



Diversity of yeasts and filamentous fungi in five fresh fruit juices in Egypt

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Abstract

The present investigation aimed at evaluating five freshly prepared juices for their contamination with yeasts and filamentous fungi. The mean pH values of juice samples ranged from 4.62 in strawberry to 6.45 in sugarcane. The general isolation medium DRBC supported more fungal species diversity in all juices, than both xerophilic media DG18 and MY50G. DRBC also supported more propagules originated from different juice types than DG18, however, more propagules were obtained on MY50G from only guava and orange juices. The highest numbers of propagules recovered on the three media were also from sugarcane followed by strawberry and guava, while the lowest from mango and orange. Moreover, yeasts almost predominated on DRBC, but filamentous fungi CFU excelled on DG18 in sugarcane and strawberry and on MY50G in orange only. Yeasts were represented by 25 genera and 48 species. *Candida* was the dominant genus of yeasts that contributed 11 species, from which *C. bentonensis*, *C. quercitrusa*, *C. saitoana*, *C. santamariae* var. *membranifaciens*, *C. smithsonii*, *C. spandovensis* and *C. tropicalis* were regularly identified from all juices on all isolation media, beside *Hanseniaspora* (2 species, from which *H. uvarum*), *Pichia* (5 spp., *P. kluyveri*, *P. terricola*), *Cryptococcus* (*C. albidus* var. *kuetzingii*), *Debaryomyces* (*D. nepalensis*), *Papiliotrema* (4 spp., *P. terrestris*), *Rhodotorula* (3 spp.), *Saccharomycopsis* (*S. crataegensis*), *Saprochaete* (*S. gigas*) and *Torulospora* (*T. delbrueckii*). Some other yeast species were recovered only from one juice but not from the others. Filamentous fungi e.g. *Aspergillus* (14 species, from which *A. flavus*), *Aureobasidium pullulans*, *Cladosporium* (8 spp., *C. cladosporioides*, *C. herbarum*, *C. sphaerospermum*), *Exophiala* (2 spp.), *Fusarium* (6 spp.) and *Penicillium* (18 spp., *P. chrysogenum* and *P. raistrickii*.) were found contaminating all juices on almost all isolation media. Notably, 29 yeasts and filamentous species were reported on the xerophilic media only. Some filamentous species were also encountered from one juice only.

Key words – Guava – ITS – Mango – Mycobiota – Orange – Phenotypic and Genotypic Characterization – Strawberry – Sugarcane.

Introduction

Fresh fruit juices are very much appreciated from their delicious taste and high vitamin content. They, however, contain a microbiota which is normally present on the surface of fruits during harvest, transport, storage, and processing (Tournas et al. 2006). Yeasts represent the main

component of fruit juices and the major genera include *Candida*, *Dekkera*, *Hanseniaspora*, *Pichia*, *Saccharomyces* and *Zygosaccharomyces*. The filamentous fungi, *Penicillium*, *Byssoschlamys*, *Aspergillus*, *Paecilomyces*, *Mucor*, *Cladosporium*, *Fusarium*, *Botrytis*, *Talaromyces* and *Neosartorya* are most frequently isolated from fresh fruits and juices (ICMSF 2005).

Yeasts and filamentous fungi play a deteriorative role in the quality and food safety which may be hazardous to human health. It is therefore essential to routinely identify mycobiota associated with different foods.

The aim of this work is to identify and evaluate the diversity of yeasts and filamentous fungi that contaminate some fresh fruit juices, at the consumption stage, collected from fruit juice shops (orange, strawberry, mango, guava and sugarcane) in Assiut City. The identification was based on phenotypic characteristics, in addition to genotypic in case of yeasts.

Materials & Methods

Isolation of yeasts and filamentous fungi from fresh fruits juices

Collection of samples

A total of 25 samples of fresh squeezed sugarcane (*Saccharum officinarum* L., family Poaceae), orange (*Citrus sinensis* L., family Rutaceae), guava (*Psidium guajava* L., family Myrtaceae), mango (*Mangifera indica* L., family Anacardiaceae), and strawberry (*Fragaria x ananassa* Duch., family Rosaceae) juices (five samples each) were collected from different shops in Assiut City during the period from February to April 2013. Samples were soon brought into the laboratory and pH and fungal analysis were determined as soon as they brought.

Determination of juices pH

To determine the pH in juices samples, a pH meter (Orior Research Model GOHL Digital Ionalyzer) was used. The electrode was immersed directly in the sugarcane, orange, guava, mango, and strawberry juices (Jackson 1958).

Isolation of juices fungi

The dilution-plate method adopted from Johnson & Curl (1972) was used for enumeration of different fungal species as follows:

1- Ten ml of juice sample were placed in a sterile 250 ml Erlenmeyer flask containing 90 ml of sterile distilled water. The flask containing the suspension was shaken on a mechanical orbital shaker for 30 minutes.

2- Ten ml of the suspension were immediately drawn (while in motion) and transferred immediately into Erlenmeyer flasks containing 90 ml of sterile water and the dilution process was repeated until the desired final dilution was reached (1:1000) which supports a total of about 25- 40 colonies per plate. The suspension was shaken by hand for few minutes, and was in motion while 1 ml of the desired dilution being drawn and transferred aseptically into each of several Petri-dishes and ~20 ml / plate of an appropriate agar medium cooled to just above solidifying temperature were added. The dishes were rotated by hand in a broad swirling motion to disperse the juice suspension in the agar.

3- The plates (5 plates for each type of 3 media for each sample) were incubated at 28°C for 1-2 weeks during which the developing fungi were counted and isolated for further identification and the number of colony forming units (CFU) was calculated per ml juice. Isolates of different fungi were maintained on yeast extract malt extract agar, YM (for yeasts), Czapek's agar and malt extract agar slants (for filamentous fungi) and stored at 5°C till confirming the identification.

Media used for isolation of fungi from juice samples

Dichloran rose bengal chloramphenicol agar, DRBC (King et al. 1979) of the following composition (g/l): peptone 5.0, potassium dihydrogen phosphate 1.0, magnesium sulphate 0.5,

glucose 10.0, agar 15.0. Dichloran (2 µg/ml), rose bengal (25 µg/ml) and chloramphenicol (100 µg/ml) were used as bacteriostatic agents.

Dichloran 18% glycerol agar base, DG18 (Hocking & Pitt 1980) of the following composition (g/l): glucose 10.0, peptone 5.0, potassium phosphate monobasic 1.0, magnesium sulfate heptahydrate 0.5, dichloran (0.2% in ethanol, w/v) 1.0 ml, agar 15.0, chloramphenicol 0.1, glycerol 220, distilled water 1000 ml.

Malt extract yeast extract 50% glucose agar, MY50G (Pitt & Hocking 1985) of the following composition was employed: (g/l): malt extract 10.0, yeast extract 2.5, agar 10.0, glucose 500.0, distilled water 500.0 ml. Agar, yeast extract, and malt extract are added to water and boiled to dissolve agar, then glucose is slowly added while stirring to avoid lumps. The medium is dispensed as required and autoclaved at 121 °C for 30 minutes.

Phenotypic identification of filamentous fungi

The filamentous fungi were identified based on their macro- and microscopical features following the keys of Raper & Fennell (1965), Booth (1971), Ellis (1971), Pitt (1979), Sutton (1980), Moubasher (1993), Samson & Frisvad (2004), Leslie & Summerell (2006), Samson & Varga (2007), Domsch et al. (2007), Seifert et al. (2011).

Identification of yeasts

Morphological characters: Formation of pseudomycelium and true mycelium (Wickerham 1951) and the ability to form ascospores on three sporulation media (corn meal agar, potato glucose agar and yeast extract malt extract agar, YM, at 25 C) were determined (Barnett et al. 2000).

Physiological characters: Biochemical characteristics were performed for yeasts (Table 2). A fermentation test of sugars was performed and oxidative utilization of carbon compounds was tested according to Barnett et al. (2000). The growth of yeast strains on nine nitrogen compounds (potassium nitrate, sodium nitrite, ethylamine-HCl, L-lysine-HCl, creatine, creatinine, D-glucosamine, imidazole, D-tryptophan) was also determined (Suh et al. 2008). Hydrolysis of urea, growth at high osmotic pressure, growth at different temperatures, growth in the presence of cycloheximide, diazonium blue B (DBB) and production of extracellular starch-like compounds were also performed. Identification keys of Kurtzman & Fell (1998) and Barnett et al. (2000) were employed to assign each isolate to species level. Confirmations of these identifications were carried out using molecular techniques.

Genotypic identification of yeast strains: The yeast strains were grown on YM plates and incubated at 25° C for 2 days. A small amount of fungal biomass was scraped off and suspended in 100 µl of distilled water and boiled at 100°C for 15 minutes following the manufacturer's protocol (SolGent Company, Daejeon, South Korea). The samples were directly sent for extraction and sequencing. Yeast DNA was extracted and isolated using SolGent purification beads at this company. Internal transcribed spacer (ITS) sequences of nuclear ribosomal DNA were amplified using the universal primer ITS1 (5'- TCC GTA GGT GAA CCT GCG G -3'), and ITS4 (5'- TCC TCC GCT TAT TGA TAT GC -3'). Then amplification was performed using the polymerase chain reaction (PCR) (The GeneAmp® PCR System 9700 thermal cycler, Applied Biosystems, Foster City, California, USA). The PCR reaction mixtures were prepared using SolGent EF-Taq as follows: 10X EF-Taq buffer 2.5 µl, 10 mM dNTP (T) 0.5 µl, primer (F-10p) 1.0 µl, primer (R-10p) 1.0 µl, EF-Taq (2.5 U) 0.25 µl, template 1.0 µl, DW up to 25 µl. Then the amplification was carried out using the following PCR reaction conditions: one round of amplification consisting of denaturation at 95°C for 15 min followed by 30 cycles of denaturation at 95°C for 20 s, annealing at 50°C for 40 s and extension at 72°C for 1 min., with a final extension step of 72°C for 5 min.

The PCR products were then purified with the SolGent PCR Purification Kit-Ultra prior to sequencing. Then the purified PCR products were reconfirmed (using size marker) by electrophoresis of the PCR products on 1% agarose gel. Then these bands were eluted and sequenced. Each sample was sequenced in the sense and antisense direction. Contigs were created from the sequence data using the CLCBio Main Workbench program. The sequence obtained from

each isolate was further analysed using BLAST from the National Center of Biotechnology Information (NCBI) website. Sequences obtained together with those retrieved from the GenBank database were subjected to the Clustal W analysis using MegAlign software version 5.05 (DNASTAR Inc., Madison, Wisconsin, USA) for the phylogenetic analysis (Thompson et al. 1994). Sequence data were deposited in GenBank and accession numbers are given for them.

Results

PH of juice samples and fungal diversity

The mean pH values of juice samples ranged from 4.62 in strawberry and 4.66 in orange to 6.45 in sugarcane (Table 1). A total of 56 genera and 136 species + 6 varieties (31 genera and 92 species + 2 varieties of filamentous fungi, and 25 genera and 44 species + 4 varieties of yeasts) were recovered from all fruit juices investigated on DRBC (89 species + 3 varieties), DG18 (79 species + 5 varieties) and MY50G (103 species + 5 varieties). In all juices the mean total CFU of all fungi and yeasts recovered on DRBC exceeded those recovered on DG18 and MY50 except for guava/orange on MY50G. The diversity of yeast species recovered on all isolation media was more (or at least equal in numbers) in sugarcane, guava and orange juices, but filamentous species diversity was more pronounced in mango and strawberry (Table 1).

The yeast isolates were characterized using phenotypic, physiological (Table 2) and molecular (Table 3) methods while filamentous fungi were identified using phenotypic characteristics based on macro- and microscopic features. Representative strains of the species recovered are deposited at Assiut University Mycological Center Culture Collection (AUMC) and ITS gene sequences of the yeast strains were deposited at NCBI and accession numbers are given for them (Table 3).

Fungi recovered from fruit juices on DRBC

Sugarcane juice was the most heavily contaminated with fungal CFU (500572 CFU/ml in all samples analyzed) followed by strawberry (117729 CFU), while orange (51220 CFU), mango (67750 CFU) and guava (76000 CFU) juices were less contaminated (Table 3). Yeasts comprised the major proportions in all juice types (93.03% of the total count of fungi in guava juice to 54.78% in orange juice) (Table 4).

