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ASSESSMENT OF PHYSICO-CHEMICAL PROPERTIES AND FUNGAL CONTAMINATION IN PLASTIC BOTTLES AND TETRA PACKED MANGO JUICES MARKETED IN KARACHI CITY

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ABSTRACT

Local market is full of various fruit juices, which are packed in different packing materials for this purpose as they keep unspoiled for a long period of time, some preservative agents and different methods of pasteurization process to keep these packages uncontaminated. In this study, physicochemical properties (pH, conductivity, density, dissolved oxygen, salinity and TDS) and fungal contamination were measured. For this task mango juices in tetra packs and bottles of 16 companies were purchased from different localities of Karachi city. The following fungal species; *Aspergillus candidus, A. fumigate, A. flavus, A. niger, A. wentii, Rhizopus sp., Penicillium sp., Saccchromyces, Curvularia lunata* were recorded in mango juice samples of tetra packs whereas none of the fungus was recovered in mango juice samples in plastic bottles. The influence of physico-chemical parameters on fungal species has also been discussed.

Key words: physico-chemical parameters, fungal species, mango juices, tetra pack, plastic bottles

INTRODUCTION

In most countries, the hot climate means that the intake of liquids must be high to compensate for the expected losses from respiration (Al-Jedah *et al.,* 2002; Victor *et al.*, 2012). Available juices are rich in nutrients, vitamins and minerals that contribute to good health. Orange juice contains high concentration of vitamin C and antioxidants phytochemicals (Franke *et al.,* 2005) and significantly improves blood lipid profiles in people affected with hypercholesterolemia (Kurowska, *et al*. 2000). Fruit juices promote detoxification in the human by means of acid which is an essential universal constituent of Juice and the most commonly citric acid is used (Deanna *et al.*, 2007). The ingredients of processed juices contain mainly water, sugar, preservatives and color and fruit pulps. The most commonly used preservatives are benzoate and sulphur dioxide (Rehman *et al.,* 2011). Fruit juices are known for their ability to raise serum antioxidant capacity and even balance the oxidative pressure and inflammation normally caused by high-fat and high-sugar meals (Ghanim *et al.,* 2010) Fruit juice consumption overall in Europe, Australia, New Zealand and the US has increased in recent years (TOC, 2008) probably due to public awareness of juices as a healthy natural source of nutrients and increased public interest in health issues. In fact, fruit juice intake has been consistently associated with reduced risk of many cancer types (Brock *et al.,* 1988; Uzeudun *et al.,* 2002; Radosavljevic *et al.,* 2004; Kwan *et al.,* 2005; Maserejian *et al.,* 2006). Contamination of fruit and fruit juices by yeasts and moulds, often occurs in fallen or damaged fruit and these should be avoided where possible. Water and other chemicals are possible sources of microbial contamination. Process machinery and filling lines are mainly problematic and strict hygiene is essential. Mould spoilage within the factory is associated with poor hygiene. Various types of heat resistant spores can be produced: ascospores, chlamydospores and sclerotia. Many of the above moulds are found on fruits pre- and post-harvest. Most moulds require oxygen to grow. The recent trend towards polyethylene terephthalate (PET) packaging presents its own problems (Wareing *et al*., 2005). Moulds produce a vast number of enzymes: lipases, proteases, carbohydrases for the degradation of complex molecules, and can utilize nitrogen and carbon sources in many forms from nitrates to proteins and from simple sugars to complex carbohydrates unable to assimilate nitrate or complex carbohydrates such as starch, and require vitamins for their growth (Pitt and Hocking, 2009). Mycotoxins are secondary fungal metabolites and are toxic to humans and animals, causing severe disorders like cancer, immune suppression, or endocrine disruption. Since mycotoxins are very stable and mainly resistant against heat treatment and acidic environment, they remain in the food during processing and storage, causing a serious food safety problem (Filtenberg *et al*., 1996). The main mould spoilers in fruits and vegetables are *Mucor* and *Rhizopus* species from Zygomycota, and *Aspergillus* and *Penicillium* species from Ascomycota. Some species of fungi have been found in processed fruit and fruit juice. When commercial

pasteurization with treatment are applied fruit and fruit product may spoil by germination and subsequent growth of fungal organisms (Faten *et al.*, 2011).

