

REGULAR ARTICLE

# Microbiological and physicochemical criteria of fruit juices sold in Egypt: Incidence of spore-forming bacteria

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## ABSTRACT

The quality and safety of several commercial fruit juices ( $n=360$ ) sold in Egypt were assessed in this study by analyzing quantitative microbiological and physicochemical parameters, and incidence of spore-forming bacteria. Three commercial brands e.g. apple (A4 & A5) and cocktail (C4) samples contained Coliforms and presumptive *Escherichia coli*; mean 1.30 to 1.85  $\log_{10}$  CFU/ml. *Staphylococcus aureus* were absent in all the samples tested. *Alicyclobacillus acidoterrestris*, *Bacillus coagulans*, *B. licheniformis* and *B. mycoides* species were the predominant organisms isolated from A4, guava (G4), mango (M3) and C4 juice samples. The A4, G4, M3 and C4 samples contained of 2.36 - 3.23  $\log_{10}$  CFU/ml from total bacterial counts and yeast/mold counts. The TSS and total sugars were within expected ranges. However, the titratable acidity, pH, and color were varied. Thus, this study emphasizes the need for the accelerated and sharp detection of spore forming bacteria in juice products, and provides information on the quality attributes of some brands of fruit.

**Keywords:** *Alicyclobacillus*; *Bacillus*; Food safety; Fruit juices; Physicochemical analysis

## INTRODUCTION

Fruit juices are well appreciated by consumers because of their taste, nutritional value, and availability at the right time. Also, they are an important part of the modern diet in many countries (Vantarakits et al., 2011). In spite of these potential benefits offered, concerns over their safety and quality have been raised. Food and Drug Administration (FDA) estimated that about 140 juice related illnesses that can be prevented yearly. Therefore, the labeling rules for juice products have increased the awareness of consumers of the hazards of drinking untreated juice (FDA, 2001). There is now increasing concern from pathogenic microorganisms among regulators regarding the safety of juices due to the potential ability of these pathogens to survive during the manufacturing process. Soil, faeces, food ingredients and processing chain might be sources of contamination in the food chain. Sporulation of spore forming bacteria is occurring in very diverse environments (Carlin, 2011). Until recently, fruit juice considered to be susceptible to spoilage by acidophilic microorganisms. However, the acidic pH

and heat treatment applied during manufacturing of fruit juices led to prevent the spoilage by lactic acid bacteria, yeasts-molds and mycelial fungi (Wineen, (2006)). There are several researches of fungal contamination in fruit juices are dwell in the literature (Mendoza et al., 1982; Parish and Higgins, 1989; Kurtzman et al., 2001; Tournas et al., 2006 and Parish, 2009). In addition, contamination by spore-forming, thermophilic and acidophilic bacteria such as *Alicyclobacillus acidoterrestris* and *Bacillus* spp. resulted in increased number of spoilage cases of fruit juices and their products (Chang and Kang, 2004). The incidence of *Alicyclo. acidoterrestris* has been reported in fruit juices e.g. pear juice, white grape, aloe vera, apples, citrus and mango (McIntyre et al., 1995; Walls and Chuyate, 1998; Wineen, 2006; Durak et al., 2010; Steyn et al., 2011). The worldwide requirements with regard to the presence of *Alicyclobacillus* spp. in juices is changeable and approximately 60% of importers require their absence for purchasing the products. The spread of *Alicyclobacillus* spp. in a container of fruit juice received in the destination country may result in economic losses and leads to distrust by buyers to suppliers

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Received: 26 April 2015;

Revised: 20 August 2015;

Accepted: 25 August 2015;

Published Online: 30 August 2015

(Oteiza, et al., 2011). This bacterium is known to produce chemicals that induce sour and off-flavors in fruit juice (Walls and Chuyate, 1998); with important implications for the relevant industries (Walker and Phillips, 2008). Fruit juices are generally treated at temperatures of about 95 °C for 2 min (Komitopoulou et al., 1999); however spores have been shown to survive such heat treatments (Splittstoesser et al., 1994) and surviving spores can germinate and grow at pH < 4 (Walker and Phillips, 2008) in fruit juice, leading to spoilage. *Bacillus licheniformis* survive pasteurization process and cause spoilage of juices, producing compounds associated with disinfectant-like odour. Other bacteria were isolated from 51 juices samples (42.5%) and fungi from 78 juices samples (65%). *Escherichia coli* O157:H7 was detected in four of the juices samples (3.34%), and *Staphylococcus aureus* was detected in four different samples (3.34) (Vantarakis, et al., 2011). Juices have become frequent vehicle for transmitting pathogens, such as *E. coli* O157:H7 (APHA, 2001), *Salmonella* (CDC, 1997) and *Cryptosporidium* (Buchanan, et al., 1998).

