MICROBIAL CONTENT STABILITY AND INHIBITORY EFFECTS OF ANTIMICROBIALS ON THE SHELF-LIFE OF HIGH MOISTURE DRIED FRUIT

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Thesis presented in fulfilment of the requirements for the degree of

MASTER OF SCIENCE IN FOOD SCIENCE

In the Department of Food Science
Faculty of Agricultural and Forestry Sciences
University of Stellenbosch

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December 2000

DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that it has not previously, in its entirety or in part, been submitted at any university for a degree.

Sanneliza Engelbrecht

UITTREKSEL

Die droging van vrugte is 'n eeu-oue preserveringstegniek wat vandag steeds baie gewild is. Daar is 'n tendens in die oorsese markte na droëvrugte wat sagter en smaakliker is. Gebaseer of hierdie tendens het die Suid Afrikaanse droëvrugtebedryf 'n nuwe "soft eating", hoë vog droëvrug (HVDV) produkreeks die lig laat sien. Hierdie produkte het 'n voginhoud van *ca* 36% (m/m) en 'n water aktiwiteit (a_w) van *ca* 0.85. Dit is die hoër voginhoud wat lei tot mikrobiologiese bederf en verbruining. Tans is die rakleeftyd van die produkte ongeveer drie maande, terwyl uitvoer standaarde 'n rakleeftyd van minstens nege maande vereis.

Tydens hierdie studie is die mikrobiese samestelling van vyf Suid Afrikaans beskikbare HVDV produkte (rosyne, pruimedante, nektariens, taaipit perskes en appelkose) bepaal. Dit is bevind dat hoë vog droë (HVD) rosyne en pruimedante meestal hoë bakterie tellings het, terwyl appelkose meer vatbaar is vir giste en misiliêre fungi. Die organismes wat meestal geïsoleer is, was lede van die endospoorvormende *Bacillus* groep en osmotolerante giste soos *Zygosaccharomyces.* Lede van die genera *Salmonella*, *Staphylococcus* en selfs anaerobiese endospoorvormers is gevind in van die HVD pruimedante en rosyne. Vanuit hierdie resultate was dit duidelik dat die huidige pasteurisasie proses van 7 h by 80°C, wat deur die industrie gebruik word, nie effektief is om bederf organisms suksesvol te elimineer nie.

Hoë vog droë nektariens an pruimedante is gebruik in uitdaging studies, waar vier indikator organismes (*B. cereus*, *Z. rouxii*, *Escherichia coli* en *Penicillium chrysogenum*) gekies is, om die rakleeftyd van die twee HVDV produkte oor 'n opbergingstydperk van 6 maande by 5° en 25°C te evalueer. Die resultate het hoër bakteriese tellings vir vrugte wat by 25°C (1 200 kve.g^{-1} versus 450 kve.g^{-1} by 5°C) opgeberg is getoon, asook dat Maillard verbruining by HVD nektariens plaasvind by die hoër opbergingstemperatuur. Verder is bevind dat die pasteurisasie metode (7 h by 80°C) nie effektief was om die endospoorevormer *B. cereus*, *P. chrysogenum* en *Z. rouxii* te elimineer nie, wat ernstige bederf implikasies tot gevolg kan hê. Die *E. coli* stam het nie in een van die twee HVDV produkte oorleef nie en geen tellings is gevind nie,

selfs nie voor pasteurisasie nie. Die resultate het duidelik getoon dat die huidige pasteurisasie proses vir HVDV nie effektief is om mikrobiese bederf te voorkom nie, terwyl 'n lae opbergingstemperatuur aanbeveel word om 'n beter kwaliteit eindproduk te verseker.

Die impak van verskillende preserveermiddels (lisosiem, pimarisien, sorbaat, bensoaat en 'n imidasool verbinding) ten opsigte van die groei en oorlewing van *B. cereus* en *Z. rouxii* is geëvalueer in nektarien puree. Lisosiem het die beste inhibisie effek (70% inhibisie) t.o.v. *B. cereus* getoon gedurende die 12 h inkubasie periode, gevolg deur bensoaat, sorbaat, die imidasool verbinding en laastens pimarisien. Die data verkry vir die inhibitoriese effek van die preserveermiddels t.o.v *Z. rouxii*, het getoon dat bensoaat en sorbaat die beste groei inhibisie getoon het (100% inhibisie na 24 h). Pimarisien het 'n 100% inhibisie getoon na 48 h, terwyl die imidasool verbinding 89% inhibisie getoon het.

Van die verskillende pasteurisasie kombinasies wat aangewend is om die endopsoorvormer *B. cereus* te elimineer, is dit bevind dat 'n pasteurisasie van 150 min by 90°C die beste inhibisie resultate getoon het. Die kleur van die nektariens was egter onstabiel. Gevolglik is daar besluit om gemodifiseerde atmosfeer verpakking (MAV) te evalueer as deel van die produk se prosessering. Dit is bevind dat die kleur van die HVD nektariens stabiel was en daar word verder aanbeveel om 'n tyd/temperatuur kombinasie van 150 min by 90°C, saam met MAV te gebruik, om 'n kwaliteit eindproduk te verseker.

ABSTRACT

Drying of fruit is an age-old preservation technique that even to this day, remains popular. On the foreign markets there is a trend towards dried fruit products that are softer and more palatable. Based on this, the South African dried fruit industry has launched a range of "soft eating", high moisture dried fruit (HMDF) products. These products have a moisture content of *ca* 36% (w/w) and a water activity (a_w) of *ca* 0.85, both of which are considerably higher than that of traditional dried fruit. It is this higher moisture content that makes the product susceptible to serious microbial spoilage and colour deterioration in spite of the use of sulphur dioxide. Currently, the shelf-life of these products is approximately three months and to ensure a quality export product, the shelf-life must be at least nine months.

During this study the microbial composition of five South African commercially available HMDF products (raisins, prunes, nectarines, cling stone peaches and apricots), was determined. It was found that high moisture dried (HMD) raisins and prunes had the highest bacterial counts, while apricots were more susceptible to yeast and mould spoilage. The most frequently isolated microbes were members of the endospore forming *Bacillus* group and osmotolerant yeasts like *Zygosaccharomyces*. Members of the genera *Salmonella*, *Staphylococcus* and even anaerobic endospore formers were present in certain samples of the HMD prunes and raisins. From these results it was concluded that the current pasteurisation method of 7 h at 80°C used by the industry is not effective in successfully eliminating spoilage microbes.

High moisture dried nectarines and prunes were used in challenging studies, where four indicator organisms (*B. cereus*, *Z. rouxii*, *Escherichia coli* and *Penicillium chrysogenum*) were selected to evaluate the shelf-stability of these two HMDF products over six months at 5° and 25°C. The results showed higher bacterial counts for the fruit stored at 25°C (1 200 cfu.g^{-1} versus 450 cfu.g^{-1} at 5°C), as well as extensive Maillard browning of the nectarines at the higher storage temperature. Furthermore, it was found that the pasteurisation method (7 h at 80°C) was not effective in eliminating the

endospore former *B. cereus*, *P. chrysogenum* and *Z. rouxii*, which may thus lead to serious spoilage implications. The *E. coli* strain did not survive in either of the HMDF products and no counts were found even before pasteurisation. From these results it was clear that the current pasteurisation method of HMDF products is not effective in terms of the prevention of spoilage, and that storage of the product at lower temperatures would lead to a better quality product.

The impact of different preservatives (lysozyme, pimaricin, sorbate, benzoate and an imidazole compound) on the survival and growth of *B. cereus* and *Z. rouxii*, were evaluated in nectarine puree prepared from dried nectarines. Lysozyme showed the best inhibitory effect (70% inhibition) on *B. cereus* over a 12 h incubation period, followed by benzoate, sorbate, the imidazole compound and lastly, pimaricin. The data obtained for the inhibitory effect of the preservatives on *Z. rouxii* showed that benzoate and sorbate showed the best growth inhibitory effect (100% inhibition after 24 h). Pimaricin gave a 100% inhibitory effect after 48 h while the imidazole compound gave a 89% inhibition.

From the different pasteurisation time/temperature combinations applied to eliminate the endopspore former *B. cereus*, it was found that pasteurisation of 150 min at 90°C showed the best inhibition results. However, the colour of the product was unstable. It was, therefore, decided to also evaluate the use of modified atmosphere packaging (MAP), as part of the product's processing. It was found that the colour of the HMD nectarines was stable and it is thus recommended that a time/temperature combination of 150 min at 90°C together with MAP, is used to ensure a shelf-stable HMDF product.

To my father and late mother, who gave me endless love and opportunities in life.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to the following persons and institutions for their invaluable contribution to the completion of this research:

Professor T.J. Britz, Study Leader and Chairman of the Department of Food Science, University of Stellenbosch, for his unremitting advice, guidance and patience during this study and preparation of this thesis;

Dr. R.C. Witthuhn, Co-Study Leader and Lecturer at the Department of Food Science, for valuable advise and ever willing assistance during the course of my research and fulfilment of this thesis;

Dr. E. Joubert, Co-Study Leader and Senior Researcher at ARC Infruitec-Nietvoorbij, for valuable advice and assistance during the course of my research;

Mr. G.O. Sigge, Mrs. L. Maas and Mr. E. Brooks, for technical assistance during the execution of the experimental procedures, and Mrs. M.T. Reeves for help with administrative work;

Dr. M. Manley, Senior Lecturer, Department of Food Science, for the use of the NIR computer and printer;

Mr. Anton Erwee, fellow post-graduate student, for his endless assistance and support in the cannery; as well as the technical personal, ARC Infruitec-Nietvoorbij, for general assistance and advise in the cannery;

Ms. C. du Toit, S. Eksteen and Mr. J. Schoeman, SAD, for advice and assistance during the course of my research;

Mr. A. Stange, Food and Beverage Manager, Seravac, for the donation of lysozyme and Mr. H. Grobelaar, Business Manager, Dairy and Beverage, Anchor Bio-Technologies, for the donation of pimaricin;

Harry Crossley Research Bursary, Stellenbosch 2000 Merit Bursary, and the Department of Food Science for financial support throughout my post-graduate studies;

My fellow post-graduate students, for their help and support during my research;

My family and close friends, for endless love, support and consideration throughout my studies.

CONTENTS

Language and style used in this thesis are in accordance with the requirements of the *International Journal of Food Science and Technology*. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

CHAPTER 1

INTRODUCTION

The sun drying of fruit is one of the oldest processing techniques applied in the preservation of food. South Africa has produced dried fruit for more than three centuries and the evidence of the drying of fruit is richly woven throughout our colourful history (Anon., 1976).

Dried fruit products fall in the intermediate moisture food (IMF) product range with a water activity (a_w) of between 0.65 and 0.90 and a moisture content of 15 – 50% (w/w) (Leistner, 1985; Robson, 1976). These foods are shelf-stable at ambient temperatures for varying periods of time (Burrows & Barker, 1976) and it is this feature that makes IMFs very convenient products, especially for developing countries where cooling and suitable storage facilities are not always available.

The preservation principle applied during the production of IMF products is based on lowering the a_w of the product and this is usually achieved by drying the food. Although some microbes are destroyed during the drying process, it is not lethal to most and many can be easily recovered from the dehydrated food (Robson, 1976). Mycelial fungi are more tolerant to a decreased a_w than yeasts, while yeasts are more tolerant to a decreased a_w than bacteria (Leistner & Rödel, 1976). According to Scott (1957), the minimum a_w required for bacterial spoilage is 0.90 and for yeasts 0.88, while the spoilage caused by mycelial fungi was observed to be at a minimum a_w of 0.80. The general a_w range of traditional IMFs makes it unlikely that Gram-negative and most Gram-positive bacteria will play a role in the spoilage, with the exception of certain Gram-positive cocci, endospore formers and lactobacilli. Most other spoilage bacteria require a_w levels above 0.90 for growth and, therefore, do not play a significant spoilage role in IMFs (El-Halouat & Debevere, 1997; Labuza *et al.*, 1975).

Traditional dried fruit is very popular, but there is a world-wide trend for dried fruit products with a higher moisture content that makes the fruit softer and more palatable. The SAD (S.A. Dried Fruit Co-Operative Ltd.) has taken note of this trend and new additions to their product range include several “soft eating”, high moisture dried fruit (HMDF) products. These include high moisture dried (HMD) nectarines, cling peaches, apricots, prunes and raisins (J. Schoeman,

1999, SAD, Wellington, personal communication). These HMDF products are produced by rehydrating dried fruit to a moisture content of 36% and an a_w of *ca* 0.85. The higher moisture content leads to a higher a_w and a subsequent change in the spoilage microbial population, as well as an increased spoilage rate, which results in a shortened product shelf-life. The discolouration of the fruit is also a serious problem with these HMDF products, since the fruit undergoes a very drastic pasteurisation for 7 h at 80°C, which clearly enhances the colour degradation reactions of the product.

The most frequent spoilers of HMDF products (a_w = *ca* 0.85) have been reported to be fungi. As early as 1968, Pitt & Christian reported that yeasts and moulds (mycelial fungi) are the main causes of HMD prune spoilage. They found that the yeast *Zygosaccharomyces rouxii* was a common spoiler and that several *Eurotium*, *Aspergillus* and *Penicillium* species are common spoilage moulds of HMD prunes. The most frequently isolated yeast from IMFs belongs to the genus *Zygosaccharomyces* (Restaino *et al.*, 1982; Tapia De Daza *et al.*, 1995). High moisture papaya products (Tapia De Daza *et al.*, 1995) and even glazed cherries (Tilbury, 1976) were also reported to be spoiled by *Zygosaccharomyces rouxii.* In contrast, HMD apricots were most frequently spoiled by *Aspergillus repens* strains (Harel *et al.*, 1978).

The problem with HMDF is that the fruit spoils far easier and faster than traditional dried fruit giving a shelf-life of only three to six months (J. Schoeman, 1999, SAD, Wellington, personal communication). The exportation of the product requires at least a nine-month shelf-life, therefore, additional or more optimised preservation techniques must be considered. Different approaches can be used, like more intense pasteurisation methods or the addition of alternative preservatives. The problem with more intense pasteurisation methods is the effect of Maillard browning that leads to the formation of unacceptable brown by-products at elevated temperatures (Ames, 1990). Dried fruit is a natural product and any addition of preservatives nullifies the concept of a “natural” product, and to market the product overseas it is of the utmost importance to minimise the addition of chemical preservatives. Thus, if the shelf-life of HMDF products can be lengthened to nine months the product will be much more acceptable for the export market and these types of exports can contribute to the SA dried fruit industry's and the country’s economy.

The objectives of this study, therefore, were:

- To determine the microbial population and content of commercially available HMDF products. From the data obtained specific HMDF products and indicator organisms, representative of the microbes found in the commercial HMDF products will be selected for subsequent "challenging" studies.
- To challenge HMD nectarines and prunes over a 6 month storage period at two different storage temperatures with at least four indicator organisms, including an endospore former, an osmotolerant yeast, a mycelial fungus and if necessary, a member of the Enterobacteriaceae. The survival of these indicator organisms will be monitored during the storage period.
- To add and evaluate different preservatives, including lysozyme, K-sorbate, Na-benzoate, pimaricin and an imidazole compound as hurdles in eliminating or minimising the microbial spoilage population.
- To apply different time/temperature steam pasteurisation combinations so as to determine the most effective pasteurisation combination to eliminate selected microbes to produce an acceptable coloured end-product.

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CHAPTER 2
LITERATURE REVIEW

A. BACKGROUND

The drying of fruit is considered to be one of the oldest processing techniques that has been used for the preservation of food. The history of sun dried foods dates back to ancient times (Taylor, 1999). It has even been reported that the Benedictine monks introduced prunes to Europe after their crusades that were subsequently planted in their monastery's gardens, especially in the region of Bordeaux, France. These prune trees were re-planted in the French Agen region and were later imported to South Africa by H.E.V. Pickstone in 1882 and one of them, the "Prune d' Agen" prune cultivar was found to be the most suited for drying purposes (Anon., 1976).

South Africa has produced dried fruit for more than three centuries. In 1658, Jan van Riebeeck ordered the planting of 1 200 grapevines, including cultivars best suited for making raisins. He also ordered the planting of apricot trees in the Company's Gardens. When the French Huguenots settled in 1688 in the Wagenmakersvallei (Wellington district), they where overwhelmed by the fact that the yellow apricots ripen much faster in South Africa. They then proceeded to dry apricots and grapes and are today considered to be the fathers of the South African dried fruit industry (Anon., 1976; Davies, 1987).

The first evidence of the planned exportation of dried fruit was reported to be during the British Empire Exhibition of 1833, when a Mr. P. Jordaan won a medal for the excellent quality raisins that he had produced (Anon., 1976). In 1875, Krone & Kie built the first packaging house for dried fruit in Worcester. This packaging house was the first supplier of dried fruit to the local market and later specialised in exporting dried products to Europe and the UK (Anon., 1976).

Prunes are presently marketed in Europe and South Africa at a moisture content of 40% (w/w) and a water activity of 0.85 (Salunkhe *et al.*, 1991). Traditional dried fruit are very popular, but there is a world-wide trend for dried fruit with a higher moisture content that are softer and easier to eat. The higher moisture content leads

to a higher water activity and a subsequent change in the spoilage population, as well as an increased spoilage rate, resulting in a shortened shelf-life.

B. INTERMEDIATE MOISTURE FOODS

Foods normally contain moisture levels of about 20 - 80% (w/w) and possess a water activity (a_w) between 0.95 and 1.00. The solute concentration in these foods is not sufficient to inhibit microbiological and biochemical activity and consequently deterioration and spoilage will occur at ambient temperatures (Robson, 1976). The concentration of the aqueous solution present in the food can, however, be increased to a point where microbial activity will be restricted. Several desirable features are then manifested in the food, including reduced spoilage, improved safety, increased shelf-life and finally a soft, moist texture is obtained (Robson, 1976).

The term, Intermediate Moisture Foods (IMFs), is applied to products with an a_w in the range of 0.65 - 0.90 and a moisture content of 15 - 50% (w/w) (Leistner, 1985; Robson, 1976). These products are shelf-stable at ambient temperatures for varying periods of time. Impetus was given to IMFs during the early 1960's with the development and marketing of intermediate moisture pet foods (Burrows & Barker, 1976) and the development of IMFs for the US space programmes. Food for human consumption that meets the basic criteria of this class has been produced for many years (Brimelow, 1985) and are referred to as traditional or conventional IMFs. A few examples are listed in Table 1.

The technology of producing IMFs has been practised since antiquity, but has only been applied industrially during the past three decades. Modern living, international economics and world-wide food and health objectives have all added to the motives for developing new IMFs (Robson, 1976). More specifically, the continued concentration of urban populations (Robson, 1976), a general food shortage in most countries and the fact that most people in developing countries do not have access to cooling facilities (Taylor, 1999) are all proof of the need for new IMFs.

Table 1. Traditional Intermediate Moisture Foods (Bradley, 1994).

Food products	a_w range	Moisture content (%) (w/w)
Dried fruits	0.60 - 0.75	18 - 25
Cake and pastry	0.60 - 0.90	35
Fruit cake	0.73 - 0.83	36
Fruit juice concentrates	0.79 - 0.84	30 - 35
Jams	0.81 - 0.91	20 - 35
Honey	0.75	17.5
Sugars, syrups	0.60 - 0.75	0.5 - 2.1

Today a wide range of interesting modern IMFs are being developed, including many meat products (Kotzekidou & Lazarides, 1991; Labuza *et al.*, 1975), guava slices (a_w = 0.84 - 0.90) (Ayub *et al.*, 1997), banana chips (a_w = 0.66 - 0.72) (Zeb *et al.*, 1996) and intermediate moisture (IM) tomato products (a_w = 0.88 - 0.90) (Shi *et al.*, 1999).

During the development of a new IMF it is extremely important to consider the following criteria: the feasibility and economics of producing the product; the a_w range of the product; the proposed packaging system; the proposed manufacturing set-up; the proposed quality assurance scheme; and the way in which the product is planned to be sold (Brimelow, 1985). Methods have been developed to achieve the necessary increase in the concentration of solutes present in the water and the consequent decrease in a_w of the dried products. Two basic processes are used, namely adsorption and desorption. During adsorption, the food is dried and then subjected to controlled rehydration until the desired a_w is obtained. Desorption, on the other hand, is the infusion of the food in a solution of higher osmotic pressure so that the desired a_w is reached at equilibrium. This process can be accelerated by higher temperatures (Robson, 1976). It was found that foods preserved by desorption have a higher moisture content at a given a_w than food preserved by adsorption. Microbes also have higher a_w requirements for growth if the food has been prepared by adsorption (Leistner & Rödel, 1976).

In their studies on the microbiological stability of two IM food products, a banana and a pork product, Labuza *et al.* (1975) used adsorption and desorption methods to prepare the products. They found that *Staphylococcus aureus* was inhibited at an a_w of 0.90 if adsorption was used, whereas values between 0.75 and 0.85 were required for desorption systems. A similar effect was noted for specific fungi (Labuza *et al.*, 1975). From a processing point of view, growth minima are much higher if the food is prepared by an adsorption technique under conditions where stress is eliminated. Although adsorption is more expensive than desorption, it can be used to create very stable IMF systems without the need for chemical growth inhibitors (Labuza *et al.*, 1975).

The use of humectants in IMFs

The production of non-conventional IMFs is based on the addition of water binding agents or humectants, which include polyhydric alcohols, sorbate, antioxidants, plasticisers, emulsifiers, stabilisers and chelators (Robson, 1976). The addition of humectants makes water biologically and chemically unavailable and, therefore, retards spoilage, lipid oxidation, and enzymatic and non-enzymatic browning. Furthermore, it improves the palatability and the stability of the product (Linko *et al.*, 1985).

Humectants, such as sugars, salts and polyhydric alcohols have good a_w lowering capabilities, good water holding capacity and a low toxicity. A problem with modern humectants is that they may lead to the production of off-flavours in foods and must be used below their characteristic off-flavour concentrations if they are to improve the organoleptic acceptability of the product (Linko *et al.,* 1985). Further research still has to be done to improve the odour and taste characteristics of humectants and to make sure that they are not toxic for human consumption (Kapsalis *et al.,* 1985).

In a study (Patil & Singh, 1998) using pumpkin syrup solids (PSS) as a humectant, it was found that PSS could be a very promising humectant for the use in IMFs intended for human consumption. PSS has a good water binding capacity, less than that of glycerol, but better than that of either sucrose, sorbitol or propylene glycol. In terms of sensory properties, PSS has a pleasant flavour and a relatively low sweetness value of 0.19 versus a value of 1.0 for sucrose. It can thus be added in large quantities, up to a maximum of 16% (w/w), without substantially influencing the flavour of the food (Patil & Singh, 1998).

C. HURDLE TECHNOLOGY

Hurdle technology has been successfully used in the preservation of many IMFs. This technology is based on the preservation of foods with not just one preservation method, but a combination of parameters, or so called "hurdles". These parameters can include: high temperature (T); low temperature (t); less water availability (a_w); sufficient acidification (pH); reduced oxygen supply (Eh); preservatives (nitrite, smoke, SO_2); competitive microbes (lactic acid bacteria); and irradiation (gamma

radiation) (Leistner, 1985). The hurdle effect is of fundamental importance for the preservation of foods, since the hurdles in a stable product inherently control microbiological spoilage and food poisoning (Alzamora *et al.*, 1999; Forcinio, 1999; Leistner, 1985).