A total of 103 species and 5 varieties belonging to 41 genera were recovered from all juice samples investigated on DRBC. From these, 40 species and 4 varieties assigned to 22 genera were yeast fungi and 62 species + 2 varieties belonging to 19 genera were filamentous fungi. The highest number of species and genera were recovered from mango juice (56 species and 4 varieties related to 28 genera) followed by strawberry (45 species + 3 varieties belonging to 25 genera), sugarcane (44 + 2 assigned to 22 genera), then guava (41 + 1 related to 23) and orange (40 + 4 assigned to 24 genera). From the 22 yeast genera recovered, *Candida* (9 species + 2 varieties), *Pichia* and *Papiliotrema* (4 species each), *Rhodotorula* (3 species), *Cutaneotrichosporon*, *Hanseniaspora* and *Wickerhamomyces* (2 species each) registered the highest number of species. *Candida bentonensis*, *C. quercitrusa*, *C. saitoana*, *C. santamariae* var. *membranifaciens*, *C. smithsonii*, *C. spandovensis*, *C. tropicalis*, *Cryptococcus albidus* var. *kuetzingii*, *Debaryomyces nepalensis*, *Hanseniaspora uvarum*, *Papiliotrema terrestris*, *Pichia kluyveri*, *P. terricola*, *Saprochaete gigas* and *Torulospira delbrueckii* were found contaminating all juice types. On the other hand, *C. cylindracea* was recovered from mango juice only, *C. boidinii* and *Pichia fermentans* from guava juice only, *P. manshurica* and *Sporobolomyces roseus* from strawberry only, while none was specific for sugarcane or orange juice (Table 4).

From 19 genera of filamentous fungi, only the genera *Aspergillus* and *Aureobasidium*, *Cladosporium* and *Penicillium* were isolated from all juice types. However, only *Aureobasidium* sp. and *C. cladosporioides* out of 62 filamentous species and 2 varieties, were recovered from all juice types. The highest numbers of filamentous species were registered in the following decreasing order: 31 species from mango > 22 from strawberry > 21 from sugarcane > 17 from orange > 16

from guava juice. High numbers of propagules were obtained from sugarcane juice than those obtained from the other four juices, with the lowest being recorded in guava juice (Table 4).

Fungi recovered from fruit juices on DG18

Sugarcane juice was the most heavily contaminated with fungal CFU (487020 CFU/ml in all samples analyzed on DG18) followed by strawberry (67350 CFU), while mango (38310 CFU), orange (45420 CFU) and guava juices (59900 CFU) were less contaminated. Yeast propagules predominated in guava (accounting for 86.73% of the total CFU), mango (68.13%), and strawberry juices (55.46%), while filamentous fungi predominated in sugarcane (62.00%) and orange juices (59.00%), (Table 5).

A total of 89 species and 3 varieties belonging to 39 genera were recovered from all juice samples investigated on DG18. From these, 31 species and 3 varieties assigned to 19 genera were yeast fungi and 57 species + 1 variety belonging to 20 genera were filamentous fungi. The broadest spectrum of species and genera were recovered from strawberry juice (41 species and 3 varieties related to 26 genera) followed by guava (36 species + 3 varieties belonging to 22 genera), mango (37 + 2 assigned to 20 genera), then orange (34 + 2 related to 23) and sugarcane juice (34 + 1 assigned to 15 genera). From 19 yeast genera recovered, *Candida* (7 species + 2 varieties), *Papiliotrema* (4 species), *Pichia* and *Rhodotorula* (3 species each) registered the highest number of species. *Candida smithsonii*, *C. spandovensis*, *Cryptococcus albidus* var. *kuetzingii*, *Debaryomyces nepalensis*, *Hanseniaspora uvarum*, *P. terricola* and *Torulospora delbrueckii* contaminated all juice types (Table 4). On the other hand, *Cystofilobasidium ferigula*, *Papiliotrema laurentii*, *P. rajasthanensis*, *Rhodotorula pacifica* and *Sporidiobolus pararoseus* were recovered from sugarcane juice only, *Cystobasidium minutum* from mango, *Pichia fermentans* from guava juice only, while none was specific for strawberry or orange juice (Table 5).

From 20 genera of filamentous fungi, only the genera *Aspergillus*, *Cladosporium*, *Fusarium* and *Penicillium* were isolated from all juice types. However, only *C. cladosporioides*, *C. herbarum* and *C. sphaerospermum* out of 57 filamentous species and 1 variety were recovered from all juice types. Sugarcane juice possessed high numbers of propagules of filamentous fungi, while the lowest contamination level was registered in guava juice, the same trend was recorded on DRBC. Filamentous fungi showed almost the same taxa diversity as yeasts in different juice types (Table 5).

Fungi recovered from fruit juices on MY50G

An almost similar trend of total counts of fungi was reported on MY50G as those recovered on DRBC and DG18, where sugarcane juice was the most heavily contaminated (380020 CFU/ml in all samples analyzed) followed by strawberry (102500 CFU), while mango (50590 CFU), orange (75820 CFU) and guava (89650 CFU) juices were less contaminated. Yeast fungi registered high proportion of total CFU in guava, mango and strawberry juices (79.71-94.26% of the total CFU of fungi), while sugarcane (55.52%) and orange (77.45%) juices were highly contaminated by filamentous fungi (Table 6).

A total of 79 species and 5 varieties belonging to 36 genera were recovered from all juice samples investigated. From these, 32 species and 4 varieties assigned to 20 genera were yeast fungi and 47 species + 1 variety belonging to 17 genera were filamentous fungi. The broadest spectrum of taxa was recovered from strawberry juice (35 species and 3 varieties related to 20 genera) and narrowest was recorded in guava (28 species + 3 varieties belonging to 18 genera). From yeast genera, *Candida* (7 species + 2 varieties), *Pichia* (4 species), *Papiliotrema* and *Rhodotorula* (3 species each), and *Hanseniaspora* (2 species) registered the highest number of species. *Candida saitoana*, *C. santamariae* var. *membranifaciens*, *C. smithsonii*, *C. spandovensis*, *Debaryomyces nepalensis*, *Hanseniaspora uvarum*, *Pichia terricola* and *Torulospora delbrueckii* contaminated all juice types. On the other hand, *Pichia membranifaciens* was recovered from sugarcane juice only, *C. intermedia* and *Papiliotrema flavescens* from mango juice only, *Cystofilobasidium ferigula* and *Rhodotorula dairenensis* from guava juice only, *Spencermartinsiella ligniputridi*,

Wickerhamomyces pijperi from orange juice, and *Barnettozyma californica* var. *californica* and *Candida tropicalis* from strawberry only (Table 6).

From 17 genera of filamentous fungi, only the genera *Aspergillus* and *Aureobasidium*, *Cladosporium* and *Penicillium* were isolated from all juice types. However, only *A. flavus*, *Aureobasidium* sp. and *P. raistrickii* out of 47 filamentous species and one variety were recovered from all juice types. The highest numbers of filamentous species were registered in strawberry and the lowest in guava juice. On the other hand, the highest number of propagules of filamentous fungi was obtained from sugarcane juice and the lowest in guava juice (Table 6).

Overview on genera and species diversity from various fruit juices on different media

In all products, the general medium DRBC supported more fungal species diversity than both xerophilic media DG18 and MY50G. Also, DRBC supported more propagules originated from different juice types than DG18, however, more propagules were obtained on MY50G from only guava and orange juices. On the other hand, the highest numbers of propagules were recovered on the three media were regularly from sugarcane followed by strawberry and guava, while the lowest numbers were recorded from mango and orange. Moreover, yeasts constituted almost the greatest part of propagules on DRBC, but filamentous fungi CFU exceeded on DG18 in sugarcane and strawberry and on MY50G from orange only.

Yeast fungi were recovered from all fruit juices on the three isolation media. *Candida* (represented by 11 species), *Hanseniaspora* (2), *Pichia* (5), followed by *Cryptococcus* (*C. albidus* var. *kuetzingii*), *Debaryomyces* (*D. nepalensis*), *Papiliotrema* (4 species), *Rhodotorula* (3), *Saccharomycopsis* (*S. crataegensis*) and *Torulospora* (*T. delbrueckii*) were recovered from all juice types on almost the three isolation media. *C. bentonensis*, *C. quercitrusa*, *C. saitoana*, *C. santamariae* var. *membranifaciens*, *C. smithsonii*, *C. spandovensis*, *C. tropicalis*, *Hanseniaspora uvarum*, *Papiliotrema terrestris*, *Pichia kluyveri*, *P. terricola* and *Saprochaete gigas* were isolated from all juice types. Some other yeast species were recovered only from one juice but not from the others such as *C. boidonii* and *Rhodosporeidiobolus odoratus* (from guava), *C. cylindracea* (mango), *Pichia manshurica* (strawberry), *Sporidiobolus pararoseus* (sugarcane) but none was specific for orange juice. Others were reported from all except sugarcane (*C. santamariae* var. *santamariae*), strawberry (*P. membraifaciens*, *Rhodotorula dairenensis*, *Saccharomyces paradoxus*, *Zygoascus meyeræ*) and guava (*R. glutinis*), while the remaining yeast species were recorded from 2 or 3 juices.

Filamentous fungi with only *Aspergillus* (13 species + 1 variety), *Aureobasidium* sp., *Cladosporium* (8 species), *Exophiala* (2), *Fusarium* (6) and *Penicillium* (18) were found contaminating all juices on almost all isolation media. More frequently isolated species from all juices comprised *Aspergillus flavus*, *Cladosporium cladosporioides*, *C. herbarum*, *C. sphaerospermum*, *P. chrysogenum* and *P. raistrickii*. Notably, some species were reported on the xerophilic media only such as *Lachancea thermotolerans*, *Alternaria brassicicola*, *Aspergillus proliferans*, *A. unguis*, *Cladosporium psychrotolerans*, *Penicillium variabile*, *P. verrucosum* and *Phaeostilbella* sp. on MY50G; *Sporidiobolus pararoseus*, *Rhodosporeidiobolus odoratus*, *Arhrinium saccharicola*, *Aspergillus nidulans*, *A. petrakii*, *Cladosporium langeronii*, *C. subtitisimum*, *Fusarium semitectum*, *Nannizziopsis vriesii*, *Phoma pimprina*, *Scopulariopsis carbonarius* and *Stemphylium sarciniforme* on DG18; and *Cystofilobasidium ferigula*, *Alternaria atra*, *Aspergillus amstelodami*, *A. oryzae*, *A. terreus*, *Penicillium lividum*, *Rhizopus stolonifer*, *Stemphylium botryosum* and *Wallemia sebi* on both DG18 and MY50G. Some filamentous species were also encountered only from one juice but not from the others.

Table 1 Means pH, mean colony forming units (CFU) and number of yeast and filamentous species recovered on DRBC, DG18 and MY50G agar media (pH and CFU are calculated as mean out of 5 replicate-samples of each fruit juice).

