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Professional Paper

PREVALENCE OF CANDIDA SPECIES IN THE FRESH FRUIT JUICES*

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Fruit juices are popular soft drinks with an important role in human nutrition. Fruit juices are often infested by yeast species that can survive different storage conditions. The aim of this study was to determine the degree of yeast contamination of freshly squeezed juices in three large supermarkets in Zagreb, Croatia. The analysis included 84 juice samples obtained from freshly squeezed orange, lemon, grapefruit, and apples. Their acidity varied between pH 2.1 and pH 4.9. Juice samples were plated directly on Sabouraud 4 % glucose Agar (Merck, 1.05438) and processed according to standardised methods (HRN ISO 7954:2002). Yeasts were isolated in all 84 samples and ranged between $0.005x10^3$ and $23x10^3$ colony forming units per mL (CFU mL^{-1}). The most common yeasts identified using the API 20C AUX yeast kit included *Candida guillermondii*, *C. krusei*, *C. famata*, *C. spherica*, *C. colliculosa*, *C. albicans, Trichosporon mucoides, Kloeckera* spp. and yeast-like fungus *Cryptococcus neoformans*. *C. guillermondii* prevailed in 55.95 % of all samples.

KEY WORDS: *health risk, soft drink, yeasts*

Fruit juices are popular soft drinks with an important role in human nutrition. They are advertised as very healthy food supplements containing a variety of vitamins necessary for the good bodily function, and of the immune system in particular.

Some supermarkets produce their own freshly squeezed fruit juices, which are often packed in attractive plastic packages and kept on ice. People who look for "healthy food" favour freshly squeezed juice without preservatives, aromas or colour over commercial brands. The most common consumers are the elderly and three to four-year-olds. Parents prefer to give their children a fresh natural beverage full of vitamins instead of water alone. Of freshly squeezed juices, citruses are the most popular (1). However, consumption of a freshly squeezed fruit juice within few days does not warrant good hygienic quality. In general, the acidity (pH) of orange or grapefruit juices between 3.5 and 3.9 and high sugar content (2) create favourable conditions for the growth of fungi. Sugar favours the development of a microbial biofilm. In addition, the fruit surface can contain different contaminants that end up in the freshly squeezed juice offered in markets. Inadequate cleaning of fruit processors can pose a risk for consumers (3). Yet, fruit juice does not undergo strict microbiological quality checks and there are no quick and simple fungal tests available.

Of more than 100,000 fungi species known today, a few hundred are opportunistic human pathogens (4).

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Since the late 20th century, the incidence of mycoses has increased as a consequence of an increasing number of people whose immunological system is weakened by chronic diseases, organ transplants, stress, or cancer. In the hospital environment, fungi can reach blood through various pathways such as catheters or directly from the gastrointestinal tract (5). A Danish epidemiological study demonstrated that *Candida albicans* caused fungemia in 63 % of clinical patients, while *C. krusei* was detected only in 3 % of patients (6).

Fungi can also spoil food and cause economic losses in the food industry. The most common yeast species that spoil soft drinks are *Zygosaccharomyces bailii* and *Brettanomyces naardenensis*. Other species associated with spoilage include *Saccharomyces, Candida, Torulopsis, Pichia, Hansenula,* and *Rodothorula* genera (7). The aim of this study was to determine fungal contamination of freshly squeezed juices sold in three large supermarkets in Zagreb, capital of Croatia.

MATERIALS AND METHODS

Eighty-four samples of freshly squeezed juices were collected at three large Zagreb supermarkets from March to August 2008. Juice varieties included orange, lemon, grapefruit, apple, and mixed fruit, all packed in 0.5 L bottles and kept on ice. Juice samples were transported to the laboratory in a handy refrigerator at +8 °C and analysed within one hour. Fungi were isolated according to the ISO 7954:2002 standard "Microbiology - General guidance for enumeration of yeasts and moulds - colony count technique at 25 °C". Samples were cultured on Sabouraud 4 % glucose agar (Merck, Germany). Plates were incubated at 25 °C for five days. The number of yeasts was expressed as colony forming units per mL (CFU mL^{-1}). Primary differentiation was based on yeast macromorphology, Gram staining, and microscopy. For biochemical identification we used commercial Api 20 C AUX kits (bioMérieux, France). Briefly, yeast suspensions were prepared to adjust turbidity to McFarland standard No. 2, and then we inoculated the commercial kit trays, and incubated them at 30 °C for 72 h. The results were compared with standards and read using the API® software (bioMérieux, France). All isolates were identified with the precision of >95 %.