In Latin America, shelf-stable whole fruit and fruit purees have been produced by using combination hurdle treatments (Taylor, 1999). The fruit products were produced by using a mild heat treatment (blanching for 1 or 2 min with saturated steam), lowering of the pH to 3.0 by the addition of citric or phosphoric acid, a slight reduction in a_w from 0.98 to 0.93 by the addition of sugars and the addition of anti-microbials, like potassium sorbate and/or sodium bisulphite.

Hurdle technology is also commonly applied to IMFs, such as dried prunes with a moisture content of 36% (El-Halouat & Debevere, 1997). In the study, three hurdles were applied during the production of IM prunes and included temperature (T) with the product being pasteurised, the addition of potassium sorbate as anti-fungicide, and a relatively low a_w of 0.80 (El-Halouat & Debevere, 1997; McBean & Pitt, 1965).

The microbiological stability of mango juice was also improved by the combined application of a mild heat treatment (55°C for 15 min) and the supplementation with extracts of ginger (4% (v/v)) and nutmeg (4% (v/v)). Microbial growth was markedly inhibited and the product had an acceptable taste (Ejechi *et al.*, 1998).

Hurdle technology has also been applied in the preservation of a tomato concentrate in Brazil (Jardim *et al.*, 2000). Samples were formulated so as to contain various combinations of acetic acid (0.1 - 3.0% (v/v), NaCl (2 - 10% (w/v)), sodium benzoate (up to 0.15% (w/v)) and potassium sorbate (up to 0.15% (w/v)). The samples were then artificially contaminated with a mixture of yeasts, filamentous fungi and lactic acid bacteria to a count of approximately 5 - 10 cfu.g^{-1}, packed in unsealed glass jars and stored at 25° ± 2°C for up to a year. All samples containing at least one chemical preservative had a shelf-life of at least one year. In a second series of studies in which only NaCl (2.0 - 8.0% (w/v)) and acetic acid (0.1 - 2.5% (v/v)) were used, four of the nine treatments without chemical preservatives gave a shelf-life of a year. These treatments without chemical preservatives are of special interest for possible industrial use (Jardim *et al.*, 2000).

In India, a shelf-stable cottage cheese product was also successfully developed by using hurdle technology. The cheese could be stored at temperatures as high as 35°C for several weeks. It was achieved by combining a mild heat treatment (T = 0.4 min), a slightly reduced a_w (0.96) and a moderately low pH (4.2) (Taylor, 1999).

Several meat products have also successfully been produced with the use of hurdle technology. The technique was especially successful in the production of intermediate moisture fermented sausages (Table 2) (Leistner, 1985). A ready-to-eat canned paneer curry was also formulated so as to have an a_w of 0.95, pH of 5.0, potassium sorbate at 0.15% and was processed at a F-value of 0.8. Changes in the rheological properties of the paneer portion, as well as chemical and sensory changes during storage at 30°C, were studied. The product kept well for approximately one month and had a better quality than the heat sterilised product (F-value = 15.0) stored under similar conditions (Rao & Patil, 1999).

It was reported by Karthikeyan *et al.* (2000) that a ground chevon meat product called "Keema" could successfully be prepared using goats meat mixed with various other additives as hurdles to prevent spoilage, and optimal levels of each was determined. The humectants used included NaCl (2.5% (w/w)), sugar (1.0% (w/w)), skim-milk powder (2.0% (w/w)) and isolated soy protein. The preservatives used were spice mix (2% (w/w), ascorbic acid (500 $mg.kg^{-1}$), sorbic acid (500 $mg.kg^{-1}$) and sodium nitrate (100 $mg.kg^{-1}$). Hurdle treated "Keema" was found to be safe and acceptable for three days at ambient storage, and still fairly acceptable by day five, whereas the control samples were only acceptable for one day after manufacture (Karthikeyan *et al.*, 2000).

Hurdle technology has also successfully been used in South Africa to develop a shelf-stable canned steamed bread. Hurdles used included the lowering of the pH; the addition of NaCl (8 – 16 $g.kg^{-1}$) and glycerol (150 and 180 $g.kg^{-1}$) to lower the a_w; fat added to retard staling; calcium propionate (15%) added as preservative and heat applied to destroy vegetative forms of heat sensitive micro-organisms (90°C internal temperature in the can). The bread showed very low total plate counts and was found acceptable for human consumption (Lombard *et al.*, 2000).

Table 2. Sequence of hurdles used in the ripening of fermented sausages and their importance for the inhibition of undesirable micro-organisms (Leistner, 1985).

Sequence of hurdles
nitrate → Eh → c.m. → t → pH → a_w → smoke
Implications
Inhibition of organisms:
nitrate : *Salmonella* spp.
pH (< 4.5) and a_w (< 0.94): *Clostridium botulinum*
pH (< 4.0), t (< 7°C) and Eh (< 83%): *Staphylococcus aureus*
smoke: undesirable mycelial fungi

Hurdles: nitrate = preservative; Eh = redox potential; c.m. = competitive microbes; t = refrigeration; pH = acidity; a_w = water activity; smoke = preservative.

D. WATER ACTIVITY

Water is one of the compounds that is always present in food (Clarke, 1990). It is normally present in three basic forms: free water, adsorbed water and hydration water. The free water acts as a dispersing agent for colloids and as a solvent for salts. The adsorbed water is absorbed in the fruit cell walls or protoplasm and is bound to proteins. Hydration water is chemically bound, for example in lactose monohydrate that contains one water molecule (Bradley, 1994).

Many properties of foods are affected by the content and nature of the contained water (Troller, 1995). Water participates in the mass transfer and chemical reactions in food, where it plays a major role in determining the texture, non-enzymatic browning reactions, enzymatic reactions and lipid oxidation. Other properties of foods may also be influenced by the manipulation of the water activity (a_w) levels. Microbial growth, and in some cases the production of microbiological metabolites, are particularly sensitive to changes in a_w (Troller & Scott, 1994).

Water activity can be defined as the ratio of vapour pressure of a solution to that of pure water at a specific temperature (Troller & Scott, 1994). This can be expressed mathematically as a function of Raoult's law, that formulates:

$$a_w = P/P_0 = n_1/(n_1 + n_2)$$

Where P_0 is the vapour pressure of pure water, P is the vapour pressure of the solution, n_1 is the number of moles of solvent and n_2 is the number of moles of solute (Troller & Scott, 1994). Since solutes in food and fruit depress the water vapour pressure, P will always be less than P_0. Therefore, the a_w always has a value of between 0 and 1.0, where a value of 0 would represent the total absence of water and a value of 1.0 would represent pure water (Bourne, 1991).

The term, water activity, is also related to the equilibrium relative humidity (ERH), which is the relative humidity of the air surrounding a food product when the vapour pressure in the air is the same as in the food at equilibrium state (Bourne, 1991). Since relative humidity is expressed as a percentage, the ERH values can easily be transformed into a_w values as follows:

$$a_w = ERH/100$$

Relative humidity (RH) usually characterises ambient atmospheric conditions that might occur in a food warehouse or processing plant (Troller & Scott, 1994).

The concept of water activity has many useful applications in the food industry. One of these applications is to determine whether food will absorb or lose water in a given environment. Although macroscopically invisible, water vapour also flows spontaneously from high-pressure regions (high a_w) to low-pressure regions (low a_w). Therefore, food with an a_w of 0.6 that is placed in a chamber where the air is at 40% relative humidity (RH), will lose water vapour to the air until the a_w in the food equals that of the air (Bourne, 1991). From this it is clear that a_w and not the moisture content, is the driving force behind the movement of water vapour. A typical example of this, as reported by Chirife (1989), is a dry soup mix containing dried chicken pieces, dried vegetables and dried noodles. When the ingredients are placed in a moisture-proof package, there will be a water transfer between the constituents, until equilibrium is reached (Chirife, 1989). Therefore, it is important to know what the a_w of the end-product is going to be, to ensure a microbiological safe product.

By measuring and controlling a_w in foodstuffs, it is possible to make the following assumptions: which micro-organisms may be potential sources of spoilage; how to maintain the chemical stability of foods; how to minimise non-enzymatic browning reactions and spontaneous auto-catalytic lipid oxidation reactions; how to prolong the desired activity of enzymes and vitamins in food and how to optimise the physical properties of foods, such as texture and shelf-life, and lastly how to choose the correct packaging material (Fontana, 2000).

The degree of resistance to the passage of water vapour is an important criterion in the selection of packaging material for foods, especially where the quality will deteriorate with the absorption of water from the atmosphere during storage. Fresh fruit and vegetables have a high water content, usually in the range of 80 - 90% and an a_w of 0.98. These fresh products would, therefore, quickly lose moisture to the air and become wilted, limp and dry. The skins of these products act as barriers to impede the loss of water vapour and the skin can even be seen as its own packaging material (Owen *et al.*, 1991).

In a recent study, Irwandi *et al.* (1998) evaluated packaging materials to obtain the highest quality intermediate moisture fruit leather. Four types of packaging materials were evaluated; laminated aluminium foil (LAF), high-density polyethylene, and low-density polyethylene and polypropylene. During a storage period of 12 weeks, LAF packed fruit leather showed the lowest changes in a_w, moisture content

and microbial growth, as well as the lowest decrease in colour quality (Irwandi *et al.*, 1998). Therefore, LAF was found to be the best packaging material for the fruit leather.

In most foods, the amount of water present will have an effect on the perception of texture. Most foods have an a_w greater than 0.8 at the moment of consumption. This is of importance, because this level of a_w promotes the growth of microbes. People consume foods with a high a_w level because they prefer their food to be in a moist and tender state for mastication (Bourne, 1987). The reason for rehydrating dried fruit, with an increase in a_w from 0.70 to 0.80 is to improve the fruit's palatability. The adjectives moist, juicy, tender and chewy describe desirable textural attributes, while hard, dry, tough and crumbly describe undesirable textural attributes. To obtain desirable textures it is usually necessary to have a high moisture content, which means the a_w is potentially high enough to support microbial growth. When food is dried to an a_w level that will not support undesirable microbial growth, the texture usually becomes too hard or tough (Bourne, 1987).

Moisture content, however, fails to describe the chemical state of the water in a particular system, while a_w gives a better reflection of the chemical state of water in a food system (Owen *et al.*, 1991). The definition proposed for textural properties by Bourne (1982) was: "the textural properties of a food are that group of physical characteristics that arise from structural elements of the food, are sensed by the feeling of touch, are related to the deformation, disintegration and flow of the food under force, and are measured objectively by functions of mass, time and distance". The term "textural properties" is preferable above "texture", because it encompasses and describes a group of properties. "Texture" cannot be completely specified by a single measurement like pH and/or moisture content (Bourne, 1987).

Rockland (1969) listed food characteristics as a function of their localised moisture sorption isotherms. The list included the following texture terms: a) local isotherm one (low moisture content) – dry, hard, crisp and shrunken; b) local isotherm two (intermediate moisture content) – dry, firm and flexible; and c) local isotherm three (high moisture content) – moist, soft, flaccid, swollen and sticky.

In their studies on the cutting resistance of a reconstructed fruit bar with different water activities, Owen *et al.* (1991) found that the product, according to texture, was most acceptable at an a_w of 0.85. It was also found that it was totally

unacceptable at an a_w of 0.30, as it became brittle and showed lower cutting resistance.

E. INFLUENCE OF DRYING AND WATER ACTIVITY ON MICROBES

The preservation principle of IMFs is based on lowering the a_w of the food and this is usually achieved by drying the food. Although some microbes are destroyed during the process of drying, it is not lethal to most, and many can easily be recovered from the dehydrated food. This is especially true when poor quality foods are used for drying and improper practises are followed during their manufacturing (Robson, 1976).

Mycelial fungi are more tolerant to a decreased a_w than yeasts, while yeasts are more tolerant to a decreased a_w than bacteria (Leistner & Rödel, 1976). According to Scott (1957), the minimum a_w for the spoilage caused by bacteria is 0.90, and for yeasts 0.88, while the spoilage caused by mycelial fungi was observed to be at a minimum a_w of 0.80. The general a_w range of traditional IMFs makes it unlikely that Gram-negative or most Gram-positive bacteria will play a role in the spoilage, with the exception of certain Gram-positive cocci, endospore formers and lactobacilli. Most spoilage bacteria require a_w levels above 0.90 for growth and, therefore, play no significant role in IMFs (El Halouat & Debevere, 1997; Labuza *et al.*, 1975). In Table 3 an overview of the a_w limits of the microbial growth of microbes significant to public health, as well as examples of foods that generally contain micro-organism levels within these ranges, are given.

In Table 4, a number of bacteria, yeasts and mycelial fungi that have been isolated from IMFs, are listed. It is important to keep in mind that a_w and the a_w inhibition range, are also strongly influenced by environmental parameters such as pH, temperature and redox potential (Eh) (Chirife & Beura, 1994). The interaction between a_w, pH and storage temperature on the growth of mycelial fungi on fruit jam was studied by Horner & Anagnostopoulos (1973). They reported that the interaction between a_w and temperature was the most significant. The thermal resistance or D-value, is also influenced by the a_w value, with a maximum resistance

Table 3. Water activity and growth of microbes in food (Beuchat, 1981).

Range of a_w	Micro-organism generally inhibited by lowest a_w in this range	Foods generally in this range
1.00 – 0.95	*Pseudomonas, Escherichia, Proteus, Shigella, Klebsiella, Bacillus, Clostridium perfringens*, some yeasts	Highly perishable (fresh) foods and canned fruits, vegetables, meat, fish and milk; foods containing up to ≈ 40% (w/w) sucrose or 7% NaCl.
0.95 – 0.91	*Salmonella, Vibrio parahaemolyticus, Clostridium botulinum, Serratia, Lactobacillus, Pediococcus*, some moulds and yeasts	Some cheeses (cheddar, Swiss, muenster, provolone); cured meats; some fruit juice concentrates; foods containing 55% (w/w) sucrose (saturated) or 12% NaCl.
0.91 - .087	Many yeasts, *Micrococcus*	Fermented sausages; sponge cakes; dry cheeses; margarine; foods containing 65% (w/w) sucrose (saturated) or 15% NaCl.
0.87 – 0.80	Most moulds, *Staphylococcus aureus*, most *Saccharomyces bailii*, *Debaromyces*	Most fruit juice concentrates; sweetened condensed milk; flour; rice; pulses containing 15 – 17% moisture.
0.80 – 0.75	Most halophilic bacteria, mycotoxigenic aspergilli	Jam; marmalade.
0.75 – 0.65	Xerophilic moulds, *Saccharomyces bisporus*	Rolled oats containing ≈ 10% moisture; fudge; jelly; some dried fruits; nuts.
0.65 – 0.60	Osmophilic yeasts, few moulds	Dried fruits containing 15 – 20% moisture; honey.
0.50	No microbial proliferation	Pasta containing ≈ 12% moisture; spices containing ≈10% moisture.
0.40	No microbial proliferation	Whole egg powder containing ≈5% moisture.
0.30	No microbial proliferation	Cookies, crackers, bread crusts, etc. containing 3 – 5% moisture.
0.20	No microbial proliferation	Whole milk powder containing 2 – 3% moisture; dried vegetables.

Table 4. Minimal a_w for microbial multiplication in IMFs (Leistner & Rödel, 1976).

Type of organism	Minimal a_w	Range
Bacteria		
Pediococcus	0.90	
Streptococcus	0.90	
Lactococcus	0.90	0.75 - 0.90
Vibrio	0.90	
Staphylococcus	0.84	
Halophillic bacteria	0.75	
Yeasts		
Hansenula	0.88	
Candida	0.88	
Hansenospora	0.88	
Torulopsis	0.88	0.65 - 0.88
Debaryomyces	0.88	
Saccharomyces	0.80	
Zygosaccharomyces	0.65	
Moulds (Mycelial fungi)		
Cladosporium	0.86	
Paecilclomyces	0.86	
Penicillium	0.80	
Aspergillus	0.72	0.62 - 0.86
Emericella	0.80	
Eremascus	0.80	
Wallemia	0.70	
Eurotium	0.62	

occurring in the IM range (Murrel & Scott, 1966). The effect of lowering the a_w below the optimum for a specific organism is to increase the growth lag phase and to decrease the growth rate and size of the final microbial population (Richard-Molard *et al.*, 1985).

In studies on the influence of a_w on the growth of *Enterobacter aerogenes* in culture media, Wodzinski & Frazier (1961) found that by lowering the a_w, the lag phase and generation time of the organism was lengthened, until growth finally stopped. It is also important to note that they reported that the minimum a_w was raised when the incubation temperature was decreased. Scott (1957) related a_w levels to the probability of spoilage in that, at a_w values of between 0.80 and 0.85, spoilage occurred readily by a variety of fungi within one to two weeks. At a_w values of 0.75, spoilage was delayed, with fewer types of organisms. At a_w values of 0.65 very few organisms are known to grow and no spoilage is likely to occur, for even up to two years.

Microbes may employ many strategies as protection against osmotic stress when food is dried, like the intracellular accumulation of compatible solutes. Other strategies employed by bacteria include the accumulation of K^+ ions, glutamate, glutamine, proline, γ-aminobutyrate, alanine, glycinebetaine, sucrose, trehalose or glucosylglycerol. Gram-negative bacteria tend to accumulate proline by the mechanism of enhanced transport (Marshall *et al.*, 1971). For example, it was found that in a high osmotic strength growth medium, the growth of *Staphylococcus aureus* was enhanced by L-proline through the use of a low affinity transport system (Townsend & Wilkinson, 1992). It has also been reported that halo-tolerant and xerotolerant fungi produce polyhydric alcohols, such as glycerol, erythritol and arabitol in response to osmotic shock (Baross & Lenovich, 1996).

F. INFLUENCE OF A_W ON FUNGI

Mycelial fungi

Mycelial fungi that spoil food products are a very heterogeneous group of micro-organisms with respect to the a_w levels that inhibit their growth. Most mycelial fungi that occur in food products require a very high a_w, close to the maximum a_w for optimal growth to develop (Magan, 1988; Marín *et al.*, 1998). However, some

species are able to grow at much lower a_w levels (Richard-Molard *et al.*, 1985). Jarvis (1976) divided fungi according to their a_w into three groups, namely the hygrophilic fungi, those that are unable to grow below an a_w of 0.90; the mesoxerophilic fungi, those that can grow at a_w values of 0.90 - 0.80; and the xerophilic fungi that are capable of growth below an a_w of 0.80.

It is important to take note that fungal sexual reproduction requires an even higher a_w level than normal growth (Pitt & Christian, 1968). Limiting a_w values to prevent sporulation is thus of great importance. Fungi that are unable to sporulate will not complete their life-cycle, which is essential for extensive growth and subsequent marked degradation of the substrate (Richard-Molard *et al.*, 1985). Thus, IMFs have to be produced and subsequently stored at a_w values of 0.85 or lower, as most hygrophilic species can be discounted at the lower a_w levels (Richard-Molard *et al.*, 1985). Examples of mycelial fungi capable of growth at low a_w values, together with their associated toxins and the limiting a_w values, are presented in Table 5.

The growth of mycelial fungi is known to be accompanied by the production of metabolites that produce diverse toxic effects in susceptible animals and humans (Bacon *et al.*, 1973). Such metabolites, known collectively as mycotoxins, range from relatively simple organic molecules (kojic acid) to substituted coumarins (aflatoxins, ochratoxins) and scirpenes (Bacon *et al.*, 1973; Li *et al.*, 2000). Already 27 years ago, Bacon *et al.* (1973) found that 200 mycelial fungi were capable of producing mycotoxins, although many of the species have not been implicated in outbreaks of clinical mycotoxinosis. Information on the a_w requirements for mycotoxin production is limited, since mycotoxins are frequently produced at sub-optimal growth temperatures (Bacon *et al.*, 1973). The most mycotoxinogenic strains are mesoxerophilic and xerophilic and are, therefore, able to grow in conditions of reduced a_w (Jarvis, 1976; Trucksess *et al.*, 1999).

In studies on the susceptibility of date fruits to aflatoxin production, Ahmed *et al.* (1997) studied 12 different date cultivars at different stages of ripening. The dates where inoculated with *Aspergillus parasiticus* and a maximum toxin problem of 300 $\mu g.g^{-1}$ was detected in the mature stage, which is the most suitable stage for human consumption. Although differences were observed between varieties and

Table 5. Xerophilic and mesoxerophilic fungi, their toxins and limiting a_w levels (Jarvis, 1976).

Microbe	Mycotoxins produced	Limiting a_w level for growth	Limiting a_w level for toxin production
Aspergillus chevalieri	Xanthocillin X	0.65	-
A. flavus	Aflatoxins, Aspergillic acid	0.78	0.83-0.87
A. fumigatus	Fumagillin, Gliotoxin	0.82	-
A. nidulans	Kojic acid Nidulline	0.78	-
A. niger	Oxalic acid	0.88	-
A. ochraceus	Ochratoxins Penicillic acid	0.76	0.85
Penicillium citrinum	Citrinin	0.80	-
P. cyclopium	Penicillic acid	0.81	-
P. expansum	Patulin	0.82	-
P. palitans	Penicillic acid	0.83	-
P. patulans	Patulin	0.81	-
P. puberulum	Penicillic acid	0.81	-
P. viridicatum	Ochratoxin Citrinin	0.81	-

- = data not available

stage of maturation, Ahmed *et al.* (1997) concluded that toxigenic *Aspergillus* spp. could proliferate on any date fruits that suffered mechanical stress in the field or during harvesting.

Penicillium expansum was found to produce the aflatoxin, patulin in apple (Siliha & Askar, 2000), blackcurrant and cherry juice (Larsen *et al.*, 1999). Patulin is a mycotoxin with the capability to disturb mitochondrial and plasma membrane functions. It has a wide spectrum of activity and can be carcinogenic and mutagenic. It can be a problem in baby foods and the maximum level in baby and dietetic foods in some countries is 10 ppb (Siliha & Askar, 2000).

Ochratoxin A, a mycotoxin formed by *Aspergillus ochraceus* and *Penicillium verrucosum*, was produced on inoculated apricot jam, with a fruit/sugar ratio of 1:1 and an a_w of 0.89. It was also isolated from various types of jam samples obtained from households. The mycotoxin was detected at concentrations ranging from 0.09 – 94.6 $ng.g^{-1}$ (Ruland *et al.*, 1998). Since Ochratoxin A can cause problems to the human nervous system (Li *et al.*, 2000), this is a serious problem because jams are also in the IMF range. It is thus important not to scrape off any moulds on top of jam and to eat the rest of the jam as it may contain the mycotoxin.

The most troublesome microbes in IM dried fruits are mycelial fungi, with the *Aspergillus glaucus* group being the most notorious spoilers at low a_w values (El-Halouat *et al.*, 1998). In contrast, it was found that IM apricots were most commonly contaminated with *Aspergillus repens* (Harel *et al.*, 1978).

The predominant spoilage mycelial fungi in dried IM prunes were reported to be members of the *Aspergillus glaucus* group and *Xeromyces bisporus*. Aleuriospores of *X. bisporus* were able to germinate within a 120 day period at an a_w level of 0.61, but it has been found that generally, higher moisture levels were required for both asexual and sexual sporulation (Pitt & Christian, 1968). In a later study, El-Halouat & Debevere (1997) found that *Penicillium chrysogenum* and *Fusarium oxysporum* are also common spoilage fungi on prunes (a_w = 0.88). This can be problematic, as it has been reported that *Penicillium chrysogenum* produces the neurotoxins, isofumigaclavines A and B, in homemade wine. These toxins are extremely toxic to humans and can cause serious neurological problems (Moeller *et al.*, 1997).

