

## Inhibitory Effects of Na-Hypochlorite and Heating on the Mycobiota Associated with Fruits or Juice of Passion (*Passiflora edulis* Sims) in Uganda

Mady A. Ismail\*

Department of Botany, Faculty of Science, Assiut University, P.O. Box 71516, Assiut, Egypt

(Received June 6, 2005)

A total of 34 species belonging to 21 genera of fungi were recorded on passion fruits of both pure and hybrid origin in Uganda, however, the pure type exhibited wider spectrum (28 species and 16 genera) than the hybrid type (21 & 15). Also, yeasts (unidentified and *Rhodotorula mucilaginosa*) were also encountered in high numbers. Moreover, the mean count of all mycobiota obtained from the pure type was higher than that of hybrid, despite the bigger size of the later. Members of yeasts and *Cladosporium* followed by *Phoma*, *Penicillium*, *Fusarium* and *Alternaria* species dominated on passion fruits of pure origin, while only *C. cladosporioides*, *F. solani* and yeasts dominated on the hybrid type. Treatment with Na-hypochlorite exhibited inhibitory effects on the total mycobiotic propagules as well as the dominant species from fruits of both types. The current results, therefore, suggest the use of Na-hypochlorite to control the post-harvest mycobiota associated with passion fruits. Regarding the mycobiota contaminating passion juice, yeasts were found to be the major contaminants with *Candida parapsilosis* being the most common. Moulds constituted only a minor proportion with *Acremonium strictum* followed by *Fusarium chlamydosporum*, *F. moniliforme*, *F. acuminatum* and *F. solani* as the most dominant species. In the heat-treated juice samples, the counts of the most commonly encountered mycobiota (both yeasts and molds) were significantly inhibited or completely eliminated. Some unidentified *Bacillus* species were also recovered from the juice, however, their counts in the heated samples were increased but insignificantly.

**KEYWORDS:** Fruits, Heat, Juice, Mycobiota, Na-hypochlorite, Passion, Uganda

A characteristic shared by most fruits is their high acidity and a pH of under 3.5 is not uncommon (Splittstoesser, 1987; Saenz *et al.*, 1998). Various organic acids are responsible for this acidity. pH is the single most important factor with respect to the type of microorganisms that can spoil this class of food. While most species of bacteria are inhibited by the hydrogen ion concentrations for various fruits, yeasts and molds are more aciduric, therefore they are the principal spoilage microorganisms of fruits and fruit products (Splittstoesser, 1987; Deak and Beuchat, 1993; Tournas, 1994; Esegbe and Bankole, 1996; Dugan and Roberts, 1997; Prada and Pagnocca, 1997).

Although the surfaces of fresh fruits harbor large numbers of both yeasts and molds, yeasts generally lack the mechanisms to invade and infect plant tissue and therefore are secondary rather than primary agents of spoilage (Dennis, 1983; Splittstoesser, 1987; Fleet and Heard, 1992). Some of the molds responsible for spoilage are true plant pathogens in that they can invade and cause an infection of intact, formerly healthy tissue. Others are saprophytic species whom become established after the fruit has been infected by a pathogenic organism or has been damaged by some physical or physiological cause. Growth of saprophytic molds generally is restricted to

dead plant tissues (Splittstoesser, 1987; Mehrotra, 1998).

In passion fruits, brown spot is caused by either *Alternaria alternata* (Fullerton, 1982) or *A. passiflorae* (Inch, 1978; Emechebe and Mukibi, 1975; Snowdon, 1990). Fruit infection by *Septoria* spot developed in leaves (caused by *Septoria passiflorae*) can occur at any stage of growth of fruits (Snowdon, 1990). A number of other fungi associated with decaying passion fruits include *Aspergillus niger*, *Cladosporium* sp., *Colletotrichum gloeosporioides*, *Fusarium oxysporum*, *Penicillium expansum*, *Phytophthora nicotianae* var. *parasitica* and *Rhizopus stolonifer* (Snowdon, 1990).

Molds and yeasts are important in fruit processing, however, in that, unless removed or destroyed, they may be introduced in large numbers into juice and other products (Splittstoesser, 1987). Some of the postharvest treatments used to destroy fungal spores are chemical (Abdel-Mallek *et al.*, 1995; Dugan and Roberts, 1997; Schirra *et al.*, 1997; Mehrotra, 1998), biological (Janisiewicz *et al.*, 1998; Mari and Guizzardi, 1998) or a combination of chemical and heat treatments (Johnson *et al.*, 1990; Schirra *et al.*, 1997; Obeta and Ugwuanyi, 1997).

On the other hand, most aciduric microorganisms were reported to possess little heat resistance and, as a result, fruits and fruit products usually require only a relatively mild thermal process to achieve commercial stability (Stokes, 1971; Splittstoesser, 1987; Schirra *et al.*, 1997).

\*Corresponding author <E-mail: madyismail@yahoo.com>

However, others can survive after heat-treatments and can grow and spoil the products during storage, which results in great economic losses (Tournas, 1994).

Little is known about the mycobiota associated with fruits and juice of passion (Lutchmeah, 1993). Thus, this investigation was designed to throw light on the mycobiota associated with both fruits and juice of post-harvest marketed purple passion in Uganda (both pure and hybrid types). Postharvest treatments were also assessed for their beneficial to control these mycobiota.

