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MICROBIAL LOAD AND NUMERICAL TAXONOMY OF YEASTS ASSOCIATED WITH HIGH SUGAR FOODS

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ABSTRACT

This study, was carried out mainly to isolate and identify the dominant yeasts in high sugar foods, as well as to have a clear picture on the associated microorganisms in these products. The total counts of bacteria, osmophilic bacteria, coliforms, fecal *Escherichia coli, Staphylococcus* spp., *Salmonella* and *Shigella,* molds and yeasts were determined in sugarcane juice, fruit juice nectars (mango and koktel), jam (strawberry and fig), honey and halawa tahenia. One hundred and thirty-four yeasts were isolated from some of these high sugar foods. These yeasts in addition to 31 standard yeast strains were classified using the morphological and biochemical tests. A Vernetzüngsdiagram was automatically calculated by subjecting the obtained results to the EDV-programme. The numerical classification of the total 134 yeast isolates besides the 31 standard yeast strains resulted in 16 groups representing 16 yeast species forming clusters with high similarity 12 ascosporogenous groups *i.e., Saccharomyces cerevisiae, Torulaspora delbrueckii, Debaryomyces hansenii, D. polymorphus, Cryptococcus albidus, Kluyveromyces phaffi, Hansenula anamala, Schizosaccharomyces pombe, Zygosaccharomyces bailii, Pichia fermentants, K. marxianus* var bulgaricus and *Zygo. rouxii*, while the other 4 groups were nonascosporogenous yeast *i.e., Candida zeylanoides, C. parapsilosis, C. sake* and *Rhodotorula glutinis*. The most dominant yeast species found in sugarcane juice were ascosporogenous (68.53%) including *S. cerevisiae* (11.57%), *T. delbrueckii* (9.91%), *D. hansenii* (9.09%), *Zygo. bailii* (6.61%), *D. polymorphus* (5.78%), *K. phaffi* (4.13%), *K. m. var bulgaricus* (4.95%), *Zygo. rouxii* (3.30%), *Crypt. albidus* (2.47%), *H. anamala* (3.30%), *Schizo. pombe* (2.47%), and *P. fermentants* (4.95%), beside nonascosporogenous (31.38%) including *C. zeylanoides* (10.74 %), *C. parapsilosis* (9.91%), *C. sake* (4.95%) and *Rh. glutinis* (5.78%). The dominant yeast species found in honey were *Crypt. albidus* (38.46%), *K. phaffi* (15.38%), *C. zeylanoides* (15.38%), *D. hansenii* (7.69%), *D. polymorphus* (7.69%), *S. cerevisiae* (7.69%) and *P. fermentants* (7.69%).

Key words: High sugar foods, numerical taxonomy, yeasts, osmophilic bacteria, coliforms.

INTRODUCTION

Other than bacterial pathogens, the control of spoilage yeast is one of most important aspect in food preservation. Factors such as low temperature, reduced water activity (a_w), addition of preservatives and low pH, are all used to inhibit or destroy yeast and other microorganisms (Marechal *et al.,* 1999). Addition of preservatives to food has been carried out for centuries. Some methods of food preservation are based on the use of natural substances, such as essential oils (Canner and Beuchat, 1984, Moleyar and Narasimham, 1992).Yeast resistance to such preservatives raises problems for food industry, causing a requirement for increased preservatives levels in low- pH foods to prevent yeast spoilage (Piper *et al.,* 2001).

Water activity a_w of foods is also a very important factor affecting yeast growth. While most yeasts grow well in 20% w/v glucose, only a limited number of yeast species are able to grow at low a_w caused by the presence of high concentrations of sugar (60% w/v) these have been referred to as osmotolerant or xerotolerant

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species (Tilbury, 1980 a,b). The relative sensitivity of yeasts to high temperature has a strong influence on the germination process. The most important microorganisms associated with the sugarcane juice are essentially the ones coming from soil and plants, in which the molds, yeast, lactic and sporulated bacteria predominate (Oliveira *et al.*, 2007). Quantitatively, sugarcane juice is basically comprise by water (80%) and total dissolved solids (20%). Among the total solids, sucrose (17%), glucose (0.4%) and fructose (0.2%); organic non-sugars, as nitrogenous substances, wax fats, pectins, organic acid, coloured materials, and inorganic non-sugars, are found. High water activity, pH range of 5.0 - 5.5, high concentration of organic and inorganic nutrients and maintenance at 25°C - 30°C are favorable conditions for a great and diverse microbial community (Oliveira *et al.*, 2006).

Honey is a product extremely rich in sugars of which glucose and levulose are outstanding. It also possesses vitamins, mineral salts and enzymes. Honey, however and despite having a high tenor in sugars and consequently a reduced water activity, contains an acid pH and bactericidal substances.

Currently, in excess of 800 yeast species have been recognized (Barnett *et al.*, 2000). However, only a small fraction of these yeast species are responsible for major losses in processed foods around the world (Pitt and Hocking, 1997) and may be described as "spoilage yeast". The importance of these spoilage yeast is increasing because, in the modern world a greater proportion of foods is being processed, preserved in some forms and stored or transported over long distances before consumption. In food and drinks industries, the most problematic spoilage yeasts encounted are those belonging to the genus *Zygosaccharomyces*. The species *Zygo. bailii* and *Zygo. rouxii* are often major spoilage organisms of fruit juices, sauces, carbonated soft drinks, sugarcane juice and jams. The unusual physiological characteristics of these yeasts are largely responsible for their ability to cause spoilage, including resistance to weak-acid preservatives, extreme osmotolerance and ability to ferment hexose sugars (James and Stratford, 2003).

