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A REPORT ON THE QUALITY ASSESSMENT OF PRESERVED FRUIT JUICES: MICROBIAL AND BIOCHEMICAL ANALYSIS

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ABSTRACT: *Fruit juices are good source of energy and vitamins. In today's busy lifestyle, various preservation techniques enabled us to preserve fruit juices for longer period of time, thereby providing us with a good opportunity to add natural products in our daily diet instead of synthetic drinks with adverse impact on our health. Mostly juice companies are marketing the preserved juices in tetra-packs. To maintain the high quality of fruit extracts in these containers and to avoid any food poisoning, the sterilization techniques applied should be accurate thus making these fruit extracts safer for health without chances of any contamination. Although most of the brands claim to bring high quality fruit juices in the market but there are many cases where these preserved fruit extracts caused severe illness to the consumer. The present study is concerned with the microbiological analysis of the locally preserved fruit juices. Quality of the juice samples was analysed through biochemical analysis by estimating their total phenolic content and antioxidant activity. Bacterial growth was observed in five juice samples while all the juice samples gave negative results for fungal growth, however, in only two samples, yeast growth was observed i.e., sample B and sample C. Twelve bacterial colonies were isolated from fruit juice samples which were further categorized morphologically. Sample E has shown maximum antioxidant activity and was recorded as the most healthy and safe mango juice having no microbial growth with higher total phenolic content (TPC).*

Keywords: Antioxidants, TPC, fruit juice.

INTRODUCTION

Fruit juices are fermentable liquids extracted from fresh fruits and preserved by various physical and chemical techniques. These fruit juices typically contain not less than 20% fruit juice [1]. In today's busy lifestyle, fruit juices are instant source of energy and are common beverages in most countries of the world being nutritious, having great taste and several health benefits. Preserved fruit juices are a good source of vitamins and minerals which are frequently consumed by a large number of people. These are particularly suitable for lactose-intolerant consumers. Juices contain both beneficial as well as harmful bacteria. Bifidobacteria are beneficial bacteria present in fruit juices. These bacteria live in human gastrointestinal tract and exert beneficial impact on human health [2]. Some important constituents of fruit juices include water dominant components, carbohydrates including sucrose, fructose, glucose and sorbitol in variable concentrations. Usually preserved fruit juices contain small amount of proteins, cholesterol and no fibers. They are rich in minerals and vitamin C. The antioxidants present in these juices generally minimize the risk of cancer and heart diseases [3]. Regular consumption of fruit juices with high polyphenols increase the protection against numerous degenerative diseases and also minimize the risk of chronic diseases [4]. Polyphenolics, flavonoids, vitamin C, carotenoids and tocopherols are found in fruits and vegetables. Antioxidant activity of phenolics is conferred by their redox properties. These usually behave as reducing agent, metal chelators and hydrogen donors. Fruit juices contain carotenoids and phenolic compounds that have antioxidant potential. Among the antioxidants found in citrus fruits, ascorbic acid lowers the chances of cardiovascular diseases, arteriosclerosis and some forms of cancers and also protects from oxidative stress [5]. Due to the consumption of unhealthy fruit juices, there are several reports for human illness around the world. Several factors are responsible for the contamination of the fruit juices. Some of these include the use of contaminated water for dilution of fruit juices, dressing with ice prepared from contaminated water, prolonged preservation without refrigerator and improper preservatives, airborne dust and other contaminants etc. Contaminated fruit juices contain different bacterial pathogens such as *Staphylococcus aureus, Shigella* spp., *Salmonella* spp. and *Escherichia coli* etc. [6]. Microbial contamination is the major concern of fruit juices. Since bacteria are ubiquitous in nature, therefore, they get enter into the juices through several ways and become the major cause of food poisoning. Unpasteurized fruit juices are the common source of Hepatitis A, *Cryptosporidium*, *E. coli*, *Clostridium* and *Salmonella* species of bacteria [3]. Lactic acid bacteria, *Acetobacter* and *Acetomonas* are found on fruit surfaces. These are the frequent spoilers of fruit juices because they feed on the materials secreted by plants and fruits. Most fruits promote the growth of yeast because of their acidic nature and sufficient amount of sugar. Molds cause less spoilage of fruit juices because they are unable to grow in the absence of air except few such as *Penicillium* and *Aspergillus* [3]. *Salmonella* and *E. coli* can survive for weeks in chilled juices with high pH range [7]. Microbiologically safe food consumption is a great concern today [1]. Improper handling contributes to the entry of pathogenic bacteria into the fruit juices. Fresh fruit juices are more in demand because of their taste and beneficial impact on health. Therefore, it is important to assess the quality of the preserved juices with respect to microbial load and physio-chemical parameters that will be helpful for public awareness as well as for the information of regulatory bodies [8]. The present work deals with microbiological and biochemical analysis of various preserved fruit juices obtained from the local market. Juice samples were analysed for the presence of microbial and fungal growth. These fruit juices were also evaluated for their antioxidant activity and total phenolic contents.