## Materials and Methods

**Isolation of mycobiota associated with passion fruits and the effectiveness of Na-hypochlorite on these mycobiota.** Purple passion (*Passiflora edulis* Sims, Family: Passifloraceae) fruit samples of both pure (*Passiflora edulis* Sims forma *edulis*, has round or egg-shaped fruits, 4–5 cm in diameter, pH range = 4.2–5.0) and hybrid (presumably it originated as a mutation from the pure type, it has somewhat larger fruits 7–8 cm in diameter, pH range = 4.2–4.8, with about double weight of that of the pure type) origins were collected from different shops and kiosks at Nakasero and Wandegya in Kampala, Uganda during February 2000. Fruits of similar size, appearance and freedom from decay and injury were selected (50 from each passion type). Furthermore, each group of samples was subdivided into two sub-groups (25 samples each). One subgroup from each passion type acted as control-, and the other as chlorine treated-samples. For the treatment, fruit samples were washed thoroughly with 2.5% sodium hypochlorite solution (approximately equal to 62.5% of the household chlorine, Jik, bleach made by Reckitt Colman, Nairobi, Kenya which was used) for two minutes, then left for 48 hours at room temperature (25°–28°C).

The technique used for enumeration and isolation of mycobiota was adopted from Abdel-Mallek *et al.* (1995). Individual samples were put in clean polyethylene bags and aliquots of 100 ml of sterilized tap water were added. The bags were then gently shaken by hand for 5 minutes. One-hundred microlitre ml of washing water was transferred aseptically onto the surface of agar plates. Czapek yeast autolysate agar (CYA) of Pitt (1973) supplemented per litre with 250 mg chloramphenicol, 25 mg rose bengal and 2 mg dichloran was used as the isolation medium. Three inoculated plates for each fruit sample were incubated at 25° ± 2°C for 7–10 days during which the growing fungi were counted, isolated and identified.

**Isolation of mycobiota from passion fruit juice and the effectiveness of heating on these mycobiota.** Twenty random samples of the freshly prepared passion juice (pH = 4.4–5) marketed for human consumption (sold within 1

or 2 days of preparation) were collected from different shops and kiosks in Kisugu, Kampala during April 2000. Each sample was subdivided into two sub-samples. One of the subsamples was heated in a water bath at 80°C for 30 minutes as recommended by Murdock and Hatcher (1978) and Pitt and Hocking (1997). For the isolation of mycobiota, 1 ml from each juice sample (heated or not heated) was then distributed on the surfaces of 4 CYA agar plates of the same composition mentioned above. Plates were incubated at 25 ± 2°C for 10–15 days during which the developing colonies were counted, isolated and identified.

**Identification of isolated fungi:** Fungi were identified on the basis of their macroscopic and microscopic features using the keys of Raper and Fennell (1965), Booth (1970), Ellis (1971), Pitt (1979), Pitt and Hocking (1997).

**Statistical analysis:** The data obtained were subjected to student T-test and significant difference at 0.01 and 0.05 level of probability was subsequently determined following the procedure of Snedecor and Cochran (1967).

## Results and Discussions

**Mycobiota associated with passion fruits and the effectiveness of Na-hypochlorite on these mycobiota.** Data presented in Table 1 showed that the total number of propagules found associated with non-treated pure passion fruits ranged between  $2.73 \times 10^4$  to  $32.3 \times 10^4$  c.f.u/fruit, while with those of hybrid origin between  $3.87 \times 10^4$  to  $23.0 \times 10^4$  with the mean for the hybrid type being lower ( $12.02 \times 10^4$ ) than that of the pure type ( $17.29 \times 10^4$ ). This suggests that the hybrid type is more resistant to be attacked with fungal species than the pure type. On the other hand, treatment of both passion types with 2.5% Na-hypochlorite solution showed statistically significant (at  $P=0.01$ ) inhibitory effects on the total mycobiota propagules. In this respect, the population of pathogenic fungi on the surface of citrus fruits and peaches arriving

**Table 1.** Minimum, maximum and mean of fungal propagules in control and Na-hypochlorite - treated passion fruit samples of both pure and hybrid origin

| Fungal propagules | Pure passion |         | Hybrid Passion |         |
|-------------------|--------------|---------|----------------|---------|
|                   | Non-treated  | Treated | Non-treated    | Treated |
| Minimum           | 2.73         | 0.00    | 3.87           | 0.17    |
| Maximum           | 32.3         | 7.33    | 23.0           | 11.47   |
| Mean              | 17.29        | 2.67**  | 12.02          | 3.03**  |
| SD                | 8.14         | 2.33    | 4.95           | 2.85    |
| T <sup>cal</sup>  | 8.6358       |         | 7.8775         |         |

Figures ( $\times 10^4$ ) are mean of the total fungal propagules found in washing water of 100 individuals of passion fruit (calculated from 25 in each case, 100 ml washing water each). The asterisks \*\* indicate statistical significance at  $P=0.01$ .

