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Occurrence of Fungi and Pectolytic Activity in Fruit Juices from Saudi Arabia

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Abstract: At 28°C for 14 days 12 species belonging to 3 genera were isolated from 26 kinds of juice. These were identified as *Curvularia lunata, Mycelia sterilia, Aspergillus* represented by *A. flavus, A. olivino viridi, A. parasiticus, A. viridi nutans, A. terreus, A. cervinus, A. ustus, A. nidulans A. fumigatus* and *A. niger,* the latter being predominant. But, no growth at 45°C for 14 days. About 74% of 50 samples could produce pectinase enzyme with different degrees. From the positive isolates 38% exhibited the highest pectinase production and these related to *A. flavus, A. fumigatus, A. niger, A. parasiticus* and *A. terreus.* Whereas, the remaining positive isolates were moderate and weak producers.

Key words: Fruit juices, spoilage fungi, beverage industry, mycotoxins, enzymes

Introduction

Fruit juices can be contaminated with mould spores, conidia and mycelium fragments from the environment. Saudi factories only pack the juices. Fruit juices contain various concentrations of sucrose, which constitute a very important media of fungi (Palou *et al.,* 1998).

Contamination can occur during packing and in handling of the end products. This contamination has been a serious and frequently disturbing some health problem and may lead to human illnesses caused by pathogenic fungi (Araya *et al.,* 1988; Oladiran and Iwu, 1993).

Few investigations have been done on fungi contaminating the fruit juices during growth and ripening of the fruit. This contamination with undesirable molds has been a serious and frequently disturbing problem (Abdel-Mallek *et al.*, 1995, Tournas, 1994; Moubasher *et al.,* 1993; Abrunhosa *et al.*, 2001).

The information on spoilage of heat-processed fruits and fruit products by these fungi is still scarce in developing country and its due to poorly developed fruit pressing industries, limited supply of processed fruit products as well as the absence of surveillance for these fungi (Obeta and Ugwuanyi, 1997).

Moulds associated with food spoilage like *Penicillium, Aspergillus, Citroyces, Byssochlams* (Gazrawey, 1989), and some of these fungi are known to produce a group of toxic metabolites known as a mycotoxins (El- Naghy and Fadal-allah, 1991; Bankole, 1993; Al-Granny and Al-Faso, 1997). Systemic infections with haematogenous spread throughout the body are serious and often fatal. They are uncommon except in immunocomporomized patients with impaired host defenses. who may develop widespread disease due to the yeast or filamentous fungi such as *Aspergillus* species (Sleigh and Timbury, 1998). Microbial pectinases play an important role within food idustry since they favour the extraction, clarification and reduction in viscosity of the fruit juices (Fogarty and Ward, 1974; Fogarty and Kelly, 1983; Solis *et al.,* 1990)

The aim of this study was to isolate and identify the common fungi occurrence in fruit juices and the ability of these fungi to produce pectolytic enzymes which are responsible for lyses of fruit cell walls and clarification of fruit juices.

Materials and Methods

Collection of samples: Twenty six kinds of fruit juices were collected from local markets in Jeddah (Pasha, Alwha, Sun top, Maaza, Areen, Karim, Rani, Saudi Sedafco, Al-Rabee, Lancor, Kortena, Partoleese, Mardena, Diamond, Jamjoom, Nada, Hoap, Tamam, Mana, Ceser, Snapple, Lancor, Danea, Vimto, Ranch)

Mycological analysis: One ml of each of tested juice using sterile Menziess (1957) dipper was transferred aseptically into each of ten plates and 12-15 ml of an appropriate glucose-Czapek's agar medium with the followiing composition, Na NO_3, 2g; K_2HPO_4, 1g, $MgSO_4.7H_2O$, 0.5 g; KCl, 0.5 g, yeast extract, 0.5g, glucose 10g; agar agar, 15g; per 1 liter. Rose- bengal (1/15000) was added as bacteriostatic agent, (Smith and Dawson, 1944), cooled to just above the solidifying temperature were added to each dish. The dishes are rotated by hand in a broad swirling motion, so that the juice is dispersed in the agar. Plates were incubated at 28° C, 45°C usually from 7-14 days (Moubasher *et al.*, 1975)

