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COMMUNICATION II

Microflora of Ciku (*Achras sapota* L.) of Variety Jantung

ABSTRAK

Keseluruhan kiraan mikrob pada ciku adalah rendah dan dikuasai oleh yis, yang didapati paling tinggi pada hari ketiga selepas tuai. Pembasuhan mengurangkan kiraan mikrob pada buah dengan menurunkan populasi yis sebanyak 89% dan bakteria sebanyak 75%. Semasa kecederaan, flora dominan ialah bakteria dan kemudiannya dalam masa penstoran ia diambil alih oleh kulat. Flora mikrob normal pada ciku terdiri daripada bakteria, yis dan kulapuk. Kerosakan semasa penstoran suhu rendah dikaitkan dengan strain-starin baru yis dan kulapuk. Sanitasi dan pengendalian lepas tuai yang baik penting bagi mengawal kerugian dari segi kerosakan buah-buahan yang disebabkan oleh mikroorganisma.

ABSTRACT

The overall microbial count of ciku is low and predominantly yeast, which peaks at day three after harvest. Washing reduced the microbial counts of the fruits, retarding the yeast population by 89% and bacteria by 75%. During injury, the dominant flora is bacteria which is replaced later by fungi as the storage time progeressed. Normal microbial flora of ciku consists of bacteria, yeast and moulds. Spoilage during cold storage is associated with new strains of yeast and moulds. Proper sanitation and post harvest handling treatments are important in controlling fruit losses associated with microorganisms.

INTRODUCTION

Ciku (*Manilkara Achras L.; Achras Sapota* L.) originated from tropical Americas probably from South Mexico. It belongs to the family Sapotaceae. Besides the name ciku, it is also known as sapota, sapotilla, chiko, bully and naseberry (Mustard, 1982). There are four varieties of ciku grown commercially in Malaysia, namely *jantung, betawi, pasir* and *subang*. Fruits are produced throughout the year but production is not consistent. Unless optimal measures are taken during storage, many fruits may be spoilt. Wastage of fruits by microorganisms during movement from harvest to consumption can be rapid and severe particularly in tropical areas where high temperatures and high humidity favour rapid microbial growth. Many bacteria and fungi cause postharvest decay of fruits mostly in the form of weak pathogens, in that they can only invade damaged produce where the relationship between the host (fruits) and the microbes is reasonably specific (Wills *et al.*, 1981). This study aims at identifying the microflora of ciku during ambient and low temperature storage as well as the effect of washing and injury on the microbial profiles. As there is very little published information available regarding the microbiology of the fruit, this will provide baseline information on the change in microbial growth patterns during storage.

MATERIALS AND METHODS

Fruits

Mature (7-month-old) ciku fruits of cv. *jantung* were mechanically harvested using a ciku harvester (Abdul Karim, 1988) from the Serdang Experimental Farm, Universiti Pertanian Malaysia (UPM). They were then separated into two portions, with one being left unwashed and the other washed with running tap water. Some of the unwashed fruits were artificially injured by dropping the fruits on to sterilized floor from a uniform height of five feet. They were then placed inside sterile

glass incubators and stored at room temperature. Low temperature storage studies were conducted on washed fruits kept at 10°C and 15°C. Aseptic procedures were used wherever posibble.

Microbial Enumeration and Isolation Procedures

The enumeration procedure was carried out over a period of six days after harvest. Surface sample measuring ten square cm. was swabbed with sterile cotton buds and rinsed with sterile distilled water. Four fruits were used at each sampling time and immediately discarded after swabbing. The number of bacteria and fungi was counted using the poured plate and spread plate methods, respectively. Plate count agar (PCA) (Oxoid) was used for the estimation of total aerobic bacteria whilst potato dextrose agar (PDA) (Oxoid) was used for the enumeration of moulds and yeasts. PCA plates were incubated at 35°C for 24 hours while PDA plates were incubated for two to five days at 30°C.

The composition of the microbial flora was determined by isolating and identifying different colonies chosen randomly from the total count plates according to Gill and Newton (1980). The different isolates were maintained at 4°C on nutrient agar (NA) (Oxoid) and PDA slopes for bacteria and fungi respectively. Subculture was performed once a month for identification procedures.

Composition oof Microbial Flora

Identification of bacteria was done according to Buchanan and Gibbons (1974). Moulds were identified from their cultural characteristics and microscopical appearance according to Samson *et al.*, (1984). Identification of yeasts was according to methods described by Deak and Beuchat (1987) and the use of API 20°C Identification System (API International S.A., France).

RESULTS AND DISCUSSION

The normal microflora of ciku consisted of bacteria, moulds and yeasts (Table 1). Bacteria were found to belong to the genera *Bacillus, Erwinia* and *Micrococcus.* Moulds were identified as those of strains of *Aspergillus niger, Geotrichum* spp., and *Mucor* spp., whilst yeasts belonged to

TABLE 1
Microbial flora of ciku during one week storage at room temperature (28°C ± 2)

Bacteria	Yeasts	Moulds
Bacillus spp.	*Pichia anomala*	*Aspergullus niger*
Erwinia spp	*Rhodotorula acheniorum*	*Geotrichum* spp.
Micrococcus spp.	*Brettanomyces custersii*	*Mucor* spp.

the species *Rhodotorula acheniorum, Brettanomyces custersii* and *Pichia anomala.* Studies on the yeast flora of citrus fruit (Recca and Mrak, 1952), apples (Marshall and Walkley, 1952), and grapes (Mrak and McClung, 1940) also indicated that the population of yeasts was about evenly divided between ascosporogenous and imperfect stages. Examples of the less common ascosporogenous genera were *Saccharomyces, Pichia* and *Hanseniaspora* and those of the more common ascosporogenous species were *Candida* and *Rhodotorula.* The predominants moulds genera were *Aspergillus, Mucor, Alternaria* and *Botrytis.*

