

Full Paper

Characterization of the yeast flora present in some Turkish high-sugar products

Sule Senses-Ergul and Zekiye Yesim Ozbas*

Hacettepe University, Faculty of Engineering, Food Engineering Department, Beytepe 06532, Ankara, Turkey

(Received July 4, 2005; Accepted February 10, 2006)

In this study, 129 Turkish high-sugar products were examined in terms of their yeast flora and 73 representative strains were isolated. Yeast isolates were identified at species level by using Api-lab Plus (bioMérieux, France), a specific computer program developed for ID 32C strips (bioMérieux, France). While one of the isolates could be identified at genus level as *Aureobasidium*, 66 of them were identified as 21 species belonging to 8 different genera. The distribution of these isolates were as follows: *Candida* (38), *Rhodotorula* (8), *Zygosaccharomyces* (7), *Cryptococcus* (6), *Saccharomyces* (3), *Debaryomyces* (2), *Pichia* (1) and *Torulasporea* (1). Approximately 70% of the isolates were found to have the ability to grow on media with 50% (w/w) glucose. Hence, they were characterized as xerotolerant strains. Although *Zygosaccharomyces rouxii* is known as the most xerotolerant yeast species, only two strains of *Z. rouxii* could be isolated from Turkish high-sugar foods. During identification studies, it was observed that ID 32C test strips should certainly be supported by morphological and physiological tests for obtaining more reliable identification results. If not, closely related yeast species such as anamorph and telemorph forms can not be distinguished.

Key Words—high sugar foods; identification; xerotolerance; yeast

Introduction

Yeasts are widely distributed in nature and have extremely diverse metabolic capabilities. They can utilize a wide range of nutrients under variable environmental conditions (Tornai-Lehoczki et al., 2003). Yeasts have been reported to be significant spoilage organisms, especially in foods with low pH, high sugar content, high salt content and in foods containing sorbate and benzoate as preservatives, as well as in the presence of alcohol where most bacterial species are inhibited (Evans et al., 2004; Paraphailong and Fleet, 1997).

Many environmental factors affect the yeast growth, but the response to any particular condition varies with the species (Paraphailong and Fleet, 1997). For example, foods containing high levels of salt and sugar will select for the yeasts capable of growing under low water activity (a_w) values, such as *Debaryomyces hansenii* in the presence of high salt and *Zygosaccharomyces rouxii* in the presence of high sugar concentrations (Betts et al., 1999).

It has previously been reported that the spoilage of various high-sugar products, such as honey, maple syrup, dried fruits, concentrated fruit juices, raw sugar cane, jams and jellies, was caused by yeast activity (Tokouka, 1993). These kinds of high-sugar products are known to be susceptible to spoilage only by xerophilic molds and xerotolerant yeasts (Deak and Beuchat, 1996). There is a clear correlation between the distribution of the yeast species and the type of

* Address reprint requests to: Dr. Zekiye Yesim Ozbas, Hacettepe University, Faculty of Engineering, Food Engineering Department, Beytepe 06532, Ankara, Turkey.

Tel: +90 312 297 71 12 Fax: +90 312 299 21 23

E-mail: yesim@hacettepe.edu.tr

their sources (Tornai-Lehoczki et al., 2003). In order to design adequate strategies to prevent spoilage, it is advantageous to know the identity of the spoilage organisms present in the products and to get an insight into the source of contamination (Loureiro, 2000).

With this study, we have intended to isolate and identify the yeasts present in some Turkish high-sugar products. In order to achieve an approach for preventing yeast spoilage in these foods, the isolates were characterized in terms of their physiological properties.