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MATERIALS AND METHODS

In the present work, five different mango juice samples were selected for the study.

SAMPLE COLLECTION

Four different brands of commercially available mango juice (sample A, B, C & E) preserved in tetra-packs were procured from the local market in the month of August, 2014. Information on the labels was recorded, including manufacture and expiry dates, type of preservative(s), etc. Mango juice obtained from fresh mango was taken as control and designated as sample D. The pH of the samples was also recorded. Samples were kept in the refrigerator at 4^{o}C before commencement of analysis.

MICROBIOLOGICAL ANALYSIS

Firstly, the mango juice samples were kept at room temperature (28±2^{o}C). From each sample, 1 ml of juice was collected and inoculated in 9ml of peptone water in a sterilized beaker by using method of Oranusi *et al*. [3] and incubated for 24 hours at 37^{o}C. After 24 hours, growth of bacteria was observed by comparing with non-inoculated peptone water taken as a control. For bacterial isolation, 50µl of the bacterial culture obtained in peptone water was used to spread on L-agar and MacConkey agar plates. The plates were kept at 37^{o}C for 24 hours. For fungal isolation, saboured dextrose agar and 2% malt extract agar plates were used. The plates were placed at 37^{o}C for 24 hours after spreading the sample juices. Growth was checked by observing the colonies developed on these plates. Bacterial isolates were purified by repeated streaking on L-agar plates. These isolates were further characterized morphologically by recording their colony and cell morphology after gram-staining following Gerhardt *et al*. [9].

BIOCHEMICAL ANALYSIS

Mango juice samples were analysed biochemically by estimating their antioxidant potential and total phenolic contents.

Antioxidant Activity

Antioxidant activity of mango juice samples was analyzed using reducing assay following Rekha *et al*. [5]. The experiment was repeated thrice.

Total Phenolic Contents (TPC)

Folin's ciocalteu method was used to determine total phenolic content (TPC) following Cindric *et al*. [10]. Three replicates for each sample were analysed.

RESULTS

In the present work, four different mango juice samples (sample A, B, C & E) were selected for microbiological and biochemical analysis in addition to the natural mango extract taken as control (sample D).

PHYSICAL PARAMETERS

Basic information of preserved mango juice samples such as manufacturing and expiry dates and preservatives etc. as mentioned on the labels were recorded. The pH of all mango juice samples was also recorded (Table 1).

MICROBIOLOGICAL ANALYSIS

For bacteriological analysis, sample juices were incubated in peptone water for 24 hours at 37^{o}C and culture obtained was spread separately on L-agar and MacConkey agar plates. Growth of bacterial colonies after 24 hours of incubation at 37^{o}C was recorded. All the samples have shown positive growth on L-agar except sample E. No growth was observed on MacConkey agar plates by any sample. Twelve bacterial isolates (A1, A2, C1, C2, C3, C4, B1, B2, B3, D1, D2 and D3) were obtained from different mango juices (Table 1). These isolates were further characterized morphologically by observing their colony and cell morphology using gram-staining technique. The isolates A1, A2, B1, B2, B3, C1, C4, D1 and D2 have produced round colonies while three isolates i.e., C2, C3 and D3 produced irregular shaped colonies. Entire margin was observed in colonies of most of the isolates i.e., A1, A2, B1, B2, B3, C1, C4 and D2 while colonies of the isolates C2, C3, D1 and D3 had dentate margins. Convex elevation was observed in the colonies of isolates A1, A2, B1, B2, C1, C3, D1, D2 and D3. The colonies of the isolates B3, C2 and C4 had concave elevation. Color of the colonies produced by the isolates A1, B1, B2, B3, C1,C3 and D2 was cream while white colored colonies were produced by the isolates A2, C2, C4, D1 and D3 (Table 2). All the isolates were gram-positive rods except D3 whose cells appeared as gram-negative rods. The cells of the isolates A1, A2, B2, C2, C3, C4 and D2 were present in chains. Cells of the isolate B1 were present as isolated rods whereas cells of the isolates B3 and D3 were present as pairs or in chains while in the bacterial strains C1 and D1, cells were arranged in groups (Table 2). No fungal growth was observed on Saboured dextrose media (SDM) which was used for fungus and dermatophytes detection, however, growth was recorded in case of the juice sample B and C on malt extract agar media which was used for yeast or other fungi (Table 1; Fig 1a, b).