Serial no.	Sugarcane	Mango	Guava	Orange	Strawberry
Mean pH	6.45	5.36	5.06	4.66	4.62
Mean of total yeast CFU/ml on DRBC	58480±28429.8 ^{ab}	10106±7221.9 ^{ab}	14140±7642.4 ^b	5612±3669.4 ^a	19204±11351.5 ^c
Mean of total yeast CFU/ml on DG18	37012±21992.7 ^a	5220±2638.3 ^{ab}	10390±8376.2 ^b	3724±2127.6 ^a	7470±3321.4 ^{ab}
Mean of total yeast CFU/ml on MY50G	33808±23261.8 ^a	8492±9432.8 ^{ab}	16900±3851.1 ^b	3420±3552.7 ^a	16340±9405.8 ^{bc}
Mean of total filamentous CFU/ml on DRBC	41634.4±22315.1 ^a	3444±4756.8 ^a	1060±913.4 ^a	4632±8149.5 ^a	4340±3937.2 ^a
Mean of total filamentous CFU/ml on DG18	60392±34518.5 ^{ab}	2442±2239.6 ^a	1590±1217.8 ^a	5360±9419.2 ^a	6000±4053.5 ^a
Mean of total filamentous CFU/ml on MY50G	42196±39879.7 ^a	1626±1839.0 ^a	1030±1204.0 ^a	11744±24252.4 ^a	4160±4542.8 ^a
Mean of total CFU/ml on DRBC	100114.4±38210.4 ^b	13550±7663.9 ^b	15200±8218.5 ^b	10244±8338.1 ^a	23544±9400.6 ^c
Mean of total CFU/ml on DG18	97404±43506.2 ^b	7662±3095.4 ^{ab}	11980±8548.7 ^b	9084±9845.4 ^a	13470±5501.8 ^{abc}
Mean of total CFU/ml on MY50G	76004±57510.6 ^{ab}	10118±8387.7 ^{ab}	17930±4283.8 ^b	15164±23698.2 ^a	20500±7820.8 ^c
No of yeast species on DRBC (44)	25	29	29	27	26
No of yeast species on DG18 (34)	18	19	23	17	21
No of yeast species on MY50G (36)	16	21	20	19	19
No of filamentous species on DRBC (64)	21	31	16	17	22
No of filamentous species on DG18 (58)	17	20	16	19	23
No of filamentous species on MY50G (48)	18	17	11	16	19
No. of yeast species on all media (48)	31	34	35	33	31
No. of filamentous species on all media (94)	31	46	25	31	39
Total no. of all species on all media (142)	62	80	60	64	70
Total no. of all genera on all media (56)	29	37	32	36	50
F value	2.451	2.308	7.163**	0.501	5.489**

Significant (* P < 0.05) or high significant (** P < 0.01), note that species varieties are calculated as species.

Table 2 Physiological comparison of the strains tested of the recorded yeast species from juices.

Ascomycetous species: 1 *Barnettozyma californica* AUMC 10783, 2 *Candida bentonensis* AUMC 10764, 3 *C. boidinii* AUMC 10756, 4 *C. cylindracea* AUMC 10770, 5 *C. intermedia* AUMC 10767, 6 *C. quercitrusa* AUMC 10768, 7 *C. quercitrusa* AUMC 10781, 8 *C. saitoana* AUMC 10772, 9 *C. santamariae* var. *membranifaciens* AUMC 10777, 10 *C. santamariae* var. *santamariae* AUMC 11201, 11 *C. smithsonii* AUMC 10751, 12 *C. spandovens* AUMC 10771, 13 *C. tropicalis* AUMC 10782, 14 *Debaryomyces nepalensis* AUMC 11208, 15 *Geotrichum phurueaensis* AUMC 10798, 16 *Hanseniaspora clermontiae* AUMC 10785, 17 *H. uvarum* AUMC 10793, 18 *H. uvarum* AUMC 10794, 19 *Hyphopichia burtonii* AUMC 10755, 20 *Lachancea thermotolerans* AUMC 10763, 21 *Metschnikowia ziziphicola* AUMC 10774, 22 *Metschnikowia ziziphicola* AUMC 10778, 23 *Pichia fermentans* (= *Candida lambica*) AUMC 10784, 24 *P. kluyveri* AUMC 10797, 25 *P. kluyveri* AUMC 11205, 26 *P. manshurica* AUMC 11210, 27 *P. membranifaciens* AUMC 10760, 28 *P. terricola* AUMC 10796.

Test /Species strain number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Fermentation																												
D-glucose	-	-	++	-	++	+	+	-	++	+	+	+	++	-	-	++	++	++	-	+	+	++	++	+	-	-	-	++
D-galactose	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Maltose	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Me- α -D-glucoside	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sucrose	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-
Lactose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Raffinose	-	-	-	-	-	-	-	-	-	-	-	w	-	-	-	+	-	-	-	w	-	-	-	-	-	-	-	-
Inulin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	w
Starch	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D-xylose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Assimilation of carbon sources																												
D-glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-galactose	-	w	w	+	+	+	+	+	-	-	+	+	+	+	+	+	-	-	+	+	+	+	-	+	+	-	-	-
L-sorbose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	-	+	+	-	-	-
D-glucosamine	-	+	+	-	+	+	+	w	+	+	+	-	w	+	-	w	-	-	+	-	-	-	-	-	-	-	w	-
D-ribose	-	-	+	-	+	+	+	-	+	+	+	-	-	+	-	+	-	-	-	-	-	-	-	w	w	-	-	-
D-xylose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	-	+
L-arabinose	-	-	+	-	+	-	-	+	-	-	+	+	w	+	-	+	-	-	+	-	-	-	-	-	+	+	-	-
L-rhamnose	+	-	-	-	w	-	-	-	-	-	-	-	-	+	-	+	-	-	+	-	w	-	+	+	+	-	-	-
Sucrose	+	+	w	w	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	w

Table 2 Continued.

Test /Species strain number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	
Maltose	+	+	w	w	+	+	+	+	+	w	+	w	+	+	w	+	+	+	+	+	+	+	+	+	+	+	+	+	
α , α -trehalose	+	+	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	w	-	-	-	-	-	
Methyl- α -D-glucoside	+	+	-	-	+	+	+	+	-	-	+	-	+	+	-	+	-	-	+	+	+	+	-	+	+	-	-	-	
Cellobiose	+	+	-	-	+	-	-	+	+	+	+	-	+	+	-	w	+	+	+	+	+	+	-	+	+	-	-	-	
Salicin	+	+	-	-	+	-	-	+	+	+	+	-	-	+	-	+	+	+	w	-	+	+	-	-	-	-	-	-	
Arbutin	+	+	-	-	+	-	-	+	+	+	+	-	-	+	-	+	+	+	+	-	+	+	-	+	+	-	-	-	
Lactose	-	-	-	-	+	-	-	+	-	-	-	-	-	+	-	w	-	-	+	-	-	-	-	-	-	-	-	-	
Raffinose	-	+	-	-	+	-	-	+	-	-	+	+	-	+	-	-	-	-	-	-	+	w	-	-	+	+	-	-	-
Melezitose	-	+	-	-	+	+	+	+	-	-	+	-	+	+	-	-	-	-	-	-	+	+	+	-	+	+	-	-	+
Inulin	w	+	+	+	+	+	+	+	w	+	+	+	+	+	+	-	w	+	+	w	w	w	+	+	+	w	w	+	
Soluble starch	w	-	-	w	+	-	-	+	-	+	-	-	+	+	-	-	w	w	+	-	-	-	+	-	-	-	-	w	
Glycerol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	
Meso-erythritol	-	-	+	-	-	-	-	w	-	+	-	-	-	+	-	w	-	-	+	-	w	-	+	-	-	-	-	-	
Ribitol	-	+	+	-	+	+	+	+	+	+	+	+	+	+	w	+	-	-	+	+	+	+	+	+	w	w	-	w	-
Xylitol	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	+	+	+	-	w	w	-	+	-	
D-glucitol	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-	-	-	-
D-mannitol	+	+	+	+	+	=	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	w	+	+	-	-	w	
Galactitol	w	-	w	w	+	w	w	w	+	+	+	+	-	+	+	w	w	w	-	-	+	+	w	+	+	+	+	+	
Myo-inositol	-	+	-	w	-	-	-	-	-	-	-	-	-	-	-	w	-	-	-	-	-	-	-	-	-	-	-	-	
Glucono-d-lactone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	w
2-keto-D-gluconate	-	+	w	+	+	+	+	+	w	-	+	w	+	+	-	w	w	+	+	+	+	+	+	w	+	+	-	-	+
D-gluconate	+	+	w	+	+	+	+	+	+	+	+	+	+	+	w	+	+	+	+	w	+	+	+	+	+	-	-	-	
D-glucuronate	-	+	+	-	+	-	-	+	-	+	-	-	-	-	-	+	-	w	-	-	-	-	+	+	+	-	-	-	
D-galacturonate	-	+	-	-	+	w	w	+	w	-	-	-	-	+	+	+	-	w	w	-	w	-	-	w	w	-	-	-	
DL-lactate	+	+	+	+	+	+	+	+	w	+	w	+	-	+	+	+	-	w	+	w	+	w	+	+	+	+	+	w	
Succinate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	

Table 2 Continued.

Test /Species strain number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Citrate	-	-	-	+	+	+	+	+	+	+	+	w	+	+	+	+	-	-	+	-	-	+	+	+	+	-	-	+
Methanol	-	+	+	-	-	-	-	-	-	-	-	-	-	w	-	-	-	-	-	-	-	-	-	w	w	-	-	-
Ethanol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+
Propane 1,2 diol	-	-	-	-	-	-	-	+	w	-	-	-	-	w	w	w	-	-	-	-	-	w	+	+	+	+	-	w
Butane 2,3 diol	+	+	-	+	+	-	-	+	-	-	+	+	-	-	+	+	-	-	w	-	-	-	+	+	+	-	-	w
Quinic acid	-	-	-	w	+	w	w	-	-	-	-	-	w	+	-	-	-	-	-	-	+	+	-	w	w	-	-	-
Nitrogen sources																												
Nitrate	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-
Nitrite	+	+	+	+	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Ethylamine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L-lysine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Creatine	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Creatinine	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D-glucosamine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Imidazole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D-tryptophane	w	+	+	+	+	+	+	+	w-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Miscellaneous																												
0.01%	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+	-	-
Cycloheximide																												
0.1 %	-	+	+	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+	-	-	-	-	-	-	-	+	-	-
Cycloheximide																												
50% D-glucose	-	+	-	-	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+	-	+	+	-	+	+	+	+	+
60% d-glucose	-	-	-	-	-	-	-	-	w	-	+	+	-	+	-	-	-	-	+	-	+	+	-	-	-	-	-	-
10% NaCl	-	w	+	-	+	-	-	+	+	+	w	-	-	+	-	-	-	-	+	-	+	+	-	-	-	w	+	-
16% NaCl	-	-	-	-	+	-	-	-	w	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	w	-	-
Starch formation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Urease	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Diazonium blue B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+: growth; w: weak growth; -: no growth.

Table 2 Continued. Ascomycetous yeasts: 29 *Saccharomyces paradoxus* AUMC 10776, 30 *Saccharomycopsis crataegensis* AUMC 11204, 31 *Saprochaete gigas* AUMC 10769, 32 *Spencermartinsiella ligniputridi* AUMC 10779, 33 *Torulasporea delbrueckii* AUMC 10762, 34 *Wickerhamomyces pijperi* AUMC 10792, 35 *W. pijperi* AUMC 10799, 36 *W. sydowiorum* AUMC 10754, 37 *Zygoascus meyeriae* AUMC 10758. Basidiomycetous yeasts: 38 *Cryptococcus albidus* var. *kuetzingii* AUMC 10753, 39 *Cutaneotrichosporon dermatis* AUMC 10790, 40 *Cutaneotrichosporon mucoides* AUMC 10800, 41 *Cystobasidium minutum* AUMC 10791, 42 *Cystofilobasidium ferigula* AUMC 10787, 43 *Papiliotrema flavescens* AUMC 10773, 44 *Papiliotrema laurentii* AUMC 10750, 45 *Papiliotrema rajasthanensis* AUMC 10795, 46 *P. terrestris* AUMC 10766, 47 *Rhodosporidiobolus odoratus* AUMC 10780, 48 *Rhodotorula dairenensis* AUMC 11207, 49 *R. glutinis* AUMC 10757, 50 *Rhodotorula pacifica* AUMC 10761, 51 *Sporidiobolus metaroseus* AUMC 10775, 52 *S. pararoseus* AUMC 10752, 53 *Trichosporon asahii* AUMC 10759.