Yeasts

Yeasts are the second most important group of IMF spoilage micro-organisms (Leistner & Rödel, 1976). Yeasts generally require small amounts of nitrogenous compounds for growth and are fermentative rather than putrefactive. Consequently, it has been found that food with high C to N ratios tend to be spoilt by yeasts, whereas foods with high N to C ratios tend to be spoilt by bacteria (Restaino *et al.*, 1983, Tilbury, 1976). Yeasts that are capable of growth in the IMF a_w range, especially below 0.86, or in high solute concentrations, have been classified as osmophilic (Christian, 1963), osmotophilic (Van der Walt, 1970), osmotolerant (Anand & Brown, 1968), osmotrophic and xerophilic (Pitt, 1975). The a_w values of the most IMFs are in the range 0.84 - 0.86 as this range represents the best palatability of the products. However, this a_w range is also the optimum growth range for osmotolerant yeasts which are the cause of the most extensive yeast spoilage in IMFs (Tilbury, 1976). Many traditional IMFs are susceptible to spoilage by osmotolerant yeasts, and examples of these are listed in Table 6. These organisms are capable of growth over a wide pH range (pH = 2.0 - 7.0), with an optimum growth pH between 4.0 and 4.5 (Tilbury, 1976).

The osmotolerant yeast *Zygosaccharomyces rouxii* is the main spoilage yeast of IM fruit products and bakery products (Tapia De Daza *et al.*, 1995). This yeast has been reported to cause serious spoilage in IM papaya products (Tapia De Daza *et al.*, 1995), in glacé cherries and fruit concentrates (Tilbury, 1976), as well as spoilage of high moisture dried prunes and raisins (El-Halouat *et al.,* 1998). It is capable of growing under extreme conditions of temperature, pH and osmotic stress (Golden & Beuchat, 1992). *Zygosaccharomyces rouxii* grows at temperatures ranging from 4° - 42°C (Jermini & Schmidt-Lorenz, 1987) and over a pH range of 1.5 to 10.5 (Restaino *et al.*, 1983). Although, this yeast does not exhibit an absolute requirement for low water activity, it has been reported to show growth at an a_w value as low as 0.62 and can even cause spoilage at this low a_w level (Golden & Beuchat, 1992). Its tolerance to extremely high sugar and salt concentrations (Abdul-Raouf *et al.*, 1994; Onishi, 1963) and its ability to grow in the presence of $\leq$ 0.1% potassium sorbate (Restaino *et al.*, 1983) makes *Z. rouxii* a serious problem to the stability and shelf-life of many IMFs.

Table 6. IMFs susceptible to spoilage by osmotolerant yeasts (Mossel, 1975; Pitt, 1975).

Product type	**Examples**	**Water content (% w/w)**	**Solute content % (w/w)**	**a_w**
Syrups,	sucrose syrup,	0.4 - 0.70	99.3 - 99.6	0.60 - 0.75
sugars	raw cane sugar	33.3	66.7	0.85
sweet spreads and preserves	jam, honey, marmalade	20.0 - 35.0	65 - 80	0.75 - 0.85
Fruit juice concentrates	orange juice,	35	65	0.80 - 0.84
	raspberry	35	65	0.79 - 0.80
Confectionery products	marzipan,	15 - 17	83 - 85	0.75 - 0.80
	glaze cherries	30	70	0.75
Dried fruits	prunes, figs,	20	80	0.68
	dates	12 - 25	75 - 88	0.60 - 0.65

Consumers are becoming more health conscious and negative about chemical preservatives. Much research has still to be done to evaluate and characterise "natural" compounds, like essential oils, spices, killer yeasts, lysozyme and bacteriocins as possible preservatives against yeasts. Interesting work has been reported on the use of lemon grass, which showed significant inhibition against *E. coli*, *S. aureus* and *A. niger* (Baratta *et al.*, 1999). The killer yeast strain, *Saccharomyces cerevisiae* strain K2M, was also found to be very effective against *Z. rouxii*, the most common spoilage yeast of IMFs (Palpacelli *et al.*, 1992). Cerrutti & Alzamora (1996) also studied the inhibitory effect of vanillin on *Zygosaccharomyces* species in fruit purees. They found that *Z. rouxii* was inhibited by 2 000 $mg.kg^{-1}$ in banana and apple puree.

G. STABILITY OF IMFs TO BACTERIAL SPOILAGE

Intermediate moisture foods cover a wide range of food products destined to be produced, stored and consumed all over the world. The microbiological standards that are applied to the manufacture and the final quality of the product should be designed to ensure that all food are safe to eat, even after several months of storage at ambient temperatures. The standard "safe" refers to the fact that the IMFs do not contain detectable levels of toxin or unacceptable counts of viable cells (Pawsey & Davies, 1976). The following bacteria can cause severe problems in IMFs and are discussed in further detail.

Staphylococcus aureus

Staphylococcus aureus is an ubiquitous organism that is normally found on the skin, skin glands and mucous membranes of warm-blooded animals and humans. It is potentially pathogenic and can cause a wide range of infections and intoxications and some strains cause food poisoning (Lancette & Tatini, 1996). The presence of this organism in IMFs is of significance for two reasons: firstly, *S. aureus* can be an indicator of unhygienic practice in the preparation of the product; and secondly, the enterotoxin that causes food poisoning may be present at levels which constitute a health hazard (Pawsey & Davies, 1976). *Staphylococcus aureus* is also the only pathogen that can grow at low a_w values of between 0.83 and 0.84 (Smith *et*

al., 1983). It can even produce enterotoxin at an a_w of 0.86 (Troller, 1973), which is released if the cell counts exceed 10^7 cfu.g^{-1} (Tatini *et al.*, 1971). However, if conditions are favourable, enterotoxin may even be produced by high populations of non-growing cells (Markus & Silverman, 1968). Although *S. aureus* grows well in media without NaCl, it can grow well in 7 - 10% concentrations and some strains can even grow in up to 20% NaCl. The osmo-adaptation of *S. aureus* is the reason why this organism can grow and produce toxins at low a_w values. In studies by Louch *et al.* (1997) and Miller *et al.* (1992), it was found that the organism accumulated proline and glycine betaine, intracellular under low a_w growth conditions as a survival mechanism.

One method of controlling microbial numbers in IMF products is to apply a heat treatment to improve the microbiological stability of the IMF products. IMFs characteristically have high solute levels and it is to be expected that the heat resistance of staphylococcal strains in these products will be different from cells heated in diluted systems (Pawsey & Davies, 1976). Although *S. aureus* is a mesophilic organism, some strains can grow at temperatures as low as 6.7°C (Angelotti *et al.*, 1961). The growth range of *S. aureus* is normally between 7.0° and 47.8°C, and enterotoxins are produced between 10° and 46°C, with the optimum growth between 40° and 45°C (Smith *et al.*, 1983). *Staphylococcus aureus* has been reported to be able to grow in a pH range of between 4.0 - 9.8, but the optimum is in the range of pH 6 to 7. As in the case of other growth parameters, pH is dependent on the degree to which all other parameters are at optimum levels.

In their studies on *S. aureus* in a model IM product (Hennican), Boylan *et al.* (1976) found that when this organism was inoculated into the food at a_w's of 0.86 and 0.90 and pH values of 5.2 and 5.6, not all inhibitors used prevented growth. Of the compounds tested, methyl-paraben, sodium benzoate, potassium sorbate and calcium propionate proved to be the most effective against *S. aureus* at low concentrations.

Intermediate moisture environments normally prevent staphylococcal growth, because the cells are probably injured. If cells are injured due to exposure to sub-lethal heat treatments and IMF conditions, the surviving cells may lose their ability to produce colonies on selective media. Such injured cells may need a holding period

in a non-stressful liquid medium to facilitate injury repair, much in the same way as pre-enrichment is used to resuscitate injured Salmonellae (Pawsey & Davies, 1976).

The risk of the contamination of IMFs by *S. aureus* is as great as for other manufactured foods of composite nature. In the a_w range of 0.65 - 0.90, the risk for rapid growth of contaminants is not high, but they could either grow slowly or survive for periods of several months. Toxin production tends to be inhibited at higher a_w values than growth, but the possibility of toxin accumulation cannot be entirely ruled out (Pawsey & Davies, 1976).

In a study on the effect of low water activity and the addition of humectants (NaCl, glycerol and sucrose) and compatible solutes (glycine, proline, betaine and carnitine) on staphylococcal enterotoxin A (SEA) and enterotoxin B (SEB) production, Ying & Miller (2000) found that SEB is more sensitive to low a_w than SEA production. SEB production was stimulated significantly at low a_w when proline was available as a compatible solute for *S. aureus*. This stimulatory effect was not observed with any of the other compatible solutes (Ying & Miller, 2000).

The popularity of non-refrigerated, ready-to-eat foods like sausage products (low or intermediate moisture level) combined with high moisture foods like cheese, is increasing. Packaging IM meat in direct contact with high moisture cheese may change the a_w of the product sufficiently to support growth of *S. aureus* at combined interfaces (Rajkowski *et al.*, 1994). In a study to evaluate this possibility, sterile salami slices (a_w 0.60 – 0.82) were surface inoculated with log 2 - 3 cfu.g^{-1} of *S. aureus*, interfaced with processed cheese slices, vacuum packed and incubated at 19°, 28° and 37°C. *Staphylococcus aureus* levels and a_w levels were determined weekly for 9 weeks and it was found that growth occurred under all test conditions when samples were stored at 28° and 37°C. At 19°C, *S. aureus* remained viable for the duration of the study. It was, therefore, concluded that to produce these products safely, halotolerant pathogens must be rigorously excluded during the manufacturing process. Alternatively the two components can be packaged in physically separated compartments to prevent moisture transfer (Rajkowski *et al.*, 1994).

The presence of coagulase positive staphylococci in IMFs is an indicator of general processing contamination and demonstrates a potential to cause food

poisoning if large numbers of cells are present, or if the food was treated abusively (Heidelbaugh *et al.*, 1973).

Salmonella

Salmonella food poisoning results from the ingestion of foods containing appropriate strains of this genus and in significant numbers (Jay, 1996). For epidemiological purposes, salmonellae can be placed into three groups. Firstly, those that infect humans only: these include *S. typhimurium, S. paratyphi* A, *S. paratyphi* C. This group also includes the agents of typhoid and paratyphoid fevers, which are the most severe of all diseases caused by salmonellae. The second group of salmonellae includes host-adapted serovars, some of which are human pathogens and may be contracted from foods, including: poultry (*S. gallinarum*), beef (*S. dublin*), mutton (*S. abortus-ovis*) and pork (*S. choleraesuis*). The third group includes unadapted serovars (no host preference). These are pathogenic for humans and other animals and they include most foodborne serovars (Jay, 1996).

The large increase in foodborne salmonellosis which was observed in England and Wales in the 1980's has levelled off to about 30 000 reported cases per year from 1990 - 1995. The most common salmonella involved is *S. enteritides*, followed by *S. typhimurium*. However, whilst the overall incidence of human salmonellosis has apparently become constant, illness caused by *S. typhimurium* DT 104 is gradually increasing: in 1991 there were about 500 reported cases and by 1996 there were 4 006 cases reported (Anon., 2000; Threlfall *et al.*, 1997). *Salmonella typhimurium* DT 104 is an emerging food pathogen and it is important that GMP and HACCP programmes are in place to control the growth of this organism in food factories.

In a study on the survival of *Salmonella typhimurium* DT 104 at low water activity, Mattick *et al.* (2000) found that at a_w values of 0.93 - 0.98, filaments were formed. When preparations were returned to higher a_w conditions, filaments formed septa, and division was complete within approximately 2 - 3 h. It was suggested that if *Salmonella* strains form filaments in food products that have relatively low a_w values (0.82 - 0.98), there may be significant implications for public health (Mattick *et al.*, 2000).

Salmonellae have been isolated from many of the basic ingredients of IMFs, like cacao, spray dried egg and milk powder (Tauxe, 1991). The moisture content of IMFs are such that the multiplication of salmonellae cannot occur, but their resistance to heating is greatly increased and they may persist in dehydrated foods and IMFs for long periods and can subsequently be the cause of outbreaks of food poisoning. The lower a_w growth limit falls between 0.92 and 0.95, depending on the strain and the method of controlling the a_w level (Marshall *et al.*, 1971). When the temperature or the pH is not at optimum, or in the presence of inhibitors such as lactic acids, other volatile fatty acids or NaCl, the limiting a_w for growth may be even higher (Corry, 1976). An excellent example of this is the outbreaks of salmonellosis that occurred in the USA in the late seventies and early eighties due to infected cacao powder (Gill *et al.*, 1983; Tamminga *et al.*, 1976).

Many IMFs usually contain a number of different ingredients such as meat and dried skimmed milk powders, with relatively high concentrations of sugars and polyhols. IMFs usually have an a_w of between 0.80 and 0.85 (Kaplow, 1970) and are intended, as many traditional IMFs, to be consumed without further cooking, so that any salmonellae present will be consumed (Corry, 1976). The problem of salmonellae in IMFs is primarily one of survival and persistence rather than growth, although there is a possibility of multiplication during the drying processes. If the temperature and other factors are favourable, in the early stages of cheese making, during the fermentation of sausages or in the drying of meat or fish, multiplication can and will occur (Corry, 1976).

Contamination sources of *Salmonella* include all substances of animal origin (Jung & Beuchat, 1999) and vegetable and fruit-derived food can also never be assumed to be free of salmonellae (Corry, 1976). Desiccated coconut, copra and cacao beans and powder, and even oil seeds can harbour salmonellae. In these cases contamination occurs because of unhygienic practices during harvesting, processing or storage; usually due to contamination from faeces of humans, farm animals, birds or rodents. The problem is made worse if salmonellae are able to multiply in the food, as can happen in coconuts before drying, or if processing machinery becomes contaminated and continuously inoculate fresh batches of the product (Corry, 1976).

The minimal infectious dose for *Salmonella* is considered to be in the order of 10^7 - 10^9 cells.g^{-1}, but outbreaks may occur where lower cell numbers are present

(Jay, 1996). There is also evidence that when taken on an empty stomach, low numbers of salmonellae can be infective. In addition, it has been reported that the infectious dose of *Salmonella* cells is reduced (e.g., 10 - 100 cells per gram) when the organism is present in low a_w foods (Rowe *et al.*, 1987). Chocolate is frequently consumed between meals, when the stomach is empty and IMF snack foods would probably have a similar consumption pattern with the same devastating effect (Angelotti, 1969; Corry, 1976).

Since salmonellae are relatively heat sensitive organisms, it can be considered that the drying process and heat applied during the drying will be sufficient to eliminate them. Unfortunately this does not appear to be the case. Salmonellae have frequently been isolated from spray-dried milk and dehydrated egg (Collins *et al.*, 1968; Jung *et al.*, 1999) and although spray drying does reduce numbers of salmonellae contamination substantially, it cannot be relied upon to eliminate them completely, even when post-process contamination is prevented (Corry, 1976). It is also significant that Enkiri & Alfred (1971) observed that salmonellae that were more resistant to drying and freezing, were also more frequently the cause of food poisoning.

Many non-conventional IMFs differ from the traditional types of IMFs because they contain high concentrations of non-ionic solutes at neutral pH, in combination with ingredients that can be frequently contaminated with salmonellae. As with dried foods, the salmonellae did not multiply, but there is evidence that they could persist for long periods. They have been shown to survive for over a year at 5°C in concentrated solutions of sucrose (up to 66% w/w) and in solutions of other polyols and in 20% NaCl in nutrient broth for over 70 days at 5°C and for up to 30 days at 20°C (Marshall *et al.*, 1971).

The heat resistance of *Salmonella weltevreden* inoculated into flour (used as a model for dried foods) and heated in hot air was determined for an initial a_w range of 0.20 - 0.60 prior to heating (Archer *et al.*, 1998). The death curves obtained were biphasic, demonstrating an initial rapid decline in the number of survivors (1.0 - 1.5 log reductions) during the first 5 - 10 min of heating for all the temperature/a_w combinations tested. Following the initial rapid decline in the cell number, a linear survivor curve was obtained where inactivation occurred at a slower rate. The initial decline in survivors coincided with a rapid decrease in the a_w of all the samples tested. Data showed that, for any temperature, as the initial a_w of the sample prior to

heating decreased, the heat resistance of the cells increased. Furthermore, the results demonstrated that the amount of available water in foods that are considered to be dry (i.e. with an a_w less than 0.6) will significantly influence the effectiveness of the heat processing of foods and in addition to the temperature, the a_w prior to heating is a critical controlling factor during these processes (Archer *et al.*, 1998).

In conclusion, the drying process cannot alone be relied on to render dried foods free from salmonellae, but heat or radiation pasteurisation, prior to drying can be used effectively to eliminate salmonellae. Salmonellae in high levels of sucrose, have an especially high heat resistance and other sugars and polyols have similar, though lesser effects. It would be difficult to remove salmonellae from high sugar food by heating the final product, and although it may be possible to reduce resistance of contaminating salmonellae by modifying the IMF formula, it would be preferable to ensure that the ingredients are free of salmonellae before mixing (Corry, 1976).

Aerobic and anaerobic endospore formers

The endospore forming genera *Bacillus* and *Clostridium* include important spoilage and food poisoning organisms. These include *C. botulinum*, *C. perfringens* and enterotoxin forming *Bacillus* species such as *B. cereus* (Dufrenne *et al.*, 1994; Jakobsen, 1985). Intermediate moisture foods are of sufficiently low a_w to prevent the growth of aerobic endospore formers, irrespective of storage temperature and pH. *Bacillus* contamination conceivably can occur during formulation and storage prior to a_w reduction. Good hygienic and manufacturing practices, particularly with respect to temperature control, will reduce the likelihood of such contamination (Roberts & Smart, 1976). With respect to IMFs, it is also of importance that bacilli and clostridia show greater tolerance to glycerol, than to sugar and salt when the a_w is lowered (Jakobsen, 1985). It is, therefore, necessary to use good manufacturing practices when glycerol is used as a humectant in IMFs.

Clostridia occur widely in nature, so widely that it is prudent to assume that they are always present. The most important clostridia species are *C. perfringens*, *C. botulinum* and the group comprising *C. sporogenes* and the putrefactive anaerobes (Roberts & Smart, 1976). The a_w requirements for growth of the clostridia causing food poisoning is strain specific. According to studies, types A, B and E of

C. botulinum differ in their growth behaviour, because the lower limits of a_w for growth and toxin production in salt adjusted media are 0.95 for type A, 0.94 for type B and 0.97 for type E (Jakobsen, 1985). The minimum a_w level for growth of *C. perfringens* in NaCl has been reported to be 0.95 (Kang *et al.*, 1969). *Clostridium botulinum* will be present in IMF from time to time and should it grow prior to a_w reduction, it is likely that the toxin will remain in the IMF. Similarly, if the product is seriously abused with respect to a_w and temperature, the clostridia initially present as endospores, will remain viable and capable of rapid growth (Roberts & Smart, 1976).

Bacillus cereus is an aerobic, endospore forming rod normally present in soil, dust and water. This bacterium has a minimum growth temperature of around 4° to 5°C, with a maximum growth between 48° and 50°C. Growth has been demonstrated over a pH range of 4.9 - 9.3 (Goepfert *et al.*, 1972). *Bacillus cereus* is an infectious agent responsible for human diseases such as diarrhoeal and emetic types of food poisoning caused by different toxins (Claus & Berkeley, 1986). Growth of *Bacillus* species can take place at significantly lower a_w values than growth of clostridia. The most osmotolerant species is *B. subtilus* which can grow at an a_w as low as 0.89 (Jakobsen, 1985) and, therefore, can be problematic in IMFs.

Enterobacter sakazakii

Enterobacter sakazakii was designated as an unique species in 1980 (Farmer *et al.*, 1980) and has been implicated in a rare but severe form of neonatal meningitis, associated with dried-infant formula as the mode of transmission (Gallagher & Ball, 1991; Noriega *et al.*, 1990). In survivors of "*Enterobacter sakazakii* meningitis", severe neurological problems can occur, as well as retarded neural development (Nazarowec-White & Faber, 1997).

During the manufacture of dried-infant formula all ingredients, i.e. fat, whey, vitamins, emulsifiers and stabilisers, are added and then blended. This mixture is pasteurised at 110°C for 60 s and then spray dried. However, if the formula is not dry-mixed both the liquid skimmed milk and the premix of skimmed milk and fat components are treated at 80° - 82°C for 20 s. Then the total mixture is heated at 107° - 110°C for 60 s and the liquid mixture is concentrated using a falling-film evaporator. The concentrate is heat treated again at 80°C and finally spray dried.

Although these temperatures are high, post-processing contamination can occur in the plant (Nazarowec-White & Faber, 1997).

It was found that the D-values of *E. sakazakii* ranged from 54.79 min at 52°C to 2.5 min at 60°C (Nazarowec-White & Faber, 1997). The resistance of this organism to high temperatures can be of great importance during the manufacturing of IMFs. Milk powder is a common product used in IMFs and if the product is not subjected to excessive heat treatment or hurdle technology, *E. sakazakii* may survive and spoil the IMF.

H. CONCLUSION

Changes in the South African consumer market have encouraged the development of new and more challenging products. The increasing concern and awareness of the impact of diet on health and disease (Smith, 1993) have also resulted in the development of good quality, more palatable higher moisture dried fruit.

All over the world a trend is developing for dried fruit with a higher moisture content (Renaud, 1997, personnel communication, 1999). The maximum moisture content of conventional dried fruit is 28% and this is in most cases not palatable and soft enough for the sales market, as consumers prefer softer dried fruit. The higher moisture content of the fruit retained after rehydration leads to a higher water activity and the foods are reclassified as high moisture dried fruit (HMDF). The higher water activity and moisture content leads to changes in the spoilage population and an increased spoilage rate.

It is clear that HMDF products can offer an enormous potential advantage in that they can be consumed without preparation and that they do not need refrigeration. The products can be consumed directly as healthy fruit snacks, or in combination with nuts and breakfast cereals. The fruit can also be incorporated into various fabricated foods such as biscuits and cookies (Irwandi *et al.*, 1998). In developing countries, like South Africa, it is also of great significance to produce higher-moisture dried fruit products so as to contribute to the reduction of post-harvest losses and diversification of the fruit industry. To optimise this preservation process, information is needed on the combined effect of stress factors on the

presence and growth of micro-organisms that are important as potential spoilage sources of the final fruit product.

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CHAPTER 3

MICROBIAL CONTENT OF COMMERCIAL HIGH MOISTURE DRIED FRUIT

Abstract

High moisture dried fruits (HMDF) are dried fruit that are rehydrated to a moisture content of 36% and an water activity (a_w) of 0.85. It is this increase in moisture content and a_w that changes the microbial spoilage population and increases the spoilage rate that leads to a much shortened product shelf-life. In this study the microbial population and content of commercially available South African HMDF products were evaluated with the use of seven specific culture media. The moisture content, a_w, SO_2 content and pH of each product was determined. It was found that the high moisture dried (HMD) prunes and raisins had the highest microbial counts. The most frequent spoilers were members of the aerobic endospore formers of the genus *Bacillus*. Yeast and mould (mycelial fungi) counts were also very high with the HMD apricots (1 400 cfu.g^{-1}) exceeding the limit of 1 000 cfu.g^{-1}. Lactobacilli were also found, but the counts were low in all the products evaluated (< 300 cfu.g^{-1}). Members of the genus *Staphylococcus* were found in the HMD raisins and *Salmonella* were found in the HMD prunes (40 cfu.g^{-1}) suggesting possible food safety implications. Thermoduric organisms were also detected in the HMD prunes with a count of 3 300 cfu.g^{-1}. The presence of these thermoduric organisms in the HMDF product indicated that the current pasteurisation process is not adequate and that the addition of preservatives would be needed as an additional method to ensure safe quality HMDF products. The data obtained from the potassium sorbate (K-sorbate) treated and untreated prunes clearly proved that it is extremely important that HMD prunes are treated with K-sorbate to minimise the presence of fungi. The prunes without K-sorbate gave counts as high as 75 000 cfu.g^{-1}, while no fungi were detected in the treated samples.