BIOCHEMICAL ANALYSIS

Antioxidant Activity

Antioxidant activity of fruit juices was also estimated to evaluate their antioxidant potential. Maximum antioxidant activity was observed in mango juice sample D with an optical density of 2.766 while minimum concentration of antioxidants was recorded in sample B with an optical density 1.318. Similarly sample E had also shown considerable antioxidant activity with an optical density of 1.996. In sample A and C, optical density recorded was 1.565 and 1.504 respectively (Fig 2).

Total Phenolic Contents (TPC)

Maximum phenolic content was observed in mango juice sample E i.e., 95 µg/ml as manifested by the dark blue coloration. The fruit juice sample A also produced significant amount of phenolic content i.e., 70 µg/ml. The presence of high phenolic contents in the sample was indicated by blue coloration. Sample C and D have also shown high phenolic contents while minimum total phenolic content (10µg/ml) was recorded in sample B (Fig 3).

DISCUSSION

Fruit juices are good for human health because they contain antioxidants and phenolic contents which are very important for good health. Fresh and healthy fruit juices are instant

source of energy. In the present work, four different preserved mango juice samples (A, B, C, E) were selected for microbiological and biochemical analysis in addition to the natural mango juice sample (D). Different physical

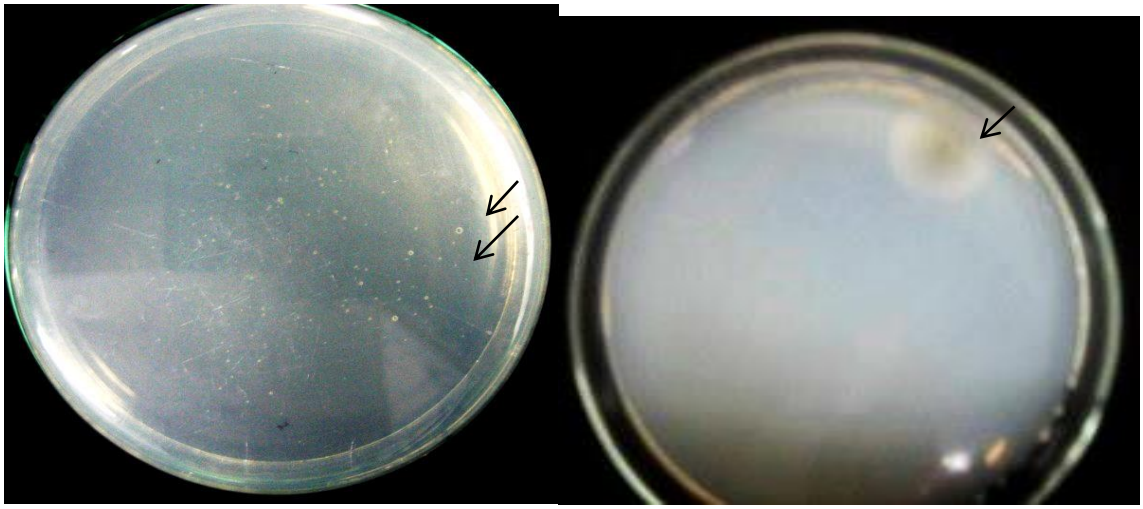
Table 1: Physical properties and growth of mango juice samples on various growth media

S#	Sample	pH	MFG.	EXP.	Preservatives	Growth on				Strains isolated
						Mac	L-agar	SDM	MEA	
1.	A	3.8	30-7-14	26-1-15	None	-	+	-	-	A1, A2
2.	B	3.9	24-7-14	24-7-15	None	-	+	-	+	B1, B2, B3
3.	C	3.6	5-8-14	04-8-15	Sodium benzonate	-	+	-	+	C1, C2, C3, C4
4.	D	4.8	-	-	-	-	+	-	-	D1, D2, D3
5.	E	4	30-7-14	26-1-15	None	-	-	-	-	-