Test /Species strain number	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53
Fermentation																									
D-glucose	++	++	++	++	++	-	+	+	++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D-galactose	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Maltose	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Me- α -D- glucoside	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sucrose	+	-	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lactose	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Raffinose	+	-	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Inulin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Starch	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D-xylose	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Assimilation of carbon sources																									
D-glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-galactose	+	-	+	-	-	w	w	+	+	w	+	+	+	+	+	+	+	+	+	w	+	+	w	+	+
L-sorbose	-	+	+	+	-	+	+	+	+	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+
D-glucosamine	-	+	+	-	w	-	-	+	+	-	+	+	-	+	-	+	+	-	w	-	-	-	-	-	+
D-ribose	-	+	+	w	-	w	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	w	w	+
D-xylose	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	w	w	+
L-arabinose	-	-	+	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+
L-rhamnose	-	-	+	-	w	-	-	+	+	-	+	+	-	+	+	+	+	+	-	-	-	+	-	-	+
Sucrose	+	-	+	+	+	-w	w	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	-w	-	+	+	-	w	w	+	+	+	+	+	w	+	+	+	+	+	+	+	+	+	+	+	+

Table 2 Continued.

Test /Species strain number	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53
α , α -trehalose	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Methyl- α -D-glucoside	+	-	+	+	-	+	-	+	+	-	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+
Cellobiose	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	w	-	+	+	+	+	+
Salicin	-	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
Arbutin	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	-	-	+	-	-	+	-	+	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+
Raffinose	+	-	+	-	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	w
Melezitose	-	-	w	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Inulin	+	+	+	w	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	w
Soluble starch	+	-	w	-	-	-	-	+	+	+	+	+	w	+	+	+	+	-	w	+	+	+	+	+	+
Glycerol	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
Meso-erythritol	-	w	-	-	+	-	-	+	-	-	+	+	-	+	+	+	+	-	-	-	-	-	-	-	+
Ribitol	-	+	-	+	+	-	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
Xylitol	-	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+
D-glucitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
D-mannitol	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
Galactitol	-	-	w	+	+	+	+	+	+	-	+	+	+	+	+	+	+	w	-	w	+	+	-	+	-
Myo-inositol	-	w	-	-	+	-	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
Glucono-d-lactone	w	+	+	+	w	+	+	+	+	-	+	w	+	+	+	+	+	-	+	+	+	+	+	+	-
2-keto-D-gluconate	+	-	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	-	w	+	+	-	-	+
D-gluconate	-	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	w	+	+
D-gluconate	-	w	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-	-	+
D-galacturonate	-	+	+	-	-	+	-	+	+	-	-	-	+	+	+	+	+	-	-	+	-	+	-	-	-
DL-lactate	+	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
Succinate	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
Citrate	-	-	+	-	+	+	w	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+
Methanol	-	-	-	-	-	-	-	-	-	-	-	w	-	-	-	-	-	-	-	-	-	-	-	-	-
Ethanol	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
Propane 1,2 diol	-	-	+	w	-	-	-	-	-	w	-	w	w	-	w	-	+	-	-	-	-	-	-	+	+
Butane 2,3 diol	-	+	+	-	+	+	w	+	-	w	w	w	+	+	+	-	w	-	+	w	w	-	-	-	+

Table 2 Continued.

Test /Species strain number	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53
Quinic acid	-	w	-	-	-	-	-	+	-	-	+	-	+	+	w	-	-	-	+	+	+	+	+	+	-
Nitrogen sources																									
Nitrate	-	-	-	-	-	-	-	+	-	+	-	-	-	+	-	-	-	w	+	+	+	+	+	+	-
Nitrite	-	-	-	-	-	-	-	+	-	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+
Ethylamine	-	+	+	+	-	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+
L-lysine	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-w	+	+	+	-	+	+
Creatine	-	-	-	-	-	-	-	w	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-
Creatinine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-
D-glucosamine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Imidazole	-	-	-	-	-	-	-	-	-	-	w	-	-	-	-	-	-	+	-	-	w	w-	-	-	-
D-tryptophane	-	+	+	+	-	-w	-w	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
Miscellaneous																									
0.01% Cycloheximide	-	+	+	+	-	-	-	-	+	-	+	+	-	+	-	-	-	+	-	-	+	-	-	-	+
0.1 % Cycloheximide	-	+	+	+	-	-	-	-	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	+
50% D-glucose	+	+	-	-	+	-	-	+	+	-	+	-	-	-	-	+	+	+	-	+	+	+	w	-	+
60% d-glucose	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	w	-	-
10% NaCl	-	w	-	-	+	-	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	-	+	+
16% NaCl	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	w	-	-	-	-	-
Starch formation	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	-	-	+	-	-	-	-	-	-	-
Urease	-	-	-	-	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Diazonium blue B	-	-	-	-	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+: growth; w: weak growth; -: no growth.

Table 3 Assiut University Mycological Centre accession number (AUMC) of yeast strains isolated from different fresh juices with accession GenBank numbers given together with the closest match in the GenBank database and sequence similarity in percent to the match as inferred from Blastn searches of ITS sequences.

AUMC number	Juice origin	Accession GenBank number	Length (bp)	Closest Genbank match # ITS		Sequencing similarity (%)	Species
				Culture code	Collection Accession no.		
10750	Sugarcane	KY445944	542	Z-b	KP337904	538/542(99%)	<i>Papiliotrema laurentii</i>
10751	Sugarcane	KY445945	598	ATCC 18803 ^T	NR_130670	521/533(98%)	<i>Candida smithsonii</i>
10752	Sugarcane	KY445946	599	ATCCMYA-4323 ^T	NR_111339	571/580(98%)	<i>Candida smithsonii</i>
				CBS484	AF417115	585/593(99%)	<i>Sporidiobolus pararoseus</i>
				CBS 491 ^T	AY015429	583/592(98%)	
10753	Mango	KY445947	626	CBS1926 ^T	HQ327002	623/625(99%)	<i>Cryptococcus albidus</i> var. <i>kuetzingii</i>
10754	Sugarcane	KY445948	609	NRRL Y-7130 ^T	HM461651	585/606(97%)	<i>Wickerhamomyces sydowiorum</i>
				NRRL Y-7130 ^T	NR_138219	532/547(97%)	
10755	Guava	KY445949	449	CBS 2352 ^T	GU246247	418/428(98%)	<i>Hyphopichia burtonii</i>
10756	Guava	KY445950	693	PMM10-1634L	KP132263	687/689(99%)	<i>Candida boidinii</i>
				ATCCMYA-4485	FJ196791	676/681(99%)	
10757	Mango	KY495725	612	CBS 20 ^T	NR_073294	608/610(99%)	<i>Rhodotorula glutinis</i>
10758	Sugarcane	KY495726	595	CBS:4075	KY106012	593/594(99%)	<i>Zygoascus meyeræ</i>
				CBS 4099 ^T	KY106013	561/561(100)	
10759	Sugarcane	KY495727	545	AUMC 10711	KX376265	543/546(99%)	<i>Trichosporon asahii</i>
				CBS 2479 ^T	NR_073341	540/543(99%)	
10760	Sugarcane	KY495728	478	CBS 82	DQ198951	470/481(98%)	<i>Pichia membranifaciens</i>
				CBS 107 ^T	NR_111195	440/447(98%)	
10761	Sugarcane	KY495729	614	SY-96 ^T	AB026006	604/609(99%)	<i>Rhodotorula pacifica</i>
10762	Sugarcane	KY495730	793	CBS 1146 ^T	NR_111257	755/756(99%)	<i>Torulaspora delbrueckii</i>
10763	Sugarcane	KY495731	664	AUMC 10224	KX015903	661/664(99%)	<i>Lachancea thermotolerans</i>
				NRRL Y-8284 ^T	NR_111334	630/633(99%)	
10764	Sugarcane	KY495732	378	NRRL YB-2364 ^T	KF360533	313/343(91%)	<i>Candida bentonensis</i>
10765	Mango	KY495733	481	CBS 107 ^T	NR_111195	436/445(98%)	<i>Pichia membranifaciens</i>
10766	Mango	KY495734	532	CBS 10810 ^T	KY104476	511/520(98%)	<i>Papiliotrema terrestris</i>
10767	Mango	KY495735	396	P-23	JQ235066	391/393(99%)	<i>Candida intermedia</i>
				CBS 572 ^T	NR_111248	345/358(96%)	

Table 3 Continued.

AUMC number	Juice origin	Accession GenBank number	Length (bp)	Closest Genbank match # ITS		Sequencing similarity (%)	Species
				Culture Collection code	Accession no.		
10768	Orange	KY495736	609	CBS 4412 ^T	AM158924	603/606(99%)	<i>Candida quercitrusa</i>
10769	Mango	KY495737	387	CBS 126.76	AY838940	318/332(96%)	<i>Saprochaete gigas</i>
10770	Mango	KY495738	544	CBS 6330 ^T	KY102038	533/542(98%)	<i>Candida] cylindracea</i>
10771	Mango	KY495739	402	CBS 6875 ^T	KY102413	268/300(89%)	<i>Candida spandovens</i>
10772	Strawberry	KY495740	614	ATCC 36584 ^T	HQ652067	600/604(99%)	<i>Candida saitoana</i>
10773	Strawberry	KY495741	574	CBS 942 ^T	NR_130696	421/486(87%)	<i>Papiliotrema flavescens</i>
10774	Strawberry	KY495742	238	CBS 10358 ^T	KY104214	232/238(97%)	<i>Metschnikowia ziziphicola</i>
10775	Strawberry	KY495743	608	CBS 486 ^T	AY015438	598/607(99%)	<i>Sporobolomyces roseus</i>
10776	Mango	KY495744	825	CBS 432 ^T	KY105208	807/817(99%)	<i>Saccharomyces paradoxus</i>
10777	Mango	KY495745	614	MUCL 29936 ^T	NR_111300	599/600(99%)	<i>C. santamariae</i> var. <i>membranifaciens</i>
				CBS 4261 ^T	KY102388	599/600(99%)	<i>C. santamariae</i> var. <i>santamariae</i>
10778	Guava	KY495746	379	XY201 ^T	DQ367882	358/366(98%)	<i>Metschnikowia ziziphicola</i>
10779	Guava	KY495747	469	NCAIMY.01992 ^T	JF909555	163/179(91%)	<i>Spencermartinsiella ligniputridi</i>
10780	Guava	KY495748	596	CBS:9115 ^T	KY104702	558/582(96%)	<i>Rhodosporidiobolus odoratus</i>
10781	Orange	KY495749	615	CBS 4412 ^T	AM158924	605/615(98%)	<i>Candida quercitrusa</i>
10782	Orange	KY495750	494	CBS 94 ^T	KY102470	441/511(86%)	<i>Candida tropicalis</i>
10783	Orange	KY495751	587	CBS 252 ^T	KY101725	577/582(99%)	<i>Barnettozyma californica</i>
10784	Orange	KY495752	406	CBS 187 ^T	KY104545	338/343(99%)	<i>Pichia fermentans (Candida lambica)</i>
10785	Guava	KY495753	750	CBS 8821 ^T	KY103516	732/745(98%)	<i>Hanseniaspora clermontiae</i>
10786	Strawberry	KY495754	804	CBS 1146 ^T	KY105617	786/790(99%)	<i>Torulaspora delbrueckii</i>
10787	Guava	KY495755	622	CBS 7202 ^T	KY103159	605/607(99%)	<i>Cystofilobasidium ferigula</i>
10788	Sugarcane	KY495756	612	CBS 9839 ^T	KY102398	584/596(98%)	<i>Candida smithsonii</i>
10789	Sugarcane	KY495757	392	AUMC 10708	KX376258	387/404(96%)	<i>Saprochaete gigas</i>
				CBS 126.76	AY838940	325/331(98%)	<i>Geotrichum gigas</i>
10790	Orange	KY495758	532	CBS 2043 ^T	NR_130667	528/529(99%)	<i>Cutaneotrichosporon dermatis</i>
10791	Orange	KY495759	588	CBS319 ^T	AF190011	586/586(100%)	<i>Cystobasidium minutum</i>
10792	Strawberry	KY495760	560	CBS 2887 ^T	KY105912	414/427(97%)	<i>Wickerhamomyces pijperi</i>
10793	Strawberry	KY495761	702	CBS 314 ^T	NR_130660	503/503(100%)	<i>Hanseniaspora uvarum</i>
10794	Strawberry	KY495762	714	CBS 314 ^T	NR_130660	632/670(94%)	<i>Hanseniaspora uvarum</i>

Table 3 Continued.