In Egypt, Few data are available concerning the microbiological quality of the fruit juices. Due to their potential in the international markets, it is necessary to evaluate if they are accomplishing the quality requirements, and to know the microbial load that can affect their safety. For this purpose, samples of national products were carefully purchased and analyzed for physical, chemical and microbiological characteristics. In this respect, *Alicyclobacillus* spp., *Bacillus* spp., *E. coli*, *Staphylococcus* spp. and yeasts should be monitored as potential spoilage microorganisms in fruit juices.

## MATERIALS AND METHODS

### Samples collection

A total of 360 fruit juices samples (200 ml), packaged in carton were carefully purchased during the first month of their production in 2013. The packaged products are belonging to six commercial brands (1, 2, 3, 4, 5 and 6). Six different juices or nectar products (apple (A), guava nectar (G), mango nectar (M), pineapple juice (P), orange (O) and cocktail (C)) from each brand were randomly sampled from supermarket chains in Zagazig, Egypt. About 36 samples from each brand and 10 samples from each kind of juice were purchased during the period of study. All were labeled as having been made from natural fruits. Samples were stored at refrigeration temperature (5 °C) from the time of purchase to delivery to the Laboratory of Microbiology and Laboratory of Food Science, Faculty of Agriculture, Zagazig University, Zagazig, Egypt. Once the samples were received, the juices type, source of fruits, location of manufacture, and purchase location were

recorded, and each sample was coded and then analyzed for microbiological and physicochemical parameters.

### Microbiological analysis

#### Microbial count

Fruit juices samples were analyzed for total bacterial count (TBC), total spore-forming bacteria count (SFC), coliforms (CF), *E. coli*, yeasts & molds (YM), and *Staphylococcus* spp. (ST) according to standard methods of APHA (2001). Briefly, 25 ml of fruit juices were weighed into sterile bags and homogenized in 225 ml of buffered peptone water (BPW, 0.1% w/v pH 7.2). Serial dilutions were made in BPW. The TBC were determined by the spread plating technique according to standard methods onto Plate Count agar (PCA, Merck, 1.05463) and incubated at 30 °C for 48 h. The SFC were counted onto PCA after immersed the serial dilution on water bath at 80 °C for 10 min. *Alicyclobacillus* spp. was counted onto Potato Dextrose agar (PDA) at pH 3.7 and incubated at 50 °C for 3 days. Staphylococci determined on Baird Parker agar (Biolife, Milano, Italy) supplemented with egg yolk and the plates incubated at 37 °C for 48 h; *S. aureus* detected by examining the plates of Baird Parker for typical black, convex colonies, with a light halo, which were tested for positive coagulase reaction (Bactident Coagulase, Biolife). Total coliforms enumerated on Violet-Red Bile agar (VRBA, Biolife, Milano, Italy) using a double layer of the medium at 35 °C for 24 h (Christen et al. 1992). For *E. coli*, the detection was done by using the selective Chromo Cult Coliform agar (Merck KGaA, Germany) according to the manufacturer's instructions and confirmed with Kovac's indole reagent. Yeasts and molds were determined onto Rose Bengal Chloramphenicol agar (RBCA, Lab M, 36) supplemented with chloramphenicol, X009 and the plates were incubated at 25 °C for 5 days. All plates were examined for typical colony types and morphological characteristics associated to each culture medium.

#### Identification of the spore-forming bacteria

The bacterial isolates onto PDA at pH 3.7 and PCA, after immersed the serial dilution on water bath at 80 °C for 10 min were identified according to Bergey's Manual of Systematic Bacteriology (Holt, et al. 1994). The tests included Gram stain, endospore formation, catalase test, starch hydrolysis (Mishra and Behera, 2008), Voges-Proskauer (VP) test (Rhodehamel, et al. 1998) and motility test (Dey, et al. 1998). After these identification tests the isolates were cultured and then confirmed by Egyptian Culture Collection at Cairo (MERCIN) Ain Shams University (Cairo, Egypt).