Many morphological and biochemical properties other than fermentation and assimilation results have been used with limited success for yeast classification (Campbell, 1975; Kockavà-Kratochvilavà and Slàvikovà, 1978). This conventional methodology requires the evaluation of some 60 to 90 tests, resulting in complex, laborious and time-consuming process (Arias *et al.*, 2002). They also, reported that classical identification relies on numerous sets of data and is still considered the standard method for yeast identification despite requiring an extended period of time and qualified personnel to achieve a proper identification. A graphical method known as a Vernetzungsdiagram was applied by (Mohamed, 1990; Mohamed and Farag, 1994 a,b and 1995; Mohamed and Fayed, 2000; El-Shourbagy; Abdel-Basit, 2009) for the identification of yeast isolated from some dairy products.

The present study aimed to classify the yeast isolated from high sugar foods (sugarcane juice, fruit juice nectars, jam, honey and halawa tahinia) using computer programme for calculating a Vernetzungsdiagram.

MATERIALS AND METHODS

Sampling

Three kinds of juice, two kinds of jam, one kind of each honey and halawa tahinia which are a high sugar food products were used in this study. The sugarcane juice samples were collected every 15 days from Zagazig, 10th of Ramadan and Belbeas cities from retail sellers in sterilized plastic bags from February 2009 to February 2010, while fruit juice nectars (mango, koktel) were collected from local markets in Sharkia Governorate during the same period, also, strawberry jam and fig jam were collected from markets in 10th of Ramadan city. Honey and halawa tahinia samples were collected from local markets in Sharkia Governorate. All samples transported in ice box at low temperature to Microbiology Laboratory at College of Agriculture, Zagazig University, they were microbiologically observed fresh and every 2 months of storage period at 5-7°C.

Microbiological Examination

Twenty five ml of either sugarcane juice or fruit juice nectar, also 25 g of each jam, honey and halawa tahinia were separately transferred to 500 ml Erlynmayer flask containing 225 ml sterilized peptone water (1.0%) and well mixed, then serial dilutions up to 10^{10} were prepared. One tenth ml of each dilution was spread on the surface of plate count solid agar medium (PC-agar, Oxoid) then incubated at 7, 30 and 37°± 2°C for 48hr for enumeration of total bacterial count (Hausler, 1972).

Osmophilic bacteria were counted according to Atlas and Parks (1997) using osmophilic agar, plates and then incubated at 7, 30 and 37± 2°C for 48hr. The total coliforms and fecal *E. coli* were counted according to Harrigan and McCance (1976) using McConkey- agar medium. Plates were incubated at 37± 2°C for 24hr for coliform, and at 45.5± 0.5°C for 24hr for fecal *E. coli*. *Staphylococcus* spp. was enumerated on Baird-parker's medium (Oxoid CM 275, Baird- Parker and Davenport, 1965). *Salmonella* and *Shigella* spp. were counted using S. S. – agar (Oxoid CM 99). All plates were incubated at 37± 2°C for 24hr (Harrigen and McCance, 1976). Molds were enumerated on Streptomycin – rosebengal - agar medium and incubated at 28± 2°C for 72hr (Baruah and Barthakur, 1997). For yeast enumeration, three different media were used,(i) yeast extract – malt extract – agar (YM – agar, Kreger – van Rij, 1984), (ii) yeast extract – glucose – chloramphenicol – bromophenol blue – agar (YGCB – agar, Perkoppovà *et al.* 1984) and (iii) Würze – bromophenol blue – agar (WB-agar, Oxoid CM 247). Petri dishes were incubated at 28 ± 2°C for 48hr, and then counted. Single colonies of yeast were picked up and streaked on slant of YM – agar tubes, then kept at 4°C for identification. The 134 yeast isolates and 31 standard yeast strains (Table 1), obtained from Centraalbureau voor Shimmel – cultures, the Netherlands (CBS) and Deutsche Sammlung von Mikroorgansimen, Germany (DEM) were subjected to identification according to Lodder (1970); Kreger-van Rij (1984) and Barnett *et al.* (2000).

Computer Identification of Yeast Isolates

The results of 65 morphological and physiological characters of 134 yeast isolates beside the 31 standard yeast strains, were introduced to the computer against the previous known results of the test for all studied yeast species by Kreger-van Rij (1984) and Barnett *et al.*, (2000).

The numerical data, the dendogram, shade diagrams and minimum spanning trees and linkage-maps were used as suggested by Ohmayer *et al.*, (1980) for computing the Vernetzungsdiagram (EDV-programmes) for yeast taxonomy. The results of morphological and biochemical reactions were recorded giving one for positive and zero for negative in the data matrix, then used for obtaining the Vernetzungsdiagram. This Vernetzungsdiagram illustrates the similarity relationship between yeasts via the continuous dark or dotted lines.

Statistical Analysis

All analyses were made in triplicate, and the data are presented as mean ± standard deviation.

RESULTS AND DISCUSSION

Microbial Population

Data of the viable counts for total bacteria, osmophilic bacteria, coliform, fecal *E. coli*, *Staphylococcus* spp., *Salmonella* and *Shigella*, molds and yeast counts of fresh sugarcane juice, fruit juice nectars (mango and koktel), jam (strawberry and fig), honey and halawa tahinia are shown in Table 2. The total counts of fresh sugarcane juice in Zagazig, 10th of Ramadan and Belbeas cities were 1.9×10^6 cfu/ml, 0.81×10^5 cfu/ml and 5.9×10^4 cfu/ml at 7°C, respectively, then increased up to 9.3×10^6, 1.0×10^6 and 4.1×10^5 at 30°C, while total bacterial count decreased at 37°C reaching 2.9×10^6, 3.4×10^5 and 1.7×10^5 cfu/ml, respectively.