AUMC number	Juice origin	Accession GenBank number	Length (bp)	Closest Genbank match # ITS		Sequencing similarity (%)	Species
				Culture Collection code	Accession no.		
10795	Strawberry	KY495763	542	CBS 10406 ^T	KY104474	530/535(99%)	<i>Papiliotrema rajasthanensis</i>
10796	Strawberry	KY495764	422	CBS2617 ^T	KY104650	418/421(99%)	<i>Pichia terricola</i>
10797	Strawberry	KY495765	448	CBS 188 ^T	KY104559	425/435(98%)	<i>Pichia kluyveri</i>
10798	Strawberry	KY495766	372	CBS 11418 ^T	KY103464	351/373(94%)	<i>Geotrichum phurueaensis</i>
10799	Strawberry	KY495767	507	CBS 2887 ^T	KY105912	466/506(92%)	<i>Wickerhamomyces pijperi</i>
10800	Orange	KY495768	532	CBS 7625 ^T	NR_073246	528/529(99%)	<i>Cutaneotrichosporon mucoides</i>
11201	Strawberry	KY495769	627	CBS 4261 ^T	KY102388	621/626(99%)	<i>C. santamariae</i> var. <i>santamariae</i>
11202	Strawberry	KY495770	611	CBS 9839 ^T	KY102398	586/597(98%)	<i>Candida smithsonii</i>
11203	Guava	KY495771	390	CBS 572 ^T	AY500373	377/391(96%)	<i>Candida intermedia</i>
11204	Guava	KY495772	627	CBS 6447 ^T	KY105255	594/600(99%)	<i>Saccharomycopsis crataegensis</i>
11205	Mango	KY495773	445	CBS 188 ^T	KY104559	430/432(99%)	<i>Pichia kluyveri</i>
11206	Guava	KY495774	614	MUCL 29936 ^T	NR_111300	609/613(99%)	<i>C. santamariae</i> var. <i>membranifaciens</i>
				CBS 4261 ^T	KY102388	609/613(99%)	<i>C. santamariae</i> var. <i>santamariae</i>
11207	Orange	KY495775	618	CBS 4406 ^T	AF444501	615/616(99%)	<i>Rhodotorula dairenensis</i>
11208	Guava	KY495776	646	JCM 2095 ^T	JN942654	635/637(99%)	<i>Debaryomyces nepalensis</i>
11209	Guava	KY495777	608	CBS 486 ^T	AY015438	601/607(99%)	<i>Sporobolomyces roseus</i>
11210	Strawberry	KY495778	439	CBS 209 ^T	NR_138211	373/430(87%)	<i>Pichia manshurica</i>

Table 4 Comparison between the colony forming units (CFU), percentage counts of CFU (%CFU) and frequency of occurrence (O) of fungal taxa recovered from the 5 types of juices analyzed on DRBC at 25° C.

Source	Sugarcane juice			Mango juice			Guava juice			Orange juice			Strawberry juice		
	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O
Taxa															
Yeasts	292400	58.41	5	50530	74.58	5	70700	93.03	5	28060	54.78	5	96020	81.57	5
<i>Barnettozyma californica</i> var. <i>californica</i>							500	0.66	2	700	1.37	1			
<i>Candida</i>	203260	40.61	5	21090	31.13	5	44600	58.68	5	12620	24.64	5	45560	38.70	5
<i>C. bentonensis</i>	3300	0.66	3	2000	2.95	2	450	0.59	3	520	1.02	3	800	0.68	1
<i>C. boidinii</i>							100	0.13	2						
<i>C. cylindracea</i>				50	0.07	1									
<i>C. intermedia</i>				540	0.80	2	1100	1.45	2	120	0.23	1			

Table 4 Continued.

Source Taxa	Sugarcane juice			Mango juice			Guava juice			Orange juice			Strawberry juice		
	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O
<i>C. quercitrusa</i>	300	0.06	2	100	0.15	2	2550	3.36	2	960	1.87	2	4500	3.82	2
<i>C. saitoana</i>	1500	0.30	2	3850	5.68	3	5200	6.84	5	2680	5.23	2	360	0.31	2
<i>C. santamariae</i> var. <i>membranifaciens</i>	23500	4.69	2	7750	11.44	3	9200	12.11	5	1420	2.77	4	9150	7.77	3
<i>C. santamariae</i> var. <i>santamariae</i>				3100	4.58	2	6150	8.09	3	40	0.08	1	13100	11.13	3
<i>C. smithsonii</i>	161620	32.29	5	1790	2.64	4	13950	18.36	5	780	1.52	4	13100	11.13	5
<i>C. spandovensis</i>	12040	2.41	3	1610	2.38	4	4500	5.92	4	4940	9.64	4	4550	3.87	4
<i>C. tropicalis</i>	1000	0.20	1	300	0.44	1	1400	1.84	1	1160	2.26	1			
<i>Cryptococcus albidus</i> var. <i>kuetzingii</i>	13000	2.60	3	210	0.31	2	250	0.33	3	1300	2.54	4	100	0.08	1
<i>Cutaneotrichosporon</i>				40	0.06	1				400	0.78	2	200	0.17	1
<i>C. dermatis</i>				40	0.06	1				200	0.39	1			
<i>C. mucoides</i>										200	0.39	1	200	0.17	1
<i>Cystobasidium minutum</i>				500	0.74	1				300	0.59	1			
<i>Debaryomyces nepalensis</i>	9300	1.86	3	1600	2.36	1	850	1.12	2	1300	2.54	4			
<i>Geotrichum phurueaensis</i>							200	0.26	1				7300	6.20	1
<i>Hanseniaspora</i>	40960	8.18	5	290	0.43	3	550	0.72	2	100	0.20	1	17450	14.82	5
<i>H. clermontiae</i>										100	0.20	1	100	0.08	1
<i>H. uvarum</i>	40960	8.18	5	290	0.43	3	550	0.72	2	80	0.16	1	17350	14.74	5
<i>Hyphopichia burtonii</i>	400	0.08	1	1250	1.85	1	300	0.39	1						
<i>Metschnikowia ziziphicola</i>				1750	2.58	1	850	1.12	11				1100	0.93	1
<i>Papiliotrema</i>	3720	0.74	3	13360	19.72	5	400	0.53	1	1720	3.36	4	2250	1.91	4
<i>P. flavescens</i>	1200	0.24	2	800	1.18	3	100	0.13	1				250	0.21	1
<i>P. laurentii</i>	720	0.14	2										800	0.68	1
<i>P. rajasthanensis</i>				50	0.07	1							600	0.51	3
<i>P. terrestris</i>	1800	0.36	1	12510	18.46	4	300	0.39	1	1720	3.36	4	600	0.51	3
<i>Pichia</i>	3920	0.78	3	750	1.11	2	6600	8.68	4	2500	4.88	4	15750	13.38	4
<i>P. fermentans</i>							50	0.07	1						
<i>P. kluyveri</i>	3080	0.62	2	100	0.15	1	400	0.53	3	2200	4.30	2	1000	0.85	2
<i>P. manshurica</i>													650	0.55	3
<i>P. membranifaciens</i>				150	0.22	1	200	0.26	1	160	0.31	1			
<i>P. terricola</i>	840	0.17	2	500	0.74	1	5950	7.83	2	140	0.27	2	14100	11.98	3
<i>Rhodotorula</i>	2880	0.58	5	300	0.44	2				120	0.23	1			
<i>R. dairenensis</i>	640	0.13	2	100	0.15	1									
<i>R. glutinis</i>	600	0.12	2	200	0.30	2									
<i>R. pacifica</i>	1640	0.33	5							120	0.23	1			
<i>Saccharomyces paradoxus</i>	200	0.04	1				2800	3.68	2	120	0.23	1			
<i>Saccharomycopsis crataegensis</i>				160	0.24	1	500	0.66	1				2300	1.95	1
<i>Saprochaete gigas</i>	800	0.16	2	400	0.59	2	250	0.33	1	520	1.02	1	1300	1.10	2

Table 4 Continued.

Source Taxa	Sugarcane juice			Mango juice			Guava juice			Orange juice			Strawberry juice		
	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O
<i>Spencermartinsiella ligniputridi</i>							150	0.20	1	280	0.55	1			
<i>Sporobolomyces roseus</i>													50	0.04	1
<i>Torulaspora delbrueckii</i>	11160	2.23	5	8780	12.96	4	11650	15.33	5	5640	11.01	5	1660	1.41	3
<i>Trichosporon asahii</i>	200	0.04	1	50	0.07	1									
<i>Wickerhamomyces</i>	1000	0.20	2										1000	0.85	2
<i>W. pipperi</i>							250	0.33	1				800	0.68	1
<i>W. sydowiorum</i>	1000	0.20	2										200	0.17	1
<i>Zygoascus meyeriae</i>	1600	0.32	1							360	0.70	1			
Filamentous fungi	208172	41.59	5	17220	25.42	5	5300	6.97	5	23160	45.22	5	21700	18.43	5
<i>Alternaria</i>				80	0.12	1							2750	2.33	5
<i>A. alternata</i>													1950	1.66	4
<i>A. botrytis</i>													100	0.08	1
<i>A. chlamydospora</i>				80	0.12	1							600	0.51	1
<i>A. tuberculata</i>													100	0.08	1
<i>Aspergillus</i>	6440	1.29	5	130	0.19	2	250	0.33	2	1140	2.23	2	150	0.13	2
<i>A. brasiliensis</i>										1140	2.23	2			
<i>A. flavus</i>	6000	1.20	5				150	0.20	2				100	0.08	1
<i>A. flavus</i> var. <i>columnaris</i>				50	0.07	1									
<i>A. niger</i>	400	0.08	1												
<i>A. ochraceus</i>	40	0.01	1	80	0.12	1									
<i>A. parasiticus</i>							100	0.13	1						
<i>A. sydowii</i>													50	0.04	1
<i>Aureobasidium</i> sp.	1000	0.20	1	660	0.97	3	1300	1.71	1	16340	31.90	3	400	0.34	1
<i>Botrytis cinerea</i>													11200	9.51	3
<i>Cladophialophora bantiana</i>													100	0.08	1
<i>Cladosporium</i>	2280	0.46	4	610	0.90	5	1100	1.45	1	600	1.17	5	3850	3.27	5
<i>C. cladosporioides</i>	200	0.04	1	250	0.37	2	500	0.66	1	100	0.20	1	2350	2.00	4
<i>C. halotolerans</i>										40	0.08	1			
<i>C. herbarum</i>										40	0.08	1	1200	1.02	1
<i>C. oxysporum</i>	1100	0.22	3										300	0.25	1
<i>C. sphaeospermum</i>	980	0.20	3	360	0.53	3	600	0.79	1	420	0.82	2			
<i>Cochliobolus australiensis</i>				1150	1.70	1									
<i>Exophiala</i>	100	0.02	1				500	0.66	3	2240	4.37	2	2000	1.70	2
<i>E. jeanselmei</i>										2240	4.37	2			
<i>E. spinifera</i>	100	0.02	1				500	0.66	3				2000	1.70	2
<i>Fusarium</i>	119972	23.97	5										50	0.04	1
<i>F. nygami</i>	24872	4.97	4												
<i>F. oxysporum</i>	18500	3.70	2												
<i>F. proliferatum</i>	2000	0.40	1												
<i>F. subglutinus</i>	18600	3.72	2												

Table 4 Continued.