#### Physicochemical analysis

##### pH and titratable acidity

The pH of samples was assessed by pH meter (pH 211 HANNA instruments Inc. Woonsocket- USA made in

Romania). An amount of 10 ml of juice was mixed with 40 ml distilled water. The electrode was inserted into the mixture and measurements taken under continuous stirring. Titratable acidity was determined in juices samples expressed as lactic acid (%) according to the standard methods (AOAC, 1997).

#### **Total soluble solids (TSS) and sugar content**

The total soluble solids (TSS) level of the juice was determined using a digital refractometer (AR-2008, Kruss, Germany) according the method of Daramola and Asunni, (2007). The measured value was expressed as °Brix. Sugar content was determined according to AOAC (1997). A substantial amount of extracted juice was dropped onto the refractometer. The reading shown was the reading of the total soluble solids for the juice.

#### **Colour**

The colour measurement was performed by using a Color Flex E Z Spectrophotometer (45/0 LAN, 45°/0°, Large, USA) with reference to illuminant D65 and a 10° observer angle to obtain values of L\*, a\*, b\*. The L\* value measures the lightness and changes from 0 (black) to 100 (white). The a\* values measures the redness where it changes from -a\* (greenness) to +a\* redness). Lastly, b\* measures the yellowness; -b\* (blueness) to +b\* (yellowness). A 50 ml sample of juice was placed in an optical cell with a path length of 20 mm (Hunter Lab setting) for the measurement. The values of a\* and b\* were used to calculate the parameters of the colour appearance (Bernalte et al., 2003).

Physicochemical determinations of the samples were analyzed in triplicate. For each replication, duplicate measurements were conducted and the results averaged.

#### **Statistical analysis**

The numerical results are given as means and standard deviations (SD) using SPSS software (SPSS for Windows), with “n” being the number of juices samples. Data were statistically analyzed using ANOVA variance analysis through the general linear models (GLM) procedure of the statistical analysis system software (SPSS for Windows). Least significant differences were used to separate means at  $p < 0.05$ .