Pectinases production: Fifty strains representing 8 species which were isolated in the current study were screened to test their ability to produce extracellular pectolytic enzymes. Stock cultures were grown on potato- dextrose agar slants at 28°C and kept at 4°C. To detect clearance zones produced by the strains, a medium containing 0. 25% pectin and 2.0 % agar was used. The colonies grown after 72 h incubation at 28°C were cut out with a cork borer (1mm) and placed in petri dishes containing the previous medium and incubated for 48 h at 28°C. After this time 2 % iodine solution was added to detect clearance zones (Solis *et al.,* 1990). The diameter of the clear zone (mm) was recorded and the data were expressed as H= high producers (3.1- 4.5 mm), M= moderate producers (2.1- 3.0), W= weak producers (1.1- 2.0).

Results and Discussion

Fungi recovered at 28°C: All juices containing sodium benzoate and stored at 4-5 °C was protected from spoilage. But, some fungi can grow at 28° C for 14 days. Twelve species belonging to 3 genera were isolated (Table 1). *Aspergillus* was the most common genus constituting 98.84% of total fungi. It was represented by 10 species which are *A. flavus, A. olivino viridi, A. parasiticus, A. niger, A. viridi nutans, A. terreus, A. cervinus* and *A. fumigatus* were the predominant species. Our results are in agreement with those of Obeta & Ugwuanyi (1995). They screened 15 kinds of Nigerian fruit juices and noticed that approximately 17% of all fruit juices are contaminated with fungi. These fungi were identified as *Pacilomyces variotii, Aspergillus tamarii, A. flavus* and *A. ochraceus. Aspergillus niger* was the most common species, it was represented by 50.87 % of total fungi in juices. Rehm (1970) reported that it was the most important fungi occurring in fruit juices and have spoilage capacity and mycotoxin production. Park *et al.* (1972) recorded it with very active enzymes clarified pineapple juice and orange juice. This species was also the dominant species isolated from deteriorating oranges by Akpomedaye and Ejechi (1998), Sahin *et al.* (1998) who isolated this fungus from the storage tanks of apple juice concentrate. Abdel-Mallek *et al.* (1995) found this fungus from 84.6% of the healthy tomato fruits. *Aspergillus flavus* was the second most frequent fungal species. It was represented by 21.39 % of total fungi contaminating the juices. It was obtained from samples of rotting sweet orange and screened for aflatoxin production in a nutrient solution, (Bankole, 1993). This species was also, the dominant one isolated from deteriorating oranges by Akpomedaye and Ejechi (1998). The rest of the *Aspergillus* species (Table 1) are *A. olivino viridi* represented by 13.29 %, *A. ustus,* represented by 9.88% and *A. nidulans* by 4.05 % which *A. parasiticus, A. fumigatus* were isolated from two samples and

Table1: Counts of fungal genera and species recovered on glucose czapek's agar in one ml.

	Types of fruit juices																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Total count	1.2	2.6	1	0.6	1.2	1.2	1.2	2.6	1.4	3.2	2	1.8	0	1	1	2.2	0.6	0.2	3	2	1.4	0
Aspergillus	1.2	2.6	1	0.4	1.2	1.2	1.2	2.6	1.4	3.2	1.8	1.8		1	1	2.2	0.6	0.2	3	2	1.4	0
A.flavus	0.4	0.6		0.2	0.2	0.2	0.4	0.2	0.2	0.2						1.6	0.4	0.2	1.2		0.2	
A.olivino viridi	0.2	0.2	0.2		0.2	0.2		0.8			0.6								0.8	0.6		
A.parasiticus				0.2																		
A. niger	0.6	1.6	0.6	0.2	0.8	0.8	0.8	1.6	1.2	0.2	0.6	1.2		0.4	0.4	0.6	0.2		2	1.4	1	
A.viridi nutans											0.6	0.6							0.2			
A. terreus		0.2																				
A.cervinus															0.6	0.6						0.2
A.nidulans											2.8									0.6		
A. ustus																					0.2	
A. fumigatus																						
Curvularia lunata					0.2																	
Mycelia sterilia											0.2											
O.R.	M	H	M	L	M	M	M	H	M	H	H	M		M	M	H	L	R	H	H	M	