Source Taxa	Sugarcane juice			Mango juice			Guava juice			Orange juice			Strawberry juice			
	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O	
<i>F. verticillioides</i>	56000	11.19	5										50	0.04	1	
<i>Leptosphaeria maculans</i>													100	0.08	1	
<i>Microsphaeropsis</i> sp.				400	0.59	1										
<i>Mucor hiemalis</i>										100	0.20	1				
<i>Necteria mauritiicola</i>				40	0.06	1										
<i>Penicillium</i>	76980	15.38	5		5250	7.75	3	1950	2.57	5	80	0.16	2	600	0.51	4
<i>P. canescence</i>	14400	2.88	4					150	0.20	1	40	0.08	1			
<i>P. chrysogenum</i>	1740	0.35	2					50	0.07	1	40	0.08	1	300	0.25	3
<i>P. citrinum</i>				50	0.07	1		50	0.07	1				100	0.08	1
<i>P. crustosum</i>	100	0.02	1													
<i>P. dauclauxii</i>	200	0.04	1					50	0.07	1						
<i>P. hirsutum</i>				50	0.07	1										
<i>P. implicatum</i>				3730	5.51	2		50	0.07	1						
<i>P. madritii</i>	400	0.08	1	700	1.03	1										
<i>P. montanense</i>				360	0.53	1										
<i>P. olsonii</i>													100	0.08	1	
<i>P. oxalicum</i>				120	0.18	1										
<i>P. quercetorum</i>								100	0.13	1						
<i>P. raistrickii</i>	60140	12.01	5	200	0.30	1		1450	1.91	3			100	0.08	1	
<i>P. viridicatum</i>				40	0.06	1		50	0.07	1						
<i>Phialophora richardsiae</i>				4720	6.97	1										
<i>Phoma</i>				3970	5.86	4		100	0.13	1	2620	5.12	3	400	0.34	2
<i>P. chrysanthemicola</i>				50	0.07	1					40	0.08	1			
<i>P. doliolum</i>				40	0.06	1										
<i>P. epicoccina</i>													300	0.25	1	
<i>P. eupyrena</i>				150	0.22	1					640	1.25	3			
<i>P. glomerata</i>				100	0.15	1										
<i>P. hedericola</i>				160	0.24	1										
<i>P. herbarum</i>				50	0.07	1										
<i>P. medicaginis</i>				2030	3.00	2										
<i>P. pinodella</i>				40	0.06	1					700	1.37	2	100	0.08	1
<i>P. pomorum</i>				1000	1.48	2		100	0.13	1	100	0.20	1			
<i>P. putaminum</i>											40	0.08	1			
<i>P. sorghina</i>				350	0.52	2					1100	2.15	2			
<i>Sarocladium</i>	200	0.04	1	50	0.07	1										
<i>S. kiliens</i>	200	0.04	1													
<i>S. strictum</i>				50	0.07	1										
<i>Talaromyces</i>				160	0.24	1					40	0.08	1	100	0.08	1
<i>T. pinophilus</i>				40	0.06	1										
<i>T. purperogenus</i>				120	0.18	1					40	0.08	1	100	0.08	1

Table 4 Continued.

Source	Sugarcane juice			Mango juice			Guava juice			Orange juice			Strawberry juice		
	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O
Taxa															
<i>Thielaviopsis</i> sp.	1200	0.24	1				100	0.13	1						
Total CFU	500572	100	5	67750	100.00	5	76000	100.00	5	51220	100.00	5	117720	100.00	5
No. of genera (41)	22			28			23			24			25		
No. of species (103+5)	44+2			56+4			41+4			40+4			45+3		

*O = occurrence out of 5 samples each.

Table 5 Comparison between the colony forming units (CFU), percentage counts of CFU (%CFU) and frequency of occurrence (O) of fungal taxa recovered from the 5 types of juices analyzed on DG18 at 25° C.

Source	Sugarcane juice			Mango juice			Guava juice			Orange juice			Strawberry juice		
	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O
Taxa															
Yeasts	185060	38.00	5	26100	68.13	5	51950	86.73	5	18620	41.00	5	37350	55.46	5
<i>Candida</i>	106980	21.97	5	14240	37.17	5	20350	33.97	5	8720	19.20	5	19250	28.58	5
<i>C. bentonensis</i>	1000	0.21	2				400	0.67	1	180	0.40	2	550	0.82	2
<i>C. intermedia</i>				150	0.39	1	50	0.08	1						
<i>C. quercitrusa</i>	600	0.12	1	50	0.13	1	200	0.33	1	140	0.31	2			
<i>C. saitoana</i>	600	0.12	2	3700	9.66	2				1780	3.92	3	6550	9.73	4
<i>C. santamariae</i> var. <i>membranifaciens</i>				100	0.26	1	8800	14.69	2	300	0.66	2	2500	3.71	3
<i>C. santamariae</i> var. <i>santamariae</i>							1950	3.26	1				4000	5.94	2
<i>C. smithsonii</i>	67500	13.86	5	5720	14.93	3	7900	13.19	4	1440	3.17	3	2000	2.97	4
<i>C. spandovensis</i>	37280	7.65	5	4520	11.80	4	1050	1.75	2	3960	8.72	2	3450	5.12	3
<i>C. tropicalis</i>										920	2.03	1	200	0.30	1
<i>Cryptococcus albidus</i> var. <i>kuetzingii</i>	200	0.04	1	690	1.80	2	650	1.09	2	1560	3.43	2	1050	1.56	2
<i>Cystobasidium minutum</i>				40	0.10	1									
<i>Cystofilobasidium ferigula</i>	400	0.08	1												
<i>Debaryomyces nepalensis</i>	3380	0.69	4	50	0.13	1	900	1.50	4	460	1.01	2	1600	2.38	2
<i>Hanseniaspora uvarum</i>	66120	13.58	5	40	0.10	1	4250	7.10	2	1040	2.29	3	7400	10.99	3
<i>Hyphopichia burtonii</i>							700	1.17	1						
<i>Metschnikowia ziziphicola</i>							1000	1.67	1				100	0.15	1
<i>Papiliotrema</i>	1440	0.30	4	2240	5.85	4	650	1.09	1	400	0.88	1	800	1.19	1
<i>P. flavescens</i>	680	0.14	2	1350	3.52	3							250	0.37	1
<i>P. laurentii</i>	400	0.08	1												
<i>P. rajasthanensis</i>	360	0.07	2												

Table 5 Continued.

Source Taxa	Sugarcane juice			Mango juice			Guava juice			Orange juice			Strawberry juice		
	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O
<i>P. terrestris</i>				890	2.32	2	650	1.09	1	400	0.88	1	550	0.82	1
<i>Pichia</i>	200	0.04	1	850	2.22	2	800	1.34	3	680	1.50	2	2650	3.93	5
<i>P. fermentans</i>							50	0.08	1						
<i>P. kluyveri</i>				800	2.09	1	450	0.75	1				550	0.82	3
<i>P. terricola</i>	200	0.04	1	50	0.13	1	300	0.50	1	680	1.50	2	2100	3.12	4
<i>Rhodospiridiobolus odoratus</i>				80	0.21	1	50	0.08	1						
<i>Rhodotorula</i>	1280	0.26	3	300	0.78	1	100	0.17	1	100	0.22	1	100	0.15	1
<i>R. dairenensis</i>	80	0.02	1	150	0.39	1	100	0.17	1	100	0.22	1			
<i>R. glutinis</i>	600	0.12	1	150	0.39	1							100	0.15	1
<i>R. pacifica</i>	600	0.12	2												
<i>Saccharomyces paradoxus</i>				100	0.26	1	3800	6.34	3	280	0.62	2			
<i>Saccharomycopsis crataegensis</i>							6000	10.02	2				300	0.45	1
<i>Sporochaeta gigas</i>							600	1.00	4	160	0.35	1	50	0.07	1
<i>Sporidiobolus pararoseus</i>	200	0.04	1												
<i>Sporobolomyces roseus</i>							200	0.33	1				100	0.15	1
<i>Torulaspora delbrueckii</i>	4860	1.00	5	7470	19.50	5	11900	19.87	4	5100	11.23	4	3750	5.57	5
<i>Wickerhamomyces pijperi</i>										120	0.26	1	200	0.30	1
Filamentous fungi	301960	62.00	5	12210	31.87	5	7950	13.27	5	26800	59.00	5	30000	44.54	5
<i>Alternaria</i>							200	0.33	1				2050	3.04	3
<i>A. alternata</i>							200	0.33	1				2000	2.97	2
<i>A. atra</i>													50	0.07	1
<i>A. chlamydospora</i>										300	0.66	1			
<i>A. tuberculata</i>															
<i>Arthrimum saccharicola</i>										100	0.22	1			
<i>Aspergillus</i>	5340	1.10	5	240	0.63	4	150	0.25	2	1000	2.20	3	350	0.52	2
<i>A. amstelodami</i>				40	0.10	1									
<i>A. brasiliensis</i>										640	1.41	2			
<i>A. flavus</i>	3940	0.81	5	100	0.26	2	50	0.08	1	360	0.79	2			
<i>A. nidulans</i>				100	0.26	1									
<i>A. niger</i>	200	0.04	1										200	0.30	1
<i>A. ochraceus</i>							100	0.17	1				100	0.15	1
<i>A. oryzae</i>	1000	0.21	1												
<i>A. petrakii</i>													50	0.07	1
<i>A. terreus</i>	200	0.04	1												
<i>Aureobasidium</i> sp.				920	2.40	3	1500	2.50	2	10440	22.99	2	2800	4.16	1
<i>Botrytis</i>							50	0.08	1				5150	7.65	4
<i>B. aclada</i>													100	0.15	1
<i>B. cinerea</i>							50	0.08	1				5050	7.50	4
<i>Cladosporium</i>	48300	9.92	5	850	2.22	2	3400	5.68	4	1060	2.34	5	16350	24.28	5
<i>C. cladosporioides</i>	4600	0.94	1	50	0.13	1	500	0.83	1	240	0.53	1	7100	10.54	2

Table 5 Continued.

Source Taxa	Sugarcane juice			Mango juice			Guava juice			Orange juice			Strawberry juice		
	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O
<i>C. herbarum</i>	8100	1.66	2	650	1.70	2	1950	3.26	3	540	1.19	3	7700	11.43	2
<i>C. langeronii</i>							850	1.42	1						
<i>C. oxysporum</i>	20560	4.22	3				50	0.08	1				100	0.15	1
<i>C. sphaerospermum</i>	15040	3.09	3	150	0.39	1	50	0.08	1	280	0.62	1	450	0.67	2
<i>C. subtilissimum</i>													1000	1.48	1
<i>Exophiala</i>				500	1.31	1				11600	25.54	2	1200	1.78	1
<i>E. jeanselmei</i>				500	1.31	1				11600	25.54	2			
<i>E. spinifera</i>													1200	1.78	1
<i>Fusarium</i>	101500	20.84	5	50	0.13	1	100	0.17	1	100	0.22	1	200	0.30	1
<i>F. nygami</i>	10000	2.05	2				100	0.17	1						
<i>F. proliferatum</i>				50	0.13	1									
<i>F. oxysporum</i>	7900	1.62	2							100	0.22	1			
<i>F. semitectum</i>	200	0.04	1												
<i>F. verticillioides</i>	83400	17.12	5										200	0.30	1
<i>Microsphaeropsis</i> sp.										900	1.98	1			
<i>Mucor hiemalis</i>													700	1.04	1
<i>Nannizziopsis vriessii</i>													350	0.52	1
<i>Penicillium</i>	146620	30.11	5	5740	14.98	4	2550	4.26	4	320	0.70	3	150	0.22	2
<i>P. canescence</i>	29100	5.98	2				1050	1.75	1						
<i>P. chrysogenum</i>				200	0.52	1	100	0.17	1	320	0.70	3			
<i>P. citrinum</i>													100	0.15	1
<i>P. crustosum</i>	400	0.08	2	360	0.94	1	50	0.08	1						
<i>P. implicatum</i>				4160	10.86	1	150	0.25	2						
<i>P. lividum</i>													50	0.07	1
<i>P. quercetorum</i>	100	0.02	1												
<i>P. raistrickii</i>	117020	24.03	5	900	2.35	2	1200	2.00	2						
<i>P. sclerotigenum</i>				120	0.31	1									
<i>Phoma</i>				3760	9.81	4				840	1.85	4	150	0.22	2
<i>P. chrysanthemicola</i>										80	0.18	1			
<i>P. eupyrena</i>										140	0.31	2			
<i>P. glomerata</i>				1000	2.61	2									
<i>P. pimprina</i>													100	0.15	1
<i>P. pinodella</i>				220	0.57	2				200	0.44	1			
<i>P. pomorum</i>				740	1.93	2				300	0.66	1			
<i>P. putaminum</i>										120	0.26	1	50	0.07	1
<i>P. sorghina</i>				1800	4.70	2									
<i>Rhizopus stolinefer</i>										40	0.09	1			
<i>Scopulariopsis cabonarius</i>										100	0.22	1			
<i>Stemphylium</i>													550	0.82	2
<i>S. botryosum</i>													450	0.67	1

Table 5 Continued.