**Table 2.** Associated mycobiota with passion fruits and the effectiveness of Na-hypochlorite on their total propagules

| Taxon*                          | Pure passion |       |     |         |       |    | Hybrid passion |       |     |         |       |     |
|---------------------------------|--------------|-------|-----|---------|-------|----|----------------|-------|-----|---------|-------|-----|
|                                 | Non-treated  |       |     | Treated |       |    | Non-treated    |       |     | Treated |       |     |
|                                 | TFP          | %TFP  | %F  | TFP     | %TFP  | %F | TFP            | %TFP  | %F  | TFP     | %TFP  | %F  |
| <i>Acremonium strictum</i>      | 1            | 0.01  | 4   | 28      | 1.40  | 8  | –              | –     | –   | –       | –     | –   |
| <i>Alternaria</i>               | 123          | 0.95  | 80  | 6       | 0.29  | 4  | 33             | 0.37  | 28  | –       | –     | –   |
| <i>A. alternata</i>             | 91           | 0.70  | 60  | 6       | 0.29  | 4  | 33             | 0.37  | 28  | –       | –     | –   |
| <i>A. passiflorae</i>           | 32           | 0.25  | 24  | –       | –     | –  | –              | –     | –   | –       | –     | –   |
| <i>Aspergillus</i>              | 13           | 0.10  | 16  | 7       | 0.35  | 16 | –              | –     | –   | 4       | 0.18  | 4   |
| <i>A. sydowii</i>               | 11           | 0.08  | 8   | 5       | 0.25  | 8  | –              | –     | –   | 4       | 0.18  | 4   |
| <i>Byssochlamys</i>             | –            | –     | –   | –       | –     | –  | 53             | 0.59  | 24  | –       | –     | –   |
| <i>B. fulva</i>                 | –            | –     | –   | –       | –     | –  | 10             | 0.11  | 12  | –       | –     | –   |
| <i>B. nivea</i>                 | –            | –     | –   | –       | –     | –  | 43             | 0.48  | 12  | –       | –     | –   |
| <i>Cladosporium</i>             | 4527         | 34.91 | 100 | 1358    | 67.83 | 96 | 5966           | 66.17 | 100 | 1544    | 67.99 | 100 |
| <i>C. cladosporioides</i>       | 4527         | 34.91 | 100 | 1342    | 67.03 | 92 | 5966           | 66.17 | 100 | 1544    | 67.99 | 100 |
| <i>C. sphaerospermum</i>        | –            | –     | –   | 16      | 0.80  | 32 | –              | –     | –   | –       | –     | –   |
| <i>Cochliobolus lunatus</i>     | –            | –     | –   | 6       | 0.29  | 16 | –              | –     | –   | 15      | 0.66  | 28  |
| <i>Fusarium</i>                 | 212          | 1.63  | 92  | 106     | 5.29  | 76 | 1154           | 12.8  | 100 | 576     | 25.36 | 100 |
| <i>F. moniliforme</i>           | 44           | 0.33  | 56  | 10      | 0.50  | 24 | 80             | 0.89  | 36  | 201     | 8.85  | 68  |
| <i>F. oxysporum</i>             | –            | –     | –   | 48      | 2.40  | 28 | –              | –     | –   | 35      | 1.54  | 40  |
| <i>F. solani</i>                | 132          | 1.02  | 68  | 31      | 1.55  | 24 | 1074           | 11.91 | 100 | 297     | 13.08 | 88  |
| <i>F. stilboides</i>            | –            | –     | –   | 8       | 0.40  | 16 | –              | –     | –   | 39      | 1.72  | 52  |
| <i>F. subglutinans</i>          | 36           | 0.28  | 16  | –       | –     | –  | –              | –     | –   | –       | –     | –   |
| <i>Geotrichum candidum</i>      | 47           | 0.36  | 36  | –       | –     | –  | 1              | 0.01  | 4   | –       | –     | –   |
| <i>Myrothecium roridum</i>      | –            | –     | –   | –       | –     | –  | –              | –     | –   | 24      | 1.06  | 4   |
| <i>Penicillium</i>              | 270          | 2.08  | 84  | 17      | 0.85  | 28 | 27             | 0.30  | 12  | –       | –     | –   |
| <i>P. chrysogenum</i>           | 3            | 0.02  | 8   | 5       | 0.25  | 16 | 20             | 0.22  | 4   | –       | –     | –   |
| <i>P. expansum</i>              | 267          | 2.06  | 84  | 12      | 0.60  | 12 | 7              | 0.08  | 8   | –       | –     | –   |
| <i>Phoma</i> sp.                | 519          | 4.00  | 76  | 213     | 10.64 | 68 | 1              | 0.01  | 4   | 1       | 0.04  | 4   |
| <i>Rhodotorula mucilaginosa</i> | 6            | 0.05  | 12  | 1       | 0.05  | 4  | 22             | 0.24  | 20  | 1       | 0.04  | 4   |
| <i>Trichoderma harzianum</i>    | 6            | 0.05  | 12  | 31      | 1.55  | 40 | –              | –     | –   | –       | –     | –   |
| Yeasts                          | 7240         | 55.83 | 100 | 226     | 11.29 | 56 | 1758           | 19.50 | 100 | 94      | 4.14  | 36  |
| Total fungi                     | 12968        | 100   | 100 | 2002    | 100   | 96 | 9016           | 100   | 100 | 2271    | 100   | 100 |

TFP = Total fungal propagules, calculated per 25 samples in each case [0.3 ml of washing water from each sample (out of 100 ml) was used].