The total osmophilic bacterial counts of sugarcane juice in Zagazig, 10th of Ramadan and Belbeas cities were 9.6×10^4, 0.26×10^5 and 4.4×10^4 cfu/ml at 7°C, then increased at 30°C being 4.2×10^6, 1.7×10^5 and 1.2×10^5 cfu/ml, then decreased gradually to be 3.1×10^5, 0.88×10^5 and 8.6×10^4 cfu/ml at 37°C. The same Table show the total counts of coliforms, fecal *E. coli*, *Staphylococcus* spp, molds and yeast, respectively where, in fresh sugarcane juice from Zagazig city were 2.0×10^4, 0.11×10^3, 0.28×10^4, 2.3×10^3 and 1.9×10^5 cfu/ml.

Salmonella & Shigella were not detected, regarding fresh sugarcane juice from 10th of Ramadan city and Belbeas, *Staphylococcus* spp, *Salmonella* and *Shigella* were not detected but contained 2.3×10^4 and 8.7×10^3 cfu/ml of coliforms, 0.35×10^4 and 0.57×10^3 cfu/ml of fecal *E. coli*, 0.53×10^3 and 1.16×10^3 fp/ml of molds and 9.0×10^4 and 3.4×10^4 cfu/ml of yeast. The same trend was observed by Oliveira *et al.* (2006); Martini and Verruma- Berrardi (2011) and Yadov *et al.* (2011).

Mango juice contained 6.9×10^4 and 5.1×10^4 cfu/ml of bacteria at 30°C and 37°C, respectively, 5.2×10^3 cfu/ml of osmophilic bacteria at 30°C and 7.2×10^2 fp/ml of molds. No osmophilic bacteria at 7°C and 37°C, coliforms, fecal *E. coli, Staphylococcus* spp., *Salmonella* and *Shigella* and yeast were detected.

The koktel juice contained 6.4×10^3, 1.1×10^4 and 3.7×10^3 cfu/ml of bacteria at 7°C, 30°C and 37°C, respectively, but contained 8.3×10^3 cfu/ml of osmophilic bacteria at 30°C and 6.0×10^3 fp/ml of molds, while no osmophilic bacteria was found at 7°C, 37°C, coliforms, fecal *E. coli, Staphylococcus* spp, *Salmonella* and *Shigella* and yeast were detected. These results are in line with those reported by Rashed *et al.* (2013).

The strawberry and fig jams contained 3.3×10^4, 7.1×10^5 cfu/g at 30°C. 2.5×10^4, 5.0×10^4 cfu/g at 37°C of bacteria and 2.0×10^3, 1.7×10^3 cfu/g of osmophilic bacteria, respectively, but strawberry contained 5.9×10^3 fp/g of molds. However non of bacteria and osmophilic bacteria were grown at 7°C. Coliforms, fecal *E. coli, Staphylococcus* spp., *Salmonella & Shigella* and yeast were not detected. These results are in agreement with those found by Ridawati *et al.* (2010).

Data set out in Table 2 show the total counts of bacteria, osmophilic bacteria, coliforms, fecal *E. coli, Staphylococcus* spp., *Salmonella & Shigella*, molds and yeast in honey from Zagazig, 10th of Ramadan and Belbeas cities. Bacteria or osmophilic ones were not detected at 7°C, also coliforms, *Staphylococcus* spp. and *Salmonella & Shigella* were not detected in honey. However total bacterial counts of honey samples collected from the 3 cities were 3.3×10^3, 3.2×10^4 and 3.7×10^4 cfu/g at 30°C, 2.5×10^3, 2.4×10^4 and 3.3×10^4 cfu/g at 37°C, respectively, osmophilic bacteria were 1.7×10^3, 9.8×10^5 and 2.9×10^3 cfu/g, respectively. While total molds and yeast were not detected in honey from Zagazig city, total molds counts were 1.5×10^2 and 1.3×10^2 fp/g also total yeast counts were 1.1×10^2 and 2.2×10^2 cfu/g in honey from 10th of Ramadan and Belbeas cities. These results support the findings reported by Gomes *et al.* (2010); Carvalho *et al.* (2010) and Pavelkovà *et al.* (2013).

The same Table shows that the halawa tahenia contained 3.0×10^5 cfu/g at 30°C and 1.1×10^5 cfu/g at 37°C of total bacteria, 1.8×10^5 at 30°C and 7.1×10^4 cfu/g at 37°C of osmophilic bacteria, and 5.2×10^3 fp/g of molds, but no coliforms, fecal *E. coli, Staphylococcus* spp., *Salmonella* and *Shigella* and yeast were detected. These results are in agreement with those found by Tolga *et al.* (2010).

Identification of yeast isolates

The isolated yeasts (134 isolates) as well as 31 standard yeast strains were subjected to 65 morphological and physiological characters (Table 3). The obtained results were analysed by single – linkage methods and numerical taxonomy then a Vernetzungsdiagram was computed (Fig. 1). Results indicated that 16 groups *i.e* 16 yeast species were isolated from sugarcane juice and honey. Twelve species of ascosporogenous yeast were also included, namely *S. cerevisiae, T. delbrueckii, D. hansenii, D. polymorphus, Crypt. albidus, K. phaffi, H. anamala, Schizo. pombe, Zygo. bailii, P. fermentants, K.m* var bulgaricus and *Zygo. rouxii.* Besides four nonascosporogenous yeast species belonging to *C. zeylanoides, C. parapsilosis, C. sake* and *Rh. glutinis* were identified according to the similarity between yeast isolates. The similarities inside groups were illustrated on the basis of differences between yeast isolates. Thus, thick line represents no difference, thin line represents one difference and stroked line represents 2-4 differences. The obtained yeast groups could be illustrated as follows: The twelve groups of ascosporogenous yeast were, the first group includes 15 strains of *S. cerevisiae* beside 13 standard strains; second group 12 strains of *T. delbreuckii,* 7 standard strains; third group 12 strains of *D. hansenii,* 6 standard strains; fourth