Juices stored under warm and cold conditions (27^{o} C and 4^{o}C) were comparatively analyzed for yeast and mould growth (Udota and Urua, 2010). In local market various kinds of fruit juice products packaged in different packaging materials some added agents and different methods of pasteurization processes to keep healthy these packages, as most of these containers filled cold. In Egypt, some fungi have been found in fruit juices. Microscopic examination of fungal isolates obtained from fruit juices samples showed the presence of the following species: *Byssochlamys nivea, Byssochlamys fulva, Neosartorya ficsheri, Penicillium italicum, Aspergillus flavus* and *Talaromyces macrosporus* packaged in Tetra Pack (Faten *et al.,* 2011). In Karachi city, mango juices packed in paper card board lined with aluminum foil (tetra pack) and plastic bottles of several companies are available for consumers. This work reports physicochemical parameters and presence of fungal species and correlates these parameters.

MATERIALS AND METHODS

Sample collection

Samples of mango juices available in tetra packs and plastic bottles within their expiry date and aseptic packaging as mentioned on the products of different brands were purchased from wholesale or retail shops in various localities in Karachi city.

Physical Parameters

Physical parameters like pH, conductivity, TDS (total dissolved solids), salinity, dissolved oxygen (DO) and temperature were determined by using HACH Sention105 multiparameter, immediately after opening the packet of sample. First of all DO was measured to avoid the solubility of atmospheric oxygen.

Isolation of fungi from juices

In direct plating method 0.01 ml of mango juice was dispersed in sterilized Petri plate and approximately 10-15 ml of molten cooled SDA dextrose agar) was poured containing 200 mg/l streptomycin and then after slightly rotating the Petri plate was left for solidification For the observation of colony forming unit of mycobiota, 2 ml juice sample was suspended in sterilized test tube containing 18 ml of sterilized distilled water was shaken well which gave dilution of 1:10. 2 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. 1 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 intensity of acidity of sample is expressed by its pH value. The range of pH of mango juices of different companies was shown in Table 1, from 3.62- 5.27. The highest pH was observed in PFCL samples, whereas lowest pH was shown by PCO but found within the reported value mentioned in Table 1 (Tasnim *et al.,* 2010).

Dissolved oxygen is the factor that determines whether the biological changes are brought about by aerobic or by anaerobic organisms (Sawyer *et al.*, 2003). DO range of mango juices of different companies are shown in Table1, from 0.35-15.9 mg/l. The highest DO was observed in SPL samples, while lowest DO was measured in BPL samples. In the reported work dissolved oxygen level in juice after gasification should be 2-9ppm (Calderon and Bolin, 1990).

Density is the measure of how close and how heavy is the particle of matter in a sample. The density of fruit juices is represented by Table1; the values of density were recorded in the range of 0.98482-1.09233 g/ml. The samples of TFCL Company showed the lowest density, while NJ exhibited the highest density. The quality of a specific fruit can be determined by its density (Kegwu and Ekwu, 2009). According to Nwanekezi and Ukagu (1999), density is an engineering feature that is applied for the preservation during separation and is regarded as an essential worth of fruit and vegetables.

Conductivity is a measure of the ability to conduct electric current (Braun, 1983). It can sometimes be used to determine the amount of total dissolved solids (TDS). In this study conductivity range was recorded as 1.017-1.917 ms/cm. The sample of NJ Company showed the lowest conductivity, while HBP exhibited the highest conductivity.

Table 1. Physico-chemical parameters of mango juices in tetra packs and plastic bottles of different companies.

Company Code	Packing Material	Physical Properties								
		pH	Temp (°C)	Conductivity ms/cm	TDS mg/l	Salinity ppt	Temp (°C)	DO mg/l	Temp (°C)	Density g/ml
NJ	Tetra Pack	3.92-4.58	24.0-27.6	1.017-1.314	472-666	0.4-0.7	23.9-27.8	0.46-0.73	23.8-27.7	1.02018-1.09233
PFI	Tetra Pack	3.82-3.92	30.3-30.8	1.511-1.524	599-698	0-0.7	29.7-30.6	0.62-15.3	29.5-30.5	1.0526-1.0715
NPL	Tetra Pack	3.86	33.5	1.289	558	0-0.5	31.6	0.73	31.6	1.03549
AFP	Tetra Pack	4.39	24.5	1.356	691	0-0.7	23.6	0.48	23.5	1.03144
BPL	Tetra Pack	3.93-4.49	23.3-33.4	1.578-2.05	584-940	0.6-0.9	24.5-31.5	0.35-0.73	21.7-31.5	1.03852-1.0726
MBL	Tetra Pack	3.77-4.25	23-24.5	1.133-1.704	565-798	0.5-0.8		0.40-0.64		1.06878-1.08542
SIL	Tetra Pack	3.99	21.5	1.359	650	0.6	27.5	0.9	26.7	1.05725
SPL	Tetra Pack	3.74	30.3	1.515	683	0.7	20	15.9	29.3	1.0306
EFL	Tetra Pack	4.94	23.7	1.531	784	0.8	23.6	0.46	23.8	1.09474
PFCL	Tetra Pack	5.27	23.6	1.753	903	0.9	23.6	0.46	23.6	1.05762
JPI	Tetra Pack	4.12-4.26	30.6-32.9	1.192-1.821	536-777	0.5-0.7	29.6-33.0	0.61-0.65	30.0-31.7	1.0626-1.067
CFL	Tetra Pack	3.68	30.3	1.177	527	0.5	29.8	15.4	29.5	1.0254
PCO	Tetra Pack	3.62	27.5	1.113	517	0.5	27.9	0.81	27.5	1.03074
TFCL	Tetra Pack	3.63	27.6	1.322	617	0.6	27.7	0.8	27.6	0.98482
HBP	Plastic Bottle	4.42	20.7	1.917	932	0.9		6.1		
SGA	Plastic Bottle	4.49	26	1.719	818	0.8		7.6		1.0003567
Reference value		3.5-4.7[a]			144-180[b]			2-9[c]		1.07068