Introduction

Intermediate moisture dried foods (IMDFs) are products with a water activity (a_w) of between 0.65 and 0.90 and a moisture content of 15 – 50% (w/w) (Leistner, 1985; Robson, 1976). These foods are shelf-stable at ambient temperatures for varying periods of time (Burrows & Barker, 1976). It is this feature that makes the IMFs very convenient products especially for developing countries where cooling and suitable storage facilities are a rarity (Taylor, 1999).

High moisture dried fruit products (HMDF) are foods with a higher a_w (0.85) and softer texture that are becoming popular all over the world (R. Renaud, 1997, IRNA, personal communication), but they still fall in the IMF product range (a_w = 0.60 - 0.90). These HMDF products are manufactured by rehydrating dried fruit to a moisture content of about 36% (w/w) and an a_w of 0.85 (J. Schoeman, 1999, SAD, Wellington, personal communication). With this increase in moisture content, the microbiological spoilage rate of the fruit may also increase dramatically leading to a much shortened shelf-life.

In the specific a_w range of the IMF products, fungi have been reported to be the most common spoilage organisms (El-Halouat *et al.*, 1998). Bacteria, in contrast, are normally not a problem as they usually do not grow in the IMF a_w range, but there are a few bacterial exceptions that can grow at these low a_w values. These include the lactic acid bacteria, the aerobic and anaerobic endospore formers (Leistner & Rödel, 1976), *Staphylococcus aureus* (Boylan *et al.*, 1976) and halophilic bacteria (a_w = 0.75). *Staphylococcus aureus* can begin to grow at a_w values of 0.83 to 0.85 and can even produce enterotoxin at an a_w as low as 0.86 (Harvey & Gilmour, 2000; Lancette & Tatini, 1996). The growth and metabolic activity of this bacterium also depends on how ideal the conditions are, making the choice of process conditions during the production of IMFs extremely important (Banwart, 1989). Conventional dried fruits, which generally have a lower a_w (*ca* 0.70), are normally resistant to spoilage by these bacteria.

As early as 1968, Pitt & Christian reported that yeasts and moulds (mycelial fungi) are the main causes of HMD prune spoilage. They found that the yeast *Zygosaccharomyces rouxii* is a common spoiler, but that *Eurotium* spp., *Aspergillus glaucus* and *A. niger*, as well as *Pencillium chrysogenum*, are common spoilage fungi in HMD prunes. The most frequently isolated yeast strains from

IMFs belong to the genus *Zygosaccharomyces* (Restaino *et al.*, 1983; Tapia De Daza *et al.*, 1995). High moisture papaya products (Tapia De Daza *et al.*, 1995) and glazed cherries (Tilbury, 1976) were reported to be specifically spoiled by *Zygosaccharomyces rouxii*. In contrast, HMD apricots are most frequently spoiled by *Aspergillus repens* (Harel *et al.*, 1978).

The aim of this study was to determine the microbial population and content of HMDF commercially available in South Africa.

Materials and methods

Fruit

Five types of commercial HMDF samples (250 g per laminated aluminium foil stand-up pouch), obtained from SAD (C. Du Toit, 1999, SAD, Worcester, personal communication) were examined and the microbial population determined. These products included: raisins, apricots, nectarines, cling stone peaches and prunes. Three production batches of each fruit type were analysed, one batch packed during July 1998 (A), one batch packed during February 1999 (B) and the third batch packed during March 1999 (C). The microbial content of batches A, B and C was determined after 8, 1 and 1 month after packaging, respectively.

Two pouches each of cling stone peaches and nectarines that had reached the expiry date, were purchased from local supermarkets and were also examined (D).

Twenty samples (250 g pouches) of untreated prunes and 20 samples (250 g pouches) of prunes that had been treated with potassium sorbate (48 $g.l^{-1}$) were received from SAD (S. Eksteen, SAD, 1999, personal communication) and were microbiologically examined on months 0 and 6. Ten samples of each treatment were evaluated at each time interval.

Microbial analyses

A 10 g sample from each HMDF product was aseptically removed and suspended in 90 ml saline solution (0.85% (w/v) NaCl) and macerated in a stomacher (BagMixer, Interscience, France). A dilution series of each sample was prepared from 10^{-1} to 10^{-6} and these were plated in triplicate into each specific medium. The nine media used in this study are listed in Table 1.

Table 1. Selective media used to evaluate the microbial content of commercial HMDF.

Medium*	Selective organisms	Incubation conditions
PCA	Viable aerobic count	37°C, 24 d
MRS	Lactic acid bacteria	30°C, 72 d
VRBA	Enterobacteriaceae	37°C, 24 h
CA	Yeasts and moulds	25°C, 5 d
PCA(°C)	Thermoduric organisms	37°C, 24 h
RCA	Clostridia	37°C, 24 h
BP	*Staphylococcus*	37°C, 48 h
SS	*Salmonella* and *Shigella*	37°C, 24 h
BG	*Salmonella*	37°C, 48 h

*Media: PCA = Plate Count Agar (Biolab)
MRS = De Man Rogosa and Sharpe Agar (Biolab)
VRBA = Violet Red Bile Agar (Biolab)
CA = Chloramphenicol Agar (Biolab)
PCA(°C) = sample heated for 10 min at 80°C (Biolab)
RCA = Reinforced Clostridial Agar (Biolab)
BP = Baird Parker Agar (Biolab)
SS = Salmonella Shigella Agar (Biolab)
BG = Brilliant Green Agar (Biolab)

Water activity, moisture content and pH determinations

The a_w levels of the samples were measured at 25°C with a hygrometer (Thermoconstanter Humidat TH-2, Novasina Defensor, EVc-26 filter). The fruit were macerated in an electric mill (Bizerba, Columbit, Freddy Hirsch & Company (Pty) Ltd., Cape Town), placed in plastic sample cups, placed in the cell of the hygrometer, and when equilibrium had been reached, the measurements were taken.

The pH of each sample was determined using a Knick pH meter (pHB-4, Merck) according to the AOAC (1990) method.

Moisture analysis was done according to the AOAC (1990) method. The fruit were macerated using a hand mill (Bizerba, Freddy Hirsch & Company (Pty) Ltd., SA), and placed in a pre-dried nickel moisture dish and weighed. The moisture dishes were placed in a preheated vacuum oven at 70°C for 16 h under constant vacuum, where after they were allowed to cool in a desiccator, before weighing and calculation of the moisture content (% w/w).

Potassium sorbate analysis

Potassium sorbate (K-sorbate) was analysed spectrophotometrically (Spectronic 20, Genesys, Spectronic Instruments, USA) at 530 nm according to the SAD standard method (C. Du Toit, 1999, SAD, Worcester, personal communication) (AOAC, 1984). A 2-thiobarbituric acid (TBA) (BDH laboratory reagents) solution was prepared by mixing 0.5 g TBA with 10 ml of a 1N NaOH solution in a 100 ml volumetric flask. Distilled water (20 ml) was added followed by 11 ml of a 1N HCl solution and made up to 100 ml with distilled water. A 0.01N $K_2Cr_2O_7$ solution was prepared and mixed with a 0.3N H_2SO_4 solution. A standard potassium sorbate solution 0.25 g K-sorbate (Saarchem) dissolved in 250 ml distilled water was prepared.

A standard curve was constructed as follows: untreated prunes were macerated and 50 g was weighed into a food processor, 450 ml distilled water added and mixed for 5 min. Of this mixture, 10 g samples were placed in five volumetric flasks. The first flask contained no K-sorbate, the second flask 0.25 ml, the third 0.5 ml, the fourth 0.75 ml and the fifth flask, 1 ml of the K-sorbate solution. Each flask was filled with distilled water to the mark. The five solutions were filtered (Whatman no 1 filter paper) and 4 ml of each filtrate removed and together with 2 ml of the $K_2Cr_2O_7$-H_2SO_4 solution, added to a set of test tubes. The solutions were vortexed and placed in a waterbath at 100°C and allowed to boil for 5 min. The tubes were removed and cooled under running tap water. Two ml of the TBA solution was added to each tube and the mixture vortexed. The tubes were again vortexed and boiled in the waterbath for 10 min at 100°C. The mixture was then cooled and the absorbence read at 530 nm.

The K-sorbate determination of the HMD prunes was done as follows: the treated prunes were macerated and a 20 g sample was weighed into the food processor, 180 ml distilled water was added and the solution was mixed for 5 min. Ten gram of this solution was weighed into a 250 ml volumetric flask and filled with distilled water to the mark. The mixture was filtered and treated as described for the standard curve. The absorbence values of the treated prunes were calculated from the standard curve.

Results and discussion

Microbial content of the commercial HMDF products

The microbial content of the four batches (A, B, C and D) of the five commercial high moisture dried fruit samples and the expired samples is summarised in Table 2, while the moisture content, a_w, pH and the SO_2 concentration of the samples are given in Table 3.

Aerobic bacteria, yeasts and moulds were found in almost all the products examined, but the counts (Table 2) were fairly low, with the exception of the raisins and the prunes, where high counts were detected on PCA. Prunes had the second highest aerobic count of 830 cfu.g^{-1}. This can be explained by the fact that prunes have a higher pH of 4.1, in comparison with the rest of the products with pH values below 4.0. Furthermore, prunes are generally not treated with SO_2. The PCA(°C) method, that indicates specifically the presence of thermoduric organisms, also showed high levels of contamination (860 cfu.g^{-1}) on the prunes. Microscopic examination of the colonies from the PCA and PCA(°C) plates indicated that the majority of these organisms were members of the aerobic endospore forming genus *Bacillus*. Since these bacteria are heat resistant it can be concluded from their presence, even after the commercial heat treatment of 7 h at 80°C (J. Schoeman, 1999, SAD, Wellington, personal communication), that the process was not as effective as expected. It must also be taken into consideration that once the sealed package has been opened the aerobic numbers will start increasing as the organisms start to grow. If the contents are consumed within a few hours, the aerobic bacteria are of no concern, but if there is a small leak in the packaging they would be responsible for product spoilage.

The data (Table 2) indicated that in most cases the aerobic counts decreased with time as can be seen when comparing the A batches (packed in July 98), the B batches (packed in February 1999) and the C batches (packed in March 1999). However, there was an increase in the case of the nectarines, but since, in this case, they were not from the same original batch, it is difficult to reach a definite conclusion.

In the case of the raisins, the moisture content and the a_w were the lowest and the SO_2 content the highest of all the samples examined (Table 3). Despite these environmental hurdles, the raisins showed the highest aerobic counts (1 800

Table 2. Microbial examination of different batches of commercial HMDF (cfu.g^{-1}) (average of triplicates).

Medium*	Raisins			Cling stone peaches				Apricots			Nectarines				Prunes		
	Batches**			Batches				Batches			Batches				Batches		
	A	B	C	A	B	C	D	A	B	C	A	B	C	D	A	B	C
PCA	80	1800	110	40	50	60	40	90	180	90	180	20	110	100	60	830	510
MRS	300	0	20	10	50	40	0	0	100	30	10	30	20	0	0	90	60
VRBA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CA	150	200	170	30	40	50	20	1440	820	130	40	50	70	30	0	10	20
RCA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3300	0
BPA	20	10	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PCA(°C)	10	0	0	0	30	0	0	0	0	0	0	0	0	0	10	860	0
SS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BGA	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	40	0

*Medium: PCA = Plate Count Agar, for viable aerobic count; MRS = De Man, Rogosa and Sharpe Agar, for Lactobacilli; VRBA = Violet Red Bile Agar, for Enterobacteriaceae; CA = Chloramphenicol Agar, for yeasts and moulds; RCA = Reinforced Clostridial Agar, for Clostridia; BPA = Baird Parker Agar, for Staphylococci; PCA(°C) = sample heated for 10 min at 80°C, for thermoduric organisms; SS = Salmonella Shigella Agar, for *Salmonella* and *Shigella* spp.; BGA = Brilliant Green Agar, for *Salmonella*.

**Batches (date packaged): A = packed July 98; B = packed February 99; C = packed March 99; D = expired at testing date

Table 3. Moisture content, a_w, SO_2 concentrations and pH of commercially available high moisture dried fruit.

Fruit type	Moisture content (% w/w)				a_w				SO_2 (mg.kg^{-1})				pH			
	Batches*				Batches				Batches				Batches			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Raisins	22.5	23.7	24.5	-	0.71	0.72	0.73	-	1 369	1 236	1 300	-	3.90	3.80	3.86	-
Cling stone Peaches	34.6	32.3	34.2	32.6	0.83	0.82	0.83	0.82	1 213	419	980	850	3.80	3.90	3.78	3.69
Apricots	35.5	36.9	35.6	-	0.83	0.85	0.84	-	1 054	1 501	1 400	-	3.65	3.52	3.60	-
Nectarines	34.8	32.1	35.2	33.4	0.83	0.81	0.86	0.84	249	1 024	1 040	910	3.75	3.88	3.74	3.52
Prunes	35.2	33.7	34.8	-	0.83	0.81	0.84	-	ND	ND	ND	-	3.90	4.01	4.10	-

*Batches (date packaged): A = packed July 98; B = packed February 99; C = packed March 99; D = expired at testing date

ND = not detected

cfu.g^{-1}), as well as the highest lactobacilli numbers (300 cfu.g^{-1}). A possible explanation for these high counts is that two types of raisins (Table 2) are used in the product, sulphured Sultanas and unsulphured Thompsons (C. Du Toit, 1999, SAD, Worcester, personal communication). The unsulphured raisins may have been the cause of the higher levels of contamination. If the final counts in the samples (Table 2) are taken into account, the original contamination, despite the pasteurisation step normally applied during processing and the presence of high concentrations of SO_2, must have been very high.

Members of the anaerobic endospore formers were only detected in one of the prune samples (batch B) and at a fairly high contamination level of 3 300 cfu.g^{-1}. Colonies were microscopically confirmed as endospore formers. The presence of anaerobic endospore forming members of the genus *Clostridium*, and at such a high contamination level, was not expected. This could be an indication of unhygienic conditions during processing, drying or possibly an intermittent contamination source.

Cling stone peaches and nectarines were found to have the lowest contamination levels, while the yeast and mould counts of the apricots were the highest of all the fruit products examined, with 1 440 and 820 cfu.g^{-1}, respectively for batch A and B (Table 2). According to recommended specifications for the dried fruit industry (Table 4), the yeast and mould counts of sample A exceeded the maximum permitted of 1 000 cfu.g^{-1}.

The data (Table 2) clearly showed that no members of the Enterobacteriaceae, coliforms or *Escherichia coli* were present in any of the samples examined. Similarly, no presence of *Salmonella* was found when using the SS-medium. However, when using the BGA-medium for *Salmonella* detection, positive counts were obtained for the raisins and prune samples. The presence of *Salmonella* in these two HMDF products can have serious health implications and their presence can impact the dried fruit industry very negatively with severe economic implications. Even though the numbers were low (10 - 40 cfu.g^{-1}), these were positively confirmed as members of the genus *Salmonella* with the API-system (API 20 E, bioMérieux, France).

Members of the genus *Staphylococcus* were only found in the raisin samples and again they were present in numbers high enough to suggest health implications. Sample A (20 cfu.g^{-1}) exceeded the maximum of 10 cfu.g^{-1} permitted

Table 4. Microbial standards applied by SAD (S. Eksteen, SAD, Wellington, 1999, personal communication).

Determination	Culture medium	Incubation temperature, time	Colonies per plate
Viable aerobic count	PCA	30°C, 3 d	30 - 300
Yeast and mould	CA	25°C, 5 d	30 - 300
Coliforms	VRBA	37°C, 24 h	0 - 300
E. coli	Brilliant Green Bile broth	37°C, 48 h 44°C, 48 h (if positive)	30 - 300
S. aureus	Baird Parker confirm: Mannitol phenolase & DNase test	37°C,48 h 37°C, 24 h	30 - 300
Salmonella	TBB medium SS Agar API 20E	43°C, 48 h 37°C, 18h	30 - 300
Streptococci	KF Streptococcus	37°C, 48 h	30 - 300
Lactobacilli	MRS	37°C, 3 d	30 - 300

Method	n	C	m (g^{-1})	M (g^{-1})
Aerobic plate count	5	2	1 000	10 000
Yeast and mould	5	2	200	1 000
Coliforms	5	2	1	10
*E. coli**	5	0	0	0
*S. aureus**	5	2	0	10
*Salmonella**	5	0	0 per 25 g	0 per 25 g

* = Determined only on request
n = number of samples
m = preferred maximum
M = maximum permitted
C = number of samples > m but < M

(Table 4), although the rest of the raisin samples were lower than 20 cfu.g^{-1}. Even though the permissible numbers are within the limits for high moisture dried fruit (Table 4), their presence and the presence of *Salmonella* could, especially if known to the general public, impact the sales of the products as a whole very negatively.

Expired HMDF

The counts of the expired fruit (batches D) (Table 2) still met the microbiological standards (Table 4) and there were only counts on the PCA and CA media. The viable counts (PCA) were the highest for nectarines with 100 cfu.g^{-1}, while the counts of the cling stone peaches were only 40 cfu.g^{-1}. The colonies were also microscopically examined and found to be members of the endospore forming genus *Bacillus*. The yeast and mould counts in these expired products were found to be low, between 20 and 30 cfu.g^{-1}. The moisture content and a_w values (Table 3) of the fruit did not differ drastically from the non-expired samples. The SO_2 content of the fruit was also generally lower than values obtained from the non-expired fruit. However, the fruit were found to be extensively discoloured.

Microbial content of the potassium sorbate treated and untreated prunes

The results of the microbial content of the prunes that had been treated with potassium sorbate and the untreated samples are given in Table 5. The data obtained showed that the viable aerobic numbers (on PCA) did not differ greatly for the treated prunes at month 0 (1 100 cfu.g^{-1}) and untreated prunes (1 300 cfu.g^{-1}). At month 6 the PCA counts of the treated and untreated prunes differed drastically. The untreated prunes had counts of 7 100 cfu.g^{-1}, while the treated prunes had counts of 500 cfu.g^{-1}. Colonies from the PCA plates were also examined microscopically and the organisms, in most cases, were found to be aerobic endospore forming members of the genus *Bacillus*. This data suggests that sorbate does influence the growth and survival of aerobic bacteria and if the sorbate concentration is decreased, aerobic bacterial numbers will increase and negatively impact the shelf-life of HMD prunes.

The numbers of the lactic acid bacteria (on MRS) were fairly low, but there was a difference between the treated and untreated prunes for month 0,

Table 5. Microbial content of HMD prunes treated with and without potassium sorbate (average of triplicates).

Medium*	Prunes treated						Prunes untreated					
	Time 0 months			Time 6 months			Time 0 months			Time 6 months		
	cfu.g^{-1}	SD	range	cfu.g^{-1}	SD	range	cfu.g^{-1}	SD	range	cfu.g^{-1}	SD	range
PCA	1 100	309.3	500 - 1 500	500	149.9	210 - 700	1 300	384.2	900 - 2 500	7 100	594.2	2 300 - 7 500
VRBA	0	-	-	0	-	-	0	-	-	0	-	-
MRS	10	9.1	0 - 30	10	6.1	0 - 20	120	22.0	8 0 -1 50	30	11.0	10 - 4 0
CA	0	-	-	0	-	-	75 000	850.2	35 000 - 80 000	110	16.3	80 - 130

*Media PCA = Plate Count Agar, for viable aerobic count;
MRS = Lactobacilli, for lactic acid bacteria;
VRBA = Violet Red Bile Agar, for Enterobacteriaceae;
CA = Chloramphenicol Agar, for yeasts and moulds;

respectively 10 cfu.g^{-1} and 120 cfu.g^{-1}. At month 6 the counts of the untreated prunes decreased to as low as 30 cfu.g^{-1}, while the counts of the treated samples were comparable.

No colonies were found on the VRBA plates for both of the experimental periods. This is a good indicator that the fruit was not spoiled by faecal contamination.

The biggest difference between treated and untreated prunes was found when using the CA-medium that is specifically selective for yeasts and moulds. No colonies were found for the treated samples for both time periods, while the counts on the untreated samples were as high as 75 000 cfu.g^{-1} at month 0. The data also showed that the counts on the untreated samples decreased after 6 months to 110 cfu.g^{-1}. This huge difference between the treated and untreated samples clearly demonstrates that K-sorbate is an effective preservative to prevent yeast and mould spoilage in HMD prunes.

The data from the treated HMD prunes (Table 5) can be compared with the data of the commercial HMD prunes given in Table 2 which had also been treated with K-sorbate. In both cases the HMD prunes had fairly similar viable bacterial counts (on PCA) and lactic acid bacterial counts (on MRS). No moulds or yeasts were detected on the CA-medium for the treated prunes, while the commercial prunes showed low counts (20 cfu.g^{-1}) (Table 2). For both HMD prune samples, no counts were detected on VRBA.

Water activity

The a_w of the commercial HMDF samples examined (Table 2), were in the range of 0.81 - 0.86. The exception was that of the three raisin samples that showed a lower a_w of between 0.71 - 0.73. These a_w values are all still in the ideal range for the growth of many yeasts and moulds (El-Halouat *et al.*, 1998) and if their numbers are high enough, the shelf-life of the product may be limited by the growth and metabolic activity of these microbes.

SO_2 content

The SO_2 content was found to vary from 249 to 1 501 mg.kg^{-1} for the HMDF products examined (Table 3), which was much lower than the permitted level of 2 000 mg.kg^{-1} for dried fruit (Anon., 1972). The data also showed the wide

variation in the SO_2 content between the different batches (A, B, C and D) of the same product for four of the HMDF products examined, either suggesting loss during storage, or lack of control during the addition of the SO_2 during processing.

Moisture, pH and K-sorbate

The average moisture content and pH of the fruit that had been treated with sorbate when sampled (n = 20), was found to be 35.3% and 3.99, respectively. For the prunes that had not undergone the sorbate treatment, the values were 35.2% and 4.01, respectively. The K-sorbate content, determined according to the SAD method, is given in Table 6. The data obtained showed that the K-sorbate level was slightly higher at 380 $mg.kg^{-1}$ at month 0, whilst at month 6 it was 350 $mg.kg^{-1}$. These values were well below the maximum permitted level of 600 $mg.kg^{-1}$ for addition of K-sorbate to dried fruit (Anon., 1972).

Conclusions

In this study the microbial composition of commercial HMDF products was determined using seven different media types. The data obtained showed that in most cases the microbial levels were within the limits set by the SAD (S. Eksteen, 1999, SAD, Wellington, personal communication), but in the case of apricots (batch A), the fungal counts exceeded the maximum permitted level of 1 000 $cfu.g^{-1}$. However, more worrisome was the presence of members of the genera *Salmonella*, *Staphylococcus* and *Clostridium* in some of the raisin and prune samples examined.

From the data it is also clear that the correct media must be used for enumeration of the specific microbes, as seen in the case of *Salmonella* detection, where two types of media were used. No counts were found on the SS-agar, while on the BG-agar the presence of *Salmonella* was detected.

The data obtained when comparing K-sorbate treated and untreated HMD prunes clearly indicated that the inclusion of K-sorbate as part of the processing of prunes did successfully contribute to the exclusion of yeasts and moulds from the product. However, the data did show that the sorbate had little or no influence on

Table 6. Potassium sorbate content of the treated HMD prunes at the different evaluation times.