%TFP = Percentage total fungal propagules, calculated per total fungi.

%F = Percentage frequency; calculated per total number of samples investigated.

\*Species isolated occasionally (having less than 10 TFP) were omitted from the table and these were either reported from pure passion fruits (*Allescheriella crocea*, *Aspergillus niger*, *A. ochraceus*, *A. tamarii*, *A. versicolor*, *Microascus cinereus*, *Mucor* sp., *Neurospora crassa*), or hybrid passion fruits (*Colletotrichum gloeosporioides*, *Hemicola grisea*, *Setosphaeria rostrata*) or from both (*Epicoccum nigrum*, *Fusarium dimerum*, and *Pestalotiopsis guepinii*).

from the orchard have been reported to be greatly reduced by surface treatment with a solution of hypochlorite or sodium O-phenyl phenate (Smith and Redit, 1968; Murdock, 1972).

Data in Table 2 showed that the most commonly encountered mycobiota from non-treated passion fruits of pure origin were yeasts (55.83% of the total propagules) and *Cladosporium* (34.91%) followed by *Phoma* (4.0%), *Penicillium* (2.08%), *Fusarium* (1.63%) and *Alternaria* (0.95%). Yeasts were also commonly reported from fruits from 20 different species of angiosperms in a tropical rain forest (Prada and Pagnocca, 1997), banana fruits (Postmaster *et al.*, 1997), as well as from apple wounds (Mercier and Wilson, 1994). *C. cladosporioides*, unidentified *Phoma* species, *P. expansum*, *F. solani*, *F. moniliforme* and *A. alternata* were the most dominant species. *F. monili-*

*forme* has been reported earlier to cause dry rot of passion fruits (Lutchmeah, 1993), while *F. solani* was reported from banana (Jimenez *et al.*, 1993) and guavas (Majumdar and Pathak, 1989). *A. alternata* was earlier isolated most commonly from brown spot of passion fruit in the Pacific Islands (Fullerton, 1982), from apple fruits and the majority of its isolates were pathogenic to apples (Wojtas-Koziel and Borecka, 1990), while *P. expansum* was predominant from rotting apples and pears (Snowdon, 1990). The remaining species (*P. chrysogenum*, *F. subglutinans* and *Alternaria passiflorae*) were infrequently encountered (Table 2). *P. chrysogenum* was one of the primary rot causing fungi of black plum in Nigeria (Eseigbe and Bankole, 1996), while *F. subglutinans* is a pathogen of pineapples (Bolkan *et al.*, 1979; Rohrbach and Taniguchi, 1984) and bananas (Jimenez *et al.*, 1993; Wade *et al.*,

1993). *A. passiflorae* is reported most commonly to cause brown spot of passion fruit in Uganda and Australia (Emechebe and Mukibi, 1975; Inch, 1978). Moreover, of the species reported in the current study, *Penicillium expansum*, *Cladosporium* sp., *F. oxysporum*, *A. niger*, *Colletotrichum gloeosporioides* were found associated with decaying passion fruits (Snowdon, 1990).

The yeast-like fungus, *Geotrichum candidum*, was isolated in moderate frequency (36% of the samples), while other species of 8 genera (*Acremonium*, *Aspergillus*, *Epicoccum*, *Microascus*, *Mucor*, *Pestalotiopsis*, *Rhodotorula* and *Trichoderma*) were reported infrequently and accounted collectively a minor proportion of the total propagules (Table 2). Some of these were reported as significant pathogens of fruits during postharvest storage such as *Pestalotiopsis* sp. and *Acremonium strictum* on banana (Wallbridge, 1981), *Geotrichum candidum* on citrus (Hall and Scott, 1977) and tomatoes (Okali and Erinle, 1989), *Epicoccum nigrum* on tomatoes, apples and pears (Bruton *et al.*, 1993), *T. harzianum* on apples (Penrose *et al.*, 1984), and species of *Mucor* on guavas (Ito *et al.*, 1979) and mangoes (Johnson *et al.*, 1990).

Statistical analysis revealed that, of the most commonly encountered fungi, yeasts, *C. cladosporioides*, *P. expansum* and *A. alternata* were highly significantly (at  $P=0.01$ ) inhibited with Na-hypochlorite - treatment. On the other hand, *F. moniliforme*, *F. solani* and *Phoma* sp. were also significantly inhibited but at  $P=0.05$  (Table 3). Also, the total fungal propagules were strongly inhibited (at  $P=0.01$ ) by about 84.6%. In this respect, the use of Na-hypochlorite has been proved to be effective for the control of passion fruit rot-development caused by fungal species most commonly encountered from passion fruits (Ismail, 2001). Also, Na-hypochlorite is reported to be lethal to fungal propagules and fungi *in vitro* (Mehrotra, 1998) and to have good curative properties against tomato fruit rots especially those caused by *Alternaria alternata*

and *Aspergillus niger* (Abdel-Mallek *et al.*, 1995). Control of brown spots of passion fruits (caused by *A. alternata* and *A. passiflorae*) is by pruning and destroying the debris and by spraying the passion trees (vines) regularly with fungicide (Snowdon, 1990).