MFG. =Manufacturing date; EXP. = Expiry date ; SDM= Saboured Dextrose Media; MEA= Malt Extract Agar Media; Mac= MacConkey agar

Table 2: Colony and cell morphology of various bacterial isolates

S#		Colony Morphology				Cell Morphology		
	Bacterial Isolates	Shape	Margin	Elevation	Color	Shape	Gram staining	Arrangement
1	A1	Round	Entire	Convex	Cream	Rods	+ ve	In Chains
2	A2	Round	Entire	Convex	White	Rods	+ ve	In Chains
3	B1	Round	Entire	Convex	Cream	Rods	+ ve	Isolated
4	B2	Round	Entire	Convex	Cream	Rods	+ve	In Chains
5	B3	Round	Entire	Concave	Cream	Rods	+ve	Pairs or in chains
6	C1	Round	Entire	Convex	Cream	Rods	+ ve	In Groups
7	C2	Irregular	Dentate	Concave	White	Rods	+ ve	In Chains
8	C3	Irregular	Dentate	Convex	Cream	Rods	+ ve	In Chains
9	C4	Round	Entire	Concave	White	Rods	+ ve	In Chains
10	D1	Round	Dentate	Convex	White	Rods	+ ve	In Groups
11	D2	Round	Entire	Convex	Cream	Rods	+ ve	In Chains
12	D3	Irregular	Dentate	Convex	White	Rods	-ve	Pairs or chains



a) **b)**

Fig 1: (a) Bacterial growth on L-agar media (sample C), (b) Fungal growth on MEA media (sample B)

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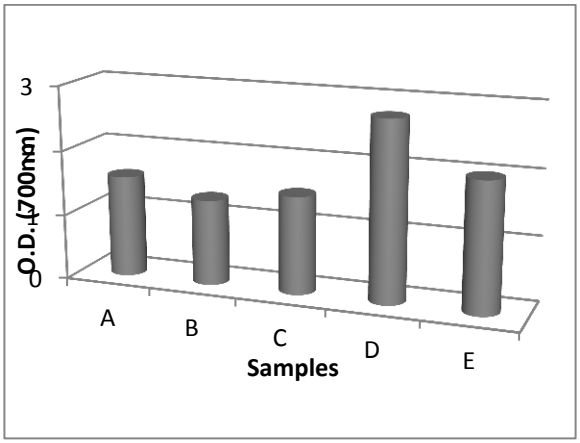

Fig 2: Antioxidant activity of sample juices (A, B, C, D, E)

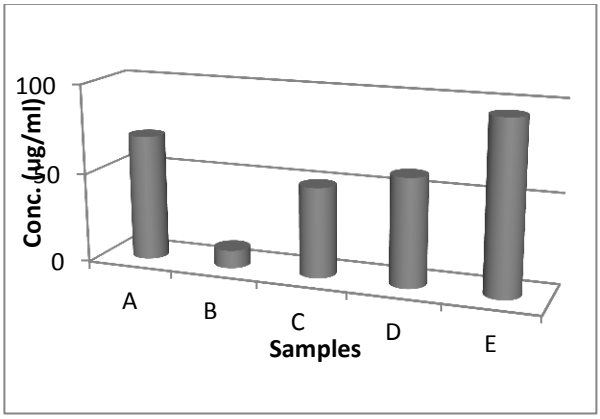

Fig 3: Total Phenolic Contents (TPC) of sample juices (A, B, C, D, E)