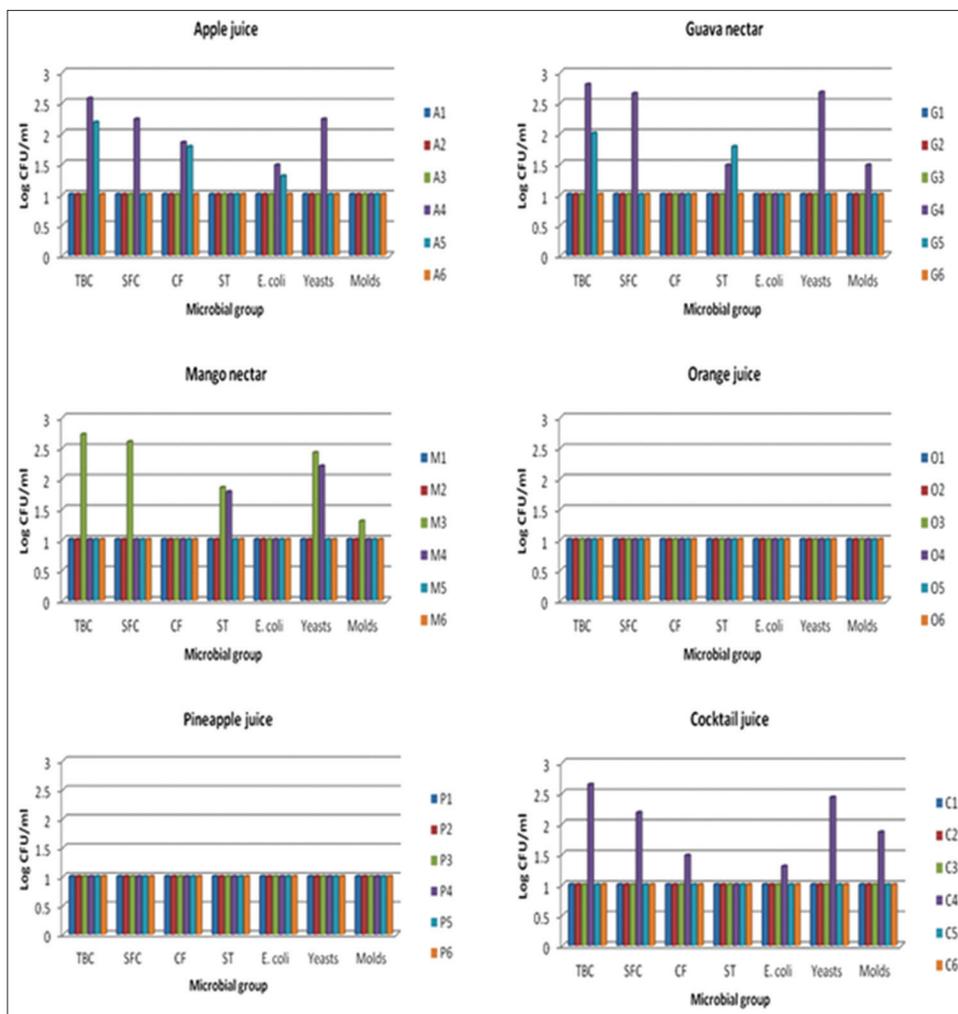
## **RESULTS AND DISCUSSION**

### **Hygiene indicators in fruit juices samples**

All 360 juice samples were tested for microbial growth on six different culture media (PCA, PDA, RBCA, VRBA, KGaA and Baird Parker) to determine hygiene quality of fruits juices samples in Zagazig, Egypt after manufacturing and within their shelf-life (Fig. 1). Total bacterial counts

(TBC), total coliforms counts and *E. coli* were considered as hygiene indicators to evaluate the microbiological profile of food samples. Additionally, they are typically measured to assess the effectiveness of sanitation practices and potential fecal contamination on products (USDA-FSIS, 1996). The results indicated that *St. aureus* were not detected in any of the samples tested. Fruit juices should not contain organisms that can cause disease for human being (EC, 2013). However, three commercial brands that produced apple and cocktail juices contained coliforms and presumptive *E. coli*; A4, A5 and C4 samples mean 1.30 to 1.85 log<sub>10</sub> CFU/ml.

The level of *E. coli* and total coliform counts in A4, A5 and C4 were all found to be significantly higher than all samples (Fig. 1). Additionally, cocktail juice from C4 exhibited significantly higher *E. coli*, coliforms, TBC, YM and SF counts compared with C1, C2, C3, C5 and C6. Thus, a number of the tested samples did not meet the Egyptian guidelines and EC (2013) for the microbiological quality of juices. Recently, Vantarakis, et al., (2011) observed that the fruit juices samples sold in Greece were negative for *Lactobacillus*, *Clostridium perfringens*, *Salmonella* spp., *B. cereus*, total coliforms, *E. coli*, and *Listeria monocytogenes*. However, in Libya *St. aureus* was detected in 8 (5.5%), *Streptococcus* spp. in 4 (2.7%), coliforms (22.6%), *E. coli* (none were of serogroup O157) in 3 (2.1%), *Klebsiella pneumonia* in 17 (11.6%), *Aeromonas* spp. in 3 (2.1%), and *Pseudomonas aeruginosa* in 6 (4.1%) in the juices tested (Ghenghesh, et al., 2005). The TBC and yeast/mold counts in A4, A4, G4, G5, M3, M4 and C4 juices samples were contained 2.18 to 2.56 log<sub>10</sub> CFU/ml. This result is in agreement with Vantarakis, et al., (2011) in Greece, about 11 juices samples (9.1%), the total number of microorganisms detected was as high as 125 colony forming units (2.09 log<sub>10</sub> CFU/ml). This is due to the acidic nature of fruit juices which probably favours the growth of yeasts. In USA, Tournas et al. (2006) during their studies on the mycological quality of packaged fruit salads and pasteurized fruit juices sold in the Washington, DC Metro area and they reported that 22% of the fruit juice samples tested showed fungal contamination. Yeasts were the predominant contaminants ranging from <1.0 to 6.83 log<sub>10</sub> CFU/ml. Also, Ndife et al. (2013) evaluated the microbial quality of different brands of orange juice in Nigerian market and stated that the viable microbial counts for the juice samples were very low (1.0x10<sup>-</sup> to 5.2x10<sup>2</sup> CFU/100 ml). A new system in sanitations and sterilization should be generally introduced into the juice industry sector to improve the hygiene quality of fruit juices especially the countries with variable in weather. However, the level of all microbial tested in pineapple and orange juice was under the detection limit. These were juices with pH below 4.0. The pH and acidity of pineapple and orange juices examined range between 2.9