Materials and Methods

Media. Four different selective media were used for the isolation of yeast flora. These were Dichloran 18% Glycerol (DG18) agar (10.0 g glucose, 5.0 g peptone, 1.0 g KH_2PO_4 , 0.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 220 g glycerol, 15.0 g agar, 0.002 g dichloran, 0.1 g chloramphenicol to 800 ml distilled water (dw), a_w : 0.96), Tryptone Glucose Yeast Extract Chloramphenicol (TGY) agar (100.0 g glucose, 5.0 g tryptone, 5.0 g yeast extract, 0.1 g chloramphenicol, 15.0 g agar per L, a_w : 0.98), Malt extract Yeast extract 50% Glucose (MY50G) agar (10.0 g malt extract, 2.5 g yeast extract, 500 g glucose, 10 g agar to 500 g dw, a_w : 0.89) and Chloramphenicol Malt extract Yeast extract 40% Sucrose (CMY40S) agar (20.0 g malt extract, 5.0 g yeast extract, 400.0 g sucrose, 0.01% (w/v) chloramphenicol, 20.0 g agar to 600 g dw, a_w : 0.95). The CMY40S medium used in this study was modified by us from that originally developed as Malt extract Yeast extract 50% Sucrose (MY50S) agar by Beuchat (1998), and this medium was successfully used in our previous studies (Senses, 2003). Tryptone Glucose Yeast extract (TGY) broth (100.0 g glucose, 5.0 g tryptone, 5.0 g yeast extract per L) was used as enrichment medium. Thirty percent (w/w) glycerol was used as dilution medium to prevent the osmotic shock of the yeast strains.

Food samples. In this study, mainly four groups of high-sugar products—fruit yoghurt and quark, dried fruits, honey in the comb and various confectionery products—were investigated for the presence of yeast microflora. For this purpose, a total of 129 food samples were examined. Fifty samples of fruit yoghurt, 4 samples of fruit quark, 10 samples of honey and 31 samples of dried fruits including apricot, fig, plum, pear, date, cherry and mulberry were collected from retail markets in Ankara. In addition to these, 34 sam-

ples of various confectionery products including molasses, jams, fruit cakes, pudding, refined sugar, fruit juice nectars and concentrates of different firms were also investigated.

Sampling procedure. Sampling was performed by aseptically transferring 10 g of food sample into TGY broth and mixing thoroughly. Then, the inoculated medium were pre-incubated at 30°C for 24 h in order to increase the chance of yeast isolation. After this enrichment period, a series of dilutions was made and aliquots (0.1 ml) of the appropriate dilutions were surface plated onto the isolation media. Inoculated plates were incubated at 30°C for 24 h. All visually different yeast colonies grown on the media were selected and pure cultures were obtained on Yeast extract Malt extract (YM) medium. In this way, 73 yeast isolates were obtained.

Identification and characterization of the isolates. Yeast isolates were identified at species level by using Apilab Plus, a specific computer program developed for ID 32C strips (bioMérieux, France). Additionally, some other physiological and morphological characteristics were also determined (Barnett et al., 2000; Kurtzman and Fell, 2000; Pitt and Hocking, 1997).

Colony morphologies of the isolates were determined on Malt Extract Agar (MEA) after 4 days of incubation at 30°C (Pitt and Hocking, 1997). Colony colors, size and the shape of well-separated colonies (regular/irregular and convex/umbonate) were recorded. Cell morphology, type of vegetative reproduction and growth characteristics of the isolates in broth medium were determined in YM broth. Ascospore formation of the cultures was examined on McClary Acetate and Gorodkova agar media. The inoculated media were incubated at 25°C for 3 weeks and examined at 7-day intervals. Formation of pseudohyphae was examined on Potato Dextrose Agar (PDA) according to the Dalmau plate technique (Yarrow, 2000).

Carbon assimilation characteristics of the isolates were determined by using ID 32C strips according to the manufacturer's instructions. ID 32C strips have 30 cupules containing different carbohydrates and one containing actidione (cycloheximide).

Nitrate assimilation of the strains was tested on Czapek agar medium (Pitt and Hocking, 1997; Yarrow, 2000). Glucose fermentation, urea hydrolysis, growth on media with 0.5% and 1% acetic acid, growth on media with 50% and 60% (w/w) glucose, growth at 37°C, growth in media with 10% NaCl–5% glucose

Table 1. Number of food samples with yeast contamination.