parameters of the mango juice samples were recorded (Table 1). In the present work, maximum pH was recorded in mango extract sample D i.e., 4.8 while minimum pH (3.6) was observed in mango juice sample C. Acidity of fruit juices is most useful for blending of fresh juice and cider [11]. Bacterial isolates were obtained by spreading the juice samples on L-agar plates. These isolates were further characterized morphologically. Twelve bacterial isolates were obtained from different fruit juice samples i.e., A1, A2, B1, B2, B3, C1, C2, C3, C4, D1, D2, D3. Two of the samples, B and C, produced fungal growth on MEA media (Malt Extract Agar) indicating the presence of fungal spores in those juice samples (Table 1; Fig 1a, b). Colonies of nine of the bacterial isolates (A1, A2, B1, B2, B3, C1, C4, D1 and D2) were round in shape while three bacterial strains i.e., C2, C3 and D3 produced irregular colonies. Colonies of the isolates A1, A2, B1, B2, B3, C1, C4 and D2 have shown entire margin while dentate margin was observed in the colonies of bacterial strains C2, C3 and D1. Majority of the bacterial strains have shown convex elevation i.e., A1, A2, B1, B2, B3, C1, C3, D2 and D3 while concave elevation was observed in the strains B3, C2 and C4. Cream colored colonies were observed in majority of the isolates (A1, B1, B2, C1, C3 and D2) while white colonies were observed in case of the isolates A2, C2, C4, D1 and D3. All the isolates were gram-positive rods except D3 which produced gram-negative rods. Cells of most of the isolates were present in chains (A1, A2, B2, C2, C3, C4 and D2) while isolated cells were observed in the strain B1. In the bacterial strains B3 and D3, cells were present in pairs or in chains while in C1 and D1, cells were present in the form of groups. Bacterial isolates were obtained both from natural (D) as well as preserved juice samples (A, B, C & E) whereas fungal growth was recorded in case of preserved juice sample B and C only (Table 1). Natural mango extract was not sterilized so presence of bacterial isolates in it was justified, however, bacterial and fungal growth in preserved juice sample indicated their contaminated nature due to unsatisfactory sterilization procedure used during preparation of the juice sample making it unsafe for human consumption. Antioxidants generally help in prevention of human diseases [12]. Various kinds of antioxidants are found in foods derived from plants and consumption of such food is responsible for protection against cancer and cardio-vascular and cerebro-vascular diseases. This property of antioxidants is due to their ability to scavenge free radicals that cause the oxidative damage to proteins, nucleic acids and lipids. Abdelhady *et al*. [13] reported that total phenolic content and antioxidant activity are closely related to each other. Phenolic content of plants directly contribute to their antioxidant action. Chemically diverse groups of antioxidants are found in nature. In the present study, antioxidant potential of mango juice samples was also estimated in the laboratory to evaluate their antioxidant activity. Maximum antioxidant activity was observed in the sample D with an optical density of 2.766 while minimum antioxidants were recorded in the preserved juice sample B with an optical density 1.318 (Fig 2). Secondary metabolites of plants are natural antioxidants. Plants produce various antioxidants such as carotenoids, flavonoids, cinnamic acid, benzoic acid, folic acid, ascorbic acid, tocopherols, tocotrienols etc. for their sustenance [14]. According to Gil *et al*. [15], red fruit juices such as grapes, different berry juices and fermented pomegranate juice have shown antioxidant potential. Fruit juice intake that contain phenolic compound is beneficial for human health [5]. In the present work, highest amount of phenolic contents (TPC) was observed in sample E i.e., 95ug/ml. Sample A and D have also shown high phenolic content while minimum level of total phenolic contents (TPC) was recorded in Sample B i.e., 10ug/ml (Fig 3). Beh *et al*. have also compared TPC of commercial and fresh fruit extracts and reported that TPC of fresh fruit extracts is high but some commercial fruit juices also contain high TPC and antioxidant activity depending on their good quality [16]. Polyphenolic compounds have shown suppressive activities against cancer cells either by inhibiting metabolic enzymes which play part in the activation of carcinogens or by altering the cell cycle. They also help in DNA protection and prevent apoptosis. [17] reported that anthocyanins are the major phenolic components in Elderberry and black current juice and is good for health. Sample E has shown high phenolic content as well as it was recorded to be free of any microbial (bacterial and fungal) growth while highest antioxidant activity was observed in

sample D. Thus sample E gave better results for biochemical parameters which depicts its better quality as compared to other sample juices. Sample A has also shown high content of phenolic compounds and antioxidants but has also shown presence of bacteria i.e., contamination in the juice which may cause harm to human health.

CONCLUSION

It is concluded from the present study that the sample E, A and D were high in antioxidant activity and total phenolic content (TPC). Natural juice extract has shown maximum antioxidant activity and high TPC indicating its better quality for human consumption. Among the preserved juice samples, sample E proved to be the best for human health showing no microbial growth with considerable antioxidant activity and highest TPC. The above study indicates the importance of various biochemical and microbiological aspects during the preparation and preservation of fruit juices so that healthy and safe juice drinks are made available in the market for human consumption. Future prospects of the present work may include investigations for better suggested protocols to achieve safer preservation of fruit juices.

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