1-Pasha coconut & pineapple	2- Al- Waha orange	3- Sun Top orange	4-Maaza Mangoo
5-Areen orange	6- Karim mixed fruit	7- Rani orange	8-Saudi orange
9- Sedafco mixed fruit	10-Al-rabee orange	11-Tamam orange	12-Hope grape
13-Partoleese apple	14- Mardena orange	15-Vimto fruit flavour	16- Ranch orange
17- Three Diamond mixed fr	18- Jamjoom apple	19- Nada orange	20- Kortena orange
21- Lacnor apple	22-Mana mangoo	23- Ceser apple	24- Snapple mangoo madness cocktail
25- Lancor peach	26-Danea strawberry		

O. R.= Occurrence remarks: H= High occurrence, 2 and more; M= Moderate occurrence, 1-1.8; L= Low occurrence, 0.4-0.8; R= Rare occurrence, less than 0.4.

Table 2: Screening of fungal isolates for pectinase production

Species	NIT	NIP	H	M	W
Aspergillus flavus	7	7	5	1	1
A. fumigatus	9	8	5	2	1
A. nidulans	3	1	-	-	1
A. niger	10	8	4	2	2
A. parasiticus	6	4	2	1	1
A. terreus	8	7	3	3	1
A. ustus	4	1	-	1	-
Curvularia lunata	3	1	-	-	1
Total isolates	50	37	19	10	8

NIT= Number of isolates tested, NIP= Number of isolates positive. H= Higher producers, M= Moderate producers, W= Weak producers.

represented 0.58 % of total fungi isolated from juices. Vermorel *et al.* (1993) noticed the *A. fumigatus* in coocked rice dishes offered to patients hospitalized in sterile units.

Curvularia (represented by *C. lunata)* and *Mycelia sterilia* represented 0.58 % of the total fungi in juices, Narciso and Parish (1997) could isolate 10 filamentous species and 3 *Mycelia sterilia* from paperboard used for packaging fruit juice.

Most recovered species were of high occurrence fungi in one ml of orange juice Al- Rabee, Nada, Saudi orange, Al- Waha, Ranch, Tamam, Kortena respectively, but some species were of moderate occurrence in orange juice and other fruit juice. Also, the rest of juices have low and rare occurrence, no growth found in Partoleese apple juice and Mana mango juice.

Fungi recovered at 45°C: No fungi were recovered at 45° C for 14 days. There are no available records thermophilic or thermotolrant. Palou *et al.* (1998) reported that most fungi are heat –sensitive and many fruit and baked products are virtually free of mould if heated in the final container.

Fifty isolates related to 8 species, which commonly contaminated the fruit juices, were checked on plate cultures to determine pectinase activity (Table 2). The results revealed that about 74% of total isolates (37 isolates) could produce pectinase enzyme with variable degrees (Table 2). In this respect, Solis *et al.* (1990) reported that, production of these enzymes in most microorganisms is limited by mechanism which regulate their synthesis. Also, most pectinases are induced by pectin and are subjects to repression due to the presence of repressor substances or of products associated with the degradation of pectin. In the current study, among the positive strains, 19 isolates (38% of total isolates) exhibited the highest pectinase production and these fungi related to *A. flavus, A. fumigatus, A. niger, A. parasiticus* and *A. terreus*. Ten isolates (20% of total isolates) could produce enzymes with moderate degree, while 8 isolates (16% of total isolates) are the weakly producers (Table 2). The present results agree to agreat extent with the findings of Saval *et al.* (1983). They indicated that the strains of *Aspergillus* spp. could produce extracellular pectinases on pectin or on agroindustrial waste products containing pectin as the sole carbon source. Also, numerous studies on several species of fungi were done (Leuchienberger and Mayer, 1991; Lourdes, 1991; Faulds, 1993; Solis *et al.*, 1993; Mehta *et al.* 1993; Dunkel, 1994; Fonseca, 1994; Channe and Shewale, 1995; Barbe, 1998).

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