Source	Sugarcane juice			Mango juice			Guava juice			Orange juice			Strawberry juice		
	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O
Taxa															
<i>S. sarraciniforme</i>													100	0.15	1
<i>Talaromyces</i>				100	0.26	1									
<i>T. purperogenus</i>				100	0.26	1									
<i>Thielaviopsis</i> sp.	200	0.04	1												
<i>Wallemia sebi</i>				50	0.13	1									
Total CFU	487020	100	5	38310	100.00	5	59900	100.00	5	45420	100.00	5	67350	100.00	5
No. of genera (40)		15			20			23			24			26	
No. of species (89+3)		34+1			37+2			36+3			34+2			41+3	

*O = occurrence out of 5 samples each.

Table 6 Comparison between the colony forming units (CFU), percentage counts of CFU (%CFU) and frequency of occurrence (O) of fungal taxa recovered from the 5 types of juices analyzed on MY50G at 25 °C.

Source	Sugarcane juice			Mango juice			Guava juice			Orange juice			Strawberry juice		
	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O
Taxa															
Yeasts	169040	44.48	5	42460	83.93	5	84500	94.26	5	17100	22.55	5	81700	79.71	5
<i>Barnettozyma californica</i> var. <i>californica</i>										280	0.37	1			
<i>Candida</i>	124200	32.68	5	29530	58.37	5	38500	42.94	5	9980	13.16	5	31650	30.88	5
<i>C. bentonensis</i>	2000	0.53	2	50	0.10	1							50	0.05	1
<i>C. intermedia</i>				150	0.30	1									
<i>C. quercitrusa</i>							200	0.22	1				250	0.24	1
<i>C. saitoana</i>	6400	1.68	3	500	0.99	2	2350	2.62	4	880	1.16	3	3900	3.80	5
<i>C. santamariae</i> var. <i>membranifaciens</i>	3300	0.87	3	13650	26.98	3	9250	10.32	3	520	0.69	1	650	0.63	2
<i>C. santamariae</i> var. <i>santamariae</i>							1750	1.95	1				16450	16.05	2
<i>C. smithsonii</i>	112300	29.55	5	5800	11.46	3	17500	19.52	4	5540	7.31	5	8500	8.29	4
<i>C. spandovensis</i>	200	0.05	1	9380	18.54	4	7450	8.31	4	3040	4.01	3	1700	1.66	2
<i>C. tropicalis</i>													150	0.15	1
<i>Cryptococcus albidus</i> var. <i>kuetzingii</i>	1200	0.32	1	100	0.20	1	650	0.73	3				50	0.05	1
<i>Cystofilobasidium ferigula</i>							50	0.06	1						
<i>Debaryomyces nepalensis</i>	8600	2.26	2	360	0.71	2	4400	4.91	4	200	0.26	1	350	0.34	1
<i>Hanseniaspora</i>	13640	3.59	2	1500	2.97	1	14100	15.73	3	160	0.21	2	3050	2.98	3
<i>H. clermontiae</i>										120	0.16	1	1350	1.32	1
<i>H. uvarum</i>	13640	3.59	2	1500	2.97	1	14100	15.73	3	40	0.05	1	1700	1.66	3

Table 6 Continued.

Source	Sugarcane juice			Mango juice			Guava juice			Orange juice			Strawberry juice		
	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O
Taxa															
<i>Hyphopichia burtonii</i>	1000	0.26	2				250	0.28	1						
<i>Lachancea thermotolerans</i>	1200	0.32	1				250	0.28	1						
<i>Metschnikowia ziziphicola</i>				50	0.10	1	250	0.28	1						
<i>Papiliotrema</i>				250	0.49	2				200	0.26	1	650	0.63	1
<i>P. flavescens</i>				50	0.10	1									
<i>P. rajasthanensis</i>										40	0.05	1			
<i>P. terrestris</i>				200	0.40	1				160	0.21	1	650	0.63	1
<i>Pichia</i>	4800	1.26	3	5450	10.77	3	12650	14.11	3	1880	2.48	3	44750	43.66	5
<i>P. fermentans</i>				50	0.10	1	100	0.11	1	120	0.16	1	100	0.10	1
<i>P. kluyveri</i>	2600	0.68	1	200	0.40	2				1400	1.85	1	50	0.05	1
<i>P. membranifaciens</i>	200	0.05	1												
<i>P. terricola</i>	2000	0.53	3	5200	10.28	3	12550	14.00	3	360	0.47	3	44600	43.51	5
<i>Rhodotorula</i>	1000	0.26	2	150	0.30	1				180	0.24	2			
<i>R. dairenensis</i>							100	0.11	1						
<i>R. glutinis</i>				50	0.10	1				180	0.24	2			
<i>R. pacifica</i>	1000	0.26	2	100	0.20	1									
<i>Saccharomyces paradoxus</i>				300	0.59	1	100	0.11	1						
<i>Saccharomycopsis crataegensis</i>	200	0.05	1	200	0.40	1	1050	1.17	2	80	0.11	1			
<i>Saprochaete gigas</i>										320	0.42	1	400	0.39	3
<i>Spenceriartinsia ligniputridi</i>										40	0.05	1			
<i>Torulaspora delbrueckii</i>	13200	3.47	2	4520	8.93	3	12000	13.39	3	3700	4.88	4	700	0.68	4
<i>Trichosporon asahii</i>													100	0.10	1
<i>Wickerhamomyces</i>										80	0.11	1			
<i>W. pijperi</i>										80	0.11	1			
<i>Zygoascus meyeriae</i>				50	0.10	1	150	0.17	2						
Filamentous fungi	210980	55.52	5	8130	16.07	4	5150	5.74	4	58720	77.45	5	20800	20.29	5
<i>Alternaria</i>													700	0.68	4
<i>A. alternata</i>													350	0.34	3
<i>A. atra</i>													50	0.05	1
<i>A. brasissicola</i>													300	0.29	1
<i>Aspergillus</i>	5180	1.36	5	290	0.57	2	350	0.39	2	540	0.71	2	350	0.34	4
<i>A. amstelodami</i>	240	0.06	2												
<i>A. brasiliensis</i>										400	0.53	1	100	0.10	1
<i>A. flavus</i>	3400	0.89	5	90	0.18	2	300	0.33	1	40	0.05	1	50	0.05	1
<i>A. flavus</i> var. <i>columnaris</i>										100	0.13	1			
<i>A. niger</i>	740	0.19	3										100	0.10	1
<i>A. ochraceus</i>													100	0.10	1
<i>A. oryzae</i>							50	0.06	1						
<i>A. proliferans</i>				80	0.16	1									
<i>A. terreus</i>	800	0.21	2												

Table 6 Continued.

Source	Sugarcane juice			Mango juice			Guava juice			Orange juice			Strawberry juice		
	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O
Taxa															
<i>A. unguis</i>				120	0.24	1									
<i>Aureobasidium</i> sp.	200	0.05	1	440	0.87	2	2600	2.90	1	48980	64.60	3	6500	6.34	1
<i>Botrytis cinerea</i>													4400	4.29	3
<i>Cladosporium</i>	14060	3.70	5	380	0.75	3	1000	1.12	1	440	0.58	2	4250	4.15	5
<i>C. cladosporioides</i>							400	0.45	1				1550	1.51	3
<i>C. herbarum</i>	5500	1.45	3	300	0.59	2	100	0.11	1				2700	2.63	3
<i>C. oxysporum</i>	7600	2.00	2				500	0.56	1						
<i>C. psychrotolerans</i>										400	0.53	1			
<i>C. sphaerospermum</i>	960	0.25	1	80	0.16	1				40	0.05	1			
<i>Exophiala</i>										6700	8.84	1	3000	2.93	1
<i>E. jeanselmei</i>										6700	8.84	1			
<i>E. spinifera</i>													3000	2.93	1
<i>Fusarium</i>	22700	5.97	5							180	0.24	2			
<i>F. proliferatum</i>	1000	0.26	1												
<i>F. verticillioides</i>	21700	5.71	5							180	0.24	2			
<i>Microsphaeropsis</i> sp.				200	0.40	1				300	0.40	1			
<i>Mucor hiemalis</i>													400	0.39	1
<i>Penicillium</i>	167340	44.03	5	3860	7.63	3	1200	1.34	4	160	0.21	3	900	0.88	3
<i>P. canescence</i>	28300	7.45	2				300	0.33	1						
<i>P. chrysogenum</i>				450	0.89	2	50	0.06	1	40	0.05	1	300	0.29	1
<i>P. citrinum</i>				150	0.30	1									
<i>P. implicatum</i>	400	0.11	1	3080	6.09	1	100	0.11	1						
<i>P. lividum</i>				80	0.16	1									
<i>P. madritii</i>	9200	2.42	2							40	0.05	1			
<i>P. olsonii</i>													200	0.20	2
<i>P. quercetorum</i>							200	0.22	1						
<i>P. raistrickii</i>	129040	33.96	5	50	0.10	1	550	0.61	2	80	0.11	1	400	0.39	2
<i>P. variabile</i>				50	0.10	1									
<i>P. verrucosum</i>	400	0.11	1												
<i>Phaeostilbella</i> sp.	900	0.24	1												
<i>Phoma</i>				2960	5.85	2				1380	1.82	2	50	0.05	1
<i>P. chrysanthemicola</i>										120	0.16	1			
<i>P. eupyrena</i>				100	0.20	1									
<i>P. hedericola</i>				360	0.71	1									
<i>P. pomorum</i>				650	1.28	1				1100	1.45	1			
<i>P. putaminum</i>										160	0.21	1	50	0.05	1
<i>P. sorghina</i>				1850	3.66	2									
<i>Rhizopus stolinefer</i>	200	0.05	1										100	0.10	1
<i>Stemphylium</i>													150	0.15	1
<i>S. botryosum</i>													150	0.15	1

Table 6 Continued.

Source	Sugarcane juice			Mango juice			Guava juice			Orange juice			Strawberry juice		
	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O	CFU	%CFU	O
Taxa															
<i>Thielaviopsis</i> sp.	400	0.11	1												
<i>Wallemia sebi</i>										40	0.05	1			
Total CFU	380020	100.00	5	50590	100.00	5	89650	100.00	5	75820	100.00	5	102500	100.00	5
No. of genera (36)		18			18			18			20			20	
No. of species (79+5)		32+2			36+2			28+3			32+3			35+3	

*O = occurrence out of 5 samples each

Discussion

The fruit juices samples analyzed in the current work have acidic pH values (4.62 – 5.36), but only cane juice near neutral (6.45). In this respect, Aneja et al. (2014) reported that the pH of freshly prepared orange juice ranged between 4.19–4.50 with a mean of 4.34. The acidic pH range (<4.5) of fruit juices serve as an important barrier for microbial growth (Aneja et al. 2014). However, food borne human pathogens (including bacteria, yeasts and filamentous fungi) survive in the acidic environment of fruit juices. Therefore, in the last two decades a number of food borne outbreaks associated with unpasteurized freshly squeezed fruit juices have been documented in many countries (Ghenghesh et al. 2005, Raybaudi-Massilia et al. 2009). According to the Gulf standards (FAO 1992) the maximum permitted counts for yeast and mould should not be more than 1×10^3 cfu ml⁻¹ in juices, however the current results revealed that cfu ml⁻¹ in all fruit juices investigated on all isolation media exceeded the permitted counts. The source of entry of microorganisms into fresh fruit juices is from environmental exposure and soil (Titarmare et al. 2009) and it was also found that strawberry is an ideal substrate for proliferation of microorganisms, fungi and yeasts such as *Botrytis cinerea* and *Saccharomyces cerevisiae* (Prasanna et al. 2007). In this respect, pH of the present fresh strawberry juice was 4.62 compared to those purchased from local market in Islamabad, Pakistan was 3.2 whose total plate count (TPC) was 2.7×10^2 CFUs g⁻¹ with no yeast or mould were found (Saddozai et al. 2012).