Foods	Yeast positive samples/Total samples
Fruit yoghurt	9/50
Quark	4/4
Dried fruit	24/31
Honey	8/10
Others	5/34
Total	50/129

and 16% NaCl–5% glucose and gelatin hydrolysis were performed according to the conventional methods of Yarrow (2000) and Pitt and Hocking (1997). Growth in media with 10% NaCl–5% glucose and 16% NaCl–5% glucose and gelatin hydrolysis tests were performed for some isolates when it was thought to be necessary.

Results and Discussion

The ratio of yeast positive food samples are shown in Table 1. A total of 129 food samples were investigated and 50 of them were found positive in terms of yeast flora. The number of the yeast positive samples were relatively low in fruit yoghurts and confectionery products. Many early studies have shown that sources of yeast contamination in fruit yoghurt were generally fruits, sugar and aroma compounds included into their composition (Deak and Beuchat, 1996; Fleet, 1992; Pitt and Hocking, 1997). Although we have mostly selected swollen packages of fruit yoghurt for investigation, only nine of them (18%) were found to be contaminated with yeasts. Of the 34 tested confectionery products only 5 of them (15%) were contaminated with yeasts. The majority of yeast-negative confectionery samples were mostly fruit juice nectars probably due to the thermal processes applied during their manufacture. During the study, 10 honey samples were investigated and eight of them (80%) were determined as yeast-positive. In addition, 31 dried fruit samples were tested for their yeast flora and the majority of them (77%) were found yeast-positive. Yeasts are a part of natural flora in these foods. So, intensive yeast contamination in dried fruit and honey samples are thought to be related with natural flora and poor handling processes applied during production.

By the end of isolation studies 73 yeast isolates

were obtained and reserved for identification. On the basis of the results obtained with ID 32C test strips, 11 yeast strains were identified at genus level and 56 of them were identified at species level. The results of ID 32C tests are stated in Table 2. For the supporting identification of the yeasts, additional identification tests were also performed. By this method a total of 66 isolates could be identified at species level and one of them at the genus level. The results of the additional tests are shown in Table 3. As a result of the tests performed, one of the strains could be identified at the genus level as *Aureobasidium*. The others were identified as 21 species belonging to 8 different genera. The majority of the isolates (38) were identified in the genus *Candida*. Other yeast strains were identified in the genera *Rhodotorula* (8), *Zygosaccharomyces* (7), *Cryptococcus* (6), *Saccharomyces* (3), *Debaryomyces* (2), *Pichia* (1) and *Torulasporea* (1). All the strains assimilated glucose. Few of the yeast strains were able to assimilate lactose, inositol, erythritol, or melibiose and few of them were able to grow in the presence of acidification (Table 2). In Table 3, it can be seen that most of the isolates had the ability to ferment glucose and to grow at 37°C. Few of the isolates identified as *Candida colliculosa*, *Candida glucosophila*, *Debaryomyces hansenii* or *Saccharomyces cerevisiae* were able to grow on 0.5% acetic acid medium. Furthermore, none of the isolates could grow on medium containing 1% acetic acid. Additionally, teleomorph and anamorph states of the isolates could be differentiated by ascospore formation test.

Because the study was performed to isolate the yeasts present in high-sugar foods, the isolates were thought to have xerotolerant characteristics. Ability to grow on media containing 50% and 60% (w/w) glucose has been defined as a distinctive feature for determining the xerotolerant characteristic of yeasts (Pitt and Hocking, 1997). Table 4 shows that the majority of the strains (70%) were able to grow on media containing 50% (w/w) glucose. However, only thirteen (19%) of the identified yeast strains had the ability to grow on 60% (w/w) glucose media. In this study, *Candida intermedia*, *Candida pelliculosa*, *Candida sake*, *Zygosaccharomyces mellis* and *Zygosaccharomyces rouxii* strains were determined to grow on both of the media and to have xerotolerant characteristics (Table 4). Deak and Beuchat (1996) have also referred to *Z. rouxii* and *Z. mellis* as xerotolerant yeast species mostly isolated from high-sugar products. The existence of the other

Table 2. ID 32C assimilation test results.