Table 1. List of 31 standard strains used for yeast identification

Species	Source and strain No.	
Debaryomyces hansenii	[a]CBS	777
	CBS	772
	CBS	3428
	CBS	70238
	CBS	70244
	CBS	767
Saccharomyces cerevisiae	CBS	1464
	[b]DSM	1333
	CBS	70449
	CBS	70470
	CBS	70471
	CBS	70508
	CBS	70509
	CBS	70511
	CBS	70514
	CBS	70547
	CBS	381
	CBS	70478
	CBS	2858
Torulaspora delbrueckii	CBS	1146
	CBS	70497
	CBS	70504
	CBS	70526
	CBS	70607
	CBS	817
	CBS	4510
Zygosaccharomyces rouxii	CBS	5717
Candida sake	CBS	159
Candida zeylanoides	CBS	619
	CBS	641
	CBS	947

[a]CBS: Central Bureau voor Schimmelcultures, the Netherlands.

[b]DSM: Deutsche Sammlung von Mikroorganismen, Germany.

Table 2. The mean values* of microbial profile in different high sugar foods from February 2009 to February 2010

High sugar foods	Total count												No. of yeast isolates
	Bacteria			Osmophilic bacteria			Coliforms (cfu/ml)	Fecal *E. coli* (cfu/ml)	*Staphylococcus spp.* (cfu/ml)	*Salmonella &Shigella* (cfu/ml)	Molds (fp/ml)	Yeasts (cfu/ml)	
	7°C (cfu/ml)	30°C (cfu/ml)	37°C (cfu/ml)	7°C (cfu/ml)	30°C (cfu/ml)	37°C (cfu/ml)							
Sugarcane juice													
Zagazig city	1.9×10^{6} ± 0.935	9.3×10^{6} ±3.560	2.9×10^{6} ± 3.937	9.6×10^{4} ± 1.177	4.2×10^{6} ± 2.832	3.1×10^{5} ± 0.497	2.0×10^{4} ± 0.494	0.11×10^{3} ± 0.064	0.28×10^{4} ± 0.304	0	2.3×10^{3}± 1.203	1.9×10^{5} ± 0.223	54
10th Ramadan city	0.81×10^{5} ± 0.423	1.0×10^{6} ± 1.797	3.4×10^{5} ± 0.845	0.26×10^{5} ± 0.232	1.7×10^{5} ± 0.529	0.88×10^{5} ± 0.602	2.3×10^{4} ± 0.663	0.35×10^{4} ± 0.402	0	0	0.53×10^{3} ± 0.071	9.0×10^{4} ± 0.121	37
Belbees city	5.9×10^{4} ± 0.223	4.1×10^{5} ± 1.272	1.7×10^{5} ± 0.376	4.4×10^{4} ± 1.204	1.2×10^{5} ± 0.378	8.6×10^{4} ± 0.230	8.7×10^{3} ± 0.785	0.57×10^{3} ± 0.070	0	0	1.16×10^{3} ± 0.423	3.4×10^{4} ± 0.060	30
Fruit juice nectars													
Mango juice	0	6.9×10^{4} ± 0.818	5.1×10^{4} ± 0.651	0	5.2×10^{3} ± 1.045	0	0	0	0	0	7.2×10^{2}± 1.454	0	0
Koktel juice	6.4×10^{3} ± 1.073	1.1×10^{4} ± 2.047	3.7×10^{3} ± 0.329	0	8.3×10^{3} ± 0.975	0	0	0	0	0	6.0×10^{3}± 1.555	0	0
Jams													
Strawbeery jam	0	3.3×10^{4} ± 0.549	2.5×10^{4} ± 0.400	0	2.0×10^{3} ± 0.404	1.4×10^{3} ± 0.357	0	0	0	0	5.9×10^{3}± 1.192	0	0
Fig jam	0	7.1×10^{5} ± 2.351	5.0×10^{4} ± 1.131	0	1.7×10^{3} ± 0.358	0.84×10^{3} ± 0.188	0	0	0	0	0	0	0
Honey													
Zagazig city	0	3.3×10^{3} ± 0.464	2.5×10^{3} ± 0.086	0	1.7×10^{3} ± 0.189	1.1×10^{3} ± 1.815	0	0	0	0	0	0	0
10th Ramadan city	0	3.2×10^{4} ± 3.79	2.4×10^{4} ± 1.78	0	9.8×10^{5} ± 1.734	8.6×10^{3} ± 0.507	0	0	0	0	1.5×10^{2}± 0.028	1.1×10^{2} ± 0.949	8
Belbeas city	0	3.7×10^{4} ± 3.243	3.3×10^{4} ± 2.866	0	2.9×10^{3} ± 0.333	2.5×10^{3} ± 0.461	0	0	0	0	1.3×10^{2}± 2.289	2.2×10^{2} ± 0.327	5
Halawa tahenia	0	3.0×10^{5} ± 0.344	1.1×10^{5} ± 1.837	0	1.8×10^{5} ± 2.593	7.1×10^{4} ± 2.215	0	0	0	0	5.2×10^{3}± 1.042	0	0
Total no. of yeasts													134

*:The sum of microbial counts in all of the samples divided by their number.

cfu: cell forming units. fp: Fungal propagules. ± Standard deviation.

