Goodrich-Schneider, R.M. and Reyes-De-Corcuera, J.I. (2009). Effects of Dissolved Oxygen in Fruit Juices and Methods of Removal. *In food Science* Vol. 8. 409-423

Tufuor, J.K. Bentu, D.K. and Essumang, J. E. (2011). Koranteng -AddoAnalysis of heavy metals in citrus juice from the Abura-Asebu- Kwamank. District, Ghana. Chem. *Pharm. Res*, 3(2): 397-402.

Wareing, P., Davenport, R.R. and Ashurst, P.R. (2005). Chemistry and Technology of Soft Drinks and Fruit Juices. (2nd Ed.) Blackwell Publishing Ltd. 11: 279-297.

Willow, L. (2006). Apples and apple juice contain antioxidants that protect cells throughout the body, particularly the brain and heart. Psychology Today. Retrieved 2010-06-19.