Tasnim *et al.,* 2010.Ashraf *et al.,* 2000.Calderon *et al.,* 1990.

Table 2. Fungal species isolated from mango juices in tetra packs and plastic bottles of different brands.

Company Code	Isolate	Range of Cfu/ml
NJ	*Aspergillus candidus, Sacchromyces, Curvularia lunata*	0-0.89
PFI	*Aspergillus fumigatus, Fusarium* sp.	0-0.55
NPL	-	-
AFP	*Sacchromyces* sp. *Rhizopus* sp.	0.66
BPL	-	-
MBL	*Monilia* sp., *Penicillium* sp., *Aspergillus wentii*	0.14-0.21
SIL	-	-
SPL	-	-
EFL	*Aspergillus flavus, A. niger*	0.42
PFCL	-	-
JPI	*A. flavus, A. niger, Rhizopus* sp., *A. wentii, Penicillium* sp	0.78-0.88
CFL	Unidentitified	0.9
PCO	-	-
TFCL	*A. niger*	0.36
HBP	-	-
SGA	-	-

Dissolved solids refer to any minerals, salts, metals, cations or anions dissolved in water. Total dissolved content are related to both sugar and fruit acid. They are main contributors of pectin, glycosidic material, salt of metals and electrolytes sodium, potassium, calcium etc. TDS content is significantly influenced by the combined effect of storage of maturity and ripening conditioned are more important quality factors for most fruit juices (Tasnim *et al.,* 2010). In the present investigation, TDS was measured in the range of 472-940 mg/l. The values of TDS in all samples were found higher than those reported in available literature.

Salinity indicates the presence of salt in the sample and is important from the viewpoint of pH. In this study salinity range was recorded as 0.4- 0.9 ppt.

Isolated fungal species from mango juices in tetra packs and plastic bottles have been mentioned in Table 2. The fungal species recovered were *Aspergillus candidus, A. fumigatus, A. flavus, A. niger, A. wentii, Rhizopus* sp., *Penicillium* sp., *Saccharomyces, Curvularia lunata*. Samples of NJ Company were exhibited highest and samples of TFCL Company were displayed comparatively least number of fungal contamination. On the other side samples in tetra pack and plastic bottle of following companies PFCL, BPL, SPL, PCO, NPL, SIL, HBP and SGA were not fungal contaminated. Samples of CFL Company was shown unidentified fungal contamination. Among these, most frequent species recorded was *A. niger* which is the identical to the fungal contamination found in mango juices in tetra packs available in Egypt (Faten *et al*., 2011). In this study *A. niger* was found in tetra packs of TFCL, JPI, EFL companies, while MBL and JPI were shown the presence of *A. wentii* and *Penicillium* sp.

A. fumigatus, *Fusarium* sp. and *Rhizopus* sp. were recorded in JPI and AFP whereas samples of PFI, NJ and AFP were shown the presence of *Saccharomyces. A. candidus* was found in NJ sample.

On the basis of data it was calculated that 50% samples showed fungal contamination, which may have low pH of fruit juices greatly limit the number and type of microorgansim that live in them, and their presence may alter pH making it favorable for pathogens (Mwambete and Peter, 2011). In processed fruit juices manufacturers commonly use sulphur dioxide and benzoate as preservative although they damage the vegetative cells. Nutrients and available oxygen in the food are the main factors determining the kind of fungal spoilage. Uses of contaminated water, long period of preservation without refrigeration for this purpose chemical presvatives are added. According to Rahman *et al.*, (2011) moulds require oxygen for their growth, but dissolved oxygen in the foodstuffs is more important than atmospheric oxygen tension (Krisch *et al.,* 2011).

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