group 8 strains of *D. polymorphus;* fifth group 8 strains of *Crypt. albidus*; sixth group 7 strains of *K. phaffi;* seventh group 3 strains of *Schizo. pombe;* eighth group 8 strains of *Zygo. bailii;* nineth group 4 strains of *H. anamala*; tenth group 7 strains of *P. fermentants*; eleventh group 6 strains of *K.m.* var bulgaricus and finally, the twelveth group 4 strains of *Zygo. rouxii*, one standard strain, all groups are of high similarity clusters. The other four groups belong to nonascosporogenous yeast species have higher similarity clusters. The thirteenth group includes 15 *C. zeylanoides,* 3 standard strains; the fourteenth group includes 12 *C. parapsilosis*; fifteenth group includes 7 *Rh. glutinis* besides one strain and sixteenth group contain 7 *C. sake.*

Results of the morphological and physiological tests for all the 16 groups of yeast isolated from different high sugar foods as shown by frequency percentage of positive reactions presented in Table 3 are in agreement with those found by Kreger-van Rij (1984); Mohamed (1990), Seiler and Busse (1990), Seiler 1991, Mohamed & Farag (1994 a,b and 1995); Mohamed and Fayed (2000); Gewaily *et al.* (2001); Hasan *et al.* (2007) and El-Shourbagy and Abdel- Basit (2009) when they applied the Vernetzungsdiagram for the identification of yeast in some dairy and food products. The classical methodology, although time consuming, proved to result in the highest correct identification percentage next to partial sequence of 26S rRNA gene method (Arias *et al.,* 2002).

Dominant Yeasts in Different High Sugar Foods

Table 4 shows the percentage distribution of 134 isolated yeast from sugarcane juice, fruit juice nectars (mango and koktel), jams (strawberry and fig), honey and halawa tahenia. The dominant yeasts were represented as *S. cerevisiae* 14 (11.57%); *T. delbreuckii* 12 (9.91%); *D. hansenii* 11(9.09%); *Zygo. bailii* 8 (6.61%), *D. polymorphus* 7(5.78%); *K.m.* var bulgaricus 6(4.95%); *P. fermentants* 6(4.95%); *K. phaffi* 5(4.13%); *Zygo. rouxii* 4(3.30%); *H. anamala* 4 (3.30%); *Crypt. albidus* 3(2.47%) and *Schizo. pombe* 3(2.47%). Fresh sugarcane juice contained 38 nonascosporogenous yeast represented by *C. zeylanoides* (10.74%); *C. parapsilosis* (9.91%); *C. sake* (4.95%) and *Rh. glutinis* (5.78%). These results confirm the results obtained and reported by Martorell *et al.* (2007) and Martini and Verruma-Berrardi (2011).

Data also showed that honey contained 11 ascosporogenous yeast *Crypt. albidus* (38.46%); *K. phaffi* (15.38%); *D. hansenii* (7.69%); *D. polymorphus* (7.69%); *S. cerevisiae* (7.69%) and *P. fermentants* (7.69%). These results are in harmony with those found by Schneider *et al.* (2003) and Beckh *et al.* (2005).

The obtained results show that yeasts were not detected in fruit juice nectars (mango and koktel), jams (strawberry and fig) and halawa tahenia. These results are in agreement with those found by Shailja *et al.* (2003); Policarpo *et al.* (2003). It is worthy noticing that *S. cerevisiae, C. zeylanoides, T. delebrueckii, C. parapsilosis, D. hansenii, Zygo. bailii, D. polymorphus, K.m.* var bulgaricus, *P. fermentants, C. sake*, and *Rh. glutinis* were among the dominant yeast species in sugarcane juice. These results are in agreement with those found by Martorell *et al.* (2007).

The dominant yeasts observed in honey namely *Crypt. albidus, K. phaffi, C. zeylanoides, D. hansenii, D. polymorphus, S. cerevisiae, P. fermentants* and *Rh. glutinis* confirmed the results obtained by Schneider *et al.* (2003).

From the results setting out in Table 4 it could be concluded that the dominant yeasts in sugarcane juice and honey belongs to *D. hansenii* (8.95%), *T. delbrueckii* (8.95%), ascosporogenous yeast, such as *S. cerevisiae* (11.19%), *D. polymorphus* (5.97%), *Crypt. albidus* (5.97%), *Zygo. bailii* (5.97%), *K. phaffi* (5.22%), *P. fermentants* (5.22%) and nonascosporogenous yeast namely *C. zeylanoides, C. parapasilosis* and *Rh. glutinis* which were recognized as osmotolerant yeast (Schneider *et al.*, 2003; Ergul and Ozbas, 2006; Ridawati *et al.,* 2010).

Finally, it could be concluded from the results of this study that the use of Vernetzungsdiagram as well as the numerical taxonomy, morphological and physiological characters, are a good tool for yeast identification.

Table 3. Frequency percentage for positive characters of osmotolerant yeast isolated from different high sugar foods