Substrate	Yeast species											
	<i>Aureobasidium</i>	<i>C. colliculosa</i>	<i>C. famata</i>	<i>C. globosa</i>	<i>C. glucosophila</i>	<i>C. guilliermondii</i>	<i>C. intermedia</i>	<i>C. kefyi</i>	<i>C. krusei</i>	<i>C. lambica</i>	<i>C. parapsilosis</i>	<i>C. pelliculosa</i>
Galactose	+	v	+	-	-	+	+	+	-	-	+	v
Actidion	+	-	-	-	-	v	-	+	-	-	-	-
Sucrose	+	+	+	-	-	+	+	+	-	-	+	+
<i>N</i> -Acetyl-glucosamine	+	v	+	+	-	+	+	v	+	+	+	-
DL-Lactate	+	+	v	-	+	v	-	v	+	+	-	-
L-Arabinose	+	-	+	-	-	+	-	v	-	-	+	-
Cellobiose	+	-	v	-	-	+	+	v	-	-	-	v
Raffinose	+	+	+	-	-	+	-	+	-	-	-	+
Maltose	+	-	+	+	-	+	+	v	-	-	+	+
Trehalose	+	v	+	-	-	+	+	v	-	-	v	v
2-Keto-gluconate	+	+	+	-	-	+	+	v	-	-	v	-
α -Methyl-D-glucoside	+	v	+	+	-	+	+	v	-	-	v	+
Mannitol	+	+	+	+	-	+	+	v	-	-	+	+
Lactose	+	v	v	-	-	-	v	v	-	-	-	-
Inositol	-	-	-	+	-	-	-	-	-	-	-	-
Sorbitol	+	+	+	+	-	+	+	+	-	-	+	+
D-Xylose	+	v	v	-	-	+	+	+	-	+	+	v
Ribose	+	-	v	-	-	v	+	v	-	-	v	-
Glycerol	+	+	+	+	+	+	+	+	+	+	+	+
Rhamnose	+	-	v	-	-	v	-	-	-	-	-	-
Palatinose	+	-	+	+	-	+	+	v	-	-	+	+
Erythritol	+	-	v	-	-	-	-	-	-	-	-	+
Melibiose	+	-	-	-	-	v	-	v	-	-	-	-
Glucuronate	+	-	-	-	-	v	-	-	-	-	-	-
Melezitose	+	-	+	-	-	v	+	v	-	-	+	-
Gluconate	+	-	v	-	-	v	+	-	-	-	+	-
Levulinate	+	-	-	-	-	v	+	-	-	-	v	-
Glucose	+	+	+	+	+	+	+	+	+	+	+	+
Sorbose	+	v	+	-	-	v	+	-	+	-	v	-
Glucosamine	-	-	-	-	-	v	+	v	+	-	+	-
Esculin	+	v	v	+	-	v	+	+	-	-	-	+

+, Growth; -, no growth; v, variable.

Table 2. (Continued).