**Fig 1.** Microbial groups total bacterial count (TBC), sporeforming count (SFC), coliforms (CF), *Staphylococcus* spp. (ST), *E. coli*, yeasts and molds (Log CFU/ml) in fruit juices (n=10).

and 4.0 and 0.26 to 0.50%, respectively. These factors could be preventing the microbial association with juices production.

Table 1 shows the types of organisms isolated from different types of fruit juices. Present study showed the presence of different species of bacteria namely, *Alicyclobacillus* sp., *Bacillus* spp., *E. coli*, *Staphylococcus* spp., *Candida* spp. and *Saccharomyces* spp.

All the isolated from spore-forming belonged to as *Alicyclo acidoterrestris*, *B. coagulans*, *B. licheniformis* and *B. mycoides* from commercially available fruit juices. Their presence may pose risks to consumers' health and should not be taken for granted. These organisms can cause spoilage and economic lose, which indicates that a noticeable number of juices tested did not meet the Egyptian guidelines for the microbiological quality of juices. It should be noted that the spore-forming bacteria were detected in apple (A4), guava (G4), mango

(M3) and cocktail (C4) juices. The other samples were under the detection limit <1.0 log CFU/ml) from these bacteria (Fig. 1). The highest spore-forming counts were detected in the G4 (mean counts: 2.65 log<sub>10</sub> CFU/ml in PCA agar and 2.45 log<sub>10</sub> CFU/ml in PDA agar). In the G4 sample, 75% of the isolated spore-forming bacteria were identified as *Alicyclobacillus* sp., *Bacillus* spp., *E. coli*, *Candida* spp. and *Saccharomyces* spp. All the isolated from spore-forming belonged to as *Alicyclo acidoterrestris*, *B. coagulans*, *B. licheniformis* and *B. mycoides* (Table 1). The results indicated that the water used for manufacturing the juices could not under control. Also, the washing step (with sanitizer) for fruits, generally considered a potential reduction step for microbial contamination, could be often not verified for its efficiency. Vantarakis, et al. (2011) reported that acidophilic microorganisms were isolated from 26 juices samples (21.7%). This result is in agreement with those obtained by Oteiza, et al., (2011) who found that *Alicyclobacillus* was found in all tested fruit juices except for orange juice marketed in

**Table 1: Isolated microorganisms and number of colonies in fruit juices samples (mean±SD)<sup>A</sup>**

Samples	No. of colonies (CFU/ml)	Identified bacteria	Identified yeasts
A4	88 x 10 <sup>1</sup> (2.94±0.32)	<i>Alicyclobacillus</i> spp., <i>E. coli</i> <i>Bacillus coagulans</i> , <i>B. licheniformis</i> ,	<i>Candida</i> spp., <i>Saccharomyces</i> spp.
A5	23 x 10 <sup>1</sup> (2.36±0.12)	<i>E. coli</i>	ND
G4	171 x 10 <sup>1</sup> (3.23±0.31)	<i>Alicyclobacillus</i> spp., <i>B. coagulans</i> <i>B. licheniformis</i> <i>B. mycoides</i> <i>Staphylococcus</i> spp.	<i>Candida</i> spp., <i>Saccharomyces</i> spp.
G5	47 x 10 <sup>1</sup> (2.53±0.35)	<i>Staphylococcus</i> spp.	ND
M3	108 x 10 <sup>1</sup> (3.03±0.23)	<i>Alicyclobacillus</i> spp., <i>B. coagulans</i> , <i>B. licheniformis</i> <i>Staphylococcus</i> spp.	<i>Candida</i> spp., <i>Saccharomyces</i> spp.
M4	63 x 10 <sup>1</sup> (2.80±0.27)	<i>Staphylococcus</i> spp.	<i>Candida</i> spp., <i>Saccharomyces</i> spp.
C4	98 x 10 <sup>1</sup> (2.99±0.37)	<i>Alicyclobacillus</i> spp., <i>B. coagulans</i> <i>B. licheniformis</i>	<i>Candida</i> spp., <i>Saccharomyces</i> spp.