Characters	Ascosporogens yeast												Nonascosporogenes yeast			
	*S. cerevisiae (28)**	*T. delbrueckii (19)*	*D. hansenii (18)*	*D. polymorphus (8)*	*Crypt. albidus (8)*	*K.phaffi (8)*	*Schizo.pombe (8)*	*Zygo.bailii (8)*	*H.anamala (8)*	*P. fermentants (7)*	*K. m. var bulgaricus (6)*	*Zygo. rouxii (5)*	*C. zeylanoides (18)*	*C. parapsilosis (12)*	*R. glutinis (7)*	*C. sake (7)*
Carbon sources																
Fermentation of																
D-Glucose	100	100	77.7	100	0	100	100	100	100	100	100	100	100	100	0	100
Lactose	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0
Assimilation of																
D-Glucose	100	100	100	100	0	100	100	100	100	100	100	100	100	100	100	100
D-Galactose	100	0	100	100	100	100	0	75	0	0	100	100	100	100	100	100
L-Sorbose	0	0	100	100	100	0	0	75	0	0	16.6	100	100	75	100	100
D-Glucosamine	0	0	100	75	100	0	0	0	0	71.4	16.6	0	100	75	100	100
D-Ribose	0	0	0	87.5	100	0	0	0	0	0	16.6	0	0	100	100	100
D-Xylose	0	0	72.2	75	100	0	0	0	100	100	83.3	100	0	100	100	100
L-Arabinose	0	0	94.4	75	100	0	0	0	0	0	0	0	0	100	0	100
D-Arabinose	0	0	0	100	100	0	0	0	0	0	100	0	0	0	100	0
L-Rhamnose	0	0	100	0	100	0	0	0	0	0	0	0	0	0	0	0
Sucrose	100	100	100	100	100	0	100	75	100	0	0	100	0	100	100	100
Maltose	100	100	100	100	100	0	100	0	100	0	0	100	0	100	100	100
Trehalose	75	100	100	100	100	0	0	75	100	0	0	100	100	100	100	100
Me α-D-Glucoside	100	100	100	100	100	0	0	0	100	0	0	0	0	100	0	100
Dextrose	0	100	100	100	100	100	0	0	100	0	100	100	0	100	100	100
Cellebiose	0	0	100	100	75	0	0	0	100	0	100	0	100	0	0	100
Salicin	0	0	94.4	100	100	0	0	0	100	0	0	0	100	0	0	100
Arbutin	0	0	100	100	100	0	100	0	100	0	100	0	100	0	0	100
Melibiose	0	0	0	75	100	0	100	0	0	0	0	0	0	0	0	0
Lactose	0	0	100	75	75	0	0	0	0	0	100	0	0	0	0	0
D-Fractose	100	100	100	100	75	100	33.3	0	100	100	100	100	0	100	100	100
Raffinose	89.2	84.2	94.4	100	75	0	0	62.5	100	0	100	0	0	0	100	0
Melezitose	100	84.2	100	100	100	0	0	0	100	0	0	0	0	100	0	100
Inulin	0	100	0	25	0	0	0	0	100	0	100	0	0	0	0	0
Glycerol	78.5	100	100	100	75	100	0	75	100	100	100	100	100	100	100	100
Erythritol	0	0	0	100	75	0	0	0	100	0	0	0	0	0	0	0
Ribitol	0	100	100	100	75	0	0	75	100	0	0	0	100	75	0	100
Xylitol	0	0	100	100	75	0	0	75	100	0	100	100	0	75	100	100
L-Arabinitol	0	0	100	75	75	0	0	0	0	0	0	0	0	75	0	0
D-Glucitol	0	0	100	100	100	0	0	75	100	0	100	100	100	100	0	0
D-Mannitol	60.7	100	100	100	100	0	0	75	100	0	100	100	100	100	71.4	100
Galactitol	0	0	0	75	75	0	0	0	100	0	0	0	0	0	0	100
m-Inositol	0	0	0	0	75	0	0	0	0	0	0	0	0	0	0	0
2-Keto-D-gluconate	0	100	100	100	75	0	100	100	0	0	0	100	100	100	0	100
D-Lyxose	0	0	0	100	62.5	0	0	0	0	100	0	0	0	0	0	100
D-Turanose	100	84.2	100	100	100	100	100	0	100	0	0	0	0	100	100	100
Gentibiose	0	10.5	100	100	100	100	100	0	0	100	100	0	0	100	100	100
N-Ac-D-glucosamins	0	0	100	100	100	100	100	0	100	100	0	0	100	100	100	100
D-Arabitol	0	84.2	83.3	100	100	71.4	100	100	100	100	100	100	100	100	100	100
Nitrogen source:																
Nitrate	0	0	0	0	100	0	0	0	100	0	0	0	0	0	100	0
Nitrite	0	0	0	0	100	0	0	0	100	0	0	0	0	0	100	0
Ethylamine	0	0	100	100	75	0	100	100	100	100	0	0	88.8	100	0	100
L-Lysine	0	100	100	100	62.5	0	100	100	100	100	100	100	88.8	100	0	100
Cadaverine	0	26.3	0	100	75	0	100	100	100	100	100	100	88.8	100	100	100
Creatine	0	0	0	87.5	0	0	0	0	0	0	0	0	0	0	0	0
Creatinine	0	0	0	0	62.5	0	0	0	0	0	100	0	0	0	0	0
Urea hydrolysis	0	0	0	0	100	0	100	0	0	0	0	0	0	0	0	0
Growth in:																
0.1%Cyclohexamid	0	0	0	100	0	0	0	0	100	0	66.6	0	100	0	100	0
Vitamine-free m	32.1	31.5	88.8	100	0	0	0	0	100	0	0	0	100	0	100	71.4
Pantothenat	53.5	100	94.4	100	100	0	100	100	100	100	100	100	100	100	100	100
Biotin	100	100	100	0	100	0	100	0	100	100	100	100	100	0	100	100
Thiamin	100	100	100	100	75.5	0	100	100	100	0	100	100	100	100	0	100
Pyridoxine	67.8	100	100	100	0	100	100	100	100	100	100	100	100	100	100	100
Riboflavine	100	100	100	100	100	100	100	100	100	100	100	100	0	100	100	100
P-Aminobenzota	7.1	100	100	100	100	100	100	100	100	100	0	0	100	100	100	100
50%Glucose	100	100	83.3	100	0	0	100	100	100	71.4	0	100	100	100	100	71.4
60%Glucose	0	100	88.8	62.5	0	0	100	75	0	0	0	100	100	0	100	28.5
4 C	100	0	77.7	75	0	100	0	0	0	0	100	0	0	100	71.4	0
37 C	0	100	0	100	0	0	100	75	0	100	100	60	100	100	100	0
Starch formation	0	0	0	100	75	0	66.6	0	0	0	0	0	0	0	0	0
Psedumycelium	0	0	50	100	100	100	100	0	0	100	100	0	0	100	100	0
True mycelium	100	100	50	0	0	0	0	100	100	0	0	100	100	0	0	100
Pellicle formation	0	0	61.1	100	100	85.7	100	100	100	100	100	100	100	100	100	0
Ascosporen	100	100	100	100	100	100	100	100	100	100	100	100	0	0	0	0