Substrate	Yeast species									
	<i>C. pulcherrima</i>	<i>C. sake</i>	<i>Cry. albidus</i>	<i>D. hansenii</i>	<i>P. anomala</i>	<i>R. glutinis</i>	<i>S. cerevisiae</i>	<i>Tr. delbrueckii</i>	<i>Z. mellis</i>	<i>Z. rouxii</i>
Galactose	+	+	v	+	+	v	+	-	v	+
Actidion	-	-	-	-	-	v	-	-	-	-
Sucrose	+	+	+	+	+	+	+	+	-	-
N-Acetyl-glucosamine	+	+	-	+	-	-	-	-	-	-
DL-Lactate	-	-	-	v	+	-	v	+	-	-
L-Arabinose	-	-	+	-	-	v	-	-	-	-
Cellobiose	+	-	+	+	-	v	-	-	-	-
Raffinose	-	-	+	+	+	+	+	+	-	-
Maltose	+	+	+	+	+	+	+	+	-	-
Trehalose	+	+	+	+	+	+	v	+	-	-
2-Keto-gluconate	+	+	+	+	-	v	-	+	-	-
α -Methyl-D-glucoside	+	+	v	+	+	-	-	+	-	-
Mannitol	+	+	v	+	+	+	-	+	v	v
Lactose	-	-	v	-	-	-	-	-	-	-
Inositol	-	-	v	-	-	-	-	-	-	-
Sorbitol	+	+	+	+	+	v	-	+	-	-
D-Xylose	+	-	+	+	+	+	-	-	-	-
Ribose	+	-	-	-	+	v	-	-	-	-
Glycerol	+	+	-	+	+	v	-	+	v	v
Rhamnose	-	-	v	v	-	v	-	-	-	-
Palatinose	+	+	+	+	+	+	v	-	-	-
Erythritol	-	-	-	+	+	-	-	-	-	-
Melibiose	-	-	-	-	-	-	-	-	-	-
Glucuronate	-	-	+	v	-	-	-	-	-	-
Melezitose	+	-	+	+	+	-	-	-	v	-
Gluconate	+	+	v	+	-	v	-	-	-	-
Levulinate	-	+	-	-	-	v	-	-	-	-
Glucose	+	+	+	+	+	+	+	+	+	+
Sorbose	+	+	-	-	-	v	v	+	-	-
Glucosamine	+	+	-	-	-	-	-	-	-	-
Esculin	+	+	+	+	-	v	-	-	-	-

+, Growth; -, no growth; v, variable.

Table 3. Results of the additional identification tests.

Tests	Yeast species																					
	<i>Aureobasidium</i>	<i>C. colliculosa</i>	<i>C. famata</i>	<i>C. globosa</i>	<i>C. glucosophila</i>	<i>C. guilliermondii</i>	<i>C. intermedia</i>	<i>C. kefyri</i>	<i>C. krusei</i>	<i>C. lambica</i>	<i>C. parapsilosis</i>	<i>C. pelliculosa</i>	<i>C. pulcherrima</i>	<i>C. sake</i>	<i>Cry. albidus</i>	<i>D. hansenii</i>	<i>P. anomala</i>	<i>R. glutinis</i>	<i>S. cerevisiae</i>	<i>Tr. delbrueckii</i>	<i>Z. mellis</i>	<i>Z. rouxii</i>
Glucose fermentation	-	+	v	+	+	v	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+
Growth at 37°C	+	-	v	-	+	+	+	+	+	+	+	+	+	-	v	+	+	v	+	+	+	-
Urea hydrolysis	+	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-
Growth on 0.5% acetic acid medium	-	v	-	-	+	-	-	-	-	-	-	-	-	-	-	v	-	-	v	-	-	-
Growth on 1% acetic acid medium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nitrate assimilation	+	v	v	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	-
Ascospore formation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	v	v
Pseudohyphae formation	+	-	-	-	+	v	-	+	-	+	+	v	-	+	-	+	+	-	-	-	-	-
Growth on 10% NaCl-5% glucose containing media	+	*	*	*	*	*	*	*	*	+	*	*	*	*	*	*	*	*	*	*	+	+
Growth on 16% NaCl-5% glucose containing media	*	*	*	*	*	*	*	*	*	-	*	*	*	*	*	*	*	*	*	*	-	+
Gelatin hydrolysis	+	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

+, Growth; -, no growth; v, variable; *, not investigated.

yeast species is thought to be related with the selective habitat present in the examined foods. Even though the yeast strains identified as *Candida kefyri*, *Candida krusei*, *Cryptococcus albidus* and *Rhodotorula glutinis* were isolated from high-sugar products, it was observed that none of them were able to grow on media containing 50% or 60% (w/w) glucose. However, these yeasts have been reported as xerotolerant species (Deak and Beuchat, 1996).