Some of the washed ciku stored at low temperatures (10°C and 15°C), after sixteen days or more were found to be infected around the calyx portion of the fruits. The bacteria were identified to be identical to that of the normal flora (Table 2). Strains of yeasts, however, had changed with the addition of *Candida bacarium, Candida silvicultrix, Klyveromyces manxianus* in place of *Rhodotorula acheniorum* and *Brettanomuces custersii.* In the case of moulds, *Fusarium* spp. replaced *Mucor* spp. It is known that *Erwinia* cause bacterial soft rot in many fruits and vegetables (Ryall and Lipton, 1979; Tugwell, 1989). *Geotrichum* had been reported to cause sour rot, whilst *Fusarium* could cause *Fusarium* rot in fruits (Ryall and Lipton, 1979).

The microbial profile of ciku is shown in *Fig. 1.* In general, the overall microbial count of ciku was rather low. The peak for total number of yeasts was achieved at day three, decreasing thereafter. The bacteria and mould counts increased throughout the enumeration

TABLE 2
Microbial flora of ciku spoiled after 16 days or more at low temperature storage of 10° and 15°C.

Bateria	Yeast	Moulds
Bacillus spp.	*Pichia anomala*	*Aspergillus niger*
Erwinia spp.	*Candida bacarium*	*Geotrichum* spp.
Micrococcus spp.	*Candida silvicultrix*	*Fusarium* spp.
	Klyveromces manxianus	

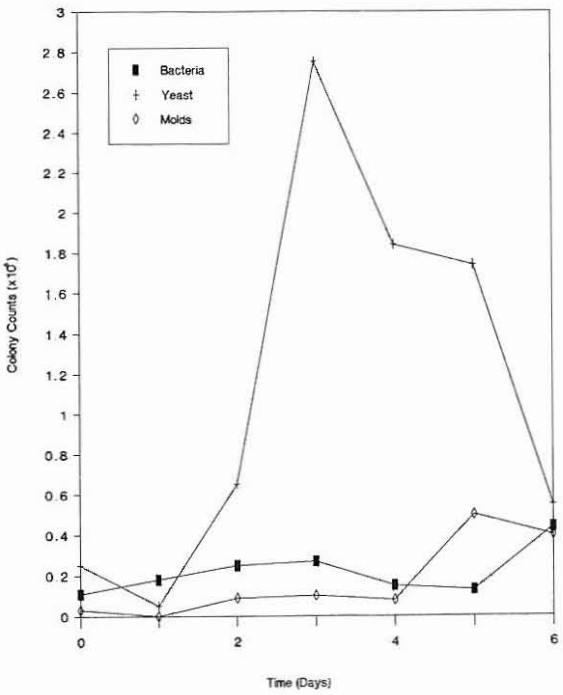

Fig. 1: Microbial profile of ciku during one week of storage at room temperature

period. According to Beuchat (1978), although the surfaces of fresh fruits harbour large number of molds and yeasts, the latter generally lack the mechanisms to invade and infect plant tissue. Therefore, they are secondary rather than primary agents of spoilage.

The proliferation of yeasts was obviously reduced by washing (*Fig. 2*). The bacterial count was also reduced for the first four days after washing, but the number started to increase by

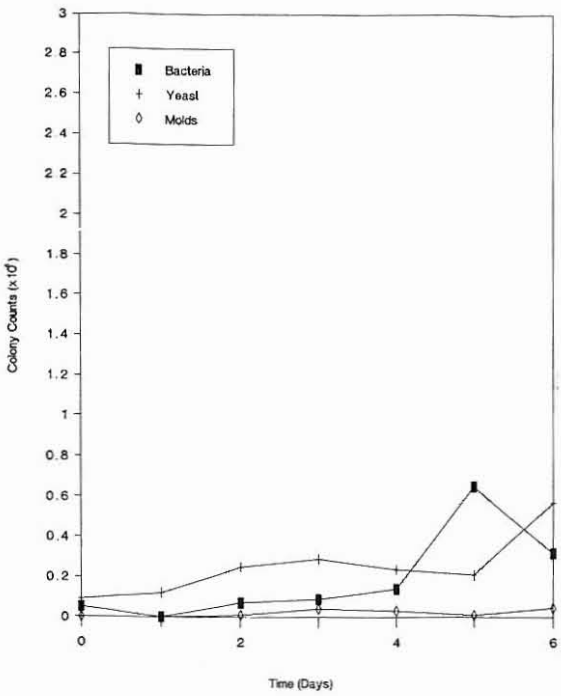

Fig. 2: Effect of washing on the microbial profile of ciku during one week of storage at room temperature

the fourth day and decreased thereafter. Moulds kept an almost constant profile throughout the enumeration period. Washing reduced the number of microorganisms attached to the fruit detaching some of the microorganisms found on the fruits. According to Beuchat (1978) washing, usually one of the first steps in fruits processing, would remove much of the original microflora. Our results (*Figs. 1* and *2*) showed that washing reduced the yeasts count by about 89%; the bacterial count by about 75% and the mould counts by only about 1%. Murdock and Brokaw (1958) reported that a brush wash followed by a rinse with chlorinated water reduced the microbial population on the surface of oranges by 95%, while a reduction in viable count ;over 99.9% was observed when apples were washed in running water (Marshall and Wakley, 1951).

Fig. 3 showed the effect of injury on the microbial profile of ciku. As can be seen, the bacterial and mould counts increased during storage. The bacterial count dropped gradually after the fourth day. The yeast population increased, peaking at day five, with a slight fall at three days of storage. The results indicated

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