AN ECOLOGICAL STUDY OF YEASTS ASSOCIATED WITH FRUITS AND VEGETABLES

by

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This thesis is dedicated to
my wife Maretha

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CHAPTER 1

SUSCEPTIBILITY OF FRUITS AND VEGETABLES TO FUNGAL SPOILAGE.

1.1 Introduction

Large and varied populations of microorganisms, including the spores of many types of fungi, contaminate the surface of fruits during the growth season. Relatively few of the fungi are capable of attacking the fruit before harvest. Many of the organisms involved are obligate parasites that are host adapted and able to infect healthy living tissues. Some of these organisms continue to grow on their host when the crop has been harvested and cause postharvest damage of the crop (Garbutt, 1997). Fruits are rich in sugars and nutrients, like organic acids, carbohydrates, vitamins and minerals making them prone to spoilage and growth of various microorganisms. The physiological state of the plant products, especially those consisting of fruits or vegetables, has a dramatic effect on susceptibility to microbiological spoilage (Hao and Brackett, 1994). Fruits and vegetables differ in the way in which they change physiologically after detachment of the plant. Nonclimacterate fruits and vegetables, such as strawberries, beans and lettuce, cease to ripen once they have been harvested. In contrast, climacterate fruits and vegetables, such as bananas and tomatoes, continue to mature and ripen after harvest (Rolle and Chism III, 1987).

Currently, synthetic fungicides are the primary means of controlling postharvest diseases (El-Ghaouth *et al.*, 1998). Consumer concerns about fungicide residues in food and possible risks associated with continuous use of synthetic fungicides to control postharvest diseases of fruits and vegetables have resulted in an intensive search for safer control options that pose minimal risk to human health and the environment (Droby *et al.*, 1999). Substantial progress has been made in identifying and developing potential biological alternatives to synthetic fungicides for the control of postharvest diseases of fruits and vegetables (Wilson and Wisniewski, 1994). Among the proposed alternatives, the use of naturally occurring, antagonistic microorganims has been the most extensively studied. The main criteria are to characterize an epiphytic yeast population that is able to survive and grow at a wide range of temperatures and diverse climatic conditions.

The purpose of this present study was to conduct an ecological study on the epiphytic yeast population developing on the surface of fruits and vegetables over a period of time at different temperatures, and to determine the characteristics of the yeasts isolated to be applied as possible biocontrol agents.

2. YEASTS AS SPOILAGE ORGANISMS

2.1 YEAST SPOILAGE OF FRUITS

Spoilage yeasts are defined as organisms that produce undesirable changes in foods or during fermentation processes. Primarily fungi (Bulgarelli and Brackett, 1991) are responsible for spoiling fresh fruit, due to the product low pH values. Yeasts are normal inhabitants on the surface of freshly harvested fruits (Last and Price, 1969; Lund, 1958). They occur in populations ranging between 10^2 and 10^6 cells/cm^2, depending on climatic conditions, degree of maturity and extent of damage. Ingram (1958) considered that the most important factors in determining the ability of yeasts to compete with moulds and bacteria on food, are the numbers and types of contaminating yeasts, available nutrients, pH, redox potential, temperature during

processing, storage and relative humidity or water activity of the surroundings of the food product. The relative proportions of species vary from commodity to commodity and are influenced by environmental, harvesting and storage conditions (Dennis and Buhagiar, 1980; Ippolito *et al.*, 2000).

The microflora of fruits are derived mainly from two sources. The primary, resident microflora consist of microorganisms adhering to the surface of the fruits. Leibinger *et al.* (1997) confirmed that *Aureobasidium pullulans* and *Rhodotorula glutinis* are common inhabitants of leaf and fruit surfaces and have a high tolerance to desiccation and irradation. Ippolito *et al.* (2000) successfully investigated the biocontrol activity of *Aureobasidium pullulans* on decay of apple fruit caused by *Botrytis cinerea* and *Penicillium expansum.* Lima *et al.* (1997) investigated the effectiveness of *Aureobasidium pullulans* and *Candida oleophila* against postharvest strawberry rots. Secondary, microflora are carried by vectors such as soil, dust and insects and vary dramatically compared to the more stable resident community (Davenport, 1976; Doores, 1983). Phaff (1957) demonstrated the presence of the yeasts *Hanseniaspora uvarum, Kloeckera apiculata* and *Pichia kluyveri,* in the alimentary canal of *Drosophila melangoster,* which oviposits the spores in cracked or damaged fruits and vegetables. The author concluded that fruit flies are important vectors in spreading fermentative yeasts. *Kloeckera apiculata* and *Candida tropicalis* were the most frequent isolates from fresh fruits.

Yeasts are mostly limited to the surface of whole sound fruits, whereas the internal tissues are generally free from contamination. There are some reports that the inner tissue of fruits also harbors viable yeasts (Beech and Davenport, 1970; Ingram, 1958 and Mrak and Phaff, 1948). Factors, which influence the susceptibility to invasion by microorganisms, include the presence of natural openings (e.g. lenticels, stomata) or wounds caused by mechanical damage. Spoilage of fresh fruits and vegetables by yeasts usually results from their fermentative activity rather than degradation of the plant tissue by the action of cell wall degrading enzymes, although some yeasts are capable of producing pectolytic, cellulolytic and xylanolytic enzymes (Vacek *et al.*,

1979). No yeast is known to secrete cellulase, and few are capable to attack insoluble complex polysaccharides or pectin (Dennis, 1972; Luh and Phaff, 1951; Stevens and Payne, 1977). Fresh fruits and vegetables are usually not infected by pectinolytic yeasts. Dennis and Buhagiar (1980), concluded that undamaged fruits, surrounded by an intact firm skin, are rarely spoiled by yeasts. Yeast species capable to produce pectinases that are able to soften and spoil fruits and vegetables include, *Kluyveromyces* spp, *Candida kefyr, Candida famata, Rhodotorula* spp, *Cryptococcus albidus* and *Pichia* spp (Dennis and Harris, 1979; Fleet, 1992; Fleming, 1982; Vaughn, 1982).

Fresh fruits and vegetables may transmit human pathogenic yeasts, such as *Cryptococcus neoformans* and *Candida albicans* (Parish and Carroll, 1985) and therefor may cause cryptococcal subacute meningitis.

Miller and Phaff (1962) showed that the apiculate yeasts comprise 90% of the spoilage of figs. Apiculate yeasts are also frequently associated with the spoilage of grapes (Guerzoni and Marchetti, 1987), tomatoes (De Camargo and Phaff, 1957) and oranges (Vacek *et al.*, 1979). *Saccharomyces* spp., however, despite being isolated regularly from fruits do not play a significant role in fruit spoilage (Fleet, 1992).

A recent and most exciting discovery has been the isolation of yeast species from fruits that exhibit antifungal activity. Such yeasts include *Cryptococcus laurentii* in posharvest biological control of gray mold of apple (Roberts, 1990), the osmophilic yeast, *Debaryomyces hansenii* has been found to be effective against the major rots of citrus (Chalutz and Wilson, 1989; Potjewijd *et al.*, 1995), like grapefruit (Droby *et al.*, 1999; Droby *et al.*, 1989) and apples (Wisniewski *et al.*, 1988) and *Candida* spp. against postharvest diseases of grapefruit (Droby *et al.*, 1999; McGuire and Baldwin, 1994) and grape, peach, apple and citrus (Arras, 1996; Droby *et al.*, 1998; El-Ghaouth *et al.*, 2000; El-Ghaouth *et al.*, 1998; Mclaughlin, *et al.*, 1992; Teixidó *et al.*, 1999; Viñas *et al.*, 1997).

2.2 Fungal spoilage of vegetables

Yeasts are not important in the spoilage of fresh vegetables, but nevertheless, substantial yeast populations have been found on the surfaces of some vegetables (Mundt, 1978). Compared to moulds and bacteria, which are considered to be the main perpetrators of fresh vegetable spoilage, yeasts play only a secondary role in the spoilage of vegetables (Dennis, 1986). In contrast to fruits, vegetables are susceptible to growth of a wider range of microorganisms. The pH of most vegetables ranges between 5.0 and 6.0, which does not inhibit the growth of most microorganisms. Several intrinsic factors, including high water content, nutrient composition and pH of most vegetables create a micro-environment which favors microbial growth and subsequent spoilage (Dennis, 1987). Spoilage can be influenced by the history of the land on which vegetables are grown. For example, repeated planting of one type of vegetable on the same land over several seasons can lead to the accumulation of plant pathogens in the soil and increased potential for spoilage (Lund, 1983).

As a general rule, vegetables become more susceptible to infection by postharvest pathogens as they ripen (Eckert, 1987). Postharvest losses of fresh vegetables primarily occur as a result of mechanical injuries, nonparasitic disorders and parasitic diseases (Harvey, 1978). Common mechanical injuries include cuts, punctures, insect scars and cracking. Nonparasitic disorders include various physiological responses of vegetables to the postharvest environment. Parasitic disorders that result in decay are usually caused by microbial invasion (Bulgarelli and Brakett, 1991). Lactic acid bacteria are usually found on fresh vegetables in relatively low numbers. Their occurrence is, however, of interest because they assume greater importance in relation to spoilage after processing (Dennis, 1987). Although lactic acid bacteria are primarily responsible for the fermentation, the reduced pH created by the production of lactic acid select for the healthy growth of yeasts during the main stages of fermentation (Fleet, 1992).

Fermentative species of yeasts grow in the depths of the brine and oxidative species grow on the surface. Major defects caused by yeasts are discoloration, softening and gaseous blistering and bloating (Brackett, 1987). Pink discoloration of sauerkraut is due to the growth of oxidative species of *Rhodotorula* spp. *Rhodotorula glutinis* was previously associated with the softening of olives (Vaughn *et al.*, 1969) and the spoiling of peas during frozen storage (Collins and Buick, 1989). Pectolytic enzymes produced by species of *Debaryomyces, Pichia, Candida, Saccharomyces* and *Rhodotorula* cause softening of product texture (Flemming, 1982). Dennis and Buhagiar, (1980) indicated the swelling, blistering and gaspocket formation in pickles and olives are due to excessive gas production by fermentative yeasts such as *Saccharomyces oleaginosus, S. kluyveri* and *Pichia anamala.* Soft rot in onions caused by *Kluyveromyces marxianus* was studied by (Johnson *et al.,* 1989; Johnson *et al.*, 1988).

Only rarely have yeasts been directly implicated as a direct cause of deterioration of vegetables (Johnson *et al.*, 1988; Moline, 1984). The most commonly occurring species are the basidiomycetous yeast species *Cryptococcus albidus, Cryptococcus laurentii, Cryptococcus macerans* and *Sporobolomyces roseus* (Geeson, 1979; King *et al.*, 1976). Török and King (1991) indicated that ascomycetous yeasts predominate on fruits, compared to vegetables, whereas more basidiomycetous yeasts occur on vegetables than on fruits. Deàk and Beuchat (1991) also detected fermenting ascomycetous related yeasts on sweet corn, although these yeasts (*Candida oleophila, Pichia guilliermondii*) constituted a minority of the population.

In a survey of fresh vegetables at harvest, Webb and Mundt (1978) observed that the moulds most commonly isolated are *Aureobasidium, Fusarium, Alternaria, Epicoccum, Mucor, Chaetomium, Rhizopus and Phoma.* There are many forms of vegetable spoilage attributed to filamentous fungi (Brackett, 1994). Some of the more common types of spoilage are listed in Table 1. One of the most widespread fungal diseases is gray mould, caused by *Botrytis cinerea.* Storage conditions at warm temperatures and high humidity favour this type of spoilage (Friedman, 1960).

TABLE 1 Common Postharvest Spoilage Fungi of Vegetables

Fungus	Commonly observed type of spoilage and representative vegetable
Alternaria	Leaf spot or nail head spot of cabbage, cauliflower, turnip, melon, cucumber, tomato, initially small, inconspicuous black spots enlarging and coalescing; superficial growth on cortical parenchyma of potato; calyx rot of peppers.
Aspergillus	*A. alliaceus* causes dry "charcoal" rots; black rot of onion, garlic.
Botrytis	*B. cinerea* causes soft, dark rots of most vegetables; *B. allii* causes neck rot of onion.
Bremia	Downey mildew of lettuce.
Ceratocystis	Black rots of vegetables; with *Rhizopus*, most serious cause of loss of stored sweet potatoes; initial brown spots enlarge, become black with perithecial formation.
Choanephora	Blossom end rot of squash.
Cladosporium	Scab on cucumber, pumpkin; black and green-black rots on aging produce.
Colletotrichum	Anthracnose or bitter rot; brown surfaces; wide host range among vegetables and fruits.
Diaporthe	*D. batatis* causes firm dry decay of sweet potato; roots turn dark brown, becoming black as pycnidia form; *D. vexans* causes fruit rot of eggplant.
Diplodia	Browning and shrivelled areas of cucurbits, sweet potatoes; stem-end rots of melons.
Fusarium	Wet weather infection of wide variety of vegetables; adventitious on aging produce; field-infecting agent, manifest through growth in storage; bulb rot of *Allium* spp.; often follows bacterial soft rots; wound infection of sweet potato; penetrating black dry rot of white potato.
Geotrichum	Sour rot of vegetables; also known as "machinery mold" or "dairy mold" in processing plants.
Mucor	Wound infections; prominent growth; filamentous; nonfermenting.
Mycosphaerella	Cucurbits; small, water-soaked spots turning black with pycnidial formation; followed by yeast's, bacteria.
Penicillium	Charcoal rot of sweet potato; common on wet and aging produce; *P. hirsutum* is a major cause of loss of stored horseradish; blue mold rots.
Phoma	Dry rot of beet.
Phomopsis	Phomopsis blight or market rot of eggplant.
Phytophtora	Buckeye rot of tomato; leathery, water-soaked rot of vegetables; downy mildews.
Pythium	Major spoilage agent of potatoes in early storage; watery, cream-gray to black rot known as "leak" is caused by *P. debaryanum*; frequently precedes *Fusarium*.
Rhizoctonia	With *Botrytis*, most common cause of mold loss of vegetables; slimy brown rots, often where vegetable is in contact with soil; forms large, black sclerotial masses.
Rhizopus	Adventitious on wet vegetables; wound-infecting on sweet potato; rapid decay; late storage rots; often characterized by fermentative odor.
Sclerotina	Water Soft rots of cucurbits; *Trichoderma* Green and black rots of brassicae.
Trichothecium	*T. roseum* causes pink rot. soft rot of celery and carrots.

Adapted from Mundt (1978)

Wisniewski *et al.* (1991) successfully applied yeast as biocontrol agent, *Pichia guilliermondii* in controlling *Botrytis cinerea* through attachment of the yeast to hyphae of the postharvest pathogen. Droby *et al.* (1993) studied the yeasts *Pichia guilliermondi* and *Candida oleophila* extensively in pilot and commercial tests for their efficacy in inhibiting the development of postharvest decay of citrus.

Another very common type of fungal spoilage is black rot, caused by *Alternaria* species. Unlike gray mould, the tissue remains quite firm, although bacterial soft rots may follow (Mundt, 1978). *Rhizopus* species also causes soft rot. This fungus cannot enter through unbroken vegetable skin. This mould gains access to internal tissues of vegetables via the common fruit flies, *Drosophila melanogaster*. The fruit fly deposits spores into growth cracks and wounds of vegetables (Brackett, 1987). In addition to these common forms of spoilage, many other forms of important fungal spoilage may exist but may only be found in specific circumstances or with certain vegetables. Most fruits differ from vegetables in that they have a more acidic pH (<4.4), as well as a higher sugar content (Brackett, 1997). Some of the moulds responsible for spoilage are true plant pathogens in that they can invade and cause an infection of intact, formerly healthy tissue (Splittstoesser, 1991). Others are saprophytic species which only become established after the fruit has been infected by a pathogenic organism or has been damaged by some physical or physiological cause (Splittstoesser, 1987). Moulds cause a number of diseases associated with fruits.

One might highlight *Penicillium italicum* that causes blue rot, *Penicillium digitatum* which cause green rot of citrus fruits and *Penicillium expansum* which is responsible for blue mould rot of apples and cherries. Wilson and Chalutz (1989) demonstrated effective control of green, blue and sour rot of citrus by the yeast strain *Debaryomyces hansenii*. This effective antagonist is also effective against *Rhizopus stolonifer, Botrytis cinerea, Alternaria alternata* (Droby *et al.,* 1989) and *Penicillium digitatum* causing green mould decay on citrus fruit (Droby *et al.*, 1999). Although the surfaces of fresh fruits harbour large numbers of both yeasts and moulds, yeasts generally lack the mechanisms to invade and infect plant tissue and therefore are

secondary rather than primary agents of spoilage. The main advantage of moulds compared to yeasts is the ability of moulds to produce a wider variety of extracellular enzymes like cellulases and pectinases causing degradation of the middellamella, resulting in detachment of cells from one another.