The diversity of the identified yeasts according to their food origin is stated in Table 5. While 32 of the yeast strains were isolated from dried fruits, 15 of them were obtained from honey samples. Nine of the strains were obtained from fruit yoghurt and 4 of them were isolated from fruit quark samples. Additionally, 7 of the isolates were obtained from various confectionery products. Since yeasts are a part of the agricultural microflora, a broad spectrum of yeasts were isolated from dried fruits, as expected. As shown in Table 5, the most dominant species in dried fruits were found to be

Candida guilliermondii (8), *Cry. albidus* (5), *C. colliculosa* (4), *C. pelliculosa* (3) or *R. glutinis* (3). Our results showing higher diversity of the yeast species isolated from dried fruits agree with the experimental evidence obtained by the other authors (Brackett, 2001; Fleet, 1992; Pitt and Hocking, 1997). In our study, most of the honey samples were determined to have yeast flora and 16 isolates were obtained. Five of the honey isolates were defined as *Z. mellis*. *Z. mellis* strains have been reported to be found mostly in habitats with low a_w values and commonly isolated from honey (Kurtzman, 1990). Only one of the strains isolated from honey was identified in the genus *Aureobasidium*. While 3 of the honey isolates were determined to be *R. glutinis*, the others were *C. colliculosa*, *Candida globosa*, *C. pelliculosa*, *D. hansenii*, *Pichia anomala*, *S. cerevisiae* and *Torulasporea delbrueckii*. In a study undertaken with Japanese honey samples, it has been reported that the yeast strains were identified in the genera *Rhodotorula*, *Debaryomyces*, *Hansenula*,

Table 4. Growth on media of high glucose content.

Yeast	MY50G	MY60G
<i>Aureobasidium</i>	1/1*	0/1
<i>Candida colliculosa</i>	5/5	0/5
<i>C. famata</i>	3/3	0/3
<i>C. globosa</i>	1/1	0/1
<i>C. glucosophila</i>	1/1	0/1
<i>C. guilliermondii</i>	9/12	0/12
<i>C. intermedia</i>	2/2	2/2
<i>C. kefyri</i>	0/2	0/2
<i>C. krusei</i>	0/1	0/1
<i>C. lambica</i>	1/1	0/1
<i>C. parapsilosis</i>	4/4	0/4
<i>C. pelliculosa</i>	4/4	4/4
<i>C. pulcherrima</i>	1/1	0/1
<i>C. sake</i>	1/1	1/1
<i>Cryptococcus albidus</i>	0/6	0/6
<i>Debaryomyces hansenii</i>	2/2	0/2
<i>Pichia anomala</i>	1/1	0/1
<i>Rhodotorula glutinis</i>	0/8	0/8
<i>Saccharomyces cerevisiae</i>	3/3	0/3
<i>Torulopsis delbrueckii</i>	1/1	0/1
<i>Zygosaccharomyces mellis</i>	5/5	4/5
<i>Z. rouxii</i>	2/2	2/2
Total	47/67	13/67

* Numbers of positive isolates/total numbers of isolates.