ND: Not detected. <sup>A</sup>Values represented as average ± standard deviation from triplicate determinations with three separately samples

Argentina. The highest percentage of positive samples was found for beetroot, strawberry, banana, peach, mango, carrot and plum juices. The percentage of positive samples for these juices ranged from 24% to 100% (Oteiza, et al., 2011). *Alicyclo acidoterrestris* being the only species associated with the spoilage of fruit juices and present in South African apple juice (Wineen, 2006). These bacteria can survive, grow and sporulate despite changes in water activity, pH and temperature (Jaquette and Beuchat, 1998; Collado et al., 2003). With regard to the detection of these bacteria in the pasteurized fruit juices, there are two possibilities. Firstly, that they survived the pasteurization process and secondly, that contamination was post-pasteurization as demonstrated by Walker and Phillips, 2007, in UK, during their studies on the growth of *Propionibacterium cyclohexanicum* in fruit juices and its survival following elevated temperature treatments. Ghenghesh, et al., (2005) recorded that *Candida albicans*, *Candida* spp. and other yeasts were found at levels 18 (12.3%), 109 (74.7%) and 85 (58%) in the juices sold in Tripoli, Libya, respectively. *C. albicans* can cause serious disease in humans (Chihab et al., 2004). Further analysis of commercially sold fruit juices 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. Recently, Parish (2009) mentioned that certain yeasts-molds and bacteria

may survive at low pH, processing and preservation conditions to cause spoilage problems in fruit juice products. Thus, spoilage of fruit juices depends on fruit quality, holding and handling conditions, grading, processing conditions, cleaning/sanitation efficiency, and temperature of product storage.

#### Physicochemical indicators in fruit juices samples

To evaluate the physicochemical properties after manufacturing and during their shelf-life, the pH, titratable acidity, total soluble solids (TSS) and total sugars as well as colour measurements of the apple, orange, guava, pineapple, mango and cocktail juices and nectars were measured (Tables 2 & 3). The pH of the tested juices ranged from 2.7 (apple juice) to 4.1 (guava and mango nectars) as shown in Table 2. The average pH of apple, guava, mango, orange, pineapple and cocktail juices was 2.96, 3.80, 3.93, 3.33, 3.68 and 3.73, respectively. There was no significant difference in pH value between the same juices compared between all companies. Similar results have been reported by Ghenghesh, et al., (2005) who found that the pH of juices examined ranged between 3.09 and 6.24 (mean = 3.82).

In this study, the values of titratable acidity of the juices ranged from 0.14 (guava nectar) to 0.50 (orange nectar). The titratable acidity was not significantly different between all the brands producing the same juice. The total sugar of tested juices ranged from 11.0 (mango nectar) to 25.0 (guava nectar).

The results of TSS are displayed in Table 2 and were expressed in °Brix. The TSS of the tested juices ranged from 10.4 °Brix (cocktail juice) to 15.0 °Brix (mango nectar). There was no significant difference in total soluble solids for the same fruit juices compared between all brands. Hence, this shows that all brands follow the same methods of production and these methods did not affect the total soluble solids of the similar juice produced by different brands.

The measurement of colour is important for the quality assessment of juice. The L value measures the lightness of the juice whereby values nearer to zero indicate the darker the juice (0=black, 100=white). In this study, there was significant difference between the similar juice producing from different brands in L value as shown in Table 3. The L value of apple juice ranged from 3.71±0.35 (A3) to 6.10±0.33 (A2), guava juice from 49.53±0.31 (G3) to 64.21±0.43 (G2), mango juice from 23.46±0.35 (M4) to 48.1±0.39 (M2), pineapple juice from 22.96±0.39 (P6) to 30.90±0.32 (P1), orange juice 27.58±0.35 (O4) to 50.72±0.34 (O2) and in cocktail juice from 15.61±0.31