K-sorbate ($mg.kg^{-1}$)	K-sorbate ($mg.kg^{-1}$)
Month 0	Month 6
380	350

the aerobic organisms and probably only a low inhibition towards the lactic acid bacteria.

The use of a wider range of microbial media also indicated the presence of other contaminating microbes including thermoduric and anaerobic endospore formers that could also survive during the processing conditions and could contribute not only to product spoilage, but also possibly, under the correct conditions, could have food safety implications. The data clearly indicated that there is scope for improvement in the processing technology and the possibility for the addition of alternative preservatives so as to eliminate the presence of both spoilage and pathogenic organisms and to produce a quality product that is microbiologically stable.

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CHAPTER 4

CHALLENGING OF HIGH MOISTURE DRIED NECTARINES AND PRUNES WITH INDICATOR ORGANISMS

Abstract

High moisture dried fruit (HMDF) is conventional dried fruit that is rehydrated to a moisture content of *ca* 36% and a water activity (a_w) of *ca* 0.85. The aim of this study was to challenge high moisture dried nectarines and prunes with four indicator organisms (*Bacillus cereus*, *Escherichia coli*, *Zygosaccharomyces rouxii* and *Penicillium chrysogenum*) over a storage period of six months. The HMDF were inoculated with 10^4 cells per gram product and pasteurised using the standard SAD method and then stored at either 5° or 25°C. The results showed higher bacterial counts for the fruit stored at 25°C, as well as extensive browning of the nectarines at the higher storage temperature due to the production of Maillard reaction by-products. Furthermore, it was found that the pasteurisation method (7 h at 80°C) was not fully effective in eliminating the endospore formers (*B. cereus)* which may have serious spoilage implications. *Penicillium chrysogenum* and *Zygosaccharomyces rouxii* counts were found to decrease favourably during the storage period of 6 months, although *P. chrysogenum* showed a slight increase after 3 months of storage in nectarines, probably due to the germination of spores. The *Escherichia coli* strain did not survive in either of the HMDF products and no counts were found even before pasteurisation. It was concluded that the environmental conditions (pH and a_w) of the fruit were too unfavourable for the organism to survive. From the results it was clear that the current pasteurisation method of HMDF products is not fully effective in terms of the prevention of spoilage, and that storage of the product at lower temperatures would lead to a better quality product.

Introduction

The increase in the popularity of high moisture dried fruit (HMDF) is a world-wide trend (R. Renaud, 1999, INRA, personal communication) as consumers favour these products because they are softer and easier to enjoy. During the production of HMDF, the dried fruit are rehydrated to a moisture content of *ca* 36% and a

water activity (a_w) of 0.85. The increase in the moisture content of the HMDF product results in a more favourable environment and subsequently a change in the dominant microbial population present on the fruit and thus microbial spoilage can occur more easily in the final product.

An increase in the water activity (a_w) of the product will direct have a impact on the growth, physiology and metabolism of all the microbes present and this may also lead to a higher resistance to inimical agents (Liu *et al*., 1998). The choice of processing conditions during the production of these types of products is extremely important in order to eliminate or minimise microbial growth and activity (Banwart, 1989). It is a known fact that there is a correlation between a_w and microbial growth. The higher the a_w, the faster the microbial growth will be within certain limits (Hanna *et al*., 1982). It has also been found that the presence of specific nutrients increase the a_w range in which the organism can grow or survive (Hanna *et al*., 1982). This increased resistance to environmental conditions, especially during processing, leads to an enhancement of the microbial activity and results in a much shortened product shelf-life, with subsequent economic losses. Therefore, it is extremely important to be familiar with the survival rate of different microbes that could play a potential spoilage role in HMDF products during the processing and storage of these products.

The aim of this study was to determine the survival rate of four selected indicator organisms (*B. cereus*, *E. coli*, *Z. rouxii* and *P. chrysogenum*) after the conventional processing (7 h at 80°C) of HMD prunes and nectarines, over a six-month storage period.

Materials and methods

Indicator organisms and culture preservation

Four organisms were chosen as indicator organisms (*Bacillus cereus* - DSM 31; *Escherichia coli* - ATCC 11775; *Zygosaccharomyces rouxii* - NRRL Y-998; and *Penicillium chrysogenum* - PPRI 4516) obtained from the University of Stellenbosch, Department of Food Science, Culture Collection. *Bacillus cereus* was chosen as representative of the aerobic heat stable endospore formers. The *E. coli* strain was chosen as indicator of the presence of members of the Enterobacteriaceae and to determine whether organisms originating from faecal

contamination would survive the HMDF processing. The osmotolerant yeast *Zygosaccharomyces rouxii* and the mycelial fungus *Penicillium chrysogenum* were chosen as potential spoilage fungi of HMDF products.

The *B. cereus* strain was cultivated at 37°C for 48 h in Nutrient Broth (NB) (Biolab) with 2% D-glucose (Merck) added. The *E. coli* strain was cultivated in MacConkey Broth (Merck) at 35°C for 48 h. *Zygosaccharomyces rouxii* was cultivated in Yeast Extract Peptone Glucose medium (YEPG), which contained 1% (w/w) yeast extract (Merck), 2% (w/w) peptone (Merck), 2% (w/w) D-glucose (Merck) and incubated at 30°C for 72 h. The *P. chrysogenum* strain was cultivated on Malt Extract Agar (MEA) (Merck) and incubated at 25°C for 5 days. Examples of typical colonies of the indicator organisms are shown in Photo 1. The pH of each medium was adjusted as recommended by the manufacturers, and the media were autoclaved for 15 min at 121°C and 100 kPa. To confirm the purity and morphology of the strains, microscopical examinations were done on the four organisms after incubation.

Growth studies

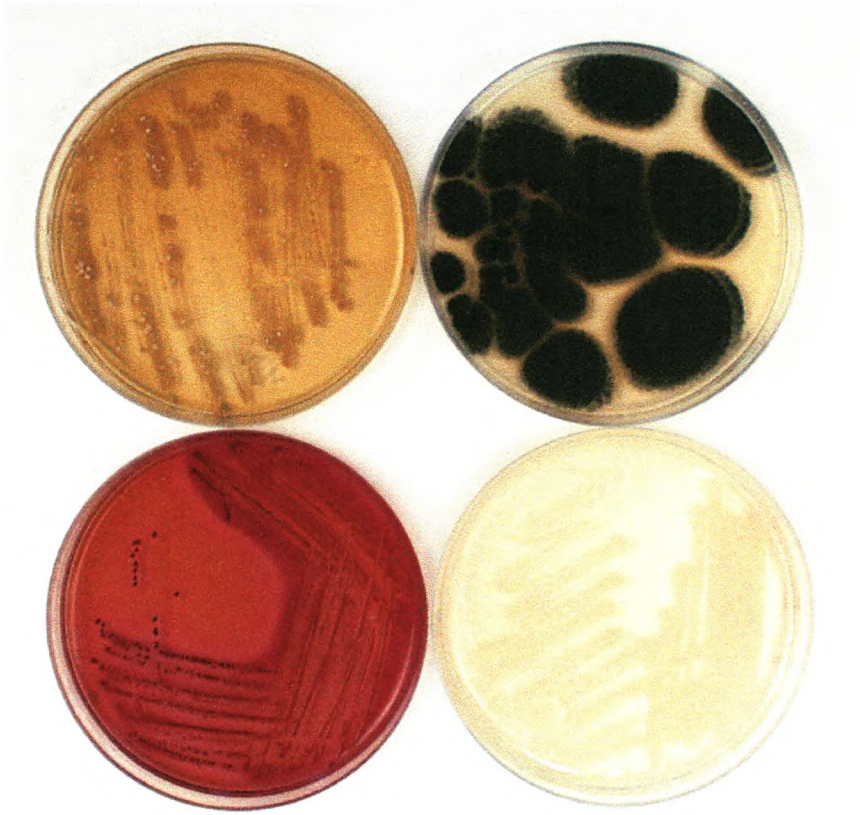
Growth profiles were determined for the *B. cereus, E. coli* and *Z. rouxii* strains using a spectrophotometer (Spectronic 20, Genesys, Spectronic Instruments, USA) at 540 nm. These profiles were also used to determine the inoculation dose of 10^4 cells per gram per 250 g fruit. The cell concentration for *P. chrysogenum* was determined using a Neubauer impaired bright light counting chamber (Neubauer D/Ruling, GSUD 105/04).

Fruit and fruit preparation

Choice grade dried nectarines and prunes were obtained from SAD, Worcester (C. Du Toit, 1999, SAD, Worcester, personal communication). The dried fruit had an initial moisture content of 18 - 19% (w/w). The nectarines were washed in tap water at 40°C for 90 sec in a steam jacketed stainless steel heating vessel and allowed to equilibrate overnight in airtight plastic containers at 0°C. The moisture content was again determined and the amount of moisture required to obtain a final moisture content of 36% (w/w), calculated.

The prunes were blanched for 13 - 14 min at 90° - 100°C in a steam tunnel and sprayed with potassium sorbate (48 $g.l^{-1}$) (Saarchem, Unilab,

Zygosaccharomyces rouxii



Penicillium chrysogenum

Escherichia coli

Bacillus cereus

Photo 1. Indicator organisms used in the challenge study.

Cape Town). The prunes were then oiled using 35 ml oil (DURKEX N.500) per 10 kg prunes. The moisture content of the prunes was then determined and the amount of moisture required to obtain a final moisture content of 36% (w/w), calculated.

Sample preparation

Samples (250 g) of each fruit type were weighed and placed in laminated aluminium foil stand-up pouches purchased from SAD. The prune and nectarine containing pouches were inoculated with one of the four indicator organisms, at a cell concentration of 10^4 cells.g^{-1} fruit. Control pouches that did not contain any indicator organisms were also included. The pouches were sealed and pasteurised at 80°C for 7 hours in a drying tunnel at ARC Nietvoorbij as recommended by SAD (J. Schoeman, 1999, SAD, Wellington, personal communication).

Product storage

The inoculated and control samples were stored at two different temperatures, 5°C and 25°C. Samples (10 g) were aseptically removed before (t_0) and after pasteurisation (t_0^1), and after 1, 3 and 6 months of storage, the microbial composition, pH and a_w of each sample was determined.

Enumeration

A duplicate 10 g sample was aseptically removed from each pouch, weighed and homogenised in 90 ml physiological saline solution (0.85% NaCl), using a stomacher (BagMixer, Interscience, France). For the pouches inoculated with *E. coli*, Violet Red Bile Agar (VRBA) (Merck) was used as enumeration media; for *B. cereus*, Plate Count Agar (PCA) (Merck); for *Z. rouxii*, Chloramphenicol Agar (CA) (Merck); and for *P. chrysogenum*, Malt Extract Agar (MEA) (Biolab). All the control samples were enumerated using PCA, VRBA, MEA and CA media.

Water activity measurements and moisture content

The water activity (a_w) levels of the fruit were measured at 25°C using a hygrometer (Thermoconstanter Humidat TH-2 Novasina Defensor, EVc-26 filter)

as described in Chapter 3 of this thesis. Moisture analysis was done according to the standard AOAC (1990) method.

Results and discussion

In this study two HMDF products, prunes and nectarines, were examined. These products were chosen, due to the fact that nectarines are especially prone to Maillard browning during heat processing and extended storage (J. Schoeman, 1999, SAD, Wellington, personal communication). The inclusion of nectarines would be an excellent indicator of whether the heat treatment used during the processing step is sufficient to eliminate the chosen indicator organisms, without enhancing the browning of the HMDF. Prunes, in contrast, were chosen as they generally have a higher microbial content (as was found in Chapter 3 of this thesis). In this case sulphur dioxide is not used, because prunes are already dark coloured and browning is not as important as in the case of nectarines. Prunes also have a higher product pH that would favour microbial survival and growth.

pH and a_w profiles

The pH values of the nectarines (Table 1) were found to be lower than those of the prunes (Table 2). The pH values for the nectarine samples was found to be in a range between 3.35 and 3.63, while the pH range for prunes was between 3.45 and 4.08. In general, the pH values of the fruit stored at 5°C were higher than those stored at 25°C, especially during the latter stages of the study. This can be explained in terms of more acidic by-products being formed either during the Maillard reaction (Eichner *et al.*, 1990) or as part of the microbial fermentations, which may have been enhanced by the higher storage temperature.

The a_w of the nectarines was found to vary between 0.85 and 0.87 as the experiment progressed (Table 3). The a_w values were usually found to be slightly higher after pasteurisation had been applied, and it was notable that less free water was retained in the pouch. This may be explained by the fact that the moisture was absorbed better by the fruit during pasteurisation.

Table 1. Average pH values of HMD-nectarines challenged with different indicator organisms during a 6-month storage period at either 5° or 25°C (duplicate values).

Indicator Organisms	Time (m)							
	t_0	t_0^1	1		3		6	
			5°C	25°C	5°C	25°C	5°C	25°C
B. cereus	3.63	3.58	3.53	3.61	3.49	3.48	3.44	3.39
E. coli	3.48	3.50	3.54	3.48	3.51	3.39	3.45	3.35
Z. rouxii	3.52	3.52	3.58	3.52	3.48	3.42	3.43	3.38
P. chrysogenum	3.51	3.49	3.50	3.53	3.49	3.47	3.46	3.46
Control	3.53	3.55	3.53	3.50	3.51	3.48	3.51	3.47

t_0 = pH-value before pasteurisation
t_0^1 = pH-value directly after pasteurisation

Table 2. Average pH values of HMD-prunes challenged with different indicator organisms during a 6-month storage period at either 5° or 25°C (duplicate values).

Indicator organisms	Time (m)							
	t_0	t_0^1	1		3		6	
			5°C	25°C	5°C	25°C	5°C	25°C
B. cereus	4.06	3.79	3.78	3.79	3.75	3.74	3.45	3.57
E. coli	4.08	4.03	4.03	4.04	4.02	4.03	3.97	3.92
Z. rouxii	4.04	4.03	4.01	4.0	4.01	4.0	3.85	3.85
P. chrysogenum	4.08	4.06	4.05	4.04	4.03	4.03	4.12	3.55
Control	4.01	4.02	4.01	4.02	4.01	4.01	3.79	3.73

t_0 = pH-value before pasteurisation
t_0^1 = pH-value directly after pasteurisation

Table 3. Average water activity values of HMD-nectarines during the 6-month storage period at either 5° or 25°C (duplicate values).

Indicator Organisms	Time (m)							
	t_0	t_0^1	1		3		6	
			5°C	25°C	5°C	25°C	5°C	25°C
B. cereus	0.85	0.86	0.86	0.86	0.86	0.87	0.86	0.87
E. coli	0.86	0.87	0.87	0.86	0.86	0.87	0.86	0.86
Z. rouxii	0.85	0.85	0.85	0.86	0.86	0.86	0.86	0.85
P. chrysogenum	0.86	0.86	0.85	0.85	0.85	0.86	0.86	0.86
Control	0.85	0.85	0.85	0.86	0.86	0.86	0.86	0.86

t_0 = a_w value before pasteurisation
t_0^1 = a_w value directly after pasteurisation

Table 4. Average water activity values of HMD-prunes during the 6-month storage period at either 5° or 25°C (duplicate values).

Indicator Organisms	Time (m)							
	t_0	t_0^1	1		3		6	
			5°C	25°C	5°C	25°C	5°C	25°C
B. cereus	0.85	0.86	0.85	0.86	0.86	0.86	0.86	0.86
E. coli	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85
Z. rouxii	0.85	0.86	0.86	0.86	0.86	0.86	0.86	0.86
P. chrysogenum	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85
Control	0.85	0.86	0.86	0.86	0.86	0.86	0.86	0.86

t_0 = a_w value before pasteurisation
t_0^1 = a_w value directly after pasteurisation

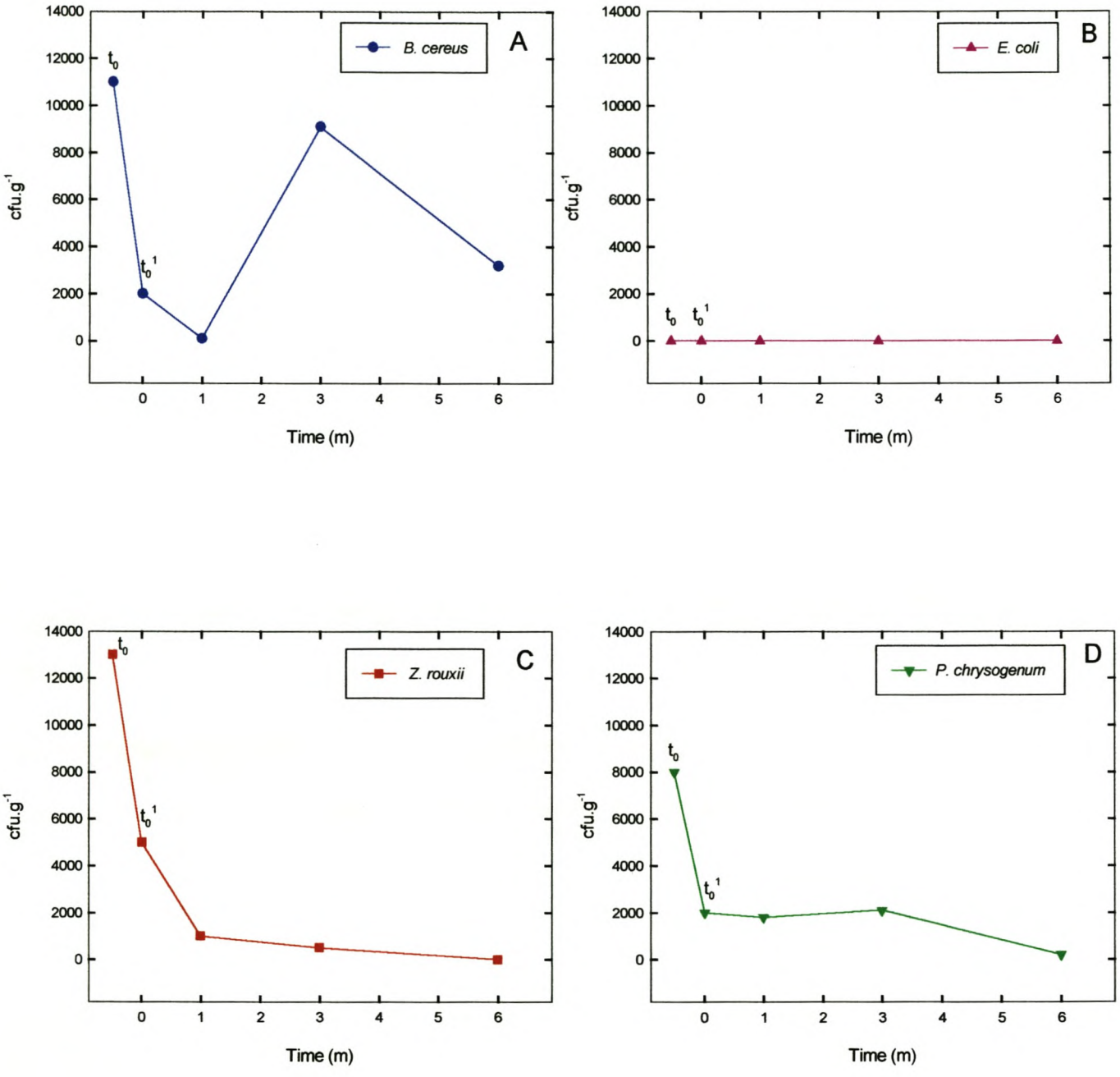


Figure 1. Changes in microbial content of HMD nectarines inoculated with the four indicator organisms over a storage period of 6 months at 5°C (t_0 = before pasteurisation; t_0^1 = after pasteurisation).

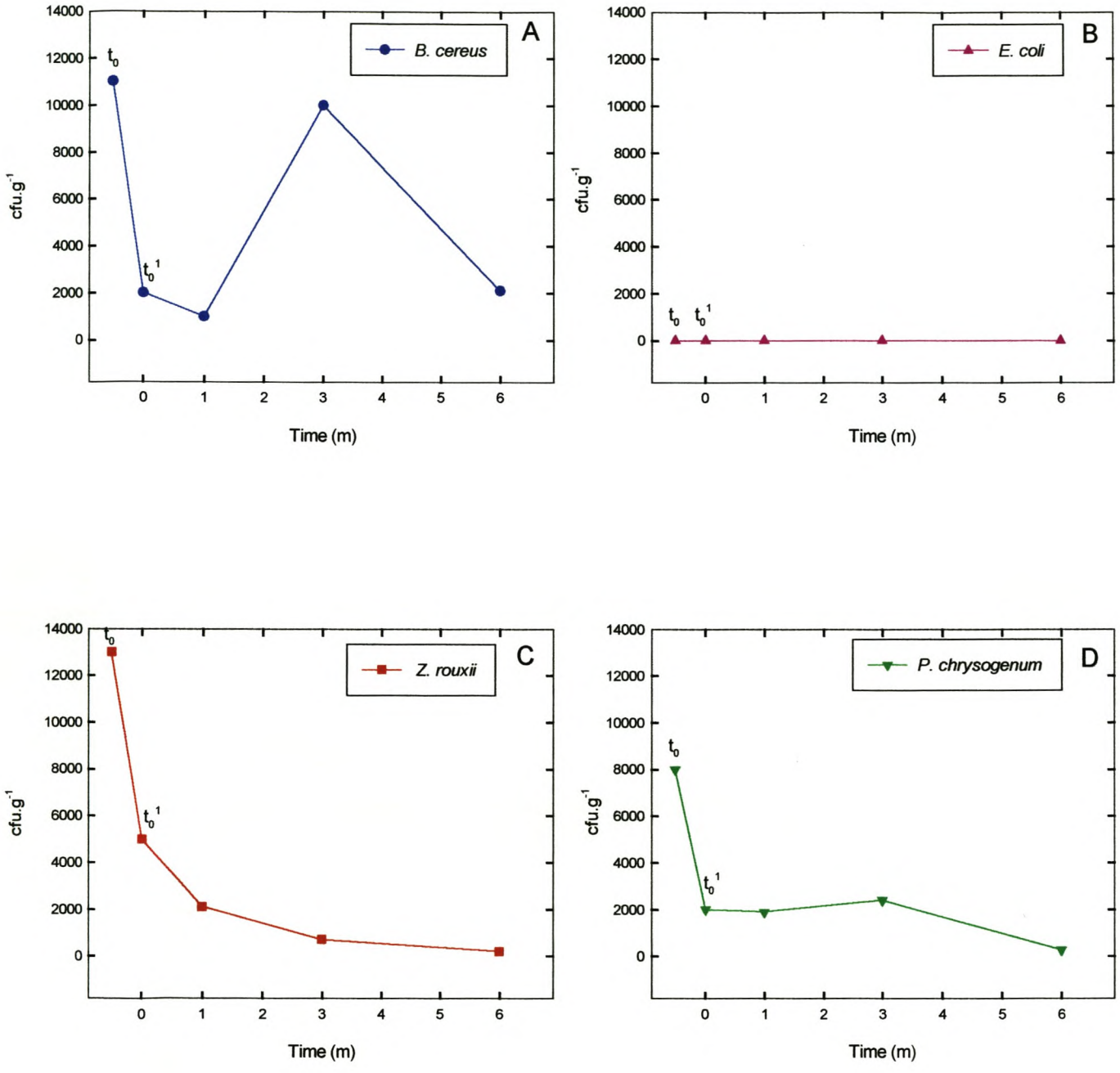


Figure 2. Changes in microbial content of HMD nectarines inoculated with the four indicator organisms over a storage period of 6 months at 25°C (t_0 = before pasteurisation); t_0^1 = after pasteurisation).

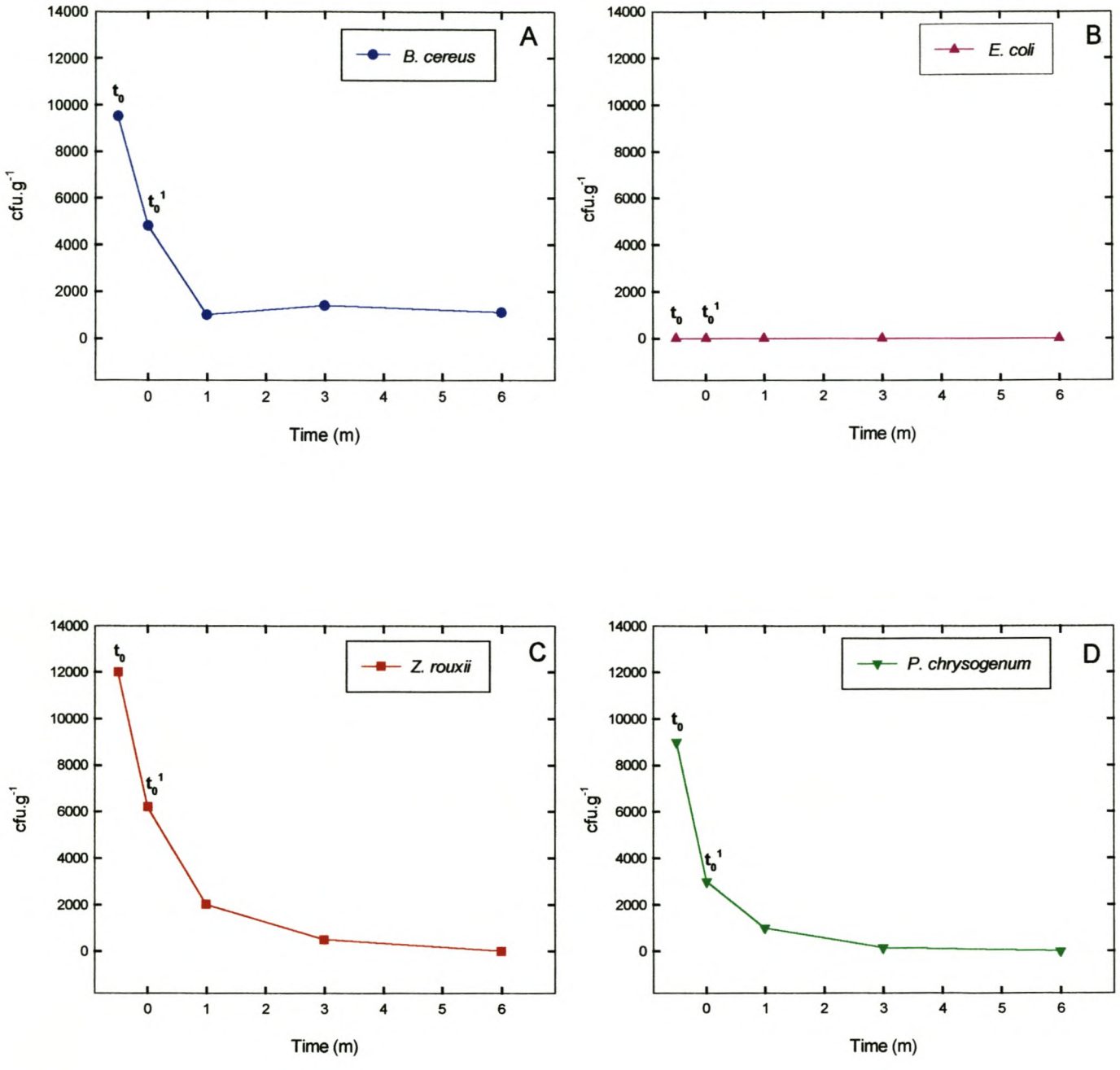


Figure 3. Changes in microbial content of HMD prunes inoculated with the four indicator organisms over a storage period of 6 months at 5°C (t_0 = before pasteurisation; t_0^1 = after pasteurisation).

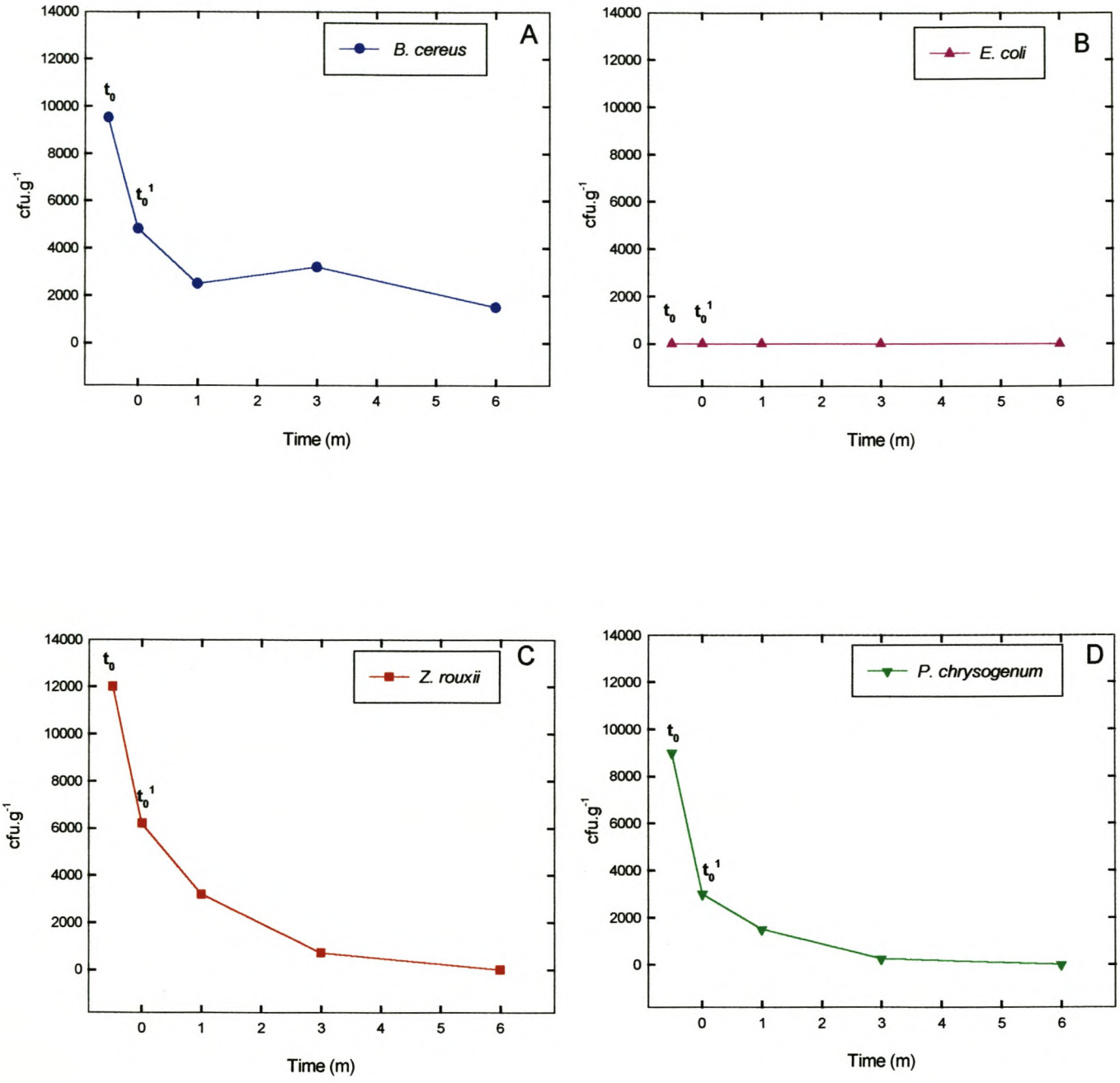


Figure 4. Changes in microbial content of HMD prunes inoculated with the four indicator organisms over a storage period of 6 months at 25°C. (t_0 = before pasteurisation; t_0^1 = after pasteurisation).

Microbial profiles

The results of the survival of the four indicator organisms in the HMD prune and nectarine products stored at either 5° or 25°C, are illustrated in Fig. 1 to 4. In all cases it was found that the microbial content of the control samples (not inoculated) was less than 30 cfu.g^{-1} and was not presented in a figure or a table.

It was found that the microbial counts during the course of the study of both the nectarine and prune samples stored at 25°C (Fig. 2 and 4) were higher than those stored at 5°C (Fig. 1 and 3). This was expected, as storage at room temperature (25°C) would be more favourable for microbial survival and growth.

In all the cases, no presence of *E. coli* was found, not even directly after inoculation (t_0). This was ascribed to the low pH and unfavourable a_w (Tables 1 - 4) of both the prunes and nectarines. It is known that *E. coli* will not survive at pH values below 5.0 and a_w values below 0.90 (Leistner & Rodel, 1976). In this study, it was found that the pH varied from 3.4 to 3.6 (Tables 1 and 2) and the a_w of both HMDF products was below 0.86 (Tables 3 and 4). Thus, it was concluded that the environmental conditions were too unfavourable for the survival and growth of this *E. coli* strain and that members of the Enterobacteriaceae would probably not cause any contamination or spoilage problems in HMDF products under these conditions.

The three other indicator organisms (Fig. 1 – 4) all showed the same drastic decreases in microbial counts after pasteurisation (period t_0 to t_0^1). This was followed by a less drastic decline up to the 6 month storage period for most of the indicator organisms. For nectarines (Fig. 1 and 2), a very sharp increase after 3 months (at 5°C, from 100 to 9 100 cfu.g^{-1}, and at 25°C, from 1 000 to 10 000 cfu.g^{-1}), with a further decline by month 6 (at 5°C from 9 100 to 3 200 cfu.g^{-1} and at 25°C from 10 000 to 2 100 cfu.g^{-1}) was found for the *B. cereus* inoculated samples. This was probably due to the germination of endospores, followed by the death of the vegetative cells in the unfavourable environmental conditions (low a_w and pH). However, the drastic increase in counts for the *B. cereus* inoculated samples was not found for the prune samples after 3 months of storage (Fig. 3 and 4). A small increase (from 2 300 to 2 700 cfu.g^{-1}) was observed in *B. cereus* counts for the prunes stored at 25°C after 3 months (Fig. 4), while the prunes stored at 5°C (Fig. 3) showed a smaller increase (from 1 000 to 1 200 cfu.g^{-1}) in *B. cereus* counts after the 3 months of storage. This was again expected as the

growth and spore germination of *B. cereus* is favoured at higher temperatures (Choma *et al.*, 2000).

In the case of *P. chrysogenum*, the nectarines (Fig. 1 and 2) showed a small increase in numbers after 3 months of storage. At 5°C, an increase from 1 800 to 2 100 cfu.g^{-1} was observed and at 25°C, a increase of 1 900 to 2 400 cfu.g^{-1} was observed. It was interesting to note that at both storage temperatures the increase was the same, namely 300 cfu.g^{-1}. Thereafter the mycelial fungi counts decreased to < 200 colonies per sample. The small increases in the case of *P. chrysogenum* was also ascribed to the germination of spores followed by a steady decline. In the case of the prunes (Fig. 3 and 4), no increases in the *P. chrysogenum* counts were found. This was ascribed to the use of potassium sorbate as it is known that this antimicrobial agent is most effective against fungi (El-Halouat *et al.*, 1998).

The *Z. rouxii samples* showed a decline in all cases, with no cfu's being detected after the 6-month storage period.

It was observed that an inoculum size of 10^4 cells per gram product could easily be standardised (Fig. 1 – 4), but a subsequent accurate enumeration leads to a large variation, probably because the fruit were very heterogeneous due to the structure of the dried fruit. Although the fruit pouches were vigorously shaken after inoculation, even distribution of the inoculum was very difficult to obtain. It was important not to damage the fruit during the mixing process, because later observation in colour changes over the storage period would be difficult.

Colour changes

After the 6-month storage period, the colour of the nectarines stored at the different temperatures was found to differ radically. In all the cases (nectarine samples) the fruit stored at 25°C were extensively discoloured as illustrated in Photo 2, while the fruit stored at 5°C still had an acceptable attractive orange colour. This discoloration can be explained in terms of the Maillard reaction that forms brown end-products, which are heat dependent. It is known that these reactions have high Q_{10} values (Labuza & Saltmarch, 1981), storage at 25°C will therefore result in more browning than at 5°C (Ames, 1990). From the colour change data (Photo 2) it is clear that storage at 5°C would greatly benefit the HMD

Photo 2. Colour changes in nectarines after a 6 month storage period at 5°and 25°C respectively.

nectarine product, not only microbiologically, but it will also contribute to better colour stability of the product.

Conclusions

Four indicator organisms (*B. cereus*, *E. coli*, *Z. rouxii* and *P. chrysogenum*) were used to determine the microbial survival and growth rate over a 6-month storage period in HMD prunes and nectarines. The HMDF samples were pasteurised using the standard tunnel pasteurisation method of SAD (7 h at 80°C). In the case of *E. coli* as indicator organism, it was found that the bacterium could not survive, even before the pasteurisation step was implemented, and it was concluded that the environmental conditions (pH and a_w) were unsuitable for *E. coli*, and related organisms, to be a spoilage or safety concern in HMD prunes or nectarines.

The data obtained in this study clearly showed that despite the 80°C for 7 h pasteurisation step, a large percentage of three of the indicator organisms survived the HMDF pasteurisation step. Even though a further decline took place over the 6 months storage period, enough microbes survived the process to indicate that their presence in high numbers must be considered a serious spoilage risk. In the case of *Bacillus cereus*, a large increase was also found after the 3-month storage period and this was followed by a decrease after the 6-month storage period. This increase/decrease profile of the *B. cereus* strain indicates that the environmental conditions were not severe enough to prevent germination and development of aerobic endospore formers in the HMD nectarines.

It can also be argued that the inoculum levels of the indicator organisms used in this study were much higher than the normal contamination levels that would occur during normal processing, as was also found during the microbial monitoring in Chapter 3 of this thesis. However, when population decimal reductions are calculated, only one decimal reduction (DR = 1) or less was found after the pasteurisation step. This clearly indicates that even if a smaller inoculum had been used at the start, sufficient numbers of cells would probably be able to survive, which could lead to a spoilage problem within a few weeks, especially at higher and more favourable storage temperatures. Therefore, it is advisable that the current pasteurisation process is optimised or that natural preservatives are included in the HMDF products to ensure a safe quality product. Furthermore, the

data obtained clearly showed that it is advisable to store HMDF products at a lower temperature, so as to minimise microbial growth and to enhance the colour acceptability, ensuring a better quality product.

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CHAPTER 5

IMPACT OF DIFFERENT PRESERVATIVES ON THE SURVIVAL AND GROWTH OF *BACILLUS CEREUS* AND *ZYGOSACCHAROMYCES ROUXII* IN NECTARINE PUREE

Abstract

In this study the impact of different preservatives (lysozyme, pimaricin, potassium sorbate, sodium benzoate and an imidazole compound) on the survival and growth of *Bacillus cereus* and *Zygosaccharomyces rouxii*, were evaluated in nectarine puree prepared from dried unsulphured nectarines. A "higher" and a "lower" concentration of each preservative was used in the study, with the "higher" concentration equivalent to the maximum levels permitted by SA legislation (Anon., 1972). At the "lower" preservative concentrations used, it was found that lysozyme showed the best inhibitory effect on *B. cereus* over the 12 h incubation period, with 51 % inhibition, followed by sodium benzoate (42% inhibition), potassium sorbate (40% inhibition), the imidazole compound (33% inhibition) and lastly pimaricin (24% inhibition). At the "higher" preservative concentrations used with *B. cereus* as indicator organism, similar trends were observed, with lysozyme again showing the best inhibition effect on *B. cereus* over the 12 h incubation period, with 70% inhibition, followed by sodium benzoate (56% inhibition), potassium sorbate (55% inhibition), pimaricin (53% inhibition) and lastly the imidazole compound (48% inhibition).

The data obtained for the inhibitory effect of the preservatives on the growth and survival of *Zygosaccharomyces rouxii* indicated that at the "lower" concentrations, sodium benzoate and potassium sorbate had the best growth inhibitory effect, with a 100% inhibition after 48 h of incubation. Pimaricin also gave a 100% inhibitory effect after 48 h, and lastly the imidazole compound with 89% inhibition. At the "higher" concentrations used it was found that all the preservatives showed a 100% inhibition after 48 h. It was interesting to observe the same trend for the "higher" and "lower" lysozyme concentrations

applied to *Z. rouxii* in that the cell numbers firstly showed a decreased by 4 h of incubation, followed by a steep increase in cell numbers up to 48 h. It was, therefore, speculated that *Z. rouxii* can probably metabolize or co-metabolize lysozyme. From the results, it was clear that the addition of additional preservatives to high moisture dried fruit could be of great benefit in making the product more shelf-stable.

Introduction

The moisture content of conventional dried fruit ranges mostly between 18 and 25%, while the moisture content of high moisture dried fruit (HMDF) is *ca* 36%. The higher moisture content of the HMDF leads to a higher water activity (a_w) and subsequently a change in the microbial spoilage population, which may result in an increased spoilage rate. To overcome this increased spoilage problem different preservatives can be employed to extend the shelf-life of the product and to increase the microbial stability.

In South Africa, sulphur dioxide (SO_2) is almost always used during the production of dried fruit (J. Schoeman, 1999, SAD, Wellington), because it is an excellent antimicrobial agent and in addition, inhibits Maillard and enzymatic browning reactions (Ames, 1990). Sulphur dioxide is also incorporated in a wide range of other foods including fruit juices and concentrates, wines and jams (Prabhakar & Reddy, 2000). The maximum level of SO_2 permitted for dried fruit in South Africa is 2 000 $mg.kg^{-1}$ (Anon., 1972). Sulphur dioxide is an extremely controversial preservative and the maximum permitted levels are either being lowered, or even being omitted all over the world, especially in European countries. The main reason for this negative public reaction to the addition of SO_2 is that it has been shown to be the cause of allergic reactions, especially in consumers suffering from asthma (Steinman, 2000). It is seen as an user unfriendly preservative, therefore, the trend to lower the SO_2 concentration in foods (Prabhakar & Reddy, 2000).

In many cases potassium sorbate (K-sorbate) and sodium benzoate (Na-benzoate) are also used as chemical preservatives in fruit products (Harel *et al.*, 1978). K-sorbate has been successfully used as preservative in low acid foods, including fruit juices, canned fruits, pickles, tomato puree and

mayonnaise (Ogbadu, 2000). Na-benzoate on the other hand is added to dried fruit, fruit juices, olives, cheese and wine (Thomas, 2000). However, only a few studies have been done to determine the influence of the addition of K-sorbate and Na-benzoate on the microbial content of HMDFs. Both these preservatives are effective against fungi at low pH values, where the pH is usually below 4.0. El-Halouat *et al.* (1998) did excellent work on the preservation of high moisture dried (HMD) prunes and raisins. They reported that the growth of *Zygosaccharomyces rouxii* was prevented by the addition of 186 $mg.kg^{-1}$ K-sorbate to HMD prunes and 153 $mg.kg^{-1}$ K-sorbate to HMD raisins, while 176 $mg.kg^{-1}$ Na-benzoate was effective in preventing the growth of *Z. rouxii* in HMD prunes and 158 $mg.kg^{-1}$ Na-benzoate in HMD raisins. In South Africa, the permitted level for K-sorbate in fruit products is 600 $mg.kg^{-1}$ and for Na-benzoate 1 000 $mg.kg^{-1}$ (Anon., 1972).

The international trend in food consumption is towards more "natural" and healthy foods. This has led to a change in the focus of modern food preservation and natural preservatives like lysozyme and bacteriocins are now more often used in the food industry. Lysozyme is an antimicrobial protein and an excellent inhibitor of Gram-positive bacteria, especially members of the genus *Clostridium* (Razavi-Rohani & Griffiths, 1997). It has also successfully been used in cheese products to prevent the late blowing of cheese by *Clostridium tyrobutyricum* (Ibrahim *et al.*, 1997). The maximum level of lysozyme permitted in South Africa is 600 $mg.kg^{-1}$ (Anon., 1972).

The bacteriocin, pimaricin, has been found to be highly effective against moulds (mycelial fungi) and is commonly known as Natamycin or Dalvocid in the industry. It is effectively used in the dairy and wine industries as a "natural" fungicide (Holley, 1981). Pimaricin is generally used for the surface treatment of cheese and dry sausages to prevent mould growth (Stark, 2000). The major advantages of pimaricin are its broad spectrum activity and the fact that it has no activity against bacteria, which makes it useful for application in products which require a sensitive starter culture and a bacterial ripening process, such as found in cheese and dry sausages (Stark, 2000). The maximum level of pimaricin permitted in South Africa is 20 $mg.kg^{-1}$ in cheese and yogurt products (Anon., 1972). However, this natural preservative has not been evaluated in intermediate moisture foods (IMFs) or

HMDF products and subsequently, no inhibitor levels have been determined for these products.

Imidazole compounds were first discovered in the 1800's and today are used in the preservation of cheese, mainly to prevent mould growth (Anon., 1992; Ellen, 1993). Although these compounds have been employed for several years in the dairy industry, imidazole compounds are not well known preservatives. No information was found in the literature on the effect of imidazole compounds on the microbial content of HMDF products.

The South African dried fruit industry has had spoilage problems in the past, especially in the HMD nectarine products (J. Schoeman, 1999, SAD, Wellington, personal communication). The product is microbiologically and colour unstable, and generally has a shelf-life of only three months. It was, therefore, decided to use nectarines as evaluation substrate in this study. The aim of this study was thus to evaluate the preservation effect of different preservatives (K-sorbate, lysozyme, Na-benzoate, pimaricin and an imidazole compound) on the survival and growth of two selected indicator organisms; an endospore forming bacterium (*Bacillus cereus*) and the osmotolerant yeast (*Zygosaccharomyces rouxii*) in nectarine puree, prepared from dried nectarines.

Materials and methods

Fruit and fruit preparation and SO_2 determination

Dried nectarines with a SO_2 content of 2 700 $mg.kg^{-1}$, obtained from SAD, Worcester (C. Du Toit, 1999, personal communication), were used in this study as evaluation substrate. The nectarines were steamed in a steam tunnel (Moolenaar, Paarl) at 100°C for 30 min to lower the excess SO_2 to 1 200 $mg.kg^{-1}$. The sulphur dioxide content was determined, using the steam distillation method (Anon., 1978).

A 100 g sample of fruit was then macerated with 400 ml of sterile distilled water in a sterile Waring commercial blender (Waring Products Corporation, USA) for 5 min until a smooth puree was formed. The fruit puree samples were then autoclaved for 15 min at 121°C and 100 kPa to eliminate any microbes present in the fruit.

Microbe and preservative addition

Strains of *Bacillus cereus* (DSM 31) and *Z. rouxii* (NRRL Y-998) from the University of Stellenbosch, Food Science Culture Collection, were respectively added to the puree at a concentration of 10^4 cells.ml^{-1} of puree. Cell concentrations were determined using a spectrophotometer (Spectronic 20, Genesys, Spectronic Instruments, USA) at 540 nm (as described in Chapter 4).

The preservatives used in this study included: lysozyme (Seravac); potassium sorbate (Saarchem); sodium benzoate (Merck); pimaricin (Anchor Yeast); and an imidazole compound. The concentrations of the preservatives added are listed in Table 1. A "higher" and a "lower" concentration of each preservative was used throughout the study, where the "higher" concentration represented the maximum concentrations permitted by SA food legislation (Anon., 1972). The "lower" concentrations were used to evaluate the effect that a lower concentration would have on the growth and survival of the indicator organisms and to observe the effectiveness of the lower concentrations in contrast with the highest permitted levels. Control samples containing no preservative, apart from SO_2 still present in the fruit, but only the indicator organisms, were also included throughout the study.

Microbial analyses

The microbial analyses for the samples were done on two specific media, Plate Count Agar (Biolab) for *B. cereus* and Chloramphenicol Agar (Biolab) for *Z. rouxii* enumeration. A dilution series (10^{-1} - 10^{-4}) was prepared of each sample and plated in duplicate in the specific media, using the pour plate technique. Microbial analyses of the samples were done just after inoculation with the specific organism (time = 0) for both organisms. *Bacillus cereus* counts were further done on time 0^1 (time just after the preservative was added) and then after 4, 8 and 12 h of incubation of the nectarine puree at 35°C, while the *Z. rouxii* counts were done at 0^1 (time just after the preservative was added) and after 4, 24 and 48 h of incubation of the nectarine at 30°C. Different incubation times for the fruit puree were chosen to suit the growth characteristics of each organism. The bacterium *B. cereus*

Table 1. Preservative concentrations added to the nectarine puree.

Preservative	"Lower" concentration $mg.kg^{-1}$	"Higher" concentration $mg.kg^{-1}$
Lysozyme	100	600
K-sorbate	100	600
Na-benzoate	100	600
Pimaricin	10	20
Imidazole compound	1	10

grows faster than the yeast *Z. rouxii* and, thus, the large difference in incubation evaluation times. The optimum growth temperatures also differ for these organisms, hence, the difference in incubation temperatures.

Inhibition calculation

The percentage inhibition was calculated using the following equation as recommended by Gilliland & Speck (1977):

$$\frac{\text{Initial inoculum (cfu.g}^{-1}\text{) - cfu.g}^{-1}\text{ found at specific test time}}{\text{Initial inoculum (cfu.g}^{-1}\text{)}} \times \frac{100}{1}$$

Results and discussion

In this study, *B. cereus* and *Z. rouxii* were used as indicator organisms to evaluate the influence of different preservatives on the survival and growth of these chosen indicator organisms in nectarine puree. *Bacillus cereus* was chosen as it is a Gram-positive endospore forming organism that is thermotolerant and difficult to eliminate. In contrast, *Z. rouxii* is an

osmotolerant yeast that is a frequent spoiler of HMDF products (El-Halouat *et al.*, 1998). For this study it was argued that these two organisms are representative of the most frequent spoilers occurring in HMDF products, as was found in Chapter 3 of this thesis.

The influence of the different preservatives on the survival and growth of *B. cereus* is shown in Fig. 1 and 2. In Fig. 1, the data represent the scenario where the preservatives, as given in Table 1, were added at the "lower" concentrations and in Fig. 2 at the "higher" concentrations. The control puree samples, for both concentrations, where no preservatives had been added but only the test organism, showed a slight decline over the 12 h of incubation (from 11 900 – 9 600 cfu.g^{-1}) (Fig. 1 and 2). The overall effect of the addition of the low concentrations of preservatives did have an inhibitory effect on the growth and survival of the *B. cereus* strain, although it was not very effective for a 12 h incubation time period (Fig. 1). The preservative that resulted in the best inhibition of *B. cereus* was lysozyme with a 51% reduction in cell numbers during the 12 h incubation time. This was expected, as lysozyme is known to be effective against Gram-positive organisms, like *B. cereus* (Razavi-Rohani & Griffiths, 1997). When all the results are taken into consideration, Na-benzoate was found to be the second best preservative (42% inhibition), followed by K-sorbate (40% inhibition), the imidazole compound (33% inhibition) and lastly pimaricin (24% inhibition).

In Fig. 1, it was also observed that all the preservatives resulted in a decline in viable bacterial numbers after 4 h of incubation and then from 4 h to 8 h an increase was observed, followed again by a decline in cfu's by 12 h. This can probably be explained by the fact that *B. cereus* is an endospore former, and after 8 h any endospores present probably germinated, leading to the increase in bacterial numbers. After this increase the *B. cereus* counts were found to decline again after 12 h of incubation.

In the study where the "higher" preservative concentrations (Fig. 2) were evaluated, lysozyme again showed the best inhibitory action on the *B. cereus* cell counts (70% inhibition over 12 h of incubation), suggesting that the preservative did have an inhibitory effect on the bacterium. It is important to note that although there was a clear decrease in cfu's over the 12 h of

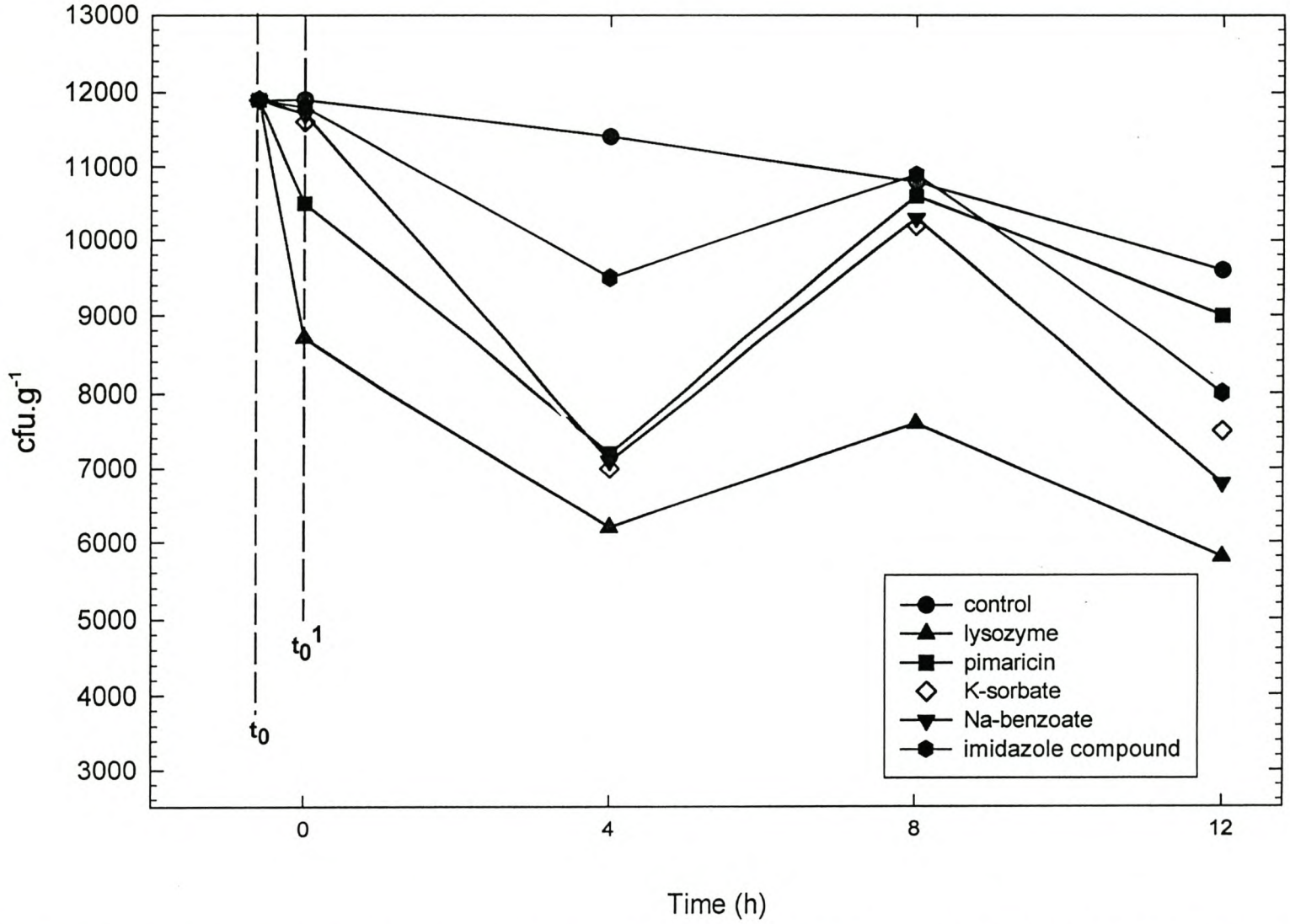

Figure 1. The influence of the lower concentrations of lysozyme (100 mg.kg^{-1}), pimaricin (10 mg.kg^{-1}), K-sorbate (100 mg.kg^{-1}), Na-benzoate (100 mg.kg^{-1}) and the imidazole compound (1 mg.kg^{-1}) on the growth and survival of *B. cereus* in nectarine puree (t_0 = after addition of indicator organism; t_0^1 = after addition of preservative).

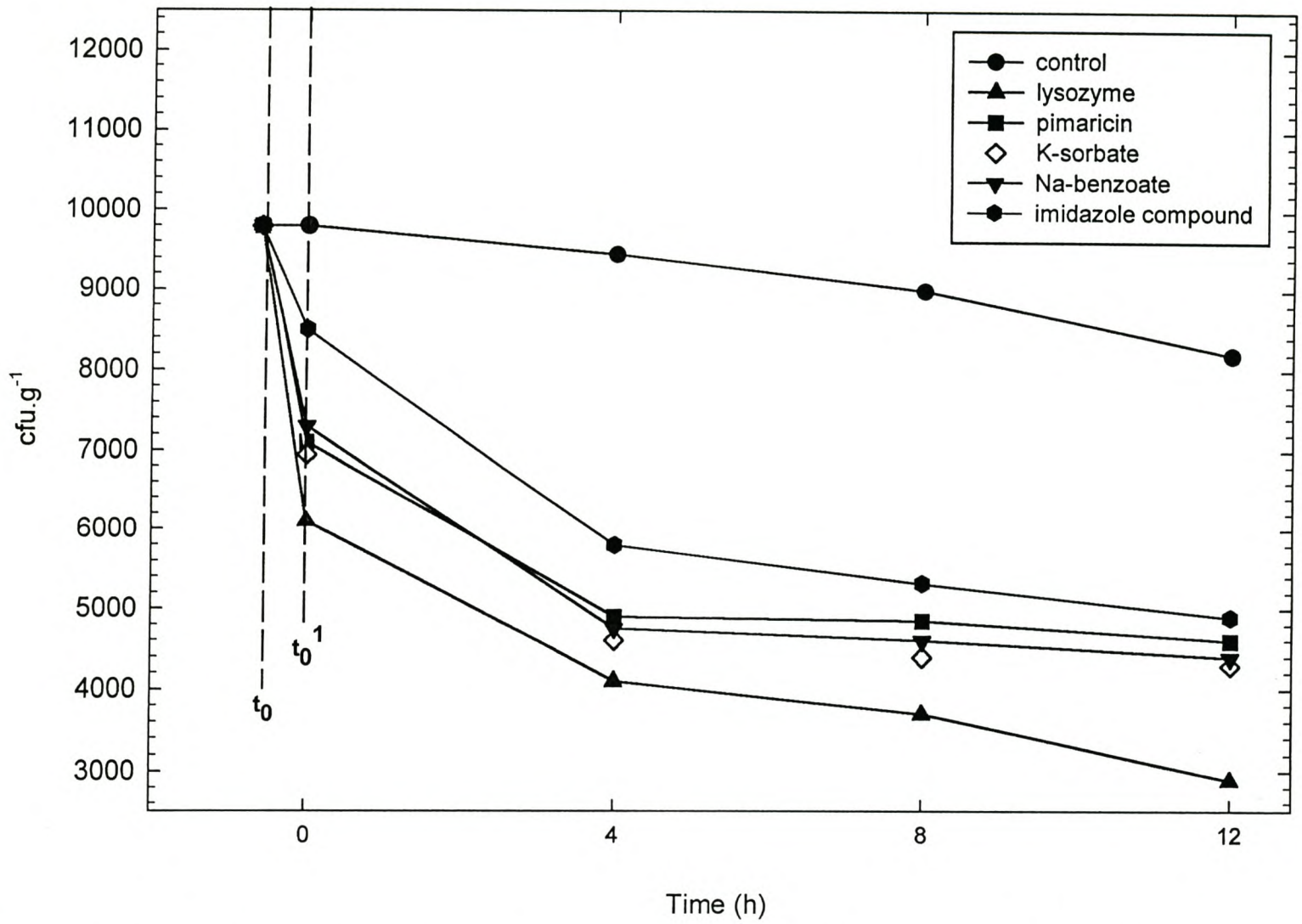

Figure 2. The influence of the higher concentrations of lysozyme (600 mg.kg^{-1}), pimaricin (20 mg.kg^{-1}), K-sorbate (600 mg.kg^{-1}), Na-benzoate (600 mg.kg^{-1}) and the imidazole compound (10 mg.kg^{-1}) on the growth and survival of *B. cereus* in nectarine puree (t_0 = after addition of indicator organism; t_0^1 = after the addition of preservative).

incubation, it was not a decimal reduction of viable cells. The cell counts only decreased from 9 800 to 2 900 cfu.g^{-1}, but it is still an effective decrease (70% inhibition). The addition of "higher" concentrations of preservatives did not show the drastic increase from 4 to 8 h as was observed in Fig 1, where the "lower" concentrations were applied.

At the "higher" concentration, the second best preservative was Na-benzoate (56% inhibition), followed by K-sorbate (55% inhibition) and pimaricin (53% inhibition). All three these preservatives showed the same general inhibition profile. This can be explained by the fact that all these preservatives are fungicides and not very effective against bacteria. The data showed that the imidazole compound resulted in the poorest inhibition effect (48% inhibition) on the *B. cereus* strain. This effect was expected, as all these preservatives (Na-benzoate, K-sorbate, pimaricin and the imidazole compound) are effective against fungi and not bacteria. It was still interesting to see that there was some inhibition by these antifungal preservatives on the bacterium, although it was not as high as the inhibition by lysozyme.

The inhibition curves for *Z. rouxii* are presented in Fig. 3 and 4. In Fig. 3, where the "lower" preservative concentrations were used, it was observed that Na-benzoate and K-sorbate had the best growth inhibitory effect (100% inhibition after 48 h). Directly after the addition of the specific preservative (t = 0[1]) there was already a decline from 11 000 cfu.g^{-1} to 7 400 cfu.g^{-1} for K-sorbate (33% inhibition) and for Na-benzoate the decline was to 7 100 cfu.g^{-1} (35% inhibition). From 24 h to 48 h there was a further decline and by 48 h no cfu's were found (100% inhibition). Pimaricin and the imidazole compound both showed the same inhibition profiles, and as for the previous two preservatives, after 48 h no counts were found for pimarcin (100% inhibition), while for the imidazole compound, 1 200 cfu.g^{-1} were still present in the puree (89% inhibition). It was interesting to note that in the puree where lysozyme was added, an increase in cfu's from 6 700 cfu.g^{-1} after 4 h to 11 300 by 48 h of incubation was seen, which was higher than the initial inoculum of 11 000 cfu.g^{-1}. It can be speculated that the organism possibly has the ability to metabolize or co-metabolize lysozyme or to use it as a growth substrate, since there was no corresponding increase in cfu's in the control sample.

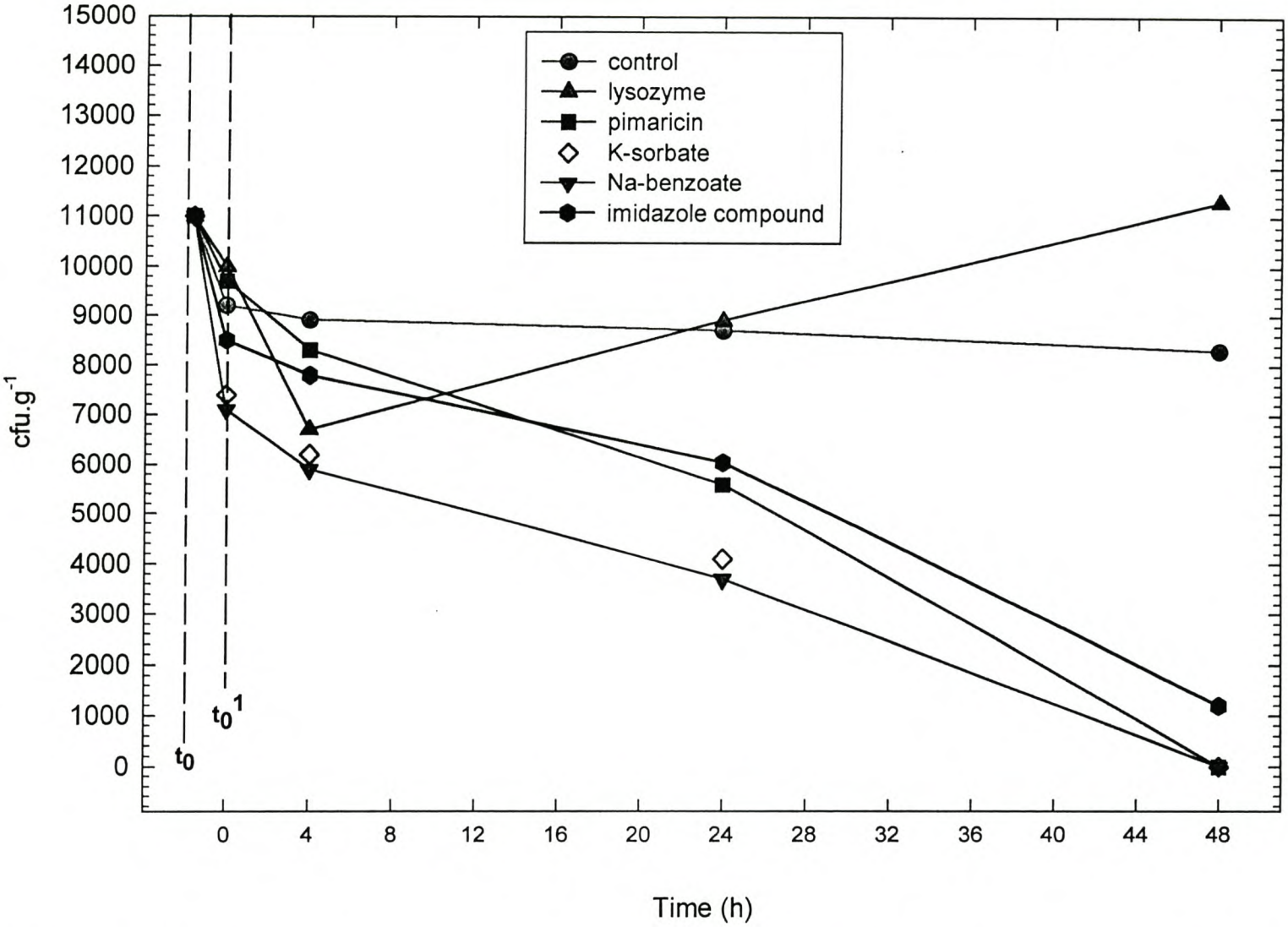

Figure 3. The influence of the lower concentrations of lysozyme (100 mg.kg^{-1}), pimaricin (10 mg.kg^{-1}), K-sorbate (100 mg.kg^{-1}), Na-benzoate (100 mg.kg^{-1}) and the imidazole compound (1 mg.kg^{-1}) on the growth and survival of *Z. rouxii* in necatrine puree (t_0 = after addition of indicator organism; t_0^1 = after addition of preservative).

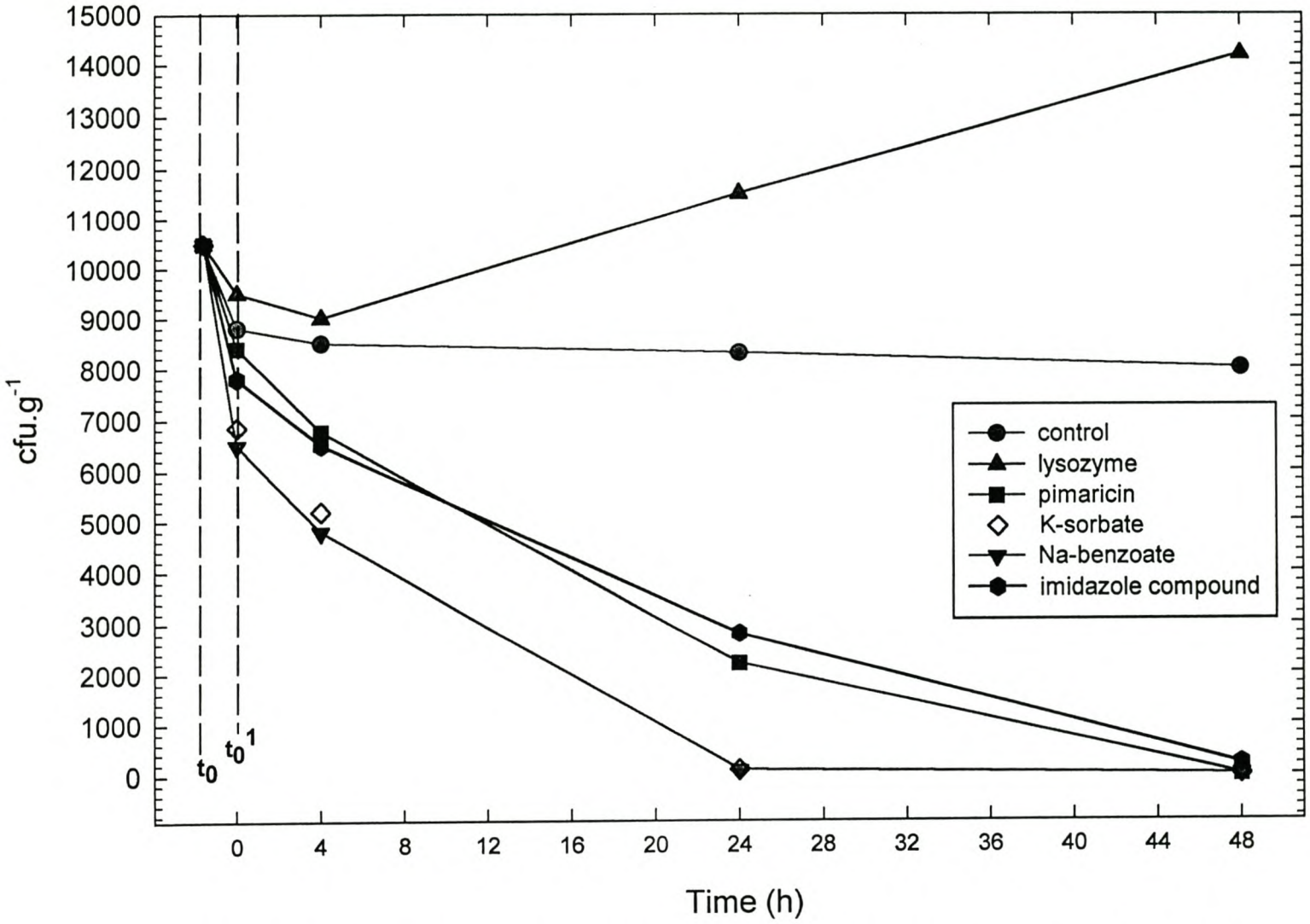

Figure 4. The influence of the higher concentrations of lysozyme (600 mg.kg^{-1}), pimaricin (20 mg.kg^{-1}), K -sorbate (600 mg.kg^{-1}), Na-benzoate (600 mg.kg^{-1}) and the imidazole compound (10 mg.kg^{-1}) on the growth and survival of *Z. rouxii* in nectarine puree (t_0 = after addition of indicator organism; t_0^1 = after addition of preservative).

the organism (100% inhibition after 48 h), followed closely by K-sorbate (100% inhibition after 48 h) that showed the same inhibition profile over 48 h.

The inhibition profile where the "higher" concentration of preservatives were used with *Z. rouxii* (Fig. 4), it was observed that K-sorbate was effective after only 24 h with 100 cfu.g^{-1} still remaining (99% inhibition) and 90 cfu.g^{-1} for Na-benzoate (99.1%). After 48 h of incubation, the K-sorbate and Na-benzoate were totally effective and no *Z. rouxii* colonies were found on the CA medium. Pimaricin and the imidazole compound were, respectively, 49% and 45% effective after 24 h of incubation and 100% effective after 48 h of incubation of the puree.

In the case where the "higher" concentration of lysozyme was used (Fig. 4), a steep increase in cfu's was observed, which was similar to the results obtained with puree sample where the "lower" concentration of lysozyme (Fig 3) was used as the preservative. In this case, the increase in viable cell counts from 4 to 48 h was even larger than observed with the addition of the "lower" concentration of lysozyme (Fig. 3). After 4 h the increase was from 9 000 cfu.g^{-1} to 14 200 cfu.g^{-1} after 48 h of incubation, which was much higher than the initial inoculum of 10 500 cfu.g^{-1}. From this it was again suspected that *Z. rouxii* could possibly be able to metabolize or co-metabolize lysozyme and that this preservative is of no use as a preservative in HMDF against *Z. rouxii* spoilage.

From the results it is clear that *B. cereus* is more difficult to eliminate than the yeast *Z. rouxii*. No preservative was effective in eliminating the *B. cereus* cells totally within 12 h, while for *Z. rouxii* a 100% inhibition after 24 h was observed with K-sorbate and Na-benzoate. However, in these studies only one bacterial inhibitor was evaluated and it is important that others are evaluated in future studies. It is also important to know which organisms are targeted in a product and to be sure that the targeted microbes can not metabolize the preservative, as the case may have been for lysozyme with *Z. rouxii*.

Conclusion

From this study, it was confirmed that endospore forming bacteria are extremely difficult to eliminate in nectarine puree. Although lysozyme showed good preservative characteristics, it was not 100% effective. It was also interesting to observe the effect of endospore germination when the "lower" preservative concentrations are used. It would, therefore, be of great importance to know the product and to set the correct specifications for preservative addition that would be effective against the targeted spoilage microbes. It can be argued that natural microbial contamination levels of HMDF products will probably never be as high as the 10^4 inoculum concentrations used in this study and, therefore, that the lysozyme would be effective at lower microbial levels.

Zygosaccharomyces rouxii was much easier to eliminate by the addition of the selected antifungal preservatives. The antifungal preservatives Na-benzoate and K-sorbate were both very effective (100% inhibition) at both the "lower" and "higher" concentrations and the fact that the added levels were not higher than the recommended maximum levels (Anon., 1972) is of value to the S.A. dried fruit industry. The possibility that the *Z. rouxii* strain can metabolize lysozyme is very interesting, and it is important to take note of this fact when applying a mixture of preservatives to eliminate yeasts and bacteria.

The effect of hurdle technology was also successfully applied in this study, as two preservatives were actually evaluated together, namely SO_2 (1 200 $mg.kg^{-1}$) and the specific preservative added. However, when studying the inhibition profiles of the controls for both indicator strains, it appears as if SO_2 was not very effective and this is something that must be evaluated in the future.

It is also important to note that nectarine puree was used as model of HMD nectarines in this study. This was done because inhibition curves are much easier to monitor in a liquid medium than a solid medium. It is from utmost importance to realise that the puree had a much higher a_w than that of HMD nectarines. The indicator organisms would be much easier eliminated in

HMDF, because *B. cereus* and *Z rouxii's* optimum a_w for growth is closer to the a_w of the nectarine puree.

From the results, it was concluded that the addition of alternative preservatives to HMDF products would be of great benefit to the dried fruit industry in enhancing the shelf-life and microbial stability of HMDF products, ensuring a better quality end-product.

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CHAPTER 6

STEAM PASTEURISATION AS AN OPTIMAL PROCESSING METHOD FOR THE PRESERVATION OF HIGH MOISTURE DRIED NECTARINES

Abstract

High moisture dried fruit (HMDF) are conventional dried fruit that are rehydrated to a moisture content of *ca* 36% (w/w) and a water activity (a_w) of *ca* 0.86. This increase in moisture content, and subsequently high, a_w makes the HMDF more prone to microbial spoilage. In addition, the colour stability of the fruit is also negatively affected by Maillard browning that is accelerated under these conditions. Pasteurisation is one of the most critical parameters that must be optimised to ensure a high quality HMDF end-product. The aims of this study, therefore, were to determine the optimal time/temperature pasteurisation combinations that would lead to the elimination of the presence of an indicator *Bacillus cereus* strain, but that would not have a negative effect on the colour stability of high moisture dried (HMD) nectarines.

It was found that a steam pasteurisation time/temperature combination of 90 min at 80° or 90°C was insufficient to eliminate the *B. cereus* strain at inoculum concentrations of 10^4 and 10^6 cfu.g^{-1}. The time/temperature combination was then changed to 120 min at 90°C and the inoculum concentrations reduced to 10^3 and 10^4 cfu.g^{-1}. The data showed that even after 120 min of pasteurisation at 90°C not all the *B. cereus* cells were eliminated and the pasteurisation time was subsequently lengthened to 150 min and evaluated at both 90° and 100°C. It was observed that the colour of the fruit was unstable at the higher pasteurisation temperature applied. Modified atmosphere packaging (MAP) was then used as Maillard browning occurred when the HMD fruit were pasteurised at 100°C, as Maillard reactions are known to be favoured by elevated temperatures.

Of the two temperatures used, 100°C gave better elimination of microbes present than 90°C for 150 min. For both of the pasteurisation time/temperature combinations no members of the genus *Clostridium* or Enterobacteriaceae were found. The yeast and mould (mycelial fungi) counts were reduced by 99.1% (at 90°C pasteurisation) and 99.8% (at 100°C pasteurisation) directly after pasteurisation, with a 100% reduction being achieved after storage for two weeks at both 5° and 40°C. The viable aerobic counts decreased after pasteurisation with a 66% reduction in counts for the 90°C pasteurisation and 74% reduction for the 100°C pasteurisation. After storage for two weeks at 5°C, a further reduction in counts was found, while for samples stored at 40°C an increase in the counts were observed. From these results it was concluded that MAP did effect the microbial population of HMD nectarines negatively and that a pasteurisation time/temperature combination of 150 min at 90°C is advised to ensure a HMDF product of excellent quality, with a shelf-life of six to nine months.

Introduction

The shelf-life of food products is one of the most critical criteria for measuring excellence in the food industry. Food is preserved to keep it consumable for longer periods of time and many conventional preserving techniques including: drying; fermentation; smoking etc., are still being used to preserve food in this high-tech century. Heat preservation is also mostly used in combination with other techniques, forming hurdles that inhibit spoilage (Alzamora *et al.*, 1999; Forcinio, 1999; Leistner, 1985).

Traditional dried fruit is an age-old preserved product and today it is still very popular. The hurdles employed during the production of dried fruit are the lowering of the water activity (a_w) to less than *ca* 0.70, the lowering of the moisture content to between 18 - 27% (w/w) and the addition of sulphur dioxide as an antimicrobial agent to prevent microbial spoilage and as a reducing agent to prevent Maillard and enzymatic browning (Salunkhe *et al.*, 1991).

High moisture dried fruit (HMDF) on the other hand, are conventional dried fruit that are rehydrated to a moisture content of between 36 and 40% (w/w) and an a_w of *ca* 0.85 (J. Schoeman, 1999, SAD, Wellington, personal communication). With this increase in moisture and a_w, microbial spoilage of the fruit occurs more rapidly and Maillard browning is an additional problem that is aggravated. Thus, in many cases it is necessary that the HMDF products are heat treated to eliminate most spoilage microbes (J. Schoeman, 1999, SAD, Wellington, personal communication). The correct time/temperature combination that is employed in this heat pasteurisation step is of utmost importance because the treatment must be long enough to eliminate the spoilage microbes, but on the other hand, short enough to prevent extensive Maillard browning that is accelerated by the higher temperatures applied during the pasteurisation step (Ames, 1990).

Maillard browning is also enhanced by free oxygen that is present in a product. Modified atmosphere packaging (MAP) is a packaging technique that is used to minimise the oxygen content of a product to inhibit enzymatic browning reactions (Davies, 1995; El-Halouat & Debevere, 1997). Different MAP gases can be used and these include carbon dioxide (CO_2), nitrogen or a combination of the two. In cases where CO_2 is used, there is the possibility of the enhancement of growth of the anaerobic endospore formers of the genus *Clostridium* that can thrive in low oxygen environments (Enfors & Molin, 1987) and can cause serious food safety problems (Tsang *et al.*, 1985).

In a previous study, Chapter 4 of this thesis, it was found that even after a pasteurisation step of 80°C for 7 hours, that is used by the industry, three (*Bacillus cereus*, *Zygosaccharomyces rouxii* and *Penicillium chrysogenum*) of the four indicator organisms that were used in the challenging tests survived in the HMDF. The survival and growth of these organisms can lead to serious spoilage problems of the HMDF. In this study, it was also found that the HMDF, especially the nectarines, were extensively discoloured. It was, therefore, essential to evaluate other pasteurisation time/temperature combinations in terms of the effectiveness in eliminating spoilage microbes and minimising the Maillard browning reactions.

The aims of this study were to evaluate different pasteurisation time/temperature combinations in terms of the survival of *Bacillus cereus*, to determine the optimal pasteurisation specifications for high moisture dried (HMD) nectarines, and to determine the effect of MAP on the microbial population of HMD nectarines.

Materials and Methods

Fruit preparation, packaging and analyses

Choice grade, dried nectarines were obtained from SAD (C. Du Toit, 1999, SAD, Worcester, personal communication). The dried fruit had an initial moisture content of 18 - 19% (w/w), an a_w of 0.75 and an average SO_2 content of 1 923 $mg.kg^{-1}$. The dried nectarines were washed in a double-jacketed steam vessel (Aluminium Plant & Vessel Company, London) for 90 sec at 45°C and the moisture content was allowed to equilibrate overnight in airtight plastic containers at 0°C. The fruit were then washed for 180 sec at 45°C. The wash-water used contained 24.8 $g.l^{-1}$ SO_2 to compensate for the loss of SO_2 during the wash process (A. Erwee, unpublished data, 2000).

The fruit (250 g) were packaged in laminated aluminium foil stand-up pouches and placed open in a steam tunnel (Moolenaar, Paarl). Different time/temperature combinations were applied, depending on the experimental study being undertaken.

Moisture analyses and a_w measurements were done according to the standard AOAC (1990) method. Sulphur dioxide determinations were done using the steam distillation method (Anon., 1978).

Experimental Study I - Effectiveness of steam pasteurisation at either 80° or 90°C for 90 min in eliminating Bacillus cereus at concentrations of 10^4 or 10^6 $cfu.g^{-1}$ in HMD nectarines

In this study the effectiveness of steam pasteurisation for 90 min at 80° or 90°C on the survival of a *Bacillus cereus* strain in HMD nectarines was evaluated (Table 1). The aerobic endospore forming *Bacillus cereus* (DSM 31) strain, obtained from the

Table 1. Pasteurisation combinations applied during this study.

Experimental Study	*B. cereus* inoculum size (cfu.g^{-1})	Pasteurisation temperature (°C)	Pasteurisation duration (min)
I	10^4	80	90
	10^6	80	90
	10^4	90	90
	10^6	90	90
II	10^3	90	120
	10^4	90	120
III	-*	90	150
	-*	100	150

*No inoculum applied, but packaged under MAP conditions

University of Stellenbosch, Food Science Culture Collection, was chosen as it was argued that if a time/temperature combination could be obtained that would eliminate the *Bacillus* counts, the other less heat stable indicator organisms would easily be eliminated.

The *B. cereus* strain was inoculated (in triplicate) into the HMD nectarines using two different inoculum concentrations (10^4 and 10^6 cfu.g^{-1}) and two pasteurisation temperatures (80º or 90ºC) for a period of 90 min were used. The inoculum concentrations were determined spectrophotometrically as described in Chapter 4 of this thesis.

For the microbiological enumeration, a duplicate 10 g sample of the HMD nectarines was aseptically removed from each pouch and suspended in 90 ml saline solution (0.85% (w/v) NaCl) and macerated in a stomacher (BagMixer, Interscience, France). A dilution series (10^{-1} - 10^{-6}) of each sample was prepared and then plated in triplicate on Plate Count Agar (PCA) (Biolab) to monitor the viable cell counts. Microbial analyses were done before pasteurisation (time = 0) and then every 30 min as the pasteurisation process proceeded.

Experimental Study II - Effectiveness of steam pasteurisation at 90ºC for 120 min on the survival of Bacillus cereus at inoculum concentrations of 10^3 or 10^4 cfu.g^{-1} in HMD nectarines

The results obtained in Experimental Study I showed that the time/temperature combinations used were not sufficient to eliminate the *B. cereus* strain at the inoculums applied. Thus, based on this data it was decided to increase the pasteurisation time/temperature combination to 120 min at 90ºC (Table 1). The *B. cereus* strain was again chosen as indicator organism because of its thermotolerance, but the inoculum concentration was reduced to 10^3 and 10^4 cfu.g^{-1} to simulate a more realistic microbial load. This decision was based on the data obtained in Chapter 3 of this thesis, where it was found that the microbial contamination of HMDF products are seldom higher than 10^4 cfu.g^{-1}. The monitoring of the survival was done in triplicate on PCA (Biolab) as described previously in Experimental Study I.

Experimental Study III - Microbial content of steam pasteurised (150 min at either 90º or 100ºC) HMD nectarines packaged under modified atmospheric conditions

In Experimental Study I and II, *Bacillus cereus* was used as an indicator to monitor the pasteurisation efficiency at different time/temperature combinations. From the data obtained in those studies it can be argued that the *Bacillus* inoculum size employed is much higher than will ever be found in good quality HMD products, and furthermore, that *Bacillus* is an endospore former, which would also influence the survival rate and thus the final microbial content in the HMD nectarines. It was also observed from the results in Chapter 4 of this thesis that the HMD fruit were heat sensitive, with Maillard browning being a serious problem. It was, therefore, decided to apply MAP to try and minimise the effect of Maillard browning, resulting from the excessive heat treatment. It was decided at this stage to evaluate time/temperature combinations of 90º and 100ºC for 150 min, but specifically on HMD nectarines that had not been inoculated with an indicator organism, to find what effect MAP and this specific time/temperature combination will have on the natural occurring microbial population of HMD nectarines.

For the MAP study the pouches were filled with 250 g HMD nectarines and the pouches were divided into two groups that were steamed in a steam tunnel (Moolenaar, Paarl) at either 90º or 100ºC for 150 min. After the steam pasteurisation, the pouches were flushed for 20 sec with CO_2 gas (Air Products, Cape Town) (30 $l.min^{-1}$ flow rate) and heat sealed using a heat sealer (Audio Futura, Portable Type no. 2, Holland).

The sealed pouches of each specific heat treatment were then further divided into two groups and a set stored at either 5º or 40ºC for 14 days. The higher temperature was chosen to accelerate the growth of any microbes present. The lower temperature was included to evaluate Maillard browning and microbial stability.

For this experimental study, the microbial content of the HMD nectarines was determined by aseptically removing a 10 g sample from each pouch, which was suspended in a 90 ml saline solution (0.85% (w/v) NaCl) and then macerated in a stomacher (BagMixer, Interscience, France). A dilution series (10^{-1} -10^{-6}) of each sample was prepared and then plated in triplicate on each specific medium. The four

media used in this study were Plate Count Agar (PCA) for viable aerobic counts, Violet Red Bile Agar (VRBA) for Enterobacteriaceae, Chloramphenicol Agar (CA) for yeasts and moulds (mycelial fungi) and Reinforced Clostridial Agar (RCA) for the detection of members of the genus *Clostridium* (all from Biolab). Samples were microbiologically monitored before pasteurisation (t_0^1) to determine the influence of the time/temperature combinations on the microbial population, and after 14 weeks at the two storage temperatures.

Results and discussion

Experimental Study I - Effectiveness of steam pasteurisation at either 80° or 90°C for 90 min in eliminating Bacillus cereus at concentrations of 10^4 or 10^6 cfu.g^{-1} in HMD nectarines

The data on the survival of the cells from the 10^4 *B. cereus* inoculum size after the 80° and 90°C pasteurisation for 90 min are summarised in Tables 2 and 3, respectively. The survival rates after the 90 min pasteurisation of the 10^6 *B. cereus* inoculum size at 80° and 90°C, are given in Tables 4 and 5. The enumeration data is also illustrated in the log form in Fig. 1 and 2.

It was found, for both inoculum concentrations that a reduction in microbial numbers took place. For the higher inoculum (10^6) (Fig. 2), the decimal reduction time (DRT) for the 80°C for 90 min pasteurisation was 67 min and for the 90°C for 90 min pasteurisation was 56 min. It was again found from the plotted data (Fig. 2), that heat penetration was not uniform throughout the product. For the lower inoculum concentration (10^4) (Fig. 1), no full decimal reduction was found for either the time/temperature combinations. However, when using the available data for calculation purposes the DRT would be 120 min for the 80°C pasteurisation and 110 min for the 90°C pasteurisation.

Table 2. Microbial enumeration of HMD nectarines inoculated with 10^4 cfu.g^{-1} *B. cereus* per gram of product and processed at 80°C for 90 min in a steam tunnel (average of triplicates).

Pasteurisation time (min)	***B. cereus* counts (cfu.g^{-1})**	**Minimum - maximum (cfu.g^{-1})**
0 (before pasteurisation)	12 000	11 200 - 12 300
30	6 200	6 000 - 6 400
60	4 400	4 200 - 4 500
90	2 800	2 700 - 2 900

Table 3. Microbial enumeration of HMD nectarines inoculated with 10^4 cfu.g^{-1} *B. cereus* per gram of product and processed at 90°C for 90 min in a steam tunnel (average of triplicates).

Pasteurisation time (min)	***B. cereus* counts (cfu.g^{-1})**	**Minimum - maximum (cfu.g^{-1})**
0 (before pasteurisation)	11 000	10 800 - 11 200
30	6 600	6 500 - 6 700
60	4 200	4 000 - 4 400
90	1 300	1 350 - 1 500

Table 4. Microbial enumeration of HMD nectarines inoculated with 10^6 cfu.g^{-1} *B. cereus* per gram of product and processed at 80°C for 90 min in a steam tunnel (average of triplicates).

Pasteurisation time (min)	***B. cereus* counts (cfu.g^{-1})**	**Minimum - maximum (cfu.g^{-1})**
0 (before pasteurisation)	1 100 000	1 000 000 - 1 200 000
30	900 000	750 000 - 910 000
60	530 000	520 000 - 550 000
90	110 000	110 000 - 112 000

Table 5. Microbial enumeration of HMD nectarines inoculated with 10^6 cfu.g^{-1} *B. cereus* per gram of product and processed at 90°C for 90 min in a steam tunnel (average of triplicates)

Pasteurisation time (min)	***B. cereus* counts (cfu.g^{-1})**	**Minimum - maximum (cfu.g^{-1})**
0 (before pasteurisation)	1 200 000	1 190 000 - 1 240 000
30	800 000	780 000 - 830 000
60	230 000	218 000 - 240 000
90	11 000	10 800 - 11 100

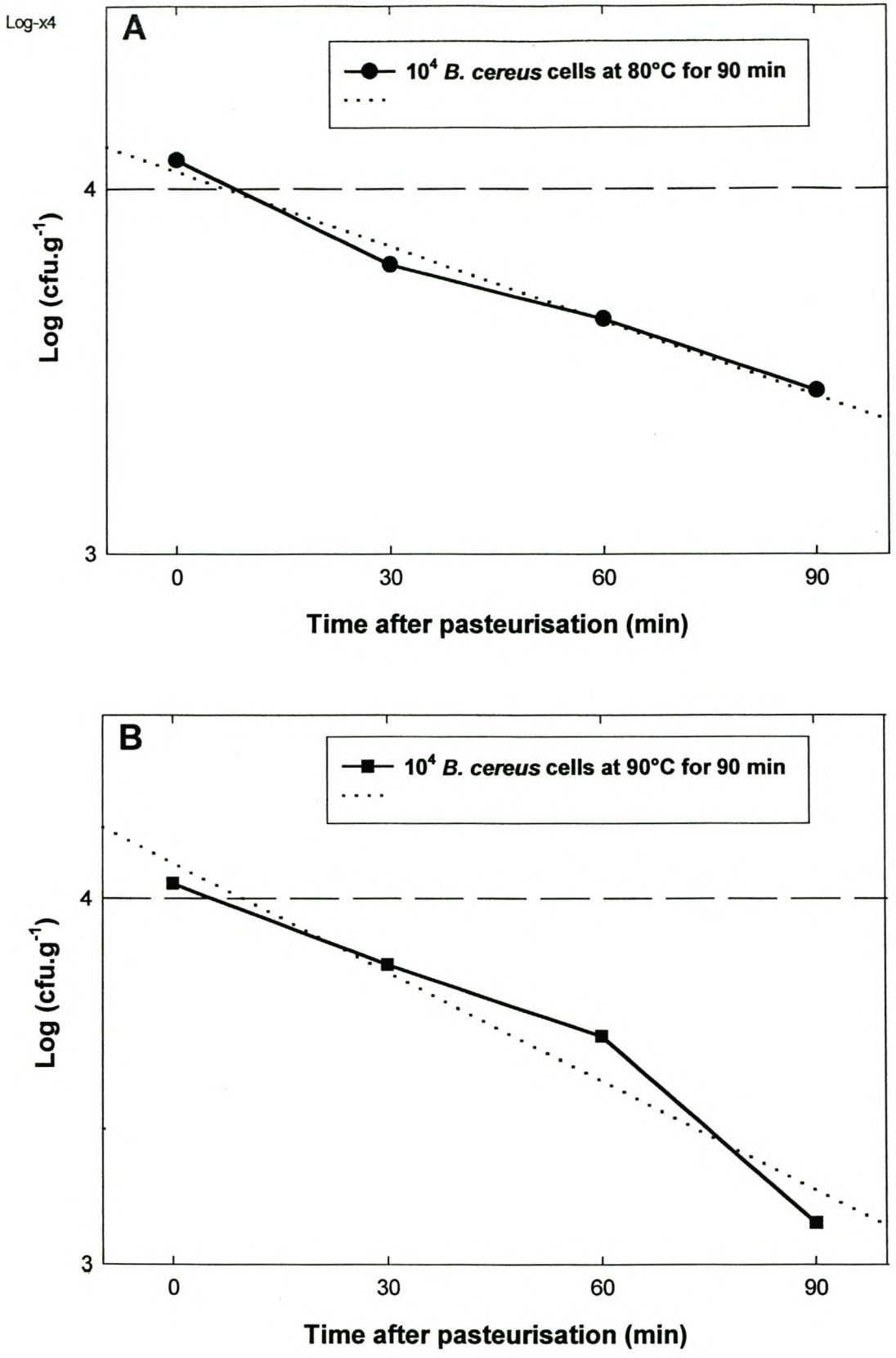

Figure 1. Influence of 80° (A) and 90°C (B) pasteurisation for 90 min on the survival of *B. cereus* (10^4 cfu.g^{-1} inoculum) in HMD nectarines (The dotted line represents the regression of the values).