*= number of isolates *S.: Saccharomyces* *T.: Torulaspora* *D.: Debaryomyces* *K.: Kluyveromyces*
Zygo.: Zygosaccharomyces *H.: Hansenula* *Schizo. : Schizosaccharomyces* *Crypt. : Cryptococcus*
P.: Pichia *Rh.: Rhodotorula* *C.: Candida*

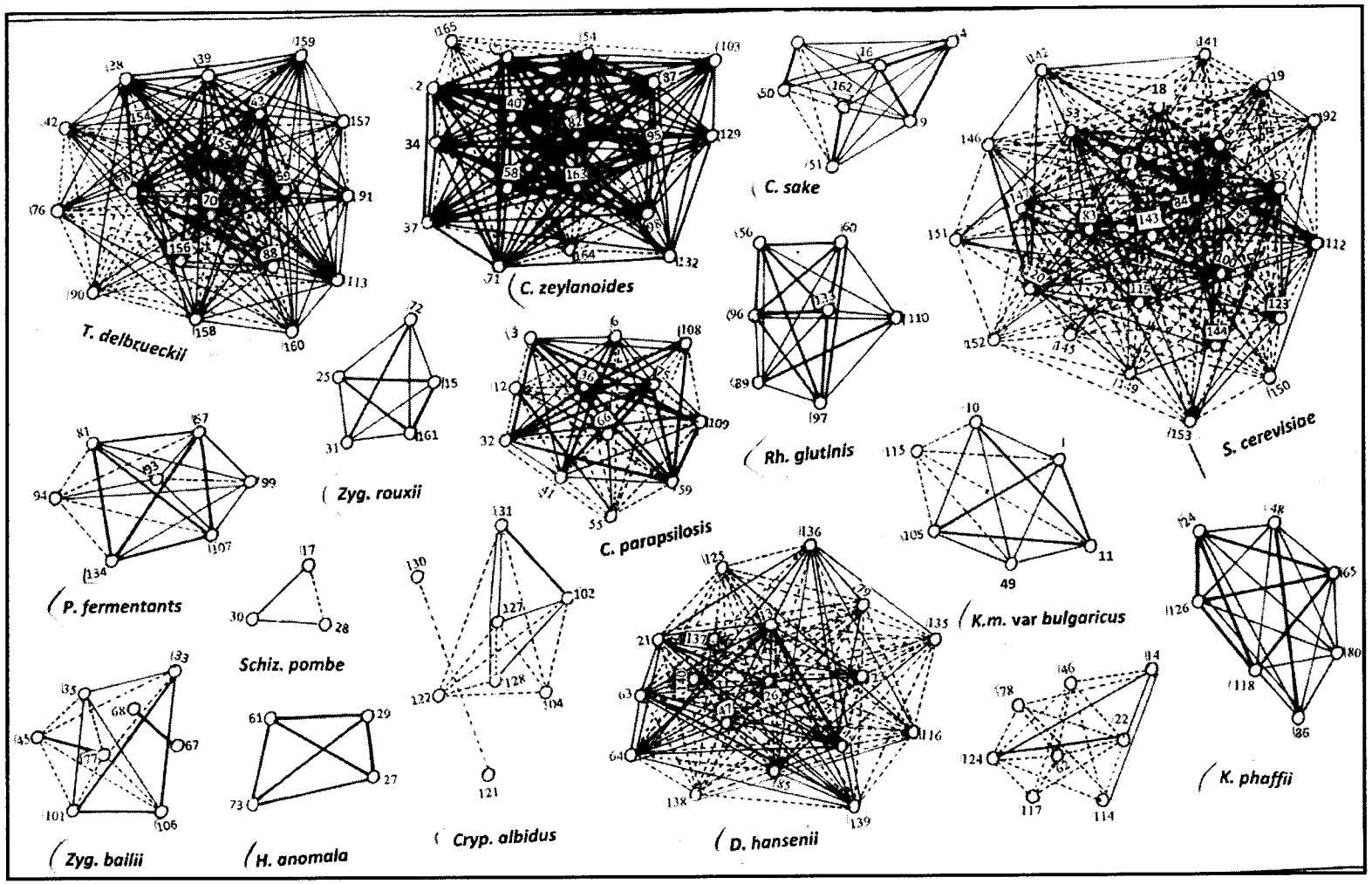

Fig. 1. Vernetzungsdiagram for 165 yeast cultures(31 standard strains and 134 isolates from high sugar foods)

——— : Zero differences ———— : One differences ---------- : 2-4 differences

Table 4. Percentage distribution of 134 yeast isolates from different high sugar foods