**Table 2: Physicochemical characteristics of fruit juices samples (mean±SD)<sup>A</sup>**

Samples	Type	Fruit	pH	Acidity (%)	TSS (°Brix)	Total sugar (%)
A1	Juice	Apple	2.7±0.11	0.47±0.14	14.0±0.13	17±0.52
A2	Juice	Apple	3.1±0.12	0.38±0.23	12.0±0.13	19±0.61
A3	Juice	Apple	3.0±0.11	0.32±0.32	13.2±0.12	15±0.61
A4	Juice	Apple	3.3±0.13	0.32±0.33	13.1±0.14	16±0.41
A5	Juice	Apple	2.9±0.41	0.34±0.11	14.3±0.14	20±0.71
A6	Juice	Apple	2.8±0.14	0.28±0.21	14.2±0.15	15±0.91
G1	Nectar	Guava	3.6±0.13	0.14±0.21	13.0±0.13	23±0.41
G2	Nectar	Guava	4.1±0.16	0.19±0.11	12.0±0.14	22±0.71
G3	Nectar	Guava	3.8±0.13	0.20±0.13	13.0±0.14	25±0.45
G4	Nectar	Guava	3.8±0.12	0.22±0.13	13.0±0.11	21±0.45
G5	Nectar	Guava	3.8±0.14	0.21±0.09	14.0±0.19	20±0.87
G6	Nectar	Guava	3.7±0.15	0.23±0.14	14.0±0.13	22±0.76
M1	Nectar	Mango	3.7±0.12	0.22±0.18	15.2±0.16	11±0.54
M2	Nectar	Mango	4.1±0.12	0.23±0.16	13.2±0.11	13±0.45
M3	Nectar	Mango	3.9±0.11	0.21±0.14	11.0±0.17	12±0.73
M4	Nectar	Mango	4.0±0.10	0.24±0.13	14.0±0.12	12±0.24
M5	Nectar	Mango	4.1±0.12	0.23±0.15	15.0±0.14	13±0.74
M6	Nectar	Mango	3.8±0.16	0.22±0.16	15.1±0.17	11±0.64
P1	Juice	Pineapple	3.9±0.15	0.29±0.12	12.0±0.14	15±0.65
P2	Juice	Pineapple	3.8±0.14	0.32±0.16	12.0±0.14	14±0.61
P3	Juice	Pineapple	3.6±0.13	0.31±0.14	13.0±0.41	16±0.67
P4	Juice	Pineapple	3.5±0.14	0.34±0.12	11.0±0.16	14±0.69
P5	Juice	Pineapple	3.7±0.13	0.29±0.16	13.0±0.17	15±0.71
P6	Juice	Pineapple	3.8±0.14	0.26±0.21	13.0±0.12	15±0.65
O1	Juice	Orange	2.9±0.12	0.46±0.15	12.2±0.17	15±0.81
O2	Juice	Orange	3.2±0.15	0.50±0.14	11.0±0.11	14±0.43
O3	Juice	Orange	3.1±0.18	0.50±0.18	13.0±0.18	13±0.63
O	Juice	Orange	3.4±0.13	0.33±0.13	14.0±0.12	15±0.6
O5	Juice	Orange	3.3±0.14	0.45±0.15	11.0±0.14	16±0.45
O6	Juice	Orange	3.6±0.13	0.43±0.13	14.3±0.17	14±0.65
C1	Juice	Cocktail	3.3±0.12	0.33±0.16	12.0±0.19	11±0.82
C2	Juice	Cocktail	3.9±0.12	0.47±0.11	12.0±0.11	11±0.54
C3	Nectar	Cocktail	3.8±0.11	0.23±0.13	12.4±0.16	12±0.54
C4	Nectar	Cocktail	3.7±0.10	0.23±0.15	10.4±0.17	11±0.34
C5	Juice	Cocktail	3.9±0.19	0.28±0.11	11.2±0.19	13±0.33
C6	Juice	Cocktail	4.0±0.15	0.30±0.18	14.2±0.12	11±0.38

<sup>A</sup>Values represented as average±standard deviation from triplicate determinations with three separately samples