The present findings revealed that *Candida* (11 species), *Hanseniaspora* (2), *Pichia* (5) followed by *Cryptococcus* (*C. albidus* var. *kuetzingii*), *Debaryomyces* (*D. nepalensis*), *Papiliotrema* (4 species), *Rhodotorula* (3), *Saccharomycopsis* (*S. crataegensis*) and *Torulospira* (*T. delbrueckii*) were prevalent in all fruit juices. This is in agreement to large extent with the results of Moss (2008) who found that yeasts genera responsible for spoilage of fruit juices include *Candida*, *Pichia*, *Rhodotorula*, *Torulopsis*, *Saccharomyces*, *Zygosaccharomyces*, *Hansenula* and *Trichosporon*. Yeast spoilage in fruit juices is characterized by production of CO₂, alcohol, turbidity, locculation, pellicles, and clumping, and may also produce organic acids and acetaldehyde which contribute to a “fermented flavor”; and pectin esterases which degrade pectin causing spoilage (ICMSF 2005, Lawlor et al. 2009).

Twenty-two yeast species were identified from fresh cane juice collected from both the crusher and from the distributing canals in Brazil (El-Tabey Shehata 1959), however all of them are different from those reported in the present investigation from cane juice. On the other hand, some of the species reported here (related to genera *Cryptococcus*, *Debaryomyces* and *Rhodotorula*) were also prevalent in sugarcane leaves, stems and rhizosphere during different phases of plant development collected in Rio de Janeiro, Brazil (de Azeredo et al. 1998). Also, none of 5 *Saccharomyces* species isolated from citrus and cane juice left in sterilized bottles after 2 days of collection from Rawalpindi, Pakistan (Qureshi et al. 2007) were recorded in the current study, but another species (*S. paradoxus*) was recorded in all juice types except strawberry. Yeasts were mainly isolated from

cane juice in Thailand (Milintawaisamai et al. 2009) and also some yeast isolates were obtained from juices of sugarcane (7 isolates), mango and orange (2 each) collected from Kolkata, West Bengal, India (Chatterjee et al. 2011).

Yeast isolates from orange fruit and juice in a spontaneous fermentation were, in the order of dominance, *Saccharomyces cerevisiae*, *Candida tropicalis*, *Hanseniaspora uvarum*, *Clavispora lusitaniae*, *Trichosporon asahii*, *Pichia fermentans*, *Saccharomyces unisporus*, *Pichia anomala* and *Rhodotorula mucilaginosa* (Las Heras-Vazquez et al. 2003). Some of these (*C. tropicalis*, *H. uvarum* from all juices, *P. fermentans* from all except cane and *T. asahii* from all except guava and orange) were recorded in the present investigation.

Orange juices are acidic beverages (pH 4.66) with high sugar content. Under these conditions, moulds and yeasts comprise the typical biota present in the juices and can therefore cause spoilage in the processed product. Typical yeast species found in citrus juices are *Candida parapsilosis*, *C. stellata*, *Saccharomyces cerevisiae*, *Torulasporea delbrueckii*, and *Zygosaccharomyces rouxii*, although species from the genus *Rhodotorula*, *Pichia*, *Hanseniaspora Metschnikowia* and *Dekkera* are also common worldwide (Hatcher et al. 2000, ICMSF 2005, Lawlor et al. 2009). Yoshida et al. (1991) clarified that ELISA was applicable for the detection of the yeasts *C. intermedia* or *C. parapsilosis* in orange juice and yeast of more than 10^3 cfu ml⁻¹. 14 species were identified from pasteurized single-strength orange juice that had been contaminated after pasteurization (PSOJ) of which *Candida intermedia* and *C. parapsilosis* were the predominant species, while only 6 species were isolated from fresh-squeezed, unpasteurized orange juice (FSOJ) of which *Hanseniaspora uvarum* and *H. occidentalis* represented up to 73% of total FSOJ isolates. *Candida stellata*, *Pichia fermentans*, *P. kluyveri* and *Saccharomycopsis crataegensis* were also isolated but less frequently from fresh juice (Arias et al. 2002). Most of these species were reported from the present juices.

Fifteen yeast species were isolated from fresh, fermenting and fermented juice of two varieties of mango with pH values 4.25 and 3.66 (Suresh et al. 1982), most of them were encountered during the present study from mango and other juices and these include species of *Metschnikowia*, *Trichosporon*, *Candida*, *Pichia*, *Hyphopichia*, *Rhodotorula* and *Aureobasidium*. *Saccharomyces cerevisiae* was isolated from mango fruits around peninsular Malaysia (Ma'aruf et al. 2011). Also, 17 different fruits (including orange and mango) in India were used as natural sources for yeast isolation. Fungal contamination was observed in plates with inocula from banana, mango, jamun and orange (Lathar et al. 2010).

Filamentous fungi with *Aspergillus* (13 species+1 variety), *Aureobasidium* sp., *Cladosporium* (8 species), *Exophiala* (2), *Fusarium* (6) and *Penicillium* (18) were found contaminating all juices on almost all isolation media. Most frequently isolated species from all juices comprised *Aspergillus flavus*, *Cladosporium cladosporioides*, *C. herbarum*, *C. sphaerospermum*, *P. chrysogenum* and *P. raistrickii*. Beside yeasts, moulds form the main biota of fruits before processing because of acidic pH. The genera *Alternaria*, *Penicillium*, *Byssoschlamys*, *Aspergillus*, *Paecilomyces*, *Mucor*, *Cladosporium*, *Fusarium*, *Talaromyces*, *Neosartorya*, *Aureobasidium*, *Rhizopus* and *Botrytis* are filamentous fungi most frequently isolated and have also been reported in spoilage of fresh fruits, juices and vegetables (ICMSF 2005, Moss 2008, Lawlor et al. 2009). They produce mycelial mats and musty, stale of flavours in juices (Lawlor et al. 2009).

Ahmed et al. (2010) isolated 22 fungal species from cane juice with and without lemon and these were *Absidia corymbifera*, *Acremonium* sp., *Aspergillus candidus*, *A. erythrocephalus*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. subolivaceus*, *A. sulphureus*, *A. tamari*, *A. terreus*, *A. wentii*, *Fusarium semitectum*, *F. sporotrichoides*, *Humicola grisea*, *Gilmanieila humicola*, *Curvularia lunata*, *Monilia* sp., *Rhizopus stolonifer*, *R. oryzae*, *Penicillium* sp., and yeast (*Saccharomyces* spp.) with *A. niger* as the dominant in cane juice. Abd-Elaah & Soliman (2005) obtained species of *Aspergillus*, *Mamaria*, *Torula*, *Trichoderma*, *Mucor* and *Pythium* as the most common genera from decayed sugarcane stems collected from Qena Governorate, Egypt. Also *Aspergillus fumigatus* was isolated from sugarcane bagasse (Sandhu et al. 1997). Probably, these stem- or bagasse-associated fungi are the main source of juice contamination.

Samples of drinking water (pH 7.4-10.25) and fruit juices (orange, mango, apple, mix fruit and soft drink) (pH 4.03-6.23) collected from Karachi, Pakistan yielded 10 species of fungi from both water and juices (*Aspergillus niger*, *A. clavatus*, *A. ustus*, *A. ochraceus*, *A. terreus*, *A. wentii*, *Drechslera australiensis*, *Fusarium moniliforme*, *Penicillium* sp. and *Trichoderma viride*) and *Aspergillus niger* was the dominant fungus in drinking water as well as in juices followed by *A. clavatus* and *A. ustus* (Nazim et al. 2008). *Aspergillus niger* and *Candida tropicalis* were associated with deteriorating orange fruits in Nigeria (Akinmusire 2011). On the other hand, *Alternaria alternata*, *A. brassicicola*, *Aspergillus flavus* var. *columnaris*, *F. semitectum* and *F. solani* were isolated from fresh orange juice in Egypt (Zohri et al. 2014).

Lemons are liable to infections caused by filamentous fungi and, to a lesser degree, by yeasts. *F. oxysporum*, *F. moniliforme*, *A. niger*, *A. flavus*, *A. clavatus*, *Geotrichum candidum*, *Rhizopus* sp., *Rhodotorula* sp., *Scopulariopsis* sp., *P. digitatum*, *Penicillium* sp., *Diaporthe citrus* and *Mucor* sp. were isolated from lemon collected from 3 different plantations in the province of Tucuman, Argentina (Maldonado et al. 2005).

Sixteen mycobiotic species including 5 yeasts and 11 moulds were isolated from freshly prepared juices (sweet orange, orange, and carrot). *Aspergillus flavus* and *Rhodotorula mucilaginosa* were observed in the maximum number of juice samples, and *Candida krusei*, *C. parapsilosis*, species of *Pichia*, *Saccharomyces*, *Aspergillus terreus*, *A. niger*, *Penicillium islandicum*, *P. digitatum*, species of *Alternaria*, *Cladosporium*, *Colleotrichum*, *Curvularia*, *Fusarium* and *Geotrichum* were observed in both orange juice samples (Aneja et al. 2014). Also, 12 filamentous species were isolated from 26 kinds of juice (mango, orange and strawberry are included) and these were *Curvularia lunata*, sterile mycelia, *Asperillus flavus*, *A. olivino-viride*, *A. parasiticus*, *A. viride-nutans*, *A. terreus*, *A. cervinus*, *A. ustus*, *A. nidulans*, *A. fumigatus* and *A. niger*, with the latter being predominant (Al-Gashgari 2002). Fruits may constitute the main source of juice contamination as many species were isolated from fruits sold in Jeddah city, Saudi Arabia (Al-Hindi et al. 2011); *Aspergillus flavus* (from mango), *Aspergillus oryzae* (orange) and *Aspergillus awamori* (lemon). Also, *Alternaria* with 9 species (*A. alternata*, *A. citri*, *A. infectoria*, *A. raphani*, *A. zinnia*, *A. arbusti*, *A. blumeae*, *A. bumstii* and *A. gaisen*) and *Fusarium* with 8 species (*F. oxysporum*, *F. chlamyosporum*, *F. equiseti*, *F. langsethiae*, *F. proliferatum*, *F. sporotrichioides*, *F. culmorum*, *Fusarium* sp.) were found to be the most predominant fungi on guava, mango and citrus fruits collected from mature fruits in the field of Egypt and from different markets of Najran region, Saudi Arabia (Ammar & El-Naggar 2014).

Tournas et al. (2006) found 97% of fruit salad samples (including citrus fruits, cut strawberries, cantaloupe, honeydew, pineapple, and mixed fruit salads) from local supermarkets in the Washington were contaminated with yeasts at levels ranging from <2.0 to 9.72 log₁₀ cfu/g. Frequently encountered yeasts were *Pichia* spp., *Candida pulcherrima*, *C. lambica*, *C. sake*, *Rhodotorula* spp. and *Debaryomyces polymorphus*. Low numbers of *Penicillium* spp. were found in pineapple salads, whereas *Cladosporium* spp. were present in mixed fruit and cut strawberry salads. Prasanna et al. (2007) found that strawberry is an ideal substrate for proliferation of yeasts and moulds such as *Botrytis cinerea*.

Helal et al. (2006) isolated 11 taxa of moulds and yeasts from freshly prepared orange, guava and banana juices from fresh fruits collected from the local market of Zagazig City, Egypt and kept in open bottles at room temperature for 7 days. Fungal counts/ml juice in descending order was 1×10^4 for guava, followed by 0.7×10^4 for banana, and 0.6×10^4 for orange. *A. niger*, *A. flavus*, *A. candidus*, *A. oryzae*, *C. herbarum*, *Mucor* sp., *P. lilacinus* and *Candida albicans* were isolated from the three juices with the first 2 species having the largest number of propagules; *P. digitatum* from orange and guava juices and *P. puberulum* from orange and banana juices and *B. cinerea* from guava and banana juices.

Conclusion

Juices squeezed from fresh fruits contain microorganisms which are potentially hazardous to public health. The level of moulds and yeasts in juices is mainly attributable to the low pH of

juices. The presence of pathogenic microorganisms in juices is clearly indication of food borne outbreaks. The selling and consumption of juices are never stopped on nutritional grounds. It is alarming situation for suitable agency to take some necessary action, make guidelines to prevent potential food poisoning from juices that contain such microorganisms (Ghenghesh et al. 2005, Raybaudi-Massilia et al. 2009).

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