Species	Sugarcane juice	fruit juice nectars		Jam		Honey	Halawa tahenia	No. of yeast isolates
Ascosporogenous yeast	No. %	Koktel juice No. %	Mango juice No. %	Strawberry jam No. %	Fig jam No. %	No. %	No. %	No. %
D. hansenii	11 (9.09)[1]	0 (0)	0 (0)	0 (0)	0 (0)	1 (7.69)[2]	0 (0)	12 (8.95)[3]
D. polymorphus	7 (5.78)	0 (0)	0 (0)	0 (0)	0 (0)	1 (7.69)	0 (0)	8 (5.97)
K. phaffi	5 (4.13)	0 (0)	0 (0)	0 (0)	0 (0)	2 (15.38)	0 (0)	7 (5.22)
K. m. var bulgaricus	6 (4.95)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	6 (4.47)
Zygo. rouxii	4 (3.30)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (2.98)
Zygo. bailii	8 (6.61)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	8 (5.97)
S. cerevisiae	14 (11.57)	0 (0)	0 (0)	0 (0)	0 (0)	1 (7.69)	0 (0)	15(11.19)
Crypt. albidus	3 (2.47)	0 (0)	0 (0)	0 (0)	0 (0)	5 (38.46)	0 (0)	8 (5.97)
H. anamala	4 (3.30)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (2.98)
Schizo. pombe	3 (2.47)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (2.23)
P. fermentants	6 (4.95)	0 (0)	0 (0)	0 (0)	0 (0)	1 (7.69)	0 (0)	7 (5.22)
T. delbrueckii	12 (9.91)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	12 (8.95)
Total ascosporogenous yeast	83 (68.53)	0 (0)	0 (0)	0 (0)	0 (0)	11 (84.60)	0 (0)	94(70.11)
Nonascosporogenous yeast								
C. sake	6 (4.95)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	6(4.47)
C. zeylanoides	13 (10.74)	0 (0)	0 (0)	0 (0)	0 (0)	2 (15.38)	0 (0)	15(11.19)
C. parapsilosis	12 (9.91)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	12 (8.95)
Rh. glutinis	7 (5.78)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	7(5.22)
Total nonascosporogenous yeast	38(31.38)	0 (0)	0 (0)	0 (0)	0 (0)	2 (15.38)	0 (0)	40(29.83)
Total No. of identified isolates	121(90.29)	0 (0)	0 (0)	0 (0)	0 (0)	13 (9.70)	0 (0)	134 (100)

D.: *Debaryomyces* ***Schizo.***: *Schizosaccharomyces* ***K.***: *Kluyveromyces*
P.: *Pichia* ***Zygo.***: *Zygosaccharomyces* ***T.***: *Torulaspora*
S.: *Saccharomyces* ***Crypt.***: *Cryptococcus* ***C.***: *Candida*
H.: *Hansenula* ***Rh.***: *Rhodotorula*

[1]:Percentage of yeast isolates from sugarcane juice [2]: Percentage of yeast isolates from honey
[3]:Percentage of total yeast isolates

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الحمل الميكروبى والتقسيم العددى للخمائر المرتبطة بالأغذية ذات التركيز العالى من السكر

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أجريت هذه الدراسة لعزل وتعريف الخمائر السائدة فى الأغذية ذات التركيز العالى من السكر وكذلك لكى يكون لدينا صورة واضحة عن الميكروبات الموجودة فى تلك المنتجات، تم تحديد العدد الكلى للبكتريا والبكتريا المحبة للسكر ومجموعة القولون Coliforms ، بكتريا *Echerichia coli* البرازية ، *Staphylococcus spp* ، *Salmonella* and *Shigella* وكذلك الخمائر والفطريات وذلك فى عصير القصب ، عصائر الفاكهة المعلبة (المانجو – الكوكتيل)، المربى (الفراولة – التين) ، عسل النحل والحلاوة الطحينية. تم عزل ١٣٤ عزلة من الخمائر من بعض هذه الأغذية وتم تصنيفها بالأضافة الى ٣١ سلالة خميرة قياسية وذلك باستخدام الأختبارات المورفولوجية والبيوكيميائية. وقد تم حساب باستخدام برنامج A Vernetzungsdiagram EDV . وقد تم تقسيم الـ ١٣٤ عزلة بجانب ٣١ سلالة قياسية إلى ١٦ مجموعة تضم ١٦ نوعا من الخميرة تشكل مجموعات عالية التشابه (١٢ مجموعة تكون جراثيم *Saccharomyces cerevisiae, Torulaspora delbrueckii, Debaryomyces hansenii, D. polymorphus, Cryptococcus albidus, Kluyveromyces phaffi, Hansenula anamala, Schizosaccharomyces pombe, Zygosaccharomyces bailii, Pichia fermentants, K. marxianus var bulgaricus* and *Zygo. rouxii* بينما ٤ مجاميع لاتكون جراثيم *Candida zeylanoides, C. parapsilosis, C. sake* and *Rhodotorula* . لقد وجد معظم الخمائر الأساسية فى عصير القصب مكونة للجراثيم (68.59%) وتشمل *S. cerevisiae glutinis* (11.57%), *T. delbrueckii* (9.91%), *D. hansenii* (9.09%), *Zygo. bailii* (6.61%) and *D. polymorphus* (5.78%), *K. phaffi* (4.13%), *K. m. var bulgaricus* (4.95%), *Zygo. rouxii* (3.30%), *Crypt. albidus* (2.47%), *H. anamala* (3.30%), *Schizo. pombe* (2.47%), *P. fermentants* (4.95%),بالإضافة لغير المكونة للجراثيم (31.38%) وتشمل : *C. zeylanoides* (10.74 %), *C. parapsilosis* (9.91%), *C. sake* (4.95%) and *Rh. glutinis* (5.78%). وكانت أنواع الخمائر السائدة في العسل هي: *Crypt. albidus* (38.46%), *K. phaffi* (14.38%), *C. zeylanoides* (15.28%), *D. hansenii*(7.69%), *D. polymorphus* (7.69%), *S. cerevisiae* (7..69%), *P. fermentants* (7.69%) ، وقد وجد إن استخدام برنامج AVernetzungsdiagram بالإضافة للتقسيم العددى والصفات المورفولوجية والكيميائية وسيلة جيدة لتصنيف الخمائر.

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