On the other hand, the most commonly encountered species on hybrid passion fruits were *C. cladosporioides* (66.17% of the total propagules), *F. solani* (11.91%) and yeasts (19.5%). Moderately encountered species included *A. alternata* and *F. moniliforme* while the remaining fungi were infrequent (Table 2).

The treatment with Na-hypochlorite strongly inhibited the total mycobiota propagules as well as the most common three taxa (at  $P=0.01$ ) (Table 3).

Noteworthy to mention that to be certain fungal species, as shown in Table 2, appeared after Na-hypochlorite treatment from either or both passion types (*Aspergillus sydowii*, *Cladosporium sphaerospermum*, *Cochliobolus lunatus*, *Fusarium oxysporum*, *F. stilboides*, *Myrothecium roridum*) or increased their numbers (*Acremonium strictum*, *F. moniliforme* and *Penicillium chrysogenum*). Of these, *F. oxysporum* was reported earlier as a postharvest pathogen of bananas (Wallbridge, 1981; Jimenez *et al.*, 1993), citrus, pome fruits, tomatoes and melons (Snowdon, 1990) and of black plum (Eseigbe and Bankole, 1996). The results presented here suggest that higher concentrations of Na-hypochlorite or other chemical compounds may be used to control such fungi (most of them were rarely reported on either or both passion types, Table 2).

**Mycobiota inhabiting passion fruit juice.** Yeasts, which were commonly isolated from passion fruits, were the major contaminants of passion juice, constituting about 91.3% of the total microflora. Two species (*Candida parapsilosis* and *Rhodotorula mucilaginosa*) could be identified while some others could not, with *C. parapsilosis* being the most common. It was isolated in all sam-

**Table 3.** The inhibitory effect of sodium hypochlorite on the mycobiota most commonly associated with pure and hybrid passion fruits

| Common taxa                         | Pure              |                     |              | Hybrid            |                   |                     |
|-------------------------------------|-------------------|---------------------|--------------|-------------------|-------------------|---------------------|
|                                     | TFP $\times 10^4$ |                     | % Inhibition | TFP $\times 10^4$ |                   | % Inhibition        |
|                                     | Non-treated       | Treated             |              | Non-treated       | Treated           |                     |
| <i>Alternaria alternata</i>         | 0.12 $\pm$ 0.17   | 0.008 $\pm$ 0.04**  | 93.33        | 1.32 $\pm$ 3.34   | 0.00              | 100                 |
| <i>Cladosporium cladosporioides</i> | 6.04 $\pm$ 3.01   | 1.79 $\pm$ 1.98**   | 70.36        | 7.96 $\pm$ 4.41   | 2.06 $\pm$ 2.62** | 74.12               |
| <i>Fusarium moniliforme</i>         | 0.059 $\pm$ 0.082 | 0.013 $\pm$ 0.03*   | 77.97        | 3.2 $\pm$ 8.28    | 8.04 $\pm$ 20.33  | 151.25 <sup>a</sup> |
| <i>F. solani</i>                    | 0.18 $\pm$ 0.22   | 0.04 $\pm$ 0.11*    | 77.78        | 1.43 $\pm$ 1.01   | 0.39 $\pm$ 0.55** | 72.73               |
| <i>Penicillium expansum</i>         | 0.36 $\pm$ 0.54   | 0.016 $\pm$ 0.067** | 95.56        | 0.28 $\pm$ 1.06   | 0.00              | 100                 |
| <i>Phoma</i> sp.                    | 0.69 $\pm$ 0.89   | 0.28 $\pm$ 0.39*    | 59.42        | 0.04 $\pm$ 0.2    | 0.04 $\pm$ 0.2    | 0.00                |
| Yeasts                              | 9.65 $\pm$ 6.22   | 0.30 $\pm$ 0.82**   | 96.89        | 2.34 $\pm$ 1.91   | 0.13 $\pm$ 0.37** | 94.44               |
| Total mycobiota                     | 17.29 $\pm$ 8.14  | 2.67 $\pm$ 2.33**   | 84.56        | 12.02 $\pm$ 4.95  | 3.03 $\pm$ 2.85** | 74.79               |

Figures are the means  $\pm$  standard deviations of the total fungal propagules (TFP), out of 25 passion fruit samples.

The asterisks \* and \*\* indicate statistical significance at  $P=0.05$  and  $P=0.01$ , respectively.

<sup>a</sup> % promotion.