RESULTS AND DISCUSSION

Table 1 shows pH values and isolated yeast colonies for different fruit juices. Apple juice was the least acidic and quite expectedly, lemon juice the most acidic. The highest number of yeast colonies was found in grapefruit juice, followed by orange juice, mixed fruit, lemon juice, and finally apple juice. Tournas et al. (8) found the highest number of yeasts in orange juice and the lowest in the apple juice.

Table 2 shows the frequency of isolated yeast species per fruit juice. The most were identified in the orange juice (*Cryptococus neoformans*, *Candida guilliermondii*, *Candida famata*, *Candida sphaerica*, *Kloeckera* spp., *Trichosporon mucoides*, *Candida krusei*, *Candida colliculosa*, and *Candida albicans*). Of sixty-six samples of orange juice *Candida guilliermondii* was found in 36 (54.55 %), *Candida famata* in 14 (21.21 %), and *Cryptococus neoformans* in seven (10.61 %). The other six yeast species were identified in only one or two samples. Of ten apple juice samples, *Candida guilliermondii* was detected in six (60 %) and *Cryptococus neoformans* and *Candida famata* in two (20 %). Three yeast species were identified in the remaining eight lemon, grapefruit, and mixed fruit juice samples (*Candida guilliermondii*, *Cryptococus neoformans*, and *Candida krusei*). Table 3 shows the distribution of yeast species in all juice samples.

Citrus fruits such as orange, lemon, and grapefruit are the most popular choices for fresh juice among consumers. A great majority of them is imported in Croatia from all over the world. Before the fruit hits the market it is exposed to a variety of contaminants and pests during transport, handling, and storage (8). However, freshly squeezed juices are often advertised and perceived as healthy and full of vitamins and natural sugar, even by people with health issues or immunodeficiency. Fungi tolerant to juice acidity include a variety of *Candida* species whose pathogenic potential is well known (9) and may pose a significant risk for immunocompromised individuals. A recent study reported that *Candida guilliermondii* caused candidemia in 19 % of examined patients with the prevalence in those suffering from haematological malignancies (9). In addition, it is known that *Candida albicans* and *Cryptococus neoformans* can cause general infection, especially in imunocompromised people (5, 6, 10, 11). Similar to our study, Limin et al. (12) also found the highest number of *Candida* isolates in unpasteurised apple juice. We identified

Table 1 *Acidity, number of yeast colonies, and species isolated from different fruit juice samples*

Fruit juice	Number of samples	Acidity / pH		Yeast number x10^3 / CFU mL^{-1}		Isolated species
		average	range	average	range	
Orange	66	3.5	3.0 to 4.1	3.3	0.21 to 23	*Cryptococcus neoformans* *Candida guilliermondii* *Candida famata* *Candida sphaerica* *Kloeckera* spp. *Trichosporon mucoides* *Candida krusei* *Candida colliculosa* *Candida albicans*
Apple	10	3.9	3.1 to 4.9	0.2	0.005 to 0.5	*Cryptococcus neoformans* *Candida guilliermondii* *Candida famata*
Lemon	3	2.2	2.1 to 2.3	1.3	1.1 to 1.6	*Cryptococcus neoformans* *Candida guilliermondii*
Grapefruit	3	3.2	3.1 to 3.2	5.4	3.3 to 8.6	*Candida guilliermondii* *Candida krusei*
Mixed fruits	2	3.7	3.5 to 3.9	1.8	0.4 to 3.1	Cryptococcus neoformans Candida guilliermondii
Total	84	3.5	2.1 to 4.9	2.9	0.005 to 23	