(C6) to  $46.56 \pm 0.36$  (C2). Hence, from this study it can be said that the different brands and different ways for sterilizing juices lead to varying lightness properties. However, all samples tested for colour still maintain the colour quality of the juices. The same trend was observed in the  $a^*$ (redness) and  $b^*$ (yellowness) values in juices samples. In general, the color intensity of the tested samples as measured by Hunter  $L^*$ ,  $a^*$  and  $b^*$  was strongly influenced by juice treatments in each company during processing and it can be seen that as brightness ( $L^*$ -values) decreased, redness ( $a^*$ -values) increased and yellowness ( $b^*$ -values) decreased in all samples, and overall color differences depends on the type of fruit. The hue angle and chroma are colour properties that are related to the  $a^*$ (redness) and  $b^*$ (yellowness). The slight varying was observed in chroma for the similar juice but producing from different brands.

## CONCLUSIONS

In general, based on the microbiological (TBC, SFC, coliforms, *E. coli*, *Staphylococcus* and yeasts & molds) and physico-chemical analyses (pH, titratable acidity, total soluble solids, total sugars and colour), all the similar fruit juices of the same type from different brands had similar physicochemical characteristics. However, microbiological characteristics in some brands did not fulfill the Egyptian guidelines for the microbiological quality of juice, due to the detection of spore-forming bacteria. Many of these organisms can cause spoilage and diseases. Hence, *Alicyclobacillus* as spoilage organism, and it should be involved into the Egyptian guidelines for microbiological quality of juices, due to it provides a better hygiene indicator for juice producing in the countries with climate changes and varying in temperatures.

**Table 3: Color of fruit juices samples (n=10)**

Samples	Color		
	L*	a*	b*
A1	5.46±0.34	0.67±0.22	2.19±0.13
A2	6.10±0.33	0.08±0.21	2.98±0.13
A3	3.71±0.35	1.03±0.23	2.41±0.14
A4	4.61±0.37	-0.48±0.00	2.67±0.15
A5	4.13±0.45	0.90±0.23	2.57±0.11
A6	6.06±0.32	0.33±0.24	2.89±0.10
G1	53.33±0.39	-3.40±0.00	9.16±0.13
G2	64.21±0.43	-1.73±0.00	15.70±0.17
G3	49.53±0.31	-2.88±0.00	13.86±0.15
G4	56.87±0.33	-2.65±0.00	14.87±0.19
G5	57.87±0.43	-2.77±0.00	15.74±0.21
G6	55.81±0.33	-2.69±0.00	13.89±0.26
M1	34.31±0.33	1.54±0.24	19.64±0.25
M2	48.10±0.39	6.11±0.43	29.36±0.37
M3	33.54±0.36	4.87±0.32	24.32±0.36
M4	23.46±0.35	-1.62±0.00	12.42±0.22
M5	41.30±0.33	7.21±0.54	25.33±0.31
M6	35.45±0.32	3.86±0.31	21.68±0.32
P1	30.90±0.32	-2.40±0.00	9.80±0.21
P2	29.84±0.33	-0.49±0.00	13.77±0.36
P3	25.97±0.23	-3.78±0.00	7.21±0.23
P4	26.80±0.33	2.54±0.32	6.67±0.26
P5	26.12±0.23	3.06±0.22	9.24±0.23
P6	22.96±0.38	-1.96±0.00	4.64±0.11
O1	42.63±0.31	-3.44±0.00	21.02±0.42
O2	50.72±0.34	1.07±0.12	29.28±0.54
O3	29.25±0.36	-0.41±0.00	16.91±0.16
O4	27.58±0.35	10.24±0.52	17.27±0.19
O5	33.75±0.34	0.65±0.11	19.19±0.26
O6	26.98±0.33	-2.14±0.00	12.99±0.43
C1	25.83±0.34	1.50±0.26	14.94±0.22
C2	46.56±0.36	3.84±0.23	25.38±0.21
C3	16.64±0.38	10.42±0.32	5.40±0.21
C4	34.65±0.34	5.34±0.22	4.11±0.32
C5	29.33±0.38	21.64±0.65	3.35±0.22
C6	15.61±0.31	15.03±0.43	2.63±0.12

### Author contributions

S.A.M.M. is the principal author of this work and design of study. He has made major contributions to this article in sampling, microbiological analysis of juices samples, counting, isolation, identification of microbes etc.). G.A.E.S. was involved in physicochemical analysis of juices samples.

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