**Table 4.** The inhibitory effect of heating on the counts (represented by TFP and mean  $\pm$  SD, out of 20 samples analysed, 1 ml each) of the microflora contaminating passion fruit juice

| Taxa                              | Not-heated |                    |     | Heated |                      |     | % Inhibition       |
|-----------------------------------|------------|--------------------|-----|--------|----------------------|-----|--------------------|
|                                   | TFP        | Mean $\pm$ SD      | NCS | TFP    | Mean $\pm$ SD        | NCS |                    |
| <b>Moulds</b>                     | 327        | 16.35 $\pm$ 17.66  | 19  | 177    | 8.85 $\pm$ 18.49     | 12  | 45.87              |
| <i>Acremonium strictum</i>        | 159        | 7.95 $\pm$ 8.85    | 17  | -      | 0**                  | -   | 100                |
| <i>Aspergillus sydowii</i>        | -          | -                  | -   | 164    | 8.2 $\pm$ 18.59      | 9   | 0.00               |
| <i>Aureobasidium pullulans</i>    | -          | -                  | -   | 3      | 0.15 $\pm$ 0.67      | 1   | 0.00               |
| <i>Fusarium</i>                   | 127        | 6.35 $\pm$ 11.21   | 13  | 3      | 0.15 $\pm$ 0.67*     | 1   | 97.64              |
| <i>F. acuminatum</i>              | 25         | 1.25 $\pm$ 2.73    | 8   | -      | -                    | -   | 100                |
| <i>F. chlamydosporum</i>          | 45         | 2.25 $\pm$ 8.03    | 2   | -      | -                    | -   | 100                |
| <i>F. moniliforme</i>             | 42         | 2.1 $\pm$ 4.95     | 5   | -      | -                    | -   | 100                |
| <i>F. solani</i>                  | 15         | 0.75 $\pm$ 1.55    | 5   | -      | -                    | -   | 100                |
| <i>Fusarium</i> sp.               | -          | -                  | -   | 3      | 0.15 $\pm$ 0.67      | 1   | 0.00               |
| <i>Monographella nivalis</i>      | -          | -                  | -   | 6      | 0.3 $\pm$ 1.34       | 1   | 0.00               |
| <i>Mucor</i> sp.                  | 3          | 0.15 $\pm$ 0.67    | 1   | -      | -                    | -   | -                  |
| Unidentified species              | 38         | 1.9 $\pm$ 8.49     | 1   | 1      | 0.05 $\pm$ 0.22      | 1   | 97.64              |
| <b>Yeasts</b>                     | 15968      | 798.4 $\pm$ 417.22 | 20  | 6044   | 302.2 $\pm$ 213.43** | 20  | 62.15              |
| <i>Candida parapsilosis</i>       | 9224       | 461.2 $\pm$ 224.28 | 20  | 4460   | 223 $\pm$ 201.99**   | 20  | 51.65              |
| <i>Rhodotorula mucilaginosa</i>   | 124        | 6.2 $\pm$ 13.03    | 8   | -      | 0*                   | -   | 100                |
| Unidentified yeasts (red)         | 6620       | 331 $\pm$ 333.82   | 18  | 1584   | 79.2 $\pm$ 114.87**  | 11  | 76.07              |
| <b>Bacteria (<i>Bacillus</i>)</b> | 1195       | 59.75 $\pm$ 123.84 | 12  | 1687   | 84.35 $\pm$ 96.06    | 16  | 41.17 <sup>a</sup> |
| <b>Total microflora</b>           | 17490      | 874.5 $\pm$ 445.49 | 20  | 7908   | 395.4 $\pm$ 267.09** | 20  | 54.79              |

TFP: Total fungal propagules, calculated per 20 ml in all juice samples examined (1 ml/sample).

NCS: Number of contaminated samples, out of 20 examined.

The asterisks \* and \*\* indicate statistical significance at P=0.05 and P=0.01, respectively.

<sup>a</sup>% promotion.

ples, accounting for 57.77% of all yeast propagules. *R. mucilaginosa* was less frequent giving rise to only a small proportion (0.78%) (Table 4). These two species were reported earlier from fruit juice concentrates (Deak and Beuchat, 1993).

The counts of *C. parapsilosis* and the unidentified yeasts as well as the total yeasts were significantly (at P=0.01) reduced by heat-treatment. Moreover *R. mucilaginosa* was completely eliminated from the heated juice (Table 4).

Molds constituted a minor proportion (1.87%) of the total microflora of passion juice (Table 4). The most commonly encountered fungi from non-treated samples were *Acremonium strictum* (48.62% of the total fungal propagules, in 17 samples out of 20 analyzed), followed by *Fusarium chlamydosporum* (13.76%, 2), *F. moniliforme* (12.84%, 5), *F. acuminatum* (7.65%, 8) and *F. solani* (4.59%, 5). *A. strictum*, *F. moniliforme* (Teleomorph: *Gibberella fujikuroi*), *F. solani* (Teleomorph: *Nectria haematococca*) were reported in small numbers from mango juice or natural apple drink, however the most commonly encountered fungi from fruit juices and drinks were members of *Aspergillus*, *Penicillium* and *Eurotium* (Abdel-Sater *et al.*, 2001).

*A. strictum*, the most dominant fungus, was completely eliminated by heating. On the other hand, Fusaria (all species) were significantly inhibited (at P=0.05) by 97.6%

(Table 4). Most fusaria are known to produce chlamydo-spores which could resist heating, that is why were not completely eliminated.

Unidentified species of *Bacillus* were also recovered from both heated and non-heated juices, however their counts were increased by about 41.2% in the heated ones (Table 4).