Table 2 *The frequency of isolated yeast species per fruit juice*

Fruit juice	Total N (%)	*Crypto-coccus neofor-mans* n (%)	*Candida guillier-mondii* n (%)	*Candida famata* n (%)	*Candida sphaerica* n (%)	*Kloeckera* spp. n (%)	*Tricho-sporon mucoides* n (%	*Candida krusei* n (%)	*Candida colliculosa* n (%)	*Candida albicans* n (%)
Orange	66 (78.6)	7 (10.6)	36 (54.6)	14 (21.2)	2 (3.0)	2 (3.0)	2 (3.0)	1 (1.5)	1 (1.5)	1 (1.5)
Apple	10 (11.9)	2 (20.0)	6 (60.0)	2 (20.0)	-	-	-	-	-	-
Lemon	3 (3.6)	1 (33.3)	2 (66.7)	-	-	-	-	-	-	-
Grapefruit	3 (3.6)	-	2 (66.7)	-	-	-	-	1 (33.3)	-	-
Mixed fruits	2 (2.4)	1 (50.0)	1 (50.0)	-	-	-	-	-	-	-

N – number of samples; n – positive samples

Kloeckera spp. in only 3.03 % of orange juice samples, while Tournas et al. reported its presence in 40 % of grapefruit juice samples (8). Additionally, they recovered *Rhodotorula rubra*, *Candida lambica,* and *Candida sake* from all fruit juices, among which *Candida lambica* prevailed (8). In contrast, Arias et al. (1) isolated completely different yeast species in orange juice (*Candida stellata, Hanseniaspora occidentalis, H. uvarum, Pichia fermentas, P. kluyveri,* and *Saccharomycopsis crataegensis*) dominated by *Hanseniaspora uvarum* and *Hanseniaspora occidentalis*.

CONCLUSION

In this study we detected a relatively high presence of yeasts in freshly squeezed juice, which suggests that the fruit had been contaminated during handling and processing. Therefore, to avoid juice spoilage

Table 3 *Isolated yeast species from all fruit juice samples (N=84)*

Isolated yeasts from juice samples	Number of samples	Percentage
Candida guilliermondii	47	55.95
Candida famata	16	19.05
Cryptococus neoformans	11	13.10
Candida krusei	2	2.38
Candida sphaerica	2	2.38
Kloeckera spp.	2	2.38
Trichosporon mucoides	2	2.38
Candida colliculosa	1	1.19
Candida albicans	1	1.19
Total	84	100

and possible health risk for immumocompromised people, it is necessary to ensure controlled and aseptic handling and processing conditions (1, 8).

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Sažetak

PRISUTNOST *CANDIDA* SP. U SVJEŽEM VOĆNOM SOKU

Voćni su sokovi tekući ekstrakti voća dobiveni cijeđenjem zrelog voća te su vrlo važan čimbenik u svakodnevnoj prehrani ljudi. Najčešće zastupljeni mikroorganizmi u svježem voćnom soku su kvasci koji preživljavaju niske temperature skladištenja. Svrha ovog istraživanja bila je odrediti prisutnost i brojnost kvasaca u svježim voćnim sokovima uzorkovanim u supermarketima na području Republike Hrvatske, odnosno glavnom gradu Zagrebu. Ukupno su uzorkovana i pregledana 84 uzorka svježe iscijeđenih naranči, limuna, grejpfruta i jabuka. pH-vrijednost se kretala od 2.1 do 4.9. Uzorci su nacijepljivani direktno na Sabouraudov agar s 4 % glukoze (Merck, Njemačka) u skladu s propisanom normom HRN ISO 7954:2002. U sva 84 uzorka utvrđena je prisutnost kvasaca u broju od $0.005x10^3$ do $23x10^3$ CFU mL^{-1}. Identifikacija je provedena testom API 20 C AUX (bioMérieux, 20 210). Najčešće su izolirani sljedeći kvasci: *Candida guillermondii, C. krusei, C. famata, C. spherica, C. colliculosa, C. albicans, Trichosporon mucoides, Kloeckera* spp. i kvascu slična gljivica *Cryptococcus neoformans*. U svim uzorcima *C. guillermondii* bila je najčešće izolirani kvasac (55.95 %).

KLJUČNE RIJEČI: *bezalkoholni napitak, kvasci, zdravstveni rizik*

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