Lipomyces, *Oosporidium*, *Pichia*, *Torulopsis* and *Trichosporon* (Snowdon and Cliver, 1996). In another study, the most dominant yeast species in honey samples were found to be *Saccharomyces* species (Deak and Beuchat, 1996). The isolates of the other samples mostly composed of confectionery products were identified as *C. guilliermondii* (2), *C. intermedia* (1), *C. parapsilosis* (1), *Cry. albidus* (1), *R. glutinis* (1) and *S. cerevisiae* (1). These strains were isolated from refined sugar, glucose syrup, a traditional Turkish desert known as cezerye and various confectionery products. Because of their tolerance to low a_w values, these yeasts have been reported to be important spoilage organisms in high-sugar products (Betts et al., 1999). Of the identified 67 yeast strains, 9 of them were isolated from fruit yoghurt and 4 of them were isolated from quark samples. The majority of these strains (12) were identified in the genus *Candida*. Only one of the isolates was identified as *R. glutinis*. *Candida famata*, *C. glucosophila* and *C. guilliermondii* have been reported to be included in *Candida* species having xerotoler-

ance and the ability to grow on foods with high-sugar concentrations. Similarly, *Rhodotorula* species including *R. glutinis*, have also been referred as yeasts which are able to tolerate low a_w values (Deak and Beuchat, 1996).

In this study, ID 32C and some additional tests were used for the identification and characterization of the yeast isolates. Although the software of the Apilab Plus computer program was mainly composed of the clinical species, we could identify the majority of our isolates (92%). However, we have also observed that ID 32C strips should certainly be supported by additional tests including morphological and physiological methods. Otherwise, it is thought that the system would not be adequate to differentiate yeast species which are closely related to each other. For example, an isolate identified as *Cryptococcus humicolus* by ID 32C was determined to be *Aureobasidium* with additional tests. Beside this, four isolates identified as *C. famata*, *C. pelliculosa*, *C. colliculosa* were included in the species of *D. hansenii*, *P. anomala* and *Tp. delbrueckii* with the help of ascospore formation test.

The presence of yeasts in swollen packages of fruit yoghurt and quark samples indicates that these foods are sensitive to spoilage caused by yeast activity. It is thought that the existence of yeast in these products is the result of unsuitable hygienic conditions in the production area. Additionally, the use of improperly prepared ingredients such as fruit puree and sugar may be another reason for the yeast contamination. The presence of yeasts in honey samples was thought to be related with the natural flora of the products. Yeasts are similarly known as a part of the natural flora of dried fruits. Considering the drying conditions of the fruits, a higher diversity and a higher degree of microbial population are expected in sundried fruits. Hence, it is thought that the level of microbial load may be reduced by drying in more hygienic conditions.

In this study, of the 66 yeast isolates only two of them were identified as *Z. rouxii*. However, *Z. rouxii* is reported to be the most xerotolerant yeast species found in high-sugar products (Deak and Beuchat, 1996; Pitt and Hocking, 1997). The yeast species such as *Zygosaccharomyces bailii*, generally known with xerotolerant characteristic and mostly isolated from high-sugar products, could not be found in the screened Turkish foods. The other identified flora were found mostly to be similar to those of previously reported studies.

Table 5. Distribution of the yeast species according to the food origin.

Yeast species	Ratio*	Fruit yoghurt	Quark	Honey	Dried fruit	Other
<i>Aureobasidium</i>	1/67	–	–	1	–	–
<i>Candida colliculosa</i>	5/67	–	–	1	4	–
<i>C. famata</i>	3/67	1	1	–	1	–
<i>C. globosa</i>	1/67	–	–	1	–	–
<i>C. glucosophila</i>	1/67	1	–	–	–	–
<i>C. guilliermondii</i>	12/67	2	–	–	8	2
<i>C. intermedia</i>	2/67	–	–	–	1	1
<i>C. kefyri</i>	2/67	2	–	–	–	–
<i>C. krusei</i>	1/67	1	–	–	–	–
<i>C. lambica</i>	1/67	1	–	–	–	–
<i>C. parapsilosis</i>	4/67	–	3	–	–	1
<i>C. pelliculosa</i>	4/67	–	–	1	3	–
<i>C. pulcherrima</i>	1/67	–	–	–	1	–
<i>C. sake</i>	1/67	–	–	–	1	–
<i>Cryptococcus albidus</i>	6/67	–	–	–	5	1
<i>Debaryomyces hansenii</i>	2/67	–	–	1	1	–
<i>Pichia anomala</i>	1/67	–	–	1	–	–
<i>Rhodotorula glutinis</i>	8/67	1	–	3	3	1
<i>Saccharomyces cerevisiae</i>	3/67	–	–	1	1	1
<i>Torulaspota delbrueckii</i>	1/67	–	–	1	–	–
<i>Zygosaccharomyces mellis</i>	5/67	–	–	5	–	–
<i>Z. rouxii</i>	2/67	–	–	–	2	–

* Number of isolates/number of total isolates.