**Conclusions.** From the current results, it could conclude that the numbers and composition of mycobiota (both yeasts and molds) tend to be more associated with passion fruits of pure origin than that of hybrid origin. However, these mycobiota were significantly inhibited on both passion types by Na-hypochlorite - treatment. So, we recommend the use of Na-hypochlorite (2.5%) to control these postharvest associated mycobiota by washing passion fruits for two minutes. On the other hand, the most commonly encountered mycobiota from passion juice were either significantly inhibited or completely eliminated on heat-treatment. These findings also suggest the use of heat-treatment (at least at 80°C for 30 minutes) to minimize or eliminate those mycobiota contaminating passion juice.

#### Acknowledgements

The author wishes to express the greatest appreciation to

the Egyptian Fund for Technical Co-operation with Africa which sponsored him by a grant to work at Makerere University, Uganda, and to Prof. Bukenya-Ziraba, the Head of Department of Botany, Faculty of Science, Makerere University, Uganda for the laboratory facilities he provided during this investigation.

## References

- Abdel-Mallek, A. Y., Hemida, S. K. and Bagy, M. M. K. 1995. Studies on fungi associated with tomato fruits and effectiveness of some commercial fungicides against three pathogens. *Mycopathologia* **130**: 109-116.
- Abdel-Sater, M. A., Zohri, A. A. and Ismail, M. A. 1996. Natural contamination of some Egyptian fruit juices and beverages by mycoflora and mycotoxins. *J. Food Sci. Technol.* **38**: 407-411.
- Bolkan, H. A., Dianese, J. C. and Cupertino, F. P. 1979. Pineapple flowers as principal infection sites for *Fusarium moniliforme* var. *subglutinans*. *Plant Dis. Rep.* **63**: 655-657.
- Booth, C. 1971. The genus *Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, England, p 237.
- Bruton, B. D., Redlin, S. C., Collins, J. K. and Sams, C. E. 1993. Postharvest decay of cataloupe caused by *Epicoccum nigrum*. *Plant Dis.* **77**: 1060-1062.
- Deak, T. and Beuchat, L. R. 1993. Yeasts associated with fruit juice concentrates. *Journal of Food Protection* **56**: 777-782.
- Dennis, C. 1983. Yeast spoilage of fruit and vegetable products. *Indian Food Packer* **37**: 38-53.
- Dugan, F. M. and Roberts, R. G. 1997. Pre-harvest fungal colonization affects storage life of Bing cherry fruit. *Journal of Phytopathology - Phytopathologische Zeitschrift* **145**: 225-230.
- Ellis, M. B. 1971. Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, England, p 608.
- Emechebe, A. M. and Mukibi, J. 1975. Fungicidal control of brown spot of passion fruit in Uganda. *Acta Horticulturae* **49**: 281-289.
- Eseigbe, D. A. and Bankole, S. A. 1996. Fungi associated with post-harvest rot of black plum (*Vitex doniana*) in Nigeria. *Mycopathologia* **136**: 109-114.
- Fleet, G. H. and Heard, G. M. 1992. Yeasts-Growth during fermentation. Pp 27-54. In: Fleet, G. H. Ed. Wine Microbiology and Biotechnology. Harwood Academic Publishers, Chur, Switzerland.
- Fullerton, R. A. 1982. Brown spot of passion fruit on Niue Island caused by *Alternaria alternata*. *New Zealand J. Agricult. Res.* **25**: 421-423.
- Hall, E. G. and Scott, K. J. 1977. Storage and market diseases of fruit. Melbourne, Australia; Commonwealth Scientific and Industrial Research Organisation.
- Inch, A. J. 1978. Passion fruit diseases. *Queensl. Agric. J.* **104**: 479-484.
- Ismail, M. A. 2001. Pathogenic ability and pectolytic enzymes of fungi most commonly associated with passion fruits with special reference to the effectiveness of Na-hypochlorite on the rot development. *Afr. J. Mycol. Biotechnol.* **8**: 13-24.
- Ito, P. J., Kunitomo, R. and Ko, W. H. 1979. Transmission of *Mucor* rot of guava fruits by three species of fruit flies. *Trop. Agric.* **56**: 49-52.
- Janisiewicz, W. J., Conway, W. S., Glenn, D. M. and Sams, C. E. 1998. Integrating biological control and calcium treatment for controlling postharvest decay of apple. *Hortscience* **33**: 105-109.
- Jimenez, M., Logrieco, A. and Bottalico, A. 1993. Occurrence and pathogenicity of *Fusarium* species in banana fruits. *J. Phytopathol.* **137**: 214-220.
- Johnson, G. I., Sangchote, S. and Cooke, A. W. 1990. Control of stem end rot (*Dothiorella dominicana*) and other postharvest diseases of mangoes (cv. Kensington Pride) during short- and long-term storage. *Trop. Agric.* **67**: 183-187.
- Lutchmeah, R. S. 1993. Common field and post-harvest diseases of passion fruit (*Passiflora edulis* f. *flavicarpa*) and the associated fungi in Mauritius. *Rev. Agric. Sucr. Ile Maurice* **72**: 55-59.
- Majumdar, V. L. and Pathak, V. N. 1989. Incidence of major post-harvest diseases of guava fruits in Jaipur markets. *Ind. Phytopathol.* **42**: 469.
- Mari, M. and Guizzardi, M. 1998. The postharvest phase-emerging technologies for the control of fungal diseases. *Phytoparasitica* **26**: 59-66.
- Mehrotra, R. S. 1998. Postharvest diseases. Pp 575-592. In: Mehrotra, R. S. Ed. Plant Pathology, 15<sup>th</sup> reprint. Tata McGraw-Hill Publishing Company Limited, New Delhi.
- Mercier, J. and Wilson, C. L. 1994. Colonization of apple wounds by naturally occurring microflora and introduced *Candida oleophila* and their effect on infection by *Botrytis cinerea* during storage. *Biol. Control* **4**: 138-144.
- Murdock, D. I. 1972. Microbiological study of a lemon packing house operation. *Proc. Fla. State Hort. Soc.* **84**: 266.
- \_\_\_\_\_ and Hatcher Jr., W. S. 1978. Effect of temperature on survival of yeast in 45° and 65° Brix orange concentrate. *J. Food Proct.* **41**: 689-691.
- Obeta, J. A. N. and Ugwuanyi, J. O. 1997. Shelf life study if some Nigerian fruit juices inoculated with ascospores of *Neosartorya* spp. *Plant Foods Human Nutr.* **50**: 325-331.
- Okoli, C. A. N. and Erinle, I. D. 1989. Factors responsible for market losses of tomato fruits in the Zaria area of Nigeria. *J. Hort. Sci.* **64**: 69-71.
- Penrose, L. J., Nicholls, M. R. and Koffmann, W. 1984. Apple fruit rot caused by *Trichoderma harzianum*. *Australas. Plant Pathol.* **13**: 46-47.
- Pitt, J. I. 1973. An appraisal of identification methods for *Penicillium* species: novel taxonomic criteria based on temperature and water relations. *Mycologia* **65**: 1135-1157.
- \_\_\_\_\_. 1979. The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. Academic Press, London, p 398.
- \_\_\_\_\_ and Hocking, A. D. 1997. Fungi and food spoilage. 2<sup>nd</sup> edition, Blackie Academic & Professional, London, UK. p 593.
- Postmaster, A., Sivasithamparam, K. and Turner, D. W. 1997. Enumeration and identify of microorganisms isolated from the surface of banana fruits at three developmental stages. *Scientia Horticulturae* **69**: 189-197.
- Prada, G. M. M. and Pagnocca, F. C. 1997. Ascomycetous yeasts associated with naturally occurring fruits in a tropical rain forest. *Folia Microbiol.* **42**: 39-46.
- Raper, K. B. and Fennell, D. I. 1965. The genus *Aspergillus*. Baltimore, Maryland: Williams and Wilkins, p 686.
- Rohrbach, K. G. and Taniguchi, G. 1984. Effects of temperature, moisture and stage of inflorescence development on infection of pineapple by *Penicillium funiculosum* and *Fusarium moniliforme*.