3. ECOLOGICAL FACTORS INFLUENCING THE GROWTH OF YEASTS

3.1. Temperature

The effects of temperature on the growth and properties of yeasts have been reviewed by Stokes (1971) and Watson (1987). The maximum and minimum temperatures for yeast growth are within the general range of 0-37^0 C, depending on the characteristics of yeast species and environmental parameters (Davenport, 1980).

Vidal-Leira *et al.* (1979) concluded that the vast majority (98%) of yeasts is mesophilic with values between 24 and 48^{0}C, a small minority (2%) is psychrophilic with temperature values below 24^{0}C and no yeasts are capable of growth above 50^{0}C. Strains of *Kluyveromyces marxianus* are capable of fermenting carbohydrates at 47°C (Deàk and Beuchat, 1996). The term cold tolerant is used to include all yeasts and yeast-like organisms capable of growth between -1^0 C and 4^0 C (Davenport, 1980; Kobatake *et al.*, 1992).

Basidiomycetous type yeast species, *Rhodotorula glutinis* and *Cryptococcus albidus,* can grow at temperatures of –2 and –12°C respectively. The ascomycetous yeast type *Debaryomyces hansenii,* has been reported to grow at temperatures of –12°C. Species of *Debaryomyces* are particularly adaptable to brines because of their extremely high salt tolerance, their ability to assimilate a large number of compounds as a source of carbon, and their ability to grow at low temperatures (Davenport, 1980). The ability to grow at these extreme conditions renders a competitive advantage to these yeasts making them important precursors in food spoilage.

3.2. Water activity

Water activity (Aw) is one of the most important factors affecting the growth of microorganims in foods (Deàk and Beuchat, 1996). The water activity of fresh fruits and vegetables is high enough to support the growth of most bacteria and fungi and is therefore not considered a limiting factor (Brackett, 1997). Yeasts can tolerate dry conditions with a water activity up to 0.62, whereas bacteria generally do not grow below $0.75a_w$. Increasing the concentration of solutes such as sugars or salt, temperature and other ecological factors further reduce water activity (Spencer and Spencer, 1997; Tokouka and Ishtani, 1991).

The most commonly isolated yeasts from high-sugar products include *Saccharomyces bisporus, Zygosaccharomyces rouxii* and *Schizosaccharomyces pombe* whereas *Debaryomyces hansenii* and *Pichia anomala* predominate in high-salt foods (Fleet, 1990). This group of yeasts has been referred to as osmophilic, osmotolerant or xerophilic (Tilbury, 1980a). The majority of food spoilage yeasts have minimum a_w values of 0.90 to 0.95 for growth. The most important physiological feature of *Debaryomyces hansenii* is its ability to grow in salt concentrations as high as 24% (Hocking and Pitt, 1997). *Rhodotorula mucilaginosa* and *Rhodotorula glutinis*, typical air contaminants, grow near a minimum a_w of 0.92 and are of widespread occurrence on fresh fruits and vegetables (Buhagiar and Barnett, 1971).

3.3. Oxygen

Another general environmental factor with respect to the growth and metabolism of yeasts in foods is oxygen concentration (Deàk and Beuchat, 1996; Phaff and Starmer, 1980). Yeasts are considered as aerobic organisms and approximately 40% of the yeast species described by Barnett *et al.* (1979) are listed as non-fermentative. Species within the genera *Rhodotorula* and *Cryptococcus* are strictly non-fermentative aerobes (Deàk and Beuchat, 1996). Barnett *et al.* (1990a) reported that yeast species are considered non-fermentative as judged by the lack of carbon dioxide formation in the classical Durham tube test. However, Van Dijken *et al.* (1986) concluded that the

absence of gas formation is not a reliable criterion for the absence of fermentation capacity.

During a comparative study on the enzymology of facultative fermentative and non-fermentative yeasts, Van Dijken *et al.* (1986) reported that a variety of yeasts, described as non-fermentative, possessed pyruvate decarboxylase, the key enzyme of alcoholic fermentation. *Saccharomyces, Schizosaccharomyces, Hanseniaspora* and some other genera, when submerged in sugar containing substrates, vigorously ferment sugars, but soon stop growing because of a lack of available oxygen. Surfaces of food products will normally be inhabited by aerobic yeast species (Rose, 1987). Investigations to determine the effect of controlled and modified atmosphere storage on the behavior of yeast flora in food products will be discussed later.

3.4. Nutrients

The most important nutrients for yeasts are carbohydrates that serve as sources of energy. A few sugars,mostly hexoses and oligosaccharides, can be fermented by yeasts (Deàk and Beuchat, 1996). Because sugars commonly occur in foods (fruits) and beverages, fermentation features significantly in the spoilage process (Fleet, 1992). Yeasts can utilize both organic and inorganic nitrogen compounds. Amino acids, amines and urea are suitable nitrogen sources for practically all yeasts (Large, 1986) as are inorganic ammonium salts. Many species synthesize all of the necessary vitamins for growth and biotin appears to be the most commonly required vitamin (Barnett *et al.*, 1983).

3.5. Acidity and pH

Yeasts can either produce or metabolize organic acids (Gancedo and Serrano, 1989), thereby changing the acidity and flavor of the product. Succinic acid is the main carboxylic acid produced by yeasts during fermentation. Oxidative utilization of organic acids can appreciably decrease the acidity of products and increase their pH to values that allow the growth of spoilage bacteria (Fleet, 1992). Yeasts show a

remarkable tolerance to pH and prefer slightly acidic medium, such as fruits, and have optimum pH values between 4.5 and 6.5 (Pitt, 1974).

The basidiomycetous yeasts, *Rhodotorula mucilaginosa, Rhodotorula glutinis* and *Cryptococcus laurentii* are alkali tolerant, whereas strains belonging to *Schizosaccharomyces* are alkali sensitive and cease to grow at levels above pH 8 (Aono, 1990). In the wine industry, *Schizosaccharomyces pombe* may be used to deacidify wine because of its ability to metabolize L-malic acid (Sousa *et al.*, 1995). The ability of yeasts to grow at low pH depends on energy-requiring systems that pump protons actively out of cells and thus prevent acidification of the cell interior (Deàk, 1978).

4. ROLE OF EXTRACELLULAR ENZYMES PRODUCED BY FUNGI

As one might expect, changes in texture introduced primarily through the action of amylolytic, proteolytic, cellulolytic and pectinolytic enzymes (Brackett, 1987) by breaking down the polysaccharides of the skin (Fleet, 1992). The coherence of the tissue of plants, including fruits and vegetables, is largely dependant on the middellamella which functions as an adhesive, binding cells together (Dennis, 1987). Fruits and vegetables respire by taking up oxygen and giving off carbon dioxide and generating heat. In fruits and vegetables respiration involves the enzymatic oxidation of sugars to carbon dioxide and water, accompanied by release of energy (Ryall and Lipton, 1979). The energy produced by the oxidation of sugars is converted into the energy of adenosine triphosphate (ATP), as an energy carrier (Ryall and Pentzer, 1982). Fruit and vegetable enzymes play a significant role in the growth, development, such as morphological structure, prematuration, maturation, ripening and senescence in fruit and vegetable ontology (Ryall and Lipton, 1979). Ethylene, the main precursor of colour and ripening, is synthesized within the cell enzymatically from methionine (Ryall and Pentzer, 1982).

The main constituents of the middellamella are the pectic substances, and cellulose (McFeeters *et al.,* 1992). Pectic substances are polymers of D-galacturonic acid residues glucosidically linked alpha-1.4 bonds (BeMiller, 1986). Therefore, the invasive ability of fungi depends primarily on their ability to degrade the middellamella of plant tissue by the action of pectolytic enzymes, leading to loss of turgor, and the accompanying softening of the fruits and vegetables. Fungi, such as *Botrytis, Alternaria, Fusarium, Monilinia* and *Rhizopus* produce both pectin methyl esterases and polygalacturonases (Bulgarelli and Brackett, 1991).

Yeasts capable to degrade starch, have been the subject of many ecological, biochemical and genetic studies in recent years, as comprehensively reviewed by several authors (De Mot, 1990; McCann and Barnett, 1986; Spencer-Martins and Van Uden, 1977). Amylolytic enzymes, α-amylase, β-amylase and glucoamylase accomplish hydrolysis of starch. The capacity to degrade starch, however, is not widespread among yeasts. Yeast amylases are mostly α-amylase and glucoamylases (Linardi and Machado, 1990). According to McCann and Barnett (1986), about 150 yeast species produce extracellular amylases. Most studies, however, have focussed on the amylases produced by *Schwanniomyces occidentalis*, *Saccharomycopsis fibuliger* and *Saccharomyces diastaticus* (De Mot, 1990).

Degradation of cellulose is generally of less importance in spoilage, since fewer moulds and no yeasts produce cellulases. Species within the yeast-like fungi, *Trichosporon, Aureobasidium, Geotrichum* (Dennis, 1972; Fleet, 1992) and *Trichoderma reesi* (Evans *et al.,* 1992) possess this property. Methods for screening yeasts for the presence of these enzymes should take into consideration that enzyme production may be constitutive and require the presence of the polysaccharide substrate for induction (Call *et al.,* 1984; Wimborne and Rickerd, 1978).

5. POSTHARVEST LOSSES OF FRUITS AND VEGETABLES

Postharvest fungal decays of fresh fruits and vegetables can result in serious financial losses. The losses of fruits and vegetables constitute approximately 25% of the harvested crop, caused to a large degree by pathogenic microorganisms that usually attack the commodity at certain points along the harvesting, handling and processing line (Droby and Chalutz, 1991).

Losses can be reduced by postharvest treatment of fruits with fungicides or microbial antagonists. Alternative methods like;

a. Biological control,

b. Modified atmospheres,

c. Heat treatment and

d. Natural antimicrobial systems in fruits and vegetables, for control of these losses have been investigated because fungicides are being removed from the market due to human health risk concerns (Board of Agriculture, 1987).

Fresh fruits and vegetables perished rapidly due to the high moisture content present, making the products vulnerable to microbial decay as well as physiological deterioration (Harvey, 1978). Several changes take place in the cell wall composition and structure resulting in the softening of the fruits and vegetables. Cellular water is lost because of respiration and transpiration, resulting in fruits and vegetables becoming soft, shriveled and limped. Major storage diseases of fruits are initiated by penetration of spores of fungi into the lenticular cavities of the fruit during periods of relatively high temperature and humidity late in the summer. These fungi develop to a very limited extend in the lenticular cavity and then become quiescent until the fruit begins to ripen during storage (Wills *et al.,* 1989). Postharvest fungi and bacteria are abundant in the atmosphere and on the surface of fruits and vegetables as they approach maturity in the field (Duckworth, 1966). Fungi such as *Penicillium, Rhizopus* and *Geotrichum* are not capable of directly penetrating the cuticle and

epidermis of the host, but if they gain entry through an injury or natural opening, these fungi may cause devastating rots of the mature produce (Sommer, 1982).

High and low temperatures which physically damage the surface cells of fresh fruits and vegetables invariably increase infection by pathogens which invade through unintentional wounds (Spencer and Spencer, 1997). A major portion of the decay found in terminal markets has been attributed to high and low temperatures (Hocking and Pitt, 1997). Anthocyanins that give the typical red, orange, blue and other pigments of some fruits and vegetables may increase after harvesting. Starchy fruits and vegetables undergo a decrease in starch and increase in sugar and acid contents after harvest (Salankhe *et al.*, 1991). Quick cooling after harvest is therefore imperative to preserve their appearance.

The one way to reduce shriveling and drying of fruits and vegetables in storage rooms is, by increasing the relative humidity. Vegetables as well as fruits can be protected from a lower relative humidity by using various types of permeable polyethylene bags or by providing moisture in the form of hydrocooling. Hydrocooling fluid should contain a fungicide to prevent microbial growth (Ryall and Lipton, 1979). The physiological storage life of many fruits and vegetables can be realized only by treating them with an antifungal agent before they are stored in an environment that is optimum for retention of the desired crop qualities. Improved market quality may also be achieved by incorporating modified or controlled atmospheric conditions.

6. THE PREVENTION OF POSTHARVEST LOSSES

6.1. Effect of Modified Atmospheres on microbial spoilage of fruits and vegetables

It is well known that sensory quality of produce can be preserved by storage under atmosphere with modified carbon dioxide, oxygen and nitrogen content (Berrang *et al.*, 1989). Usually, controlled atmosphere storage of vegetables employs a lower O_2

concentration and higher CO_2 concentration than normally found in air (Shewfelt, 1986). More generally, CO_2 concentrations of 15-20% can effectively delay the development of pectinolytic microorganisms and reduce the extent of decay during storage of whole fruits and vegetables (El-Goorani and Sommer, 1981).

Shifting the reaction equilibrium towards the original carbohydrate reserve slows respiration. This in turn decreases the rate of respiration, retards the ripening process and facilitates retention of higher quality for a period of time that would otherwise be possible (Lieberman, 1954). Yeasts on fruits and vegetables, are generally unaffected by modified atmospheres. Moulds are typical aerobic microorganisms and modified atmospheres with high CO_2 and low O_2 concentrations inhibit their growth (El-Goorani and Sommer, 1981). The choice of treatment used for disinfecting fruits and vegetables will depend on the type of commodity, processing conditions and the desired shelf life (Beuchat, 1992).

6.2. Effect of heat treatment on fresh fruits and vegetables for decay control

Heat treatment is the application of heat at temperatures above 40°C for control of postharvest spoilage organisms. High temperatures weaken the pathogens and might stimulate resistance in fruit (Spotts and Chen, 1987). Fruits and vegetables commonly tolerate temperatures of 50-60°C for five to ten minutes, but shorter exposure at these temperatures controls many postharvest plant pathogens (Smith *et al.*, 1964). Post-harvest rot fungi generally grow best at about 20-25°C. The maximum temperatures at which fungi can grow are typically about 27-32°C, although some species can grow at higher temperatures (Eckert and Sommer, 1967). In general, yeasts possess little heat resistance and would not be able to survive the above mentioned heat treatment (Beuchat, 1982). Temperatures as low as 46°C are lethal to some strains and in general, basidiomycetous yeasts are more sensitive to heat compared to ascomycetous species. When in dry form, yeasts possess much higher degrees of heat resistance (Scott and Bernard, 1985). The most effective heat treatment for disease control is usually close to that which can be tolerated by the product and injury may

be manifested by increased susceptibility to wound pathogens such as *Penicillium* and *Alternaria* (Daines, 1970).

Postharvest heat treatment to control decay is often applied for only three to five minutes because the target pathogens are found on the surface or within the few outer cell layers of the produce. Heat treatments have the advantage of low cost, relatively simple application equipment and chemical residues on the treated commodity. The water content of air is greatly influenced by heat transfer and the heated moist air usually kills pathogens, more effectively than dry air at the same temperature (Teitel *et al.*, 1989). When the air is saturated with water, condensation forms on surfaces that are cooler than the air and heat is transferred rapidly to the surface (Edney and Burchill, 1967). The hot water inactivates spores and hyphae located on the skin (Smith, 1971).

6.3. Natural Antimicrobial Systems in fruits and vegetables

Antimicrobial compounds either naturally present in fruits and vegetables, or formed in response to physical or chemical stresses can contribute to extending shelf life. Many of these antimicrobials contribute to the foodstuffs natural resistance to deterioration (Marsh, 1966). The antimicrobial agent is most effective when the hosts possess intrinsic resistance to infection and the environmental conditions are least favorable for the growth of the pathogen.

Organic acids naturally present in raw fruits and vegetables are considered useful in controlling yeast growth. Eschenbecher and Jost (1977) demonstrated that other plant substances including alkaloids, phenols, glycosides, essential oils and tannins are involved. Acetic, citric, succinic, malic, tartaric, benzoic and sorbic acids are the major organic acids naturally occurring in many fruits and vegetables. The mode of action of organic acids is attributed to direct pH reduction and the depression of internal pH values of microbial cells by ionization of the undissociated acid molecule. The undissociated portion of the acid molecule is primarily responsible for the

antimicrobial activity. Effectiveness depends upon the dissociation constants (pKa) of the acid (Beuchat, 1992). The pKa of most of the organic acids is between pH 3 and pH 5. Surface application would therefore be more effective on fruits.

Organic acid washes to the surface of fruits and vegetables for the purpose of reducing populations of viable microorganisms has potential. Lysozyme was more active in vegetables than in animal-derived foods tested, while quinic acid may have a role in the formation of resistance against pathogens in berries (Kallio *et al.*, 1985). A more promising group of plant antimicrobials is the phytoalexins. Phytoalexins are metabolites produced by plants as a defense reaction when exposed to stress (e.g., injuries, cold, fungal infestation). Phytoalexins alter the properties of plasma membranes (Weinstein and Albersheim, 1983) and inhibit electron transport in the mitochondria (Boydston *et al.*, 1983).

Antimicrobial activities were detected in two yeast genera against bacteria. The yeast species, *Kluyveromyces thermotolerans* and *Kloeckera apiculata* were found to produce zones of inhibition against bacterial growth of *Lactobacillus plantarum* and *Bacillus megaterium.* Both yeasts were found to express maximal antimicrobial activity when cultivated at initial pH values of six. Neither strain inhibited bacterial growth when cultivated at initial pH values of four. No antimicrobial activity was evident against four gram negative bacteria (*Acetobacter aceti, Alcaligenes spp., Enterobacter.,* and *Flavobacterium spp.).* Therefore, the antimicrobial activities appeared to be not only gram-specific but species specific (Bilinski *et al.*, 1985).

6.4. Biological control of postharvest diseases of fruits and vegetables

Fruits and vegetables suffer significant losses from parasitic diseases after harvest (Snowdon, 1992). Some postharvest pathogens cause infection when the fruits are still attached to the plant (Harvey, 1960). With others, infection is initiated through injuries incurred at the time of harvesting or mechanical wounds (Smoot, 1971). Some of the most devastating postharvest pathogens enter through mechanical and physiological injuries created during and after harvest. In less developed countries,

postharvest losses are enhanced due to the lack of poor storage, inadequate refrigeration and food handling technologies.

Fungicides are a primary means of controlling postharvest diseases after harvest or prior for shipping to markets (Eckert and Ogawa, 1985). Pathogen resistance to fungicides (Spotts, 1986) and hazards concerning human health and the environment (Dekker, 1982) in combination increased the interest in alternative methods to fungicidal treatment in controlling fruit diseases. This has led to the development of biological control (Janisiewicz, 1987) of postharvest diseases as a promising alternative procedure (Fokkema, 1993).

Postharvest treatment of fruits with microorganisms recovered from fruit surfaces is currently implemented as a alternative method for the control of postharvest diseases of fruits and vegetables (Wilson, 1989). Baker and Cook (1983) defined biological control as the reduction of the amount of inoculum or disease-producing activity of a pathogen accomplished by or through one or more organisms other than man.

Two basic approaches are available for using microorganisms to control postharvest diseases; use and management of the beneficial microflora that already exist on fruit and vegetable surfaces, since yeasts appear to be the major component of the flora of fruit surfaces (Wisniewski, 1991). This suggests that a form of biological control occurs on fruits in nature and that some of these organisms may be potential biocontrol agents for fruit pathogens (Janisiewicz, 1991). A number of factors must be considered in the selection of biocontrol agents:

- The type of organism to be used is of primary importance.
- Antibiotic-producing microorganisms are also potential candidates as biocontrol agents.
- Survivability of the agent is a major factor in determining its usefulness.

- Biocontrol agents must be compatible with chemicals used for fruit treatment and for handling in storage with fungicides used to control other diseases (Janisiewicz, 1991).

Yeasts have a number of attributes, which make them suitable as biocontrol agents of postharvest diseases,

- They can rapidly colonize and survive on fruit surfaces for long periods under different conditions.
- They produce extracellular polysaccharides that enhance their survivability and restrict both colonization sites and the flow of germination cues to pathogen propagules.
- They use available nutrients to poliferate rapidly and
- They are effected minimally by pesticides (Droby *et al.,* 1999).

Treatment of fruits with certain yeast strains that exhibit antifungal activity (Droby *et al.*, 1989) has been shown to be effective for control of postharvest decay. Such yeasts include *Debaryomyces hansenii*, *Candida* spp. and *Sporobolomyces roseus* (Beuchat, 1992). *Candida guilliermondii, Candida sake* and *Candida oleophila* have shown to be very effective against green mould decay caused by *Penicillium digitatum* (Droby *et al.*, 1999).

In this regard, it is noteworthy that the yeast *Candida guilliermondii* was the most effective and predominant species on grapefruit as well as other fruits (unpublished data). Currently, Aspire, a biocontrol product containing the yeast *Candida oleophila* as the active ingredient, is registered in the United States for commercial use as a postharvest biofungicide (El-Ghaouth *et al.*, 2000). Antagonists have been reported for a few major field and postharvest pathogens (Pusey, 1984). Antagonistic microorganisms appear to be exceptionally effective as biological control agents in the postharvest environment, mainly because they can be targeted where they are needed when they are applied to harvested commodities (Wilson, 1989). Since antagonists will be applied to food, special consideration should be given to their potential toxicity to man and animals.

Desirable characteristics of an "ideal antagonist" for postharvest environment would be:

- Genetically stable.
- Effective at low concentrations.
- Able to survive well under adverse environmental conditions.
- Efficacious against a wide range of pathogens on a variety of fruits and vegetables.
- Non-productive of secondary metabolites that may be deleterious to humans.
- Resistant to pesticides.
- Compatible with other chemical and physical treatment of the commodity, and
- Non-pathogenic against the host (Wilson and Wisniewski, 1989)

Antagonists that already exist on fruit and vegetable surfaces, hold promise as "living fungicides" for the control of postharvest diseases (Wilson *et al.,* 1991). The disadvantages of naturally occurring antagonists are, when fruits and vegetables are washed they could remove a microbial population and protective waxes that impart resistance to rotting (Droby, 1991). Application of the antagonist begins at bloom time and might continue until near harvest. The antagonists are generally applied as a conidial suspension often supplemented with nutrients such as carboxyl-methyl-cellulose, yeast extract, and sucrose, which improve conidial germination and allow them to adhere better to plant surfaces (Janisiewicz, 1991).

Furthermore, antagonists that produce antibiotics may be prone to failure due to the development of resistant strains of the pathogen (Droby, 1991). To evaluate the use of yeasts as a biocontrol agent, an understanding of the antagonistic interaction between the yeasts and postharvest pathogens is needed (Wisniewski *et al.*, 1991). Although nutrient competition has been suggested as the principle mode of antagonism (Droby *et al.*, 1989), induction of host defence mechanisms and direct interaction with the pathogen are other important factors in the mode of action (Droby *et al.*, 1991).

Competition for nutrients is a widespread phenomenon in the interaction between microorganisms on the phylloplane. They effectively utilise nutrients at low concentrations and survive and develop on the surface of the commodity, or at the infection site under temperature, pH and osmotic conditions that are unfavourable for the growth of the pathogen (Wilson *et al.*, 1991). The antagonist may induce wound healing processes and other defence reactions of the host tissue (Droby *et al.,* 1991). An effective antagonist could also contribute to the resistance of the host indirectly by changing the chemical and osmotic environment at the wound site to favour the antagonist over the pathogen (McLaughlin *et al.,* 1990).

Antagonistic yeast cells may also effect the pathogen directly or by the production of antibiotics, thereby decreasing fungal infectivity. The antagonistic cells, which tend to attach to the mycelium of the fungus, produce possible glucanases or chitinases and cause dissolution of mycelial cell walls that effect the vital processes of the fungus (Wisniewski *et al.,* 1988). Enhancing biological control by using mixtures of mutually compatible antagonists have the following advantages;

- It may broaden the spectrum of activity e.g. various fruit, cultivars and maturity stages.
- It may enhance the efficacy and reliability of the biological control and
- It allows the combination of various traits without employment of genetic engineering (Janisiewicz, 1996).

Recently, (El-Ghaouth *et al.*, 2000) developed a biocontrol product called " a bioactive coating" consisting of a unique combination of an antagonistic yeast with chemically modified chitosan. Laboratory studies shown, the combination of *Candida saitoana* with chitosan were more effective in controlling decay of apples and citrus fruit than *Candida saitoana* or the chitosan treatment alone.

Combining antagonistic yeasts with chitosan can be expected to provide more effective disease control and management of fungicide-resistant isolates of

Penicillium digitatum and *Penicillium expansum.* Antagonists can be artificially introduced onto plant surfaces to impart resistance against pathogens (Wisniewski *et al.,* 1988). Several factors indicate that postharvest biological control with the use of artificially introduced antagonists prove to be an effective technology. First, environments for the storage of harvested commodities are often controlled and maintained. Secondly, antagonists can be more easily targeted to where they are needed when they are applied to harvested commodities, compared with field or soil applications (Wisniewski *et al.*, 1988). Third, cost-effectiveness. It would be advantageous to find and develop antagonists with a broad spectrum of activity against a large number of pathogens or a wide variety of commodities (Wilson, 1989). The success of biological control of postharvest diseases in the future will not only depend on its effectiveness, but also on the competitiveness of its costs and the lack of side effects such as toxicity to mammals arising from the applied organisms.

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CHAPTER 2

The incidence of yeasts associated with fruits and vegetables

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Abstract

An ecological survey was conducted on ten different fruit and vegetable commodities to determine the levels of yeast populations on the surface and flesh of fruits and vegetables. Vegetables in general, yielded higher bacterial counts (10^7 cfu g^{-1}) while fruits contributed to higher yeast counts (10^5 cfu g^{-1}). Yeasts were predominantly generated from the surface of the products. A total of 90 yeast strains were isolated from fruits and vegetables with *Schizosaccharomyces pombe, Debaryomyces hansenii* and *Hanseniaspora guilliermondii* predominating. *Debaryomyces hansenii* proved to be the dominant species isolated in both commodities. Other species frequently encountered were *Candida tropicalis, Cryptococcus laurentii* and *Rhodotorula spp.*

1. Introduction

Biological control of postharvest diseases of fruits and vegetables has been proven feasible in numerous studies (Wisniewski and Wilson, 1992). The fruit surface supports the growth of interacting microorganisms (Janisiewicz, 1991). A wide variety of bacteria, yeasts and other fungi, typically reside on the surfaces of fruits before they are picked from the tree and undergo normal processing (Andrews, 1992). This suggests that a form of biological control occurs on fruits in nature and that some of these organisms may be potential biocontrol agents for fruit pathogens (Janisiewicz, 1991). Although some of these microorganisms may be potential pathogens, the majority are harmless epiphytes, living and dying with little or no effect on the plant itself (McGuire and Baldwin, 1994).

Although the application of yeasts species proved to be effective in postharvest biological control (McGuire, 1994; McLaughlin *et al.,* 1992; Roberts, 1990), the natural occurring surface flora of yeasts remain relatively inactive. These yeasts do not produce appropriate amounts of cellulolytic or pectinolytic enzymes to degrade the skin of the fruit and induce infection (Dennis and Buhagiar, 1980; Ingram, 1958). However, physical damage of the skin by overweening, mechanical injury or fungal attack, exposes the fruit tissue upon which yeasts can rapidly grow. Only a few yeast species produce extracellular cellulases, pectinases or xylanases in initiating the spoilage of fruits and vegetables by breaking down the polysaccharides of the skin (Fleet, 1992). No yeast has been reported to degrade cellulose, although species within the yeast-like fungi, *Trichosporon* and *Aureobasidium* possess this property (Zimmermann and Emeis, 1989).

Fruits are rich in nutrients (soluble carbohydrates) and the lower pH levels (higher organic acid content) and the high moisture content make them a primary target for growth of yeasts (Deàk, 1979). Yeasts however, rarely spoil undamaged fruits. As reviewed by Lund (1958), yeasts commonly occur on the surfaces of freshly harvested

fruits at populations of 10^3 to 10^5 cells/ cm^2. Damaged fruits frequently harbor yeast populations of 10^6 to 10^8 cells / g (Fleet, 1992). Spoilage of fresh fruits usually results from the fermentative activity of yeasts. Miller and Phaff (1962) and Spencer *et al.* (1992) found that apiculate yeasts (species of *Hanseniaspora* and *Kloeckera*), *Candida stellata, Pichia membranaefaciens, Candida guilliermondi, Pichia kluyveri* and *Candida krusei* were the most common fermentative spoilage yeasts associated with fruits. Pectolytic enzymes produced by species of *Debaryomyces, Pichia, Candida, Saccharomyces, Rhodotorula* and *Kloeckera* contribute to the softening of product texture (Vaughn *et al.,* 1972).

The yeast populations associated with fresh vegetables are generally low (10^3 to 10^6 cells/g), but may increase during storage, especially if the product is sliced or shredded (Brackett, 1987). Yeast counts of 10^8 cells/g have been observed in stored cabbages (Geeson, 1979). Bulgarelli and Brackett (1991) reported that the high carbohydrate contents and low acid contents of many vegetables favor the growth of the lactic acid bacteria. Also, the close proximity of vegetables to the soil during development facilitates contamination. According to Fleet (1990) the lactic acid bacteria lower the pH of the commodity and thereby enhance the competitiveness for yeasts to grow. The predominant types of yeasts identified on fresh vegetables are usually asporogenous pigmented varieties which belong to the genera *Cryptococcus, Rhodotorula*, *Sporobolomyces* and *Tilletiopsis* (Mundt, 1978).

An ecological study was performed to differentiate between yeasts found on the surfaces of fruits and vegetables and the inner layer. Ten different fruits and vegetables were selected from a retail supermarket in the Free State for compiling this study.

2. MATERIALS AND METHODS

2.1. Media used:

Yeast extract glucose chloramphenicol agar (YGC)(Merck, Biolab Diagnostics, South Africa, pH 6.6) was used for the isolation and enumeration of yeasts. Plate count agar (PCA)(Merck, Biolab Diagnostics, South Africa, pH 7.0) was used for the enumeration of total bacterial counts.

2.2. Sampling and isolation:

2.2.1. Surface sampling

Two sets of counts were performed for microbiological analysis on the inner tissue and outer surfaces of fruits and vegetables to distinguish between the microflora of the two layers.

For determination of outer surfaces, duplicate samples of each fruit and vegetable were dipped and rubbed individually in 100ml sterile water in sterile Whirl-pak (Nunc, Germany) plastic bags for two min. Ten ml are extracted from the sterile bags and the liquid portion diluted. Further decimal dilutions of the suspensions were carried out as required for microbiological assays in 9ml sterile peptone water. Aliquots (0.1ml) of the dilutions were spread plated, in duplicate, over the surface of plates of the appropriate media.

2.2.2. Inner tissue sampling

For the determination of microbial analysis of the inner tissue, four different samples of each fruit and vegetable were dipped individually in 0.85% sodium hypochloride for two min as described by Johnson *et al.* (1988) to eliminate the surface microflora.

Fruit and vegetable samples were cut with a sterilized knife, weighed and 20g portions were suspended in 180ml sterile Bacto peptone water and homogenized in a Warring Blender for two min. Ten ml were extracted and the liquid portion diluted in 90 ml sterile peptone water. Aliquots (0.1ml) of the serial dilutions as required for microbiological assays were prepared and spread plated, in duplicate, onto Yeast Extract Glucose Chloramphenicol agar (Merck) and Plate Count agar (Merck).

2.3. Microbial enumeration:

Plates for the detection of yeasts were incubated at 25°C for 5 days under aerobic conditions after which visually distinguishable yeast colonies on the highest dilution between 30 and 300 cfu/g were counted, purified and stored on yeast extract-malt extract (YM) slants at 4°C until characterization. The data were converted to the number of yeasts per gram of fruits and vegetables. Total viable bacterial counts on the Plate Count agar were determined after incubation at 25°C for 48h.

2.4. Yeast identification:

The representative yeast isolates were identified by using the methods described by Van der Walt and Yarrow (1984) and the computerized identification system of Barnett *et al.* (1990). Each isolate was inoculated into 6 sugar fermentation media, 32 carbon source assimilation media and vitamin free medium (Van der Walt and Yarrow, 1984). Additional tests performed included growth at 37°C, in 50% D-glucose medium, urea hydrolysis, splitting of arbutin, 0.01 and 0.1% cycloheximide and staining of 4-week-old cultures with Diazonium Blue B salt reagent. Assimilation of nitrogen compounds, as performed by means of the auxanographic method (Lodder and Kreger-van Rij, 1952), was also included.

Ascospore formation was examined on McClary's acetate agar, potato glucose agar, Gorodkowa agar, corn meal agar and malt extract agar (Van der Walt and Yarrow, 1984). The inoculated media were incubated at 18°C for 4 weeks and examined at 4-day intervals. Cell morphology and mode of reproduction were examined on malt extract agar (Difco) and on Dalmau plates (Van der Walt and Yarrow, 1984). The formation of pseudomycelium and true mycelium was examined on corn meal agar according to the Dalmau plate technique (Van der Walt and Yarrow, 1984).

3. Results and discussion

3.1. Microbial enumeration

Bacteria represented the major component of the microflora isolated from the surface of vegetables (Table 1). The high incidence of bacterial loads on the surfaces corresponds with results obtained by Brackett and Splittstoesser, 1992., Deàk and Beuchat, 1996 and Dennis, 1987. Deàk and Beuchat (1996) reported that several intrinsic factors such as high water content, adequate nutrient composition and a more neutral pH predispose vegetables to microbial spoilage. In general, vegetables harbored higher bacterial counts (10^7 cfu. g^{-1}) than yeasts (10^5 cfu. g^{-1}) on the surface. On the other hand, microbial counts obtained from the inner surfaces yielded substantial lower bacterial and yeast counts (10^2cfu.g^{-1}), which are in correspondence with previous reports (Brackett and Splittstoesser, 1992; Fleet, 1992; Lund, 1992). Potatoes and squashes yielded the highest bacterial numbers, exceeding counts of 10^7cfu.g^{-1} whereas beetroot and greenbeans yielded counts of 10^6cfu.g^{-1}.

Bulgarelli and Brackett (1991) reported that the close proximity of vegetables to the soil during development facilitates contamination. As a general rule, vegetables become more susceptible to infections as they ripen, and mechanical injuries resulting from harvesting and handling also facilitate entry of invading microorganisms

(Bulgarelli and Brackett, 1991). Onions yielded the lowest bacterial counts of 10^4cfu.g^{-1}. Beuchat and Golden (1989) indicated on the possibility of the enzymically conversion of cysteine sulphoxides to thiosulphinates when an onion is crushed. This process might have an anti-microbial effect on the growth of bacteria on onions. Higher yeast counts were detected on sweet potatoes and baby marrows yielding counts of 10^4cfu.g^{-1} and 10^5cfu.g^{-1} respectively. The higher carbohydrate composition of sweet potatoes (27.9%) might explain the higher yeast counts on this commodity (Watt and Merrill, 1950). No yeast population was obtained on onions and cucumbers.

Fruits, on the other hand yielded higher yeast counts than bacteria (Table 2). Yeast numbers reaching counts of 10^5 cfu. g^{-1} were observed whereas bacterial counts of 10^4 cfu. g^{-1} were detected. According to Brackett (1997), Dennis (1987) and Fleet (1992) moulds and yeasts tend to predominate in fresh fruits causing spoilage. Hocking and Pitt (1997) reported that the pH levels of the living tissue are responsible for the enhanced spoilage of fresh fruits compared to vegetables. Fruits are usually more acid-like and many yeasts and moulds therefore competed better against the bacteria at lower pH values (Hocking and Pitt, 1997; Splittstoesser, 1987). Substantial lower inner microbial counts were detected in all the fruit samples, and in some cases, no microbial organisms was detected from the fruits, which are in agreement with previous reports (Fleet, 1992; Lund, 1992; Samish *et al.,* 1963).

According to Splittstoesser (1987) fruits are usually washed immediately after harvest that plays an important role in reducing microbial contamination. The higher bacterial counts observed on oranges and mandarins (10^4cfu.g^{-1}) might be field contamination and not properly washed in the supermarket before purchasing. The high yeast counts obtained on strawberries (10^4cfu.g^{-1}) are reluctant to the theory of Buhagiar and Barnett (1971) with the proximity of the strawberries to soil, could well explain this unusual occurrence compared to yeasts associated with tree fruits. According to

Rosini *et al.* (1982) mechanical injury during harvesting leads to increased microbial numbers in the area surrounding the stem. The high counts on the softer fruits, like cherries (10^5cfu.g^{-1}), figs (10^4cfu.g^{-1}) and prickly pears (10^5cfu.g^{-1}) could be due to mechanical injury especially the high inner counts of bacteria on cherries (10^4cfu.g^{-1}). The higher carbohydrate and lower pH values in fruits support the growth of yeasts rather than bacteria (Fleet, 1992).

3.2. Yeast identification

A total of 90 yeast strains were isolated and identified from ten different fruit and vegetable commodities. The most predominant species isolated from vegetables were *Debaryomyces hansenii* (59%) and *Schizosaccharomyces pombe* (26%), while *Rhodotorula spp.* were present in lower numbers (Fig. 1). The occurrence of high numbers of yeast isolates, especially *Schizosaccharomyces pombe* and *Debaryomyces hansenii* on sweet potatoes (Table 3) might be due to the higher sugary content of this vegetable that presents a most conductive environment for their growth, as reported by Fleet (1992). *Schizosaccharomyces pombe* and *Debaryomyces hansenii* were frequently isolated from the ten vegetable commodities, with sweet potatoes yielding 19 different yeast strains and beetroot (Table 3) yielding four isolates with *Debaryomyces hansenii* constituting 65% of the strains isolated. *Schizosaccharomyces pombe* formed 22% of the strains isolated. The higher carbohydrate content of sweet potatoes (27%) and beetroot (10%) respectively explains the species dominance (Watt and Merrill, 1950; Fleet, 1992). Cucumbers and potatoes yielded two isolates respectively, with *Debaryomyces hansenii* predominating. Despite that *Rhodotorula* spp. are common air contaminants, only three strains were isolated from sweet potatoes and one from green peppers indicating that the vegetables were washed or suggest the cleanness of the supermarket where it was purchased. No yeast strains were isolated from carrots and squashes.

One of the limiting factors in vegetables for the growth of yeasts, is the environment (lower carbohydrate and higher pH) which consequently favors the growth of bacteria, especially the lactic acid bacteria (Fleet, 1992).

More yeasts were isolated from fruits (Table 4). *Schizosaccharomyces pombe* (28%), *Hanseniaspora guilliermondii* (26%) and *Debaryomyces hansenii* (24%) predominated, while *Candida tropicalis* (9%) and *Cryptococcus laurentii* (9%) were present in lower numbers (Fig. 2). According to Hocking and Pitt (1997), *Schizosaccharomyces pombe* is a relatively uncommon spoilage yeast species. Onishi (1990) and Corry (1987), however, reported the frequent occurrence of *Schizosaccharomyces pombe* associated with sugary environments. *Debaryomyces hansenii* has been isolated from spoiled foods (Spencer and Spencer, 1997) but simultaneously exhibits antifungal activity (Droby *et al.,* 1989; Wilson and Chalutz, 1989).

The predominance of *Debaryomyces hansenii* strains in the present study is consistent with results presented elsewhere. Tokouka *et al.* (1985) reported on the frequent isolation of the species from high sugar foods. Goto and Yokotsuka (1977) reported on the presence of the species in fresh grape musts while Spencer *et al.* (1992) indicated on the dominance of *Debaryomyces* strains associated with the rotting of citrus fruits. However, Droby *et al.* (1999) indicated that *Debaryomyces hansenii* exhibiting the highest biocontrol activity against green mould decay caused by *Penicillium digitatum.* According to Barnett *et al.* (1990), this species is capable of utilizing a wider range of carbon sources than most other spoilage yeasts, which explains its frequent incidence. In this study *Debaryomyces hansenii* strains were present on most of the fruit and vegetable commodities. *Hanseniaspora guilliermondii,* the anamorph of the fermentative spoilage yeast, *Kloeckera apiculata,* is associated with the spoilage of pineapples (Robbs *et al.,* 1989). Juven *et al.* (1984) also showed the dominance of *Hanseniaspora guilliermondii* associated with a Mediterranean fruit, pomegranate.

The fermentative ability of *Hanseniaspora guilliermondii* is not as strong as *Kloeckera apiculata,* and is therefore not considered as a spoilage yeast in fruits and vegetables (Suresh *et al.,* 1982). According to previous studies, *Candida tropicalis* and *Cryptococcus laurentii* are associated with tropical fruits (Rale and Vakil, 1984) and strawberries (Buhagiar and Barnett, 1971) respectively. Strawberries (22%) and prickly pears (22%) harbored the widest variety of yeast isolates comprising *Hanseniaspora guilliermondii* (38%), *Debaryomyces hansenii* (17%), *Schizosaccharomyces pombe* (29%) and *Cryptococcus laurentii* (13%) on both the commodities (Table 4).

Doores (1983) indicated that weakly fermentative yeasts, rather than moulds or bacteria typify the predominant primary flora of apples. Apples harbored two isolates of *Debaryomyces hansenii.* Cherries and apricots yielded thirteen yeast isolates in total, with *Hanseniaspora guilliermondi, Debaryomyces hansenii, Candida tropicalis* and *Schizosaccharomyces pombe* dominating on the surfaces of these two commodities. These species are common surface inhabitants and according to Tokouka *et al.* (1985) commonly found on other fruits.

The environment of fruits favors the growth of yeasts and is substantiated by the enhanced number of yeast strains isolated. The majority of the species isolated, is normal inhabitants of the surface microflora of fruits and is not usually associated with the spoilage. Spoilage of fresh fruits usually results from the fermentative activity of yeasts. Davenport (1976) concluded that yeasts may differ quantitatively and qualitatively according to the parts examined, and the microflora also differed according to the season, cultivar and site.

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Table 1
Mean microbial counts present on vegetables

Vegetable		Log counts (cfu.g^{-1}) Yeasts	Bacteria
Baby marrows	Surface	5.43	6.2
	Internal	2	3
Sweet potatoes	Surface	4.47	5.95
	Internal	2	2.35
Carrots	Surface	4.39	5.47
	Internal	2	2.11
Beetroot	Surface	4.3	6.38
	Internal	0	0
Cucumbers	Surface	0	5.3
	Internal	0	0
Greenbeans	Surface	4.17	6.49
	Internal	3	3.32
Squashes	Surface	4.84	7.17
	Internal	2.41	2.81
Potatoes	Surface	3.17	7.04
	Internal	2.15	3
Onions	Surface	0	4.94
	Internal	0	2.11
Greenpeppers	Surface	3.69	6.93
	Internal	2	2.62

Table 2
Mean microbial counts present on fruits

Fruit		Log counts (cfu.g^{-1}) Yeasts	Bacteria
Mandarins	Surface	5	4.6
	Internal	0	2.69
Oranges	Surface	4.77	4.84
	Internal	3	2.61
Apples	Surface	4.6	3.07
	Internal	0	3.3
Pears	Surface	3.23	3.14
	Internal	0	0
Figs	Surface	4.73	3.09
	Internal	2.14	2.04
Strawberries	Surface	4.83	4.2
	Internal	2.69	3.69
Apricots	Surface	4.69	3.25
	Internal	0	2.77
Cherries	Surface	5.34	4.38
	Internal	2.11	4.69
Prickly pears	Surface	5	3.2
	Internal	2.79	2.65
Peaches	Surface	3.14	3.17
	Internal	0	2.77

Table 3
Yeasts isolated from ten different vegetable commodities

Vegetable		Yeasts present	Isolates
Baby marrows	Internal		0
	Surface	*S. pombe* (1)	1
Sweet potatoes	Internal	*S. pombe* (1); *D. hansenii* (1)	2
	Surface	*S. pombe* (3); *D. hansenii* (13); *R. mucilaginosa* (3)	19
Carrots	Internal		0
	Surface		0
Beetroot	Internal		0
	Surface	*S. pombe* (2); *D. hansenii* (2)	4
Cucumber	Internal		0
	Surface	*D. hansenii* (2)	2
Green beans	Internal		0
	Surface	*D. hansenii* (1); *S. pombe* (1)	2
Squashes	Internal		0
	Surface		0
Potatoes	Internal		0
	Surface	*D. hansenii* (2)	2
Onions	Internal		0
	Surface	*S. pombe* (1); *H. guilliermondii* (1)	2
Greenpeppers	Internal		0
	Surface	*R. glutinis* (1)	1
Total			35

Table 4
Yeasts isolated from ten different fruit commodities

Fruit		Yeasts present	Isolates
Mandarins	Internal		0
	Surface	*C. laurentii* (1); *S. pombe* (1)	2
Oranges	Internal		0
	Surface	*S. pombe* (1)	1
Apples	Internal		0
	Surface	*D. hansenii* (2)	2
Pears	Internal		0
	Surface	*S. pombe* (1); *D. hansenii* (1)	2
Figs	Internal		0
	Surface	*S. pombe* (2); *H. guilliermondii* (2); *C. tropicalis* (1)	5
Strawberries	Internal		0
	Surface	*S. pombe* (3); *H. guilliermondii* (6); *D. hansenii* (1) *C. laurentii* (1); *C. tropicalis* (1)	12
Apricots	Internal	*S. pombe* (1)	1
	Surface	*H. guilliermondii* (2); *D. hansenii* (3); *S. pombe* (1)	6
Cherries	Internal		0
	Surface	*S. pombe* (2); *H. guilliermondii* (1); *C. tropicalis* (3) *C.laurentii* (1)	7
Prickly pears	Internal	*D. hansenii* (1)	1
	Surface	*S. pombe* (4); *D. hansenii* (3); *H. guilliermondii* (3) *C. laurentii* (2)	12
Peaches	Internal		0
	Surface	*D. hansenii* (2); *R. glutinis* (2)	4
Total			55

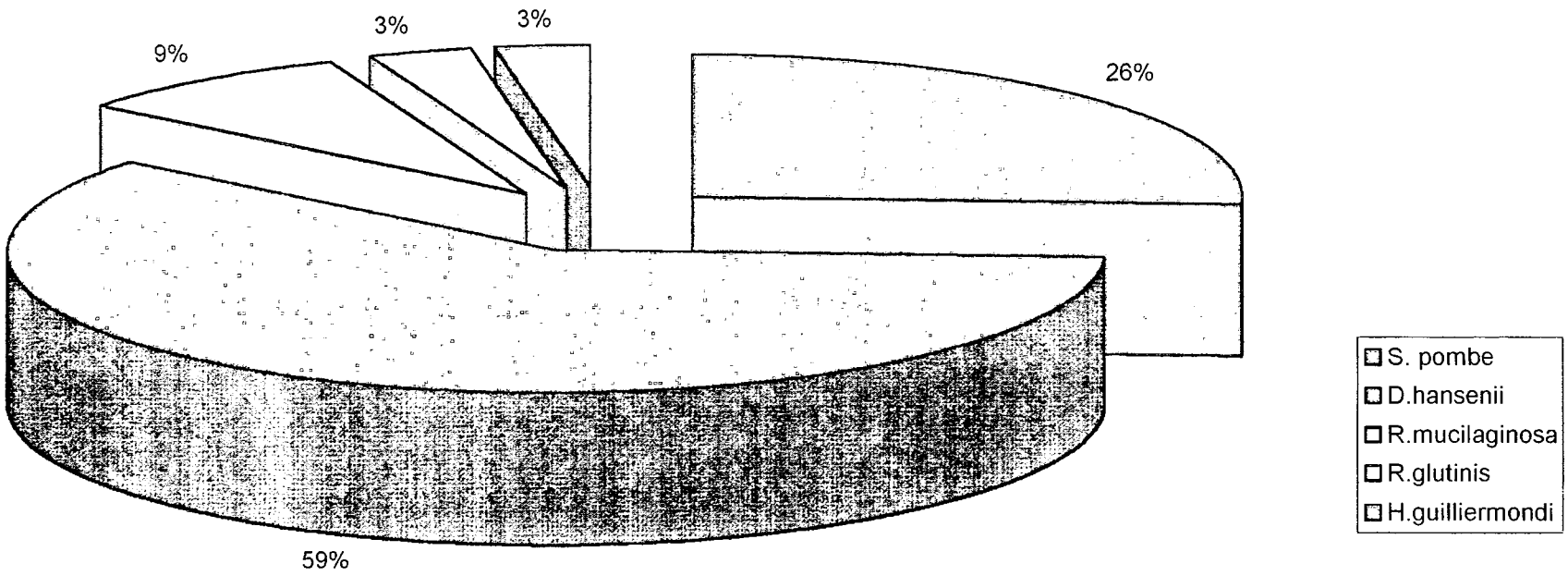

Figure 1

Proportional percentage yeasts isolated from 10 different vegetables

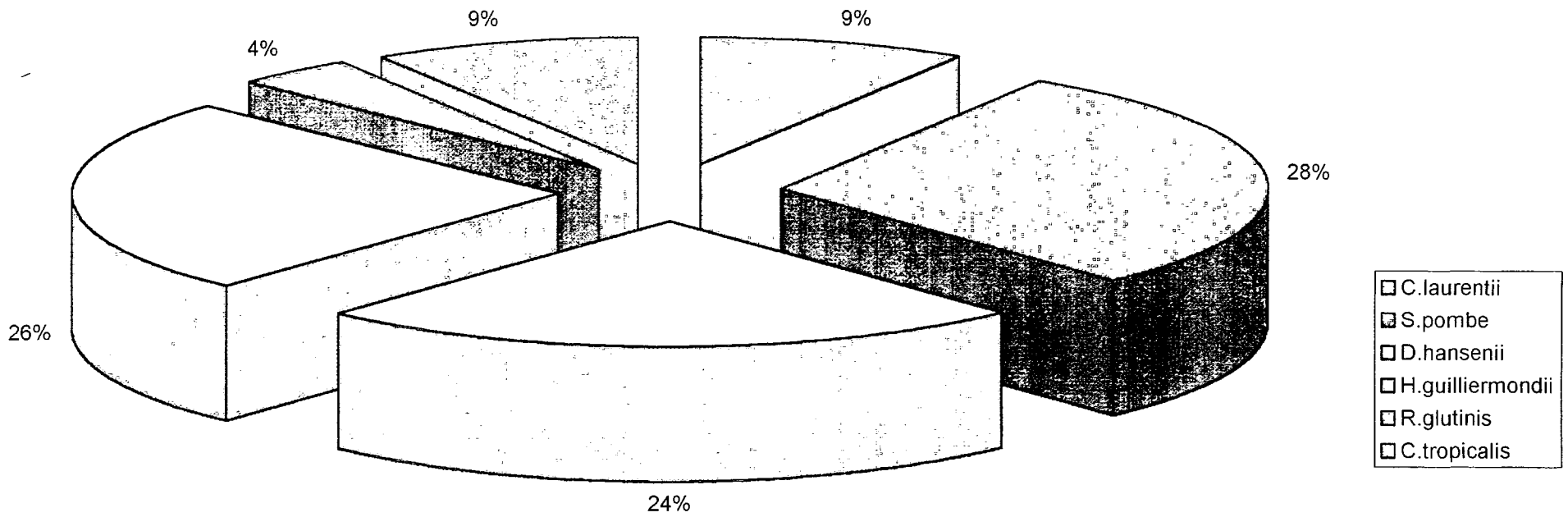

Figure 2

Proportional percentage yeasts isolated from 10 different fruits

CHAPTER 3

The survival of yeasts in fruits and vegetables stored at different temperatures

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ABSTRACT

The effect of storage temperature, chemical composition and enzymes on the survival of yeasts over a 30-day period on fruits and vegetables were investigated. Sixty-five yeast isolates were obtained from fruits, and 55 yeast isolates from vegetables. Five different fruits and vegetable commodities were individually stored at three different temperatures (5, 15 and 25°C) and monitored on a weekly basis. A general decrease in organic acids and increase in sugars were detected while the ethanol contents showed no significant changes. The basidiomycetous related yeast species dominated on vegetables stored at 5^0C while ascomycetous yeast species dominated on the surface of fruits and vegetables stored at 15 and 25°C. The highest yeast counts were observed when fruits and vegetables were stored at 5°C. *Schizosaccharomyces pombe* and *Debaryomyces hansenii* were the dominant species isolated from fruits. A new species was isolated on day 20 at 25^0C showing cellulase activity. Other species encountered were *Rhodotorula mucilaginosa, Cryptococcus albidus* and *Candida tropicalis.* Vegetables yielded higher bacterial counts (10^9 cfu.g^{-1}), whereas fruits yielded higher yeasts and mould counts of 10^6 cfu.g^{-1} and 10^4 cfu.g^{-1} respectively.

1. INTRODUCTION

The natural microflora of vegetables represent a diverse group of genera including bacteria, yeasts and moulds (Brackett, 1997). The number of microbial populations on vegetables vary widely and often depends on the type of vegetable (Splittstoesser, 1970). Bacteria are more frequently isolated from the initial spoilage defects of vegetables (Brackett, 1993; Lund, 1983). The pH levels of vegetables are usually in the range that encourage the growth of bacteria and therefore render ideal circumstances for bacterial spoilage (Lund, 1992). Gram-negative bacteria and the pseudomonads are representatives of the most dominant bacteria found on the surface of vegetables (Dennis, 1987; Pederson and Fisher, 1944). Currently, two strains of *Pseudomonas syringae* were commercialized as postharvest biofungicides (El-Ghaouth *et al.*, 2000). The inner tissue of sound fruit and vegetables is often considered to be sterile, however, several studies reported the presence of low numbers of bacteria within healthy produce such as potatoes, cucumbers and tomatoes (Brackett and Splittstoesser, 1992; Smith and Niven, 1957). If microorganisms are present in the internal tissues, they are limited to a few species (Doores, 1983).

Chemical reactions of respiration are controlled by temperature, and a rise in temperature results in doubling of the respiration. Rapidly growing, young tissue respires faster than those that develop slowly (Ryall and Lipton, 1979). The respiration rate is therefore an index of the storage life of fruits and vegetables; a higher rate of respiration equals a shorter storage, whereas a lower rate of respiration results in a longer storage life. Fruits and vegetables exhibit an enhanced shelflife at low temperatures (0-4.4°C) compared to higher temperatures (21-27°C). Consequently, rapid cooling after harvesting is imperative to conserve perishable soft fruits such as berries and leafy vegetables. The storage at low temperatures not only reduces metabolic activity of fruits and vegetables, but also controls fruit decay (Ryall and Pentzer, 1982).

The most serious consequences of holding fruit and vegetables at high temperatures are the hastening of ripening, and shortening of storage and marketing life (Ryall and Lipton, 1979). A large number of fruits and vegetables show a sudden and sharp rise in respiratory activity called the climacteric rise during the life cycle such as apples and pears, whereas others which do not show climacteric rise are called nonclimacteric fruits and vegetables such as grapes and oranges. The time of harvest for climacteric fruits and vegetables is critical for their maximum storage life and quality. Nonclimacteric fruits and vegetables are allowed to ripen on plants and the resulting maturity is regulated by storage (Biale, 1950).

As with vegetables, the normal microflora of fruits are varied and include numerous bacterial and fungal species (Goepfert, 1980). Although moulds are often identified as a cause of spoilage before and after harvest of fruits, yeasts also play an important role in the spoilage of fruits by reaching counts as high as 10^6 cells/g (Deàk and Beuchat, 1996). Davenport (1980) indicated that many yeast species survive better at low temperatures and therefore making the commodities prone to yeast spoilage. Yeasts, commonly occur on the surfaces of fruits at harvest, and the low pH and sugary composition of the fruit tissue render a most conductive environment for their growth (Fleet, 1992). Various organic acids naturally present in the fruits are responsible for the acidity. Citric acid is the principal acid present in citrus fruits, whereas malic acid predominates in apples and pears. Grapes contain almost equal quantities of tartaric and malic acids. Other organic acids found in fruits, like quinic, succinic and fumaric acid, are usually present in trace amounts (Brackett, 1987).

The predominant yeast species associated with fruits are *Hanseniaspora, Kloeckera, Pichia, Saccharomyces, Candida* and bacidiomycetous yeast species (Fleet, 1992; Spencer and Spencer, 1997; Splittstoesser, 1987). Yeasts have a more favorable surface-to-volume ratio than filamentous fungi, which allow them to absorb nutrients

and colonize fruit surfaces more rapidly (Splittstoesser, 1991). Undamaged fruits are seldomly spoiled by yeasts (Spencer and Spencer, 1997). In order for yeasts to cause spoilage, they must have the ability to produce enzymes capable of breaking down the polysaccharides of the skin (Dennis, 1987; Fleet, 1992). Pectin and cellulose represent the main structural components of plant cells (Brackett, 1987) whereas pectinases and cellulases are the most important degradative enzymes involved in spoilage. In many cases, active degradation of pectic substances leads to a rapid loss of tissue coherence as a result, of cell separation, increased liquification of the tissue and ultimately cell death (Splittstoesser, 1991).

Five classes of microbial enzymes are primarily responsible for degradation of plant materials, namely pectinases, cellulases, proteases, phosphatidases and dehydrogenases (Lund, 1971). A few food-associated yeasts like *Cryptococcus laurentii, Debaryomyces hansenii, Rhodotorula glutinis* and *Cryptococcus albidus* are able to produce some of these extracellular enzymes (Fleet, 1990). However, no yeast species have been reported to degrade cellulase (Fleet, 1992).

The purpose of this study was to investigate the development of yeast populations on vegetables over a period of 30 days stored at three different temperatures. Enzyme screening was preformed on the dominant yeast isolates to determine the cause of spoilage.

2. Materials and methods

2.1. Media used

Yeast extract glucose chloramphenicol agar (YGC)(Merck, Biolab Diagnostics, South Africa, pH 6.6) was used for the enumeration of yeasts. Plate count agar (PCA)(Merck, Biolab Diagnostics, South Africa, pH 7.0) was used for the enumeration of bacteria and Rose Bengal Chloramphenicol agar (RBCA)(Merck, Biolab Diagnostics, South Africa, pH 7.2) was used for the enumeration of moulds.

2.2. Sampling methods, selection of isolates and enumeration

Samples were taken from fruits and vegetables periodically at 5-day intervals over a period of 30 days while stored at three different temperatures 5, 15 and 25°C. Day 1 is indicative of the first sampling, when the fruits and vegetables were considered fresh. Five different types of fruits and vegetables were used for microbiological analysis as indicated in Table 1. Fruit and vegetable samples were cut with a sterile knife and 20 g portions weighed and homogenized in 180 ml sterile peptone water in a Warring Blender for 2 min and the liquid portion diluted.

Further decimal dilutions of the suspensions were carried out as required for microbiological assays in 9-ml sterile peptone water. Aliquots (0.1 ml) of the dilutions were spread plated in duplicate, over the surface of plates of the appropriate media. The plates for the enumeration of yeasts and moulds were incubated at 25°C for 72h. Yeast colonies were isolated from the highest dilutions showing growth on YGC plates. Plates for the enumeration of total counts were incubated at 25°C for 48 h.

2.3. Physical and Chemical analysis

The pH, alcohol content and percentage organic acids and sugars were measured at intervals as indicated in Table 1. The pH of the homogenized fruit and vegetable samples was measured at 24°C with a HI 9321 Microprocessor pH meter (HANNA Instruments). The organic acid contents were measured by means of a Waters HPLC system with a Biorad-Aminex HPX 87 Ion exclusion column (Molnár-Perl and Morvai, 1992). The sugar contents were measured by means of a Waters Sugar Analyzer 1 Liquid Chromatograph with a Waters Sugar Pak 1 column according to the method described by Molnár-Perl and Morvai. (1992). The alcohol content was determined by gas-liquid chromatography according to the method of Stackler and Christensen (1974).

2.4. Enzyme screening

The dominant yeast species isolated were used in the screening of five different extracellular enzymes normally associated with the possible maceration of fruits and vegetables. The extracellular enzymes, namely amylase, pectinase and cellulase were determined according to the methods described by Call *et al.* (1984).

2.4.1. Proteolytic activity

Casein digestion was determined according to the methods of Adhearn *et al.* (1968).

2.4.2. Lipolytic activity

Screening for lipase production was performed by means of agar plates containing olive oil as carbon source and Rhodamine B (pH 7.0) and agar plates containing Tween 80 as carbon source and CaCl2 (pH 7.0) (Kouker and Jaeger, 1987). The deep agar-diffusion test for the preliminary screening of lipolytic activity on Tributyrin agar plates was preformed according to the methods of Lima *et al.* (1991).

2.5. Identification

Individual yeast isolates were tentatively identified according to cellular long-chain fatty acid analysis (Viljoen *et al.,* 1986) by comparing the long-chain fatty acid profiles of the unknown with results obtained in a databank containing the yeast species long-chain fatty acid profiles. The identity of each yeast isolate was verified with the aid of conventional identification methods (Kreger-van Rij, 1984). Each isolate was inoculated into 6 sugar fermentation media, 32 carbon source assimilation media and vitamin free medium (Van der Walt and Yarrow, 1984). Additional tests performed included growth at 37°C, in 50% D- glucose medium, urea hydrolysis, splitting of arbutin, 0.01 and 0.1% cycloheximide and staining of 4-week old cultures

with Diazonium Blue B salt reagent. Assimilation of nitrogen compounds, as performed by means of the auxanographic method (Lodder and Kreger-van Rij, 1952), was also included.

Ascospore formation was examined on McClary's acetate agar, potato glucose agar, Gorodkowa agar, corn meal agar and malt extract agar (Van der Walt and Yarrow, 1984). The inoculated media were incubated at 18°C for 4 weeks and examined at 4-day intervals. Cell morphology and mode of reproduction were examined on malt extract agar (Difco) and on Dalmau plates (Van der Walt and Yarrow, 1984). The formation of pseudomycelium and true mycelium was examined on corn meal agar according to the Dalmau plate technique (Van der Walt and Yarrow, 1984).

2. RESULTS AND DISCUSSION

3.1. Physical and chemical composition

The comparative analytical data and the chemical composition of fruits and vegetables are shown in Table 1 and 2 during storage for a 30-day period at three different temperatures. A general decrease in organic acid contents and increased sugar contents were detected at 5, 15 and 25°C over the 30 days of storage in all five of the fruit commodities. The rapid increase in sugar content initially might be due to maturity, ripeness and storage conditions (Morvai *et al.,* 1991). According to Potter and Hotchkiss (1986) the organic acids of fruits generally decrease during storage and ripening. This decrease in acids resulted in increased pH values over the 30-day storage period (Table 3). Apples showed increased pH values of 4.03 at day 5 to 4.23 at day 30. The pH of fruits generally increased more rapidly when stored at 25°C correlating with the decline in organic acids which shortened the shelf life of these fruit commodities compared to fruits stored at 5°C where enhanced shelf lifes were

observed. According to Hocking and Pitt (1997), an increase in pH levels leads to skin layers to soften and soluble carbohydrates build up to weaken defense barriers.

According to Fleet (1992), utilization of organic acids and sugars by yeasts can appreciably decrease the acidity of products and increase their pH levels. Frazier and Westhoff (1988) reported that the acidity is reduced by film yeasts and moulds surviving on the surface due to oxidative metabolism of sugars, alcohol's and organic acids (Fleet, 1990). Some species, especially those related to the genera *Rhodotorula* and *Cryptococcus* and a few species of *Candida, Pichia,* and *Debaryomyces* typically utilize sugars under aerobic conditions (Gancedo and Serrano, 1989). No significant changes in the production of ethanol were detected over the 30 days of storage. A general non-changeable ethanol concentration suggested that no fermentative activity took place by the developing yeast population on the surface of the fruits.

The pH levels of vegetables stored at 15 and 25°C increased over time and the products deteriorated after 10-days, while vegetables stored at 5°C deteriorated after 20-days. The pH of potatoes increased from a pH of 6.0 at day 5 to 6.31 at day 30. The other vegetable commodities all showed the same elevated tendency in pH levels (Table 4). Vegetables, however contain lower percentages of organic acids and sugars with higher pH levels compared to fruits (Brackett, 1987). Consequently, bacteria tend to dominate on the surface of vegetables due to more favorable environmental conditions. According to Brackett (1997), bacteria are able to grow more rapidly than yeasts in most vegetables and therefore have a competitive advantage. Table 2 shows decreases in organic acids and sugars, mainly due to the utilization of these components by bacteria (Lund, 1992). No significant alcohol levels were detected, which corresponds with the results of Lund (1992). Potatoes and onions showed decreases in the organic acids and increased sugar values at day 30, but the trace amounts of lactic acid produced in some of the vegetable commodities could indicate the presence of lactic acid bacteria. Brackett (1987) reported that the low available carbohydrates and low acid content of many vegetables encourage the growth of lactic

acid bacteria. According to Fleet (1990) the lactic acid bacteria lower the pH of the commodity, which enable yeast growth to compete against bacterial growth. An overall decrease in acids and increase in sugars which led to increased pH levels over the 30-day period were observed in all the vegetable commodities stored at different temperatures.

3.2 Microbial enumeration

Enhanced growth of yeasts at higher temperatures was visible. Counts ranging between 10^3cfu.g^{-1} at 5°C and 10^6cfu.g^{-1} at 25°C were encountered. Fruits stored at 15 and 25°C deteriorated after 15-days while fruits stored at 5°C deteriorated after 30-days. The highest bacterial count of 10^8cfu.g^{-1} was observed on pears stored at 5°C. Pears, however showed higher yeast (10^6cfu.g^{-1}) and bacterial counts throughout the study (Table 3). They contain the highest carbohydrate percentage (15.8%) of all the fruit commodities studied (Watt and Merill, 1950). Chand-Goyal and Spotts (1996), reported on the identification of epiphytic yeasts on pears thriving in the elevated carbohydrate contents. According to Fleet (1992) physical damage of the skin exposes the fruit tissue upon which yeasts can rapidly grow. Mould counts ranging between 10^3cfu.g^{-1} and 10^4cfu.g^{-1} were encountered with the highest counts obtained at 25°C. Dennis (1987) concluded that bacterial counts on fresh fruits and vegetables usually range between 10^4 and 10^8 cfu.g^{-1}, yeasts between 10^2 and 10^6 cfu.g^{-1} and moulds between 10^2 and 10^4 cfu.g^{-1} with a higher number of bacteria on vegetables, and higher numbers of fungi and yeasts on fruits. Fruits and vegetables stored at 5°C remained fresh for longer periods compared to higher temperatures. Grapes deteriorated after 30 days and greenpeppers after 20 days when stored at 5°C.

Lower yeast counts were detected on oranges and mandarins. The thicker skin might play an important role (Brackett and Splittstoesser, 1992). Extensive research has been conducted on the spoilage of grapes by *Botrytis cinerea* (Coley-Smith, 1980; Ryall and Pentzer, 1982; Snowdon, 1990). Vegetables on the other hand showed higher bacterial numbers (Table 4), as expected especially at 5^0C. Significant increases in bacterial counts on day 5 at 5^0C were visible. Counts of 10^8cfu.g^{-1} were obtained. According to Brackett and Splittstoeser (1992) microorganisms will normally increase dramatically during storage conditions. Ultimate populations of bacteria often reach 10^6 to 10^7 cfu.g^{-1} before the product appears spoiled. According to Lund (1992) the predominant organisms on the vegetables were aerobic Gram-negative rods, mostly pseudomonads and the yellow pigmented bacteria. The higher water content of vegetables and the relatively low acid, carbohydrate and fat contents suggest that much of this water is in available form for the growth of spoilage bacteria (Jay, 1992). Vegetables stored at 15 and 25°C deteriorated after 10-days while vegetables kept at 5°C deteriorated after 20-days. Higher yeast counts were obtained at 5°C with the basidiomycetous related yeast species predominating on the surfaces of vegetables. A general increase in mould counts of 10^4cfu.g^{-1} to 10^6cfu.g^{-1} was encountered with the highest mould counts obtained at 25°C which corresponds with the results of Brackett (1987) indicating that bacterial and fungal spoilage of vegetables are of equal importance.

3.3 Yeast identification

A total of 119 yeast strains were isolated from fruits and vegetables over the 30-day period. Fruits yielded 65 different yeast strains and vegetables 54 strains. The basidiomycetous related yeast species dominated on vegetables at 5°C, whereas ascomycetous yeast species dominated on the surface of fruits and vegetables at 15 and 25°C. The most predominating species isolated from fruits, were *Schizosaccharomyces pombe* and *Debaryomyces hansenii* that developed after day 10 on the surface of the fruits.

According to Hocking and Pitt (1997), *Schizosaccharomyces pombe* is a relatively uncommon spoilage yeast. The predominance of this species in the present study is consistent with results presented elsewhere. Gao and Fleet (1995) reported on the frequent occurrence of the species on the deacidification in the wine industry and Tokouka *et al.* (1985) indicated the dominance of *Schizosaccharomyces* strains in high-sugar products. In this study *Schizosaccharomyces pombe* strains were present on both the fruit (32%) and vegetable (19%) commodities, with the highest percentage of strains isolated from apples (45%) and pears (29%). Chand-Goyal and Spotts (1996) reported on the dominance of yeasts present on pear surfaces. During this study 29% of the isolates were obtained from pear fruits. The highest number of strains on vegetables was isolated from potatoes (67%), including a new species. The potato-associated yeasts, *Candida solani* was also obtained (Barnett *et al.*, 1990). According to Watt and Merrill (1950) potatoes contain 19.1% of carbohydrates, explaining the dominance of *Schizosaccharomyces pombe* on this vegetable commodity. Fleet (1992) reported that the sugary composition of the fruit and vegetable tissue presents a most conducive environment for their growth.

Other species encountered included *Debaryomyces hansenii,* which predominated on apples. The highest number of yeast isolates was obtained from apples (45%). Previous studies by Davenport (1976) and Doores (1983) conclude the dominance of *Debaryomyces* strains on apples. Spoilage of fresh fruits usually results from the fermentative activity of yeasts, and since *Debaryomyces hansenii* is a weak fermenting yeast, is not capable of causing spoilage of fruits and vegetables (Suresh *et al.,* 1982). However, the developing of off-flavours in pomegranate juice were reported by Juven *et al.*, (1984).

The basidiomycetous yeast species, *Rhodotorula glutinis, Rhodotorula mucilaginosa, Cryptococcus laurentii* and *Cryptococcus albidus* predominated on vegetables contributing to 61% of the microflora present, which are in agreement with previous reports (Deàk, 1991; Fleet, 1992; Lund, 1992). *Rhodotorula* and *Cryptococcus* spp. were frequently isolated from the surface of fruits and vegetables during the study.

Roberts (1990) confirmed the natural occurrence of *Cryptococcus laurentii* on apple leaves and fruit. *Rhodotorula* and *Cryptococcus* spp. started to dominate at 5^0C on fruits from day 20 (Table 1). Investigations cited by Ingram (1958) indicated that pink and red-pigmented cells of *Rhodotorula* and *Cryptococcus* species are common airborne yeasts. The occurrence of basidiomycetous yeasts on fruits was slightly lower (37%) despite the dominance of *Rhodotorula* and *Cryptococcus* spp. on oranges and mandarins. Parish and Higgins (1989) also reported on the presence of *Rhodotorula* spp. isolated from citrus fruits. Reports of spoilage are rare, because of its non-fermentative capacity (Van Dijken, 1986) but the ability of *Rhodotorula* spp. to produce pectinases, enable them to soften and spoil fruits and vegetables (Vaughn *et al.*, 1969).

During this study no yeasts was isolated from grapes, despite being a major component of the natural flora on the surface of fresh grapes (Hocking and Pitt, 1997). Fermentative species such as *Saccharomyces cerevisiae* are usually present in low numbers (Fleet and Heard, 1992). The types of yeasts growing depend on the temperature, but usually wild yeasts like the apiculate species will carry out the first fermentation (Frazier and Westhoff, 1988). The temperature range for growth of most yeasts is likewise broad (5 to 35^0C), with some species capable of growth above or below this range (King *et al.,* 1986). Most of the basidiomycetous species were isolated at 5^0C which corresponded with results obtained by Fleet, (1992) and Spencer and Spencer, (1997). Collins and Buick (1989) reported the flavour changes caused by *Rhodotorula glutinis* on frozen peas at -18^0C. According to Spencer and Spencer (1997) refrigeration is ineffective in preventing spoilage of many foods by yeasts. The

ascomycetous yeast species dominated on fruits at 5^0C with *Debaryomyces hansenii* predominating. The presence of *Debaryomyces hansenii* at 5^0C on the fruit and vegetable commodities is in agreement with reports by Kobatake *et al.*, (1992).

Schizosaccharomyces pombe predominance at 15 and 25^0C explains the growth range of this species between 25 and 37^0C (Kreger-van Rij, 1984). Török and King (1991) also reported on the dominance of ascomycetous species on fruit. A new yeast species was isolated from potatoes on day 20 at 25^0C. Based on the results obtained, storage time and temperature played a significant role in the spoilage of fruits and vegetables. Fruit and vegetable commodities stored exhibited a longer shelf life at 5°C while fruits and vegetables stored at 15 and 25°C deteriorated after 15 and 10 days respectively. The dominant yeast species *S.pombe* and *D. hansenii* are known to have fermentative activity but their fermentative ability to cause spoilage of fruits and vegetables is not as strong as *Kloeckera* and *Pichia* spp. which may cause serious spoilage.

3.4 Enzyme screening

The dominant yeast species used for enzyme screening are shown in Table 5. Only *Rhodotorula glutinis* showed pectinase activity that corresponds with results presented by other workers (Vaughn *et al.*, 1969; 1972). Both *Debaryomyces hansenii* and *Schizosaccharomyces pombe* showed no pectinolytic activity. Further studies of more *Debaryomyces* strains are needed to determine amylolytic and pectinolytic activity. *Rhodotorula glutinis, Rhodotorula mucilaginosa* and *Cryptococcus laurentii* showed amylolytic activity (Deàk and Beuchat, 1996; Fleet, 1992; Linardi and Machado, 1990). The dominant yeast species isolated, all showed lipolytic activity while only two species *Rhodotorula glutinis* and *Rhodotorula mucilaginosa* showed proteolytic activity. The new species showed both amylolytic and cellulose activity but no proteolytic and lipolytic activity.

According to Fleet (1992), no yeasts have been reported to degrade cellulose. Further research on the possibility of cellulose activity by the new species seems very promising.

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Table 1
Comparative analytical data, chemical composition and yeasts present on fruits over a period of 30 days stored at different temperatures

Days	Fruit	Organic acids (%)								Sugars (%)					Ethanol (%)	*Yeasts present*
		Tartaric	Citric	Malic	Succinic	Ascorbic	Fumaric	Quinic	Piruvate	Sucrose	Glucose	Rhamnose	Xylose	Fructose		
Day 1*	Pears	0.18		0.01						0.82	1.22			3.29	0.03	
	Apples	0.11		1.03						0.88	0.79		0.03	2.78	0.01	*C. laurentii*
	Oranges	0.14			0.15					3.09	2.08			2.15	0.05	*C. laurentii*
	Mandarins	0.14	0.306							3.99	1.71	0.06		1.94	0.03	*C. laurentii*
	Grapes	0.18	0.034							0.26	4.22			3.78	0.01	
Day 5 5°C	Pears	0.11								0.95	2.05			4.33		
	Apples	0.20		0.61	0.09					1.27	0.72			2.36		
	Oranges	0.15		0.07						2.88	1.38			1.74	0.06	
	Mandarins	0.16	0.33							3.24	1.53			1.79	0	
	Grapes	0.16	0.03							0.19	5.14			5.25	0	
15°C	Pears	0.12	0.06							0.87	1.34		0.17	4.42	0.14	
	Apples	0.12		0.27						5.14	1.29			3.55		
	Oranges	0.14		0.08						2.34	1.19			1.21	0.06	
	Mandarins	0.16	0.32							4.91	0.88		0.03	1.16	0.14	*R. glutinis*
	Grapes	0.15	0.08							0.35	4.81			4.11	0.05	
25°C	Pears	0.14	0.07							0.6	1.87		0.1	6.24	0.15	
	Apples	0.11		0.33						1.19	1.13		0.04	3.87		
	Oranges	0.14	0.3							2.83	1.26			1.27	0.11	
	Mandarins	0.06	0.04							3.77	0.78		0.04	1	0.07	
	Grapes	0.07	0.03							0.27	3.36			2.69	0.05	
Day 10 5°C	Pears	0.08		0.43						0.69			0.12	1.17	0.17	*S. pombe; D. hansenii;*
	Apples	0.09		0.09						0.83	0.78		0.02	2.14		*C. laurentii; D hansenii; D. hansenli; D hansenii*
	Oranges	0.09	0.22							2.07	0.77			0.87	0.03	
	Mandarins	0.11	0.08							1.62	0.5			0.79	0	
	Grapes	0.09	0.03								2.47			2.24		
15°C	Pears	0.09		0.14						2.75	1.56		0.14	4.9	0.19	*P. onychis*
	Apples		0.04	0.19	0.1	0.07	0.01			1.39	1.42			3.17	0	*P. angusta; S. pombe*
	Oranges	0.09	0.24							2.06	0.97			1.15	0.09	
	Mandarins	0.13	0.08							1.61	0.37			0.55	0.01	
	Grapes	0.03	0.03								2.62			2.35	0.03	
25°C	Pears	0.09		0.24	0.05						1.89		0.12	5.54	0.25	*S. pombe; C. tropicalis; S. pombe; S. pombe; S.pombe*
	Apples	0.05		0.05	0.02					4.16	0.46		0.02	1.9	0	*S. pombe; S. pombe*
	Oranges	0.12	0.19							2.27	0.84			0.94	0.11	*C. tropicalis; S. pombe*
	Mandarins	0.17	0.07							3.94	0.28			0.64	0.06	
	Grapes	0.14	0.05								2.44			1.8	0.06	

Table 1 (continued)

Days	Fruit	Organic acids (%)								Sugars (%)					Ethanol (%)	*Yeasts present*
		Tartaric	Citric	Malic	Succinic	Asccorbic	Fumaric	Quinic	Piruvate	Sucrose	Glucose	Rhamnose	Xylose	Fructose		
Day 15 5°C	Pears	0.04	0.17	2.23						2.23	1.81	0.07	0.07	3.51	0	*P. angusta; S. pombe; S. pombe; S. pombe*
	Apples	0.05								3.17	1.13		0.05	4.12		*S. pombe; D. hansenii*
	Oranges	0.06	0.13							3.03	0.41			0.57	0.02	
	Mandarins	0.15	0.01							1.04	0.58			0.72	0.07	
	Grapes	0.08	0.06								1.56			1.6	0.07	
15°C	Pears	0.11									1.5		0.16	3.8	0.35	
	Apples	0.11								3.22	0.89		0.08	2.28	0	*S. pombe*
	Oranges	0.14								2.58	1.07			1.14	0.1	
	Mandarins	0.07								2.69	0.52			0.69	0.03	
	Grapes	DETERIORATED														
25°C	Pears	0.09		0.52							2.37			3.62	0.03	*S.pombe*
	Apples		0.03	0.11	0.01	0.05	0.01			2.95	0.76		0.11	1.57		
	Oranges	0.13		0.38	0.13					1.56	1.21			1.28	0.15	
	Mandarins	0.01		0.03						1.7	0.34		0.08	0.43	0.03	
	Grapes	0.03	0.18							0.61	2.1			1.74	0.08	
Day 20 5°C	Pears	0.11	0.04							2.59	1.47		0.2	4.34	0.01	*R. glutinis; S. pombe; D. hansenii; C. tropicalis; S. pombe*
	Apples	0.08								3.36	0.74		0.04	2.96	0	*R. glutinis; D. hansenii; S. pombe; D. hansenii; C. tropicalis*
	Oranges		0.53				0		0.04	3.26	0.87			0.96	0.01	
	Mandarins		0.29	0.18	0.07		0			2.34	0.3			0.69	0.03	
	Grapes	0.12	0.03	0.12	0.09		0				1.26			1.18		
15°C	Pears	DETERIORATED														
	Apples		0.18	0.09	0.12		0			5.2	0.62	0.06		2.02		*P. ohmeri*
	Oranges		0.59	0.29	0.2		0	0.62		2.71	1.25			1.34	0.2	
	Mandarins		0.32	0.27	0.22		0			4.95	0.98		0.06	1.12	0.23	*R. glutinis*
	Grapes	DETERIORATED														
25°C	Pears	DETERIORATED														
	Apples		0.1	0.03	0		0			3.04	0.17		0.01	0.97	0	
	Oranges		0.64	0.2			0.02	0.15		3.52	1.56			1.69	0.23	
	Mandarins		0.18	0.19			0			4.02	0.75		0.14	0.88	0.08	
	Grapes	DETERIORATED														
Day 25 5°C	Pears		0.13	0.32	0.49		0			0.66	1.64			4.02	0.02	*R. glutinis; R. glutinis;*
	Apples	0.049	0.05	-						3.18	0.42	0.03		1.62	0	*R. glutinis; S. pombe; R. glutinis; R. glutinis; H. guilliermondii*
	Oranges		0.67	0.39			0			2.41	1.08			1.06	0.04	
	Mandarins		0.11	0.17	0.03		0			0.92	0.28			0.45		
	Grapes		0.01	0.03	0.01	0.04	0				0.14			0.13		
15°C	Pears	DETERIORATED														
	Apples		0.17	0.17			0.01			5.56	1.24			3.81	0	*S. pombe; S. pombe; D. hansenii*
	Oranges		0.45	0.14			0.02		0.08	1.9	1.1			1.09	0.08	
	Mandarins		0.21	0.12			0			4.45	0.61			0.65	0.24	*R. mucilaginosa; C. tropicalis*
	Grapes	DETERIORATED														
25°C	Pears	DETERIORATED														
	Apples		0.09	0.25		0.23	0.01		0.01	5.31	2.21			4.61	0	*R. mucilaginosa*
	Oranges		0.41	0.12			0		0.05	2.97	1.66			1.78	0.14	
	Mandarins		0.13	0.04	0.02		0			4.59	0.88			0.76	0.06	
	Grapes	DETERIORATED														

Table 1 (continued)

Days	Fruit	Organic acids (%)								Sugars (%)					Ethanol	*Yeasts present*
		Tartaric	Citric	Malic	Succinic	Asccorbic	Fumaric	Quinic	Piruvate	Sucrose	Glucose	Rhamnose	Xylose	Fructose	(%)	
Day 30 5°C	Pears	DETERIORATED														
	Apples		0.14	0.3	0.17		0			1.98	1.36			4.03	0.01	*D. hansenii; R. glutinis; R. mucilaginosa*
	Oranges		0.67	0.15			0		0.08	2.37	1.31			1.3	0.04	*R. glutinis; R. mucilaginosa*
	Mandarins		0.08	0.13			0			0.98	0.46	0.29		0.53	0.01	*R. glutinis;*
	Grapes	DETERIORATED														
15°C	Pears	DETERIORATED														
	Apples	DETERIORATED														
	Oranges		0.27	0.05			0	0.21		1.44	1.32			1.22		*R. glutinis; R. glutinis*
	Mandarins		0.15	0.06	0.01		0	0.03		0.91	0.69			0.49	0.02	
	Grapes	DETERIORATED														
25°C	Pears	DETERIORATED														
	Apples	DETERIORATED														
	Oranges	DETERIORATED														
	Mandarins		0.82	0.03	0.03		0.01	0.11		0.87	0.77			0.58	0.02	*R. mucilaginosa; C. laurentii*
	Grapes	DETERIORATED														

* Day 1 = directly after purchasing

Table 2
Comparative analytical data, chemical composition and yeasts present on vegetables over a period of 30 days stored at different temperatures

Time(days)	Vegetable	Organic acids (%)									Sugars (%)						Ethanol	Yeasts present
		Tartaric	Citric	Malic	Succinic	Ascorbic	Fumaric	Quinic	Piruvate	Lactic	Sucrose	Glucose	Rhamnose	Xylose	Fructose	Arabinose	(%)	
Day 1*	Greenbeans	0.02	0.06	0.15	0.05		0.08				0.02	0.12			0.12		0.01	
	Potatoes	0.03	0.16		0.05	0.04	0.02	0.1				0.21			0.12		0	
	Onions		0.12	0.27	0.1		0.01				0.33	1.16			0.39		0	*R. mucilaginosa; R. glutinis*
	Greenpepper	0.06	0.07	0.12	0.22		0.01					0.51	0.01		0.4		0.03	*R. glutinis; C. laurentii*
	Carrots	0.03	0.05	0.11	0.21		0.01	0.03			0.48	0.83	0.23		0.79		0	
Day 5 5°C	Greenbeans		0.04	0.03	0.02		0	0.01		0.13		0.13				0.03		
	Potatoes		0.08	0.02	0.02	0.05	0.01	0.1		0.06		0.09		0.19				*S. pombe*
	Onions		0.04	0.15	0.04		0				0.19	0.39			0.24			
	Greenpepper	0.03	0.07			0.16	0			0.05	0.07	0.17	0.12		0.22		0	
	Carrots		0.14	0.03			0.01	0.18		0.11	0.17	0.43		0.17	0.36		0	
15°C	Greenbeans		0.03	0.01	0.02	0.02	0.01	0.02		0.07		0.04		0.06		0.01		
	Potatoes		0.04	0.15	0.03		0.01		0		0.7			0.03	0.01			
	Onions		0.02	0.03	0.02		0	0.02		0.015	0.25	0.4			0.43		0	
	Greenpepper		0.07	0.03	0.03	0.09	0					0.03			0.08		0.01	
	Carrots	0.01				0.04	0.01		0.01	0.01	0.2		0.18		0.14		0	
25°C	Greenbeans					0.11	0	0.01			0.42		0.31			0.31	0.01	
	Potatoes		0.01	0.03	0.01		0.01				0.48	0.16						
	Onions		0.03	0.01	0		0.01		0		0.08	0.19			0.13			
	Greenpepper		0.06	0.21	0.01	0.11	0.01					0.06						
	Carrots	0.01	0.05	0.01	0.01		0	0.02		0.02	0.24	0.38	0.06		0.41		0	
Day 10 5°C	Greenbeans		0.15	0.03			0	0.18		0.02					0.09	0.03			*C. laurentii; D. hansenii; D. hansenii*
	Potatoes		0.03	0.18	0.03		0						0.06			0		*C. laurentii; D. hansenii; C. albidus; R. mucilaginosa; E. crataegenis*
	Onions		0.03	0.02	0.01	0.06	0				0.21	0.54			0.3			*R. glutinis*
	Greenpepper	0.01	0.01	0.03	0.01		0				0.49		0.05	0.08	0.14			
	Carrots		0.03	0.01			0	0.04			0.12		0.09	0.13				
15°C	Greenbeans	DETERIORATED																
	Potatoes		0.02	0.12	0.02		0			0.02	0.5			0.11				*C. albidus*
	Onions		0.01	0.06	0.014	0.01	0				0.32	0.47			0.22			*R. glutinis*
	Greenpepper	DETERIORATED																
	Carrots		0.12	0.05	0.01		0.01	0.11			0.1				0.26	0.02		
25°C	Greenbeans	DETERIORATED																
	Potatoes		0.02	0.04			0								0.05			
	Onions		0.01	0.02		0.03	0				0.16	0.27			0.15			*R. mucilaginosa*
	Greenpepper	DETERIORATED																
	Carrots					0.09	0.01		0.01		0.1	0.05		0.02	0.06		0	*D. hansenii*

Table 2 (continued)

Time(days)	Vegetable	Organic acids (%)									Sugars (%)						Ethanol (%)	Yeasts present
		Tartaric	Citric	Malic	Succinic	Ascorbic	Fumaric	Quinic	Piruvate	Lactic	Sucrose	glucose	Rhamnose	Xylose	Fructose	Arabinose		
	Greenbeans		0.1	0.03			0.01	0.06			0.12	0.04	0.11		0.04	0.08		*S. pombe; S. pombe*
Day 15	Potatoes		0.02	0.1	0.01		0						0.2					*C. albidus*
5°C	Onions	0.06				0.11	0.01	0.23		0.12	0.19	0.6			0.28			
	Greenpepper		0.03	0.14	0.03	0.09	0.01					0.16	0.06	0.06	0.31			
	Carrots		0.06	0.01			0	0.09			0.02	0.31	0.07	0.07	0.34			
	Greenbeans	DETERIORATED																
	Potatoes		0.05	0.16	0.04		0				0.74		0.07					
15°C	Onions	0.04	0.02	0.01	0.01	0.03	0				0.21	0.68			0.42			*R. glutinis*
	Greenpepper	DETERIORATED																
	Carrots		0.12	0.02			0.01	0.33			0.14	0.1	0.1	0.11				
	Greenbeans	DETERIORATED																
	Potatoes		0.02			0.04	0	0.11		0.02	0.99		0.08	0.14				*P. ohmeri*
25°C	Onions	0.02	0.05		0.01	0.03	0	0.03			0.09	0.22			0.23			
	Greenpepper	DETERIORATED																
	Carrots		0.09	0.02	0.03		0.01	0.09		0.08	0.2		0.17					*C. tropicalis*
	Greenbeans		0.04	0.02			0.02	0.06		0.16	0.25	0.15	0.07		0.13			*C. laurentii*
Day 20	Potatoes		0.02	0.01			0.01		0	0.01	0.75	0.06	0.11					*C. laurentii; S. pombe; C. albidus; R. glutinis; R. mucilaginosa*
																		S. pombe; C. laurentii; S. pombe; D. hansenii; D. hansenii; C. laurentii
5°C	Onions	0.02	0.03	0.1	0.04		0.01				0.23	0.7			0.34		0	*S. pombe*
	Greenpepper	DETERIORATED																
	Carrots		0.07	0.01			0	0.07			0.26	0.2	0.08	0.06	0.16			
	Greenbeans	DETERIORATED																
	Potatoes		0.03	0.08	0.02		0					0.01	0.01					*R. glutinis*
15°C	Onions	0.01		0.08							0.14	0.31			0.2			
	Greenpepper	DETERIORATED																
	Carrots	DETERIORATED																
	Greenbeans	DETERIORATED																
	Potatoes	0.04									1.1		0.18	0.19				*C. laurentii; New specie*
25°C	Onions	0.01	0.01	0.03							0.16	0.15	0.05		0.15			
	Greenpepper	DETERIORATED																
	Carrots	DETERIORATED																
	Greenbeans	DETERIORATED																
Day 25	Potatoes	0.085									0.76				0.03			*D. hansenii; C. solani*
5°C	Onions	0		0.024							0.3	0.48	0.05		0.25		0	*S. pombe*
	Greenpepper	DETERIORATED																
	Carrots	DETERIORATED																
	Greenbeans	DETERIORATED																
	Potatoes	0.03		0.01							0.69	0	0.02					*C. solani; S. pombe; R. glutinis; R. mucilaginosa; S. pombe*
15°C	Onions	0		0.05	0.000						0.21	0.09	0.11		0.18		0	
	Greenpepper	DETERIORATED																
	Carrots	DETERIORATED																
	Greenbeans	DETERIORATED																
	Potatoes	0.02									0.73	0.07	0.09					
25°C	Onions	0		0.01							0.13	0.23	0.04		0.1			*R. glutinis*
	Greenpepper	DETERIORATED																
	Carrots	DETERIORATED																

Table 2 (continued)

Time(days)	Vegetable	Organic acids (%)									Sugars (%)						Ethanol (%)	Yeasts present	
		Tartaric	Citric	Malic	Succinic	Ascorbic	Fumaric	Quinic	Piruvate	Lactic	Sucrose	glucose	Rhamnose	Xylose	Fructose	Arabinose			
Day 30 5°C	Greenbeans	DETERIORATED																	
	Potatoes										0.67		0.02					*R. glutinis; R. glutinis*	
	Onions	0.34	0.22		0.14						0.09			0.16					
	Greenpepper	DETERIORATED																	
	Carrots	DETERIORATED																	
15°C	Greenbeans	DETERIORATED																	
	Potatoes	0.02	0.02	0.07	0.01						0.81	0.03	0.05						
	Onions	0.07			0.03						0.33	0.43			0.3				
	Greenpepper	DETERIORATED																	
	Carrots	DETERIORATED																	
25°C	Greenbeans	DETERIORATED																	
	Potatoes	0.01					0.01						0.03					*C. laurentii; C. laurentii; R. mucilaginosa; R. mucilaginosa*	
	Onions	0.04									0.19	0.15	0.04		0.22				
	Greenpepper	DETERIORATED																	
	Carrots	DETERIORATED																	

* Day 1 = directly after purchasing

Table 3

Changes in microbial counts(log cfu.g^{-1}) and pH on fruits over a period of 30 days stored at different temperature

Time(days)	Fruit	YGC	PCA	DRBC	pH
Day 1	Pears	3	5.02	3	3.51
	Apples	3	4.46	3.48	2.93
	Oranges	3	4.63	3.3	2.86
	Mandarins	3	4.11		3.24
	Grapes		3.9		2.85
Spoilage	Pears	6.6	5.3	4.44	4.55
Day 5	Apples	4.43	5.18	3.3	4.03
5°C	Oranges	3.47	4.46		3.84
	Mandarins	3.3	5.4	3	3.68
	Grapes	3	4.74		3.81
	Pears	5.06	7.27		4.59
	Apples	4.6	4.86		4.28
15°C	Oranges	3	5.37		3.93
	Mandarins	3	4.69		4.08
	Grapes	3	5.6	3	3.91
	Pears	4.56	5.25	3.6	4.74
	Apples	3.69	spreader	3.3	4.01
25°C	Oranges		4.49		4.02
	Mandarins	3.3	4.51		4.23
	Grapes	3.3	6.83		3.49
Day 10	Pears	6.38	7.35	4.56	4.97
5°C	Apples	3.02	5.77	4.04	4.19
	Oranges	2.49	5.77		3.61
	Mandarins	2.14	5.77		3.79
	Grapes	2.36	5.26		3.86
Spoilage	Pears	5.08	6.36	4.97	4.81
	Apples	3.03	5.46	4	4.21
15°C	Oranges	2.27	5.69	3.3	4.42
	Mandarins	2.32	5.29	3.03	3.97
	Grapes	1.84	6.68	4.86	3.9
Spoilage	Pears	6.27	6.79	6	4.8
	Apples	2.79	5.23	3.77	4.25
25°C	Oranges	2.39	5.19	3.3	4.2
	Mandarins	2.47	5.24	3	4.46
	Grapes	2.96	5.38	4.11	3.69
Day 15	Pears	2.5	7.84	4.3	4.56
	Apples	2.68	6.11	3.3	4
5°C	Oranges	2.3	5.77	3	4.39
	Mandarins	2.54	5.84	3.3	3.86
	Grapes	1.47	5.84	3	3.92
	Pears	4.81	6.7	4.51	4.85
	Apples	3.04	5.35	4.43	4.44
15°C	Oranges	2.65	5.77	4.23	4.14
	Mandarins	2.23	5.84		4.29
	Grapes	ETERIORATED			
	Pears	4.96	6.25	4.89	4.59
	Apples	2.77	5.4	3.47	4.29
25°C	Oranges	2.43	6.25	3.77	4.53
	Mandarins	2.25	5.34	3.3	4.41
Spoilage	Grapes	4.96	5.38	4.98	4.23

Table 3 (continued)

Day 20	Pears	3.31	7.33	3.69	4.63
	Apples	3.1	6.81	3.3	3.98
5°C	Oranges	3.04	6	3	3.93
	Mandarins	2.57	6.23	3	4.07
	Grapes	2.17	6.43	3	4.02
	Pears	DETERIORATED			
	Apples	2.6	6.49	3.47	4.49
15°C	Oranges	2.47	5.41	3.77	4.31
	Mandarins	2.65	5.37	3	4.3
	Grapes	DETERIORATED			
	Pears	DETERIORATED			
	Apples	3.69	6.27	3.84	4.34
25°C	Oranges	3.95	6.44	3.77	4.09
	Mandarins	2.3	5.47	3.3	4.45
	Grapes	DETERIORATED			

Day 25	Pears	6.14	8.2	3.88	4.34
	Apples	4.85	6.75	4	4.15
5°C	Oranges	3.38	6.3	3.3	3.84
	Mandarins	3.18	6.11	3.3	3.99
	Grapes	3.38	6.64	3.47	4.02
	Pears	DETERIORATED			
	Apples	4.77	6.59	4.07	4.33
15°C	Oranges	4.54	6	3.6	4.45
	Mandarins	3.25	6.11	3.47	3.94
	Grapes	DETERIORATED			
	Pears	DETERIORATED			
Spoilage	Apples	4.55	6.51	4.69	4.46
25°C	Oranges	4.54	6.39	4.14	4.42
	Mandarins	2.94	5.3	3.3	4.67
	Grapes	DETERIORATED			

Day 30	Pears	DETERIORATED			
	Apples	4.87	7	3.47	4.23
5°C	Oranges	3.25	6.14	3.3	4.07
	Mandarins	3.4	5.84		4.04
	Grapes	DETERIORATED			
	Pears	DETERIORATED			
	Apples	DETERIORATED			
15°C	Oranges	4.36	5.95	3.69	4.6
	Mandarins	3.6	5.29	3.84	4.68
	Grapes	DETERIORATED			
	Pears	DETERIORATED			
	Apples	DETERIORATED			
25°C	Oranges	DETERIORATED			
	Mandarins	4.76	5		5.03
	Grapes	DETERIORATED			

Table 4

Changes in microbial counts(log cfu.g^{-1}) and pH on vegetables over a 30 day period stored at different temperatures

Time(days)	Vegetable	YGC	PCA	DRBC	pH
Day 1	Greenbeans	4.17	8.5	3.6	6.22
	Potatoes	4.54	5.37	3	5.37
	Onions	3.47	4.37	3.47	5.27
	Greenpepper	3.47	7.44	3.69	5.72
	Carrots	3.52	6.26	4.51	5.97
Day 5	Greenbeans	4.52	8.55	4.13	6.42
	Potatoes	3.38	8.94	5.14	5.99
5°C	Onions	3.2	8.43	4.23	5.85
	Greenpepper	3	7.38	4.51	6.4
	Carrots	4.42	7.61		6.29
	Greenbeans	3.78	7.12	6.69	6.56
	Potatoes	4.2	8.23	5.3	6.02
15°C	Onions	3.69	7.26	4.67	5.82
	Greenpepper	3.52	7.78	5.23	6.51
	Carrots	5.34	8.91	5.23	6.35
	Greenbeans	3.47	6.91	6.61	6.58
	Potatoes	4.17	8.78	5.33	5.86
25°C	Onions	3.47	7.26	4.46	5.88
	Greenpepper	3.56	7.46	5.65	6.34
	Carrots	4.95	8.95	5.56	6.39
Day 10	Greenbeans	2.92	7.46	5.23	6.62
	Potatoes	3.16	6.5	4.38	6.02
5°C	Onions	3.02	5.77	3.9	5.74
	Greenpepper	3.34	7.13	4.11	6.01
	Carrots	3.9	6.86	4.85	6.4
	Greenbeans	DETERIORATED			
	Potatoes	4.14	6.55	4.27	6.09
15°C	Onions	2.98	5.84	4	5.47
	Greenpepper	DETERIORATED			
	Carrots	3.47	6.87	4.63	6.37
	Greenbeans	DETERIORATED			
	Potatoes	3.77	6.47	4.55	5.94
25°C	Onions	2.65	5.77	3.6	5.53
	Greenpepper	DETERIORATED			
	Carrots	3.6	7.06	4.84	6.27
Day 15	Greenbeans	4.17	8	4.56	6.65
	Potatoes	4.25	6.71	4.27	6.02
5°C	Onions	2.25	5.95	3	5.63
	Greenpepper	4.57	8.96	4.3	6.79
	Carrots	4.77	6.79	4.65	6.38
	Greenbeans	DETERIORATED			
	Potatoes	3.9	6.43	4.27	6.21
15°C	Onions	2.58	5.84	4.99	5.59
	Greenpepper	DETERIORATED			
	Carrots	4.6	7.23	4.57	6.26
	Greenbeans	DETERIORATED			
	Potatoes	3.95	6.5	4.9	6.41
25°C	Onions	3.69	5.47	4.25	5.78
	Greenpepper	DETERIORATED			
	Carrots	4.61	8.3	5.07	6.28

Table 4 (continued)

Day 20	Greenbeans	5.33	8	4.88	6.72
	Potatoes	4.54	6.57	4.14	6.11
5°C	Onions	3.19	6.69	3.95	5.89
	Greenpepper	DETERIORATED			
	Carrots	5.47	7.17	4.77	6.34
	Greenbeans	DETERIORATED			
	Potatoes	3.84	6.36	4.3	6.05
15°C	Onions	3.9	6.34	4.54	5.76
	Greenpepper	DETERIORATED			
	Carrots	DETERIORATED			
	Greenbeans	DETERIOATED			
	Potatoes	4.38	6.61	4.3	6.1
25°C	Onions	4.21	6.04	5.12	5.88
	Greenpepper	DETERIORATED			
	Carrots	DETERIORATED			
Day 25	Greenbeans	DETERIORATED			
	Potatoes	5.26	8.2	4.85	6.18
5°C	Onions	4.81	6.43	3.9	5.76
	Greenpepper	DETERIORATED			
	Carrots	DETERIORATED			
	Greenbeans	DETERIORATED			
	Potatoes	4.83	6.96	4.38	6.03
15°C	Onions	5	6.27	4.97	5.96
	Greenpepper	DETERIORATED			
	Carrots	DETERIORATED			
	Greenbeans	DETERIORATED			
	Potatoes	5	6.56	5.19	6.95
25°C	Onions	4.54	6.23	3.6	5.9
	Greenpepper	DETERIORATED			
	Carrots	DETERIORATED			
Day 30	Greenbeans	DETERIORATED			
	Potatoes	4.76	7.05	4.9	6.2
5°C	Onions	4.57	5.22	3.47	5.89
	Greenpepper	DETERIORATED			
	Carrots	DETERIORATED			
	Greenbeans	DETERIORATED			
	Potatoes	4.36	6.23	4.96	6.27
15°C	Onions	4.9	5.18	5.12	5.25
	Greenpepper	DETERIORATED			
	Carrots	DETERIORATED			
	Greenbeans	DETERIORATED			
	Potatoes	4.49	6	3.77	6.31
25°C	Onions	5.1	6.13	4.54	5.56
	Greenpepper	DETERIORATED			
	Carrots	DETERIORATED			

Table 5

Enzyme activities of the dominant yeasts isolated from fruits and vegetables

Yeast	Isolates	Amilase	Pectinase	Cellulase	Proteases	Lipases
Rodotorula mucilaginosa	12	+	-	-	+	+
Rodotorula glutinis	25	+	+	-	+	+
Debaryomyces hansenii	15	-	-	-	-	+
Schizosaccharomyces pombe	30	+	-	-	-	+
Cryptococcus laurentii	15	+	-	-	-	+
New species	1	+	-	+	-	-
Total	98					

CHAPTER 4

The identification of a new basidiomycetous related yeast species isolated from potatoes

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Abstract

One strain of an undescribed, potato-born species was recovered. A description of the new basidiomycetous yeast species, is given.

Introduction

During a survey for the recovering of yeasts from fruits and vegetables over a 30 day period at three different temperatures, one strain of an undescribed species was recovered from potatoes at 25°C. The description of the new species follows.

Materials and methods

The morphological and physiological characteristics of the potato-born yeast strain were determined by the conventional techniques as described by Van der Walt and Yarrow (1984).

New basidiomycetous related yeast species

Growth on malt agar

After 3 days at 25°C the cells are ellipsoidal, oval, botuliform to cilindrical, budding enteroblastically, and occur singly or in pairs. The streak culture is butryous, orange, smooth with an entire to undulating margin. Sexual reproduction by teliospores.

Fermentation

Glucose	+
Galactose	+
Sucrose	+
Lactose	+

Utilization of carbon sources

D-glucose	+	D-ribose	-
D-galactose	+	L-rhamnose	-
L-sorbose	+	Ethanol	+
Sucrose	+	Methanol	+
Maltose	+	Glycerol	+
Cellobiose	-	Erythritol	-
Trehalose	+	Adonitol	+
Lactose	+	Dulcitol	-
Melibiose	+	Sorbitol	+
Raffinose	+	D-mannitol	+
Melizitose	+	Me-α-D-glucoside	+
Inulin	-	Salicin	+
Starch	-	DL-lactate	-
D-xylose	+	Succinate	+
L-arabinose	+	Citrate	-
D-arabinose	+	m-Inositol	-
Arbutin	+	D-glucunate	+

Utilization of nitrogen sources

Potassium nitrate	+
Sodium nitrite	-
Ethylamine hydrochloride	-
L-Lysine	+
Cadaverine dihydrochloride	-
Creatine	-
Creatinine	+

Growth at different temperatures:	-1 ^{0}C +; 25°C +; 37°C +
Growth with 0.01% cycloheximide:	+
Growth on 50%m/m glucose-yeast extract-agar:	-
Hydrolysis of urea:	+
Growth in vitamin-free medium:	+
Diazonium Blue B reaction:	+

DISCUSSION AND CONCLUSIONS

The new basidiomycetous yeast species showed interesting characteristics in assimilating lactose as a carbon source. One of the most distinguishable characteristics of the new yeast is the ability to ferment carbon sources, similar to another basidiomycetous yeast with this ability, i.e. *Phaffia rhodozyma.* However, the new yeast can be distinguished from the latter by the ability to ferment lactose. Its sexual reproduction by teliospores verifies the characteristically difference to *Cryptococcus* and *Rhodotorula* spp. where no sexual reproduction is noted. During an enzyme screening, (Call *et al.,* 1984) the new species showed amylase and cellulase degrading properties. Further research on this possibility seems very promising.

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CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

Farmers suffer significant losses of fruits and vegetables from fungal diseases after harvest (Snowdon, 1990, 1992). Currently, synthetic fungicides are the primary means of controlling postharvest diseases. Consumer concerns about fungicide residues in food and possible risks associated with continuous use of synthetic fungicides to control postharvest diseases of fruits and vegetables have resulted in an intensive search for safer control options that pose minimal risk to human health and the environment (Droby *et al.*, 1999).

The use of microorganisms, particularly yeasts, occurring naturally on the surface of fruits and vegetables usually have been preferred for the control of postharvest diseases by most investigators. Consequently fruits and vegetables were investigated to determine the diversity of yeasts associated with fruits and vegetables. In addition, the survival and possible spoilage ability were also determined when stored at different temperatures.

1. THE INCIDENCE OF YEASTS ASSOCIATED WITH FRUITS AND VEGETABLES.

In this study ecological surveys was preformed to differentiate between yeasts found on the surface and inner layer of ten different fruit and vegetable commodities.

1.1. *Microbial enumeration.* Bacteria represented the major component of the microflora isolated on the surface of vegetables (10^7 cfu.g^{-1}). The higher water content, more neutral pH and the close proximity to the soil during development facilitated

contamination and favored bacteria to dominate on vegetables (Bulgarelli and Brackett, 1991). The latter explained why potatoes and squashes yielded the highest bacterial numbers, exceeding counts of (10^7 cfu. g^{-1}). Simultaneously, microbial counts obtained from the inner layer yielded lower microbial counts, which are in correspondence with previous reports (Fleet, 1992). Fruits, on the other hand yielded higher yeast counts (10^5 cfu. g^{-1}). The intrinsic factors of lower pH values and higher carbohydrate content verify the predominance of yeasts on fruits.

1.2. *Yeast identification. Schizosaccharomyces pombe* and *Debaryomyces hansenii* were the predominant species isolated from vegetables while *Rhodotorula* spp. was present in lower numbers. The higher yeast counts on sweet potatoes might be due to the higher sugar content which presented a more conductive environment for their growth on this commodity. The dominant species isolated from fruits were *Schizosaccharomyces pombe, Hanseniaspora guilliermondii* and *Debaryomyces hansenii.* Spoilage of fresh fruits usually results from the fermentative activity of yeasts, but no such species was isolated during this study.

2. THE GROWTH AND SURVIVAL OF YEASTS IN FRUITS AND VEGETABLES STORED AT DIFFERENT TEMPERATURES.

In the present study the effect of temperature, time, chemical composition and enzymes on the growth of yeasts over a 30-day period on fruits and vegetables, were investigated.

2.1. *Physical and chemical composition.* The incubation time of fruits stored at three different temperatures over a 30-day period played a significant role in the changing of the chemical and physical composition of these commodities. Storage and ripening conditions were mainly responsible for the changes in organic acids and pH (Potter and Hotchkiss, 1986). The pH of fruits generally increased more rapidly at 25°C as the organic acids decreased, shortening the shelf life of these fruit commodities compared to fruits stored at 5°C where enhanced shelf life were observed. Vegetables, however contained lower percentages organic acids and sugars with higher pH levels compared to fruits. The pH levels of vegetables stored

at 15 and 25°C increased over time and the products deteriorated after 10-days, while vegetables stored at 5°C deteriorated after 20-days. A general non-changeable ethanol concentration suggested that no fermentative activity took place by developing yeast populations on the surfaces of fruits and vegetables.

2.2. *Microbial enumeration.* Enhanced growth of yeasts at higher temperatures was visible. Yeast counts ranging between 10^3cfu.g^{-1} at 5°C and 10^6cfu.g^{-1} at 25°C were encountered. Fruits stored at 15 and 25°C deteriorated after 15-days while fruits stored at 5°C deteriorated after 30-days. Bacterial counts as high as 10^8cfu.g^{-1} were encountered while mould counts ranging between 10^3cfu.g^{-1} and 10^4cfu.g^{-1}. Vegetables showed higher bacterial counts as expected, especially at 5°C. The predominant organisms on vegetables were aerobic Gram-negative rods, mostly pseudomonads and the yellow pigmented bacteria. General increases in mould counts of 10^4cfu.g^{-1} to 10^6cfu.g^{-1} were encountered with the highest counts obtained at 25°C.

2.3. *Yeast identification.* A total of 119 yeast strains were isolated from fruits and vegetables over the 30-day period. Fruits generally harbored more ascomycetous yeast species at 5°C, while the basidiomycetous yeast species dominated on the surfaces of vegetables. The most predominating species isolated from fruits were *Schizosaccharomyces pombe* and *Debaryomyces hansenii.* Previous studies by Hocking and Pitt (1997) and Suresh *et al.*, (1984) confirmed the incapability of S. *pombe* and *D. hansenii* to cause spoilage of fruits and vegetables. The wild yeasts such as the apiculate ones *Kloeckera apiculata* usually cause spoilage of fruits. No such species was isolated during this study. A new yeast species showing cellulase activity was isolated from potatoes on day 20 at 25°C. The dominance of S. *pombe* and *D. hansenii* on both the fruit and vegetable commodities might be explained by the fact that the present study was performed in an enclosed environment, while many of the previous studies were preformed in the field where environmental factors played a significant role in the isolation of different yeast species from the surfaces of fruits and vegetables.

2.4. *Enzyme screening*. The dominant yeast species isolated from fruits and vegetables were used in enzyme screening. The main aim was to determine if the yeast species isolated were responsible for the spoilage of fruits and vegetables stored at the different temperatures. The dominant yeast species were tested for amylolytic, proteolytic, lipolytic, pectinolytic and cellolytic activity. Both *Schizosaccharomyces pombe* and *Debaryomyces hansenii* showed no pectinolytic activity. *Rhodotorula glutinis, Rhodotorula mucilaginosa* and *Cryptococcus laurentii* showed amylolytic activity while the new yeast species showed cellulose activity.

3. THE IDENTIFICATION OF A NEW BASIDIOMYCETOUS YEAST SPECIES ISOLATED FROM POTATOES.

The new basidiomycetous yeast species showed interesting characteristics in assimilating lactose as a carbon source. It's sexual reproduction by teliospores verifies the characteristically difference to *Cryptococcus* and *Rhodotorula* spp. where no sexual reproduction is noted.

4. FUTURE RESEARCH.

It is recommended that future research should include the following:

A. A basic understanding of the composition of the natural microflora on the surface and wounded areas of fruits and vegetables are crucial for the development of a viable biocontrol procedure.

B. Manipulating specific epiphytic microbial populations of fruit surfaces by a preharvest or postharvest application of single or multiple biocontrol agents.

C. More research is needed to determine the effects of various pre- and postharvest practices on the population dynamics and biological control activity of the antagonist.

D. To apply yeasts isolated during this study from the surfaces of fruits and vegetables as probible biological control agents.

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CHAPTER 6

SUMMARY

Currently, fruits and vegetables suffer significant losses from fungal diseases after harvest. The use of synthetic fungicides which pose a risk to human health and the environment, have resulted in an intensive search for safer control of postharvest diseases by the use of microorganisms, particularly yeasts, occurring naturally on the surfaces of fruits and vegetables.

An ecological survey was preformed on five different fruit and vegetable commodities to determine the diversity of yeast species associated with these commodities and the cause of spoilage at different temperatures.

A. THE INCIDENCE OF YEASTS ASSOCIATED WITH FRUITS AND VEGETABLES.

Different fruits and vegetables were surveyed to determine the diversity of yeasts and to differentiate between yeasts found on the surface and inner layer of the different fruit and vegetable commodities. Bacterial growth dominated on the surface of vegetables due to factors such as higher water content, more neutral pH and close proximity to the soil facilitating their growth.

Yeasts represented the major component of the microflora on the surface of fruits due to a lower pH and higher carbohydrate content that encouraged their growth. The inner layer of both fruits and vegetables yielded lower microbial counts. *Schizosaccharomyces pombe* and *Debaryomyces hansenii* were the predominant species isolated on both fruits

and vegetables. Spoilage of fresh fruits usually results from the fermentative activity of yeasts, but no such species were isolated during this study.

B. THE GROWTH AND SURVIVAL OF YEASTS IN FRUITS AND VEGETABLES STORED AT DIFFERENT TEMPERATURES.

The growth and survival of yeasts were carried out at different temperatures over a 30-day period to determine an epiphytic yeast population developing on the surfaces of fruits and vegetables. The chemical and physical composition were monitored over a 30-day period to determine if the changes in pH and organic acids contributed to the developing yeast population on the surface of fruits and vegetables.

A total of 119 yeast strains were isolated from fruits and vegetables with bacteria dominating on the surface of vegetables and yeasts on the surface of fruits. The most predominating species isolated were *S. pombe* and *D. hansenii*. A new yeast species, showing cellulase activity, was isolated from potatoes on day 20 at 25°C.

C. THE IDENTIFICATION OF A NEW BASIDIOMYCETOUS YEAST SPECIES ISOLATED FROM POTATOES.

The new basidiomycetous related species showed interesting characteristics in assimilating lactose as a carbon source. Its sexual reproduction by teliospores verifies the characteristically difference compared to *Cryptococcus and Rhodotorula* spp. where no sexual reproduction is noted. Accordingly, the new species was described.