Acknowledgments

This study was financially supported by Scientific Research Unit of Hacettepe University (Project number: 0101602003).

References

- Barnett, J. A., Payne, R. W., and Yarrow, D. (2000) Yeasts: Characteristics and Identification, Cambridge University Press, UK, 1139 pp.
- Betts, G. D., Linton, P., and Betteridge, R. J. (1999) Food spoilage yeasts: Effects of pH, NaCl and temperature on growth. *Food Control*, **10**, 27–33.
- Beuchat, L. R. (1998) Progress in conventional methods for the detection and enumeration of foodborne yeasts. *Food Tech. Biotech.*, **36/4**, 267–272.
- Brackett, R. E. (2001) Microbial spoilage of foods: Fruits, vegetables and grains. In *Food Microbiology. Fundamentals and Frontiers*, ed. by Doyle, M. P., Beuchat, L. R. and Montville, T. J., ASM Press, Washington, pp. 127–138.
- Deak, T. and Beuchat, L. R. (1996) *Handbook of Food Spoilage Yeasts*, CRC Press, USA.
- Evans, D. G., Everis, L. K., and Betts, G. D. (2004) Use of survival analysis and classification and regression trees to model the growth/no growth boundary of spoilage yeasts as affected by alcohol, pH, sucrose, sorbate and temperature. *Int. J. Food Microbiol.*, **92**, 55–67.
- Fleet, G. (1992) Spoilage yeasts. *Crit. Rev. Biotech.*, **12(1/2)**, 1–44.
- Kurtzman, C. P. (1990) DNA relatedness among species of the genus *Zygosaccharomyces*. *Yeast*, **6**, 213–219.
- Kurtzman, C. P. and Fell, J. W. (2000) *The Yeasts: A Taxonomic Study*, Fourth revised and enlarged edition, Elsevier, Amsterdam, 1035 pp.
- Loureiro, V. (2000) Spoilage yeasts in foods and beverages: Characterisation and ecology for improved diagnosis and control. *Food Res. Int.*, **33**, 247–256.
- Paraphailong, W. and Fleet, G. H. (1997) The effect of pH, sodium chloride, sucrose, sorbate and benzoate on the growth of food spoilage yeasts. *Food Microbiol.*, **14**, 459–468.
- Pitt, J. I. and Hocking, A. D. (1997) *Fungi and Food Spoilage*, University Press, Cambridge, 577 pp.
- Senses, S. (2003) Media optimization for xerotolerant yeasts and isolation-identification of these yeasts from some high-sugar products. M.Sc. thesis, Hacettepe University, Turkey.
- Snowdon, J. A. and Cliver, D. O. (1996) Microorganisms in honey. *Int. J. Food Microbiol.*, **31**, 1–26.
- Tokouka, K. (1993) Sugar- and salt tolerant yeasts. *J. Appl. Bacteriol.*, **74**, 101–110.
- Tornai-Lehoczki, J., Péter, G., and Dlačny, D. (2003) CHRO-Magar *Candida* medium as a practical tool for the differentiation and presumptive identification of yeast species isolated from salads. *Int. J. Food Microbiol.*, **86**, 189–200.
- Yarrow, D. (2000) Methods for the isolation, maintenance and identification of yeasts. In *The Yeasts: A Taxonomic Study*, ed. by Kurtzman, C. P. and Fell, J. W., Elsevier, Amsterdam, pp. 77–100.