- forme var. subglutinans. Phytopathology* **74**: 995-1000.
- Saenz, C., Sepulveda, E., Navarrete, A. and Rustom, A. 1998. Influence of harvest season on the characteristics of purple passion fruit (*Passiflora edulis* Sims) and its juice. *Food Science Technol. Int.* **4**: 45-51.
- Schirra, M., Cabras, P., Angioni, A., Dhallewin, G., Ruggiu, R. and Minelli, E. V. 1997. Effect of heated solutions on decay control and residues of imazalil in lemons. *J. Agri. Food Chem.* **45**: 4127-4130.
- Smith, W. L. and Redit, W. H. 1968. Post-harvest decay of peaches as affected by hot water treatments, cooling methods, and sanitation. USDA, MRR, p 807.
- Snedecor, G. W. and Cochran, W. G. 1967. Statistical methods. 6<sup>th</sup> ed. Iowa State University Press, Ames, IA.
- Snowdon, A. L. 1990. A colour atlas of post-harvest diseases and disorders of fruits and vegetables. 1. General introduction and fruits. London: Wolfe Scientific.
- Splittstoesser, D. F. 1987. Fruits and fruit products. Pp 101-128. *In*: Beuchat, L. R. Ed. Food and Beverage Mycology, 2<sup>nd</sup> edition. Van Nostrand Reinhold, New York.
- Stokes, J. L. 1971. Influence of temperature on the growth and metabolism of yeasts. *In*: Rose, A. H. and Harrison, J. S. Eds. The Yeasts. Vol. 2, Academic Press, New York.
- Tournas, V. 1994. Heat-resistant fungi of importance to the food and beverage industry. *Crit. Rev. Microbiol.* **20**: 243-263.
- Wade, N. L., Kavanagh, E. E. and Sepiah, M. 1993. Effects of modified atmosphere storage on banana postharvest diseases and the control of bunch main - stalk rot. *Postharvest Biol. Technol.* **3**: 143-154.
- Wallbridge, A. 1981. Fungi associated with crown-rot disease of boxed bananas from the Windward Islands during a two-year survey. *Trans Br. Mycol. Soc.* **77**: 567-577.
- Wojtas-Kozziel, B. and Borecka, H. 1990. The pathogenicity of *Alternaria* spp. isolated from various apple tree organs to apple fruit. *Acta Agrobot.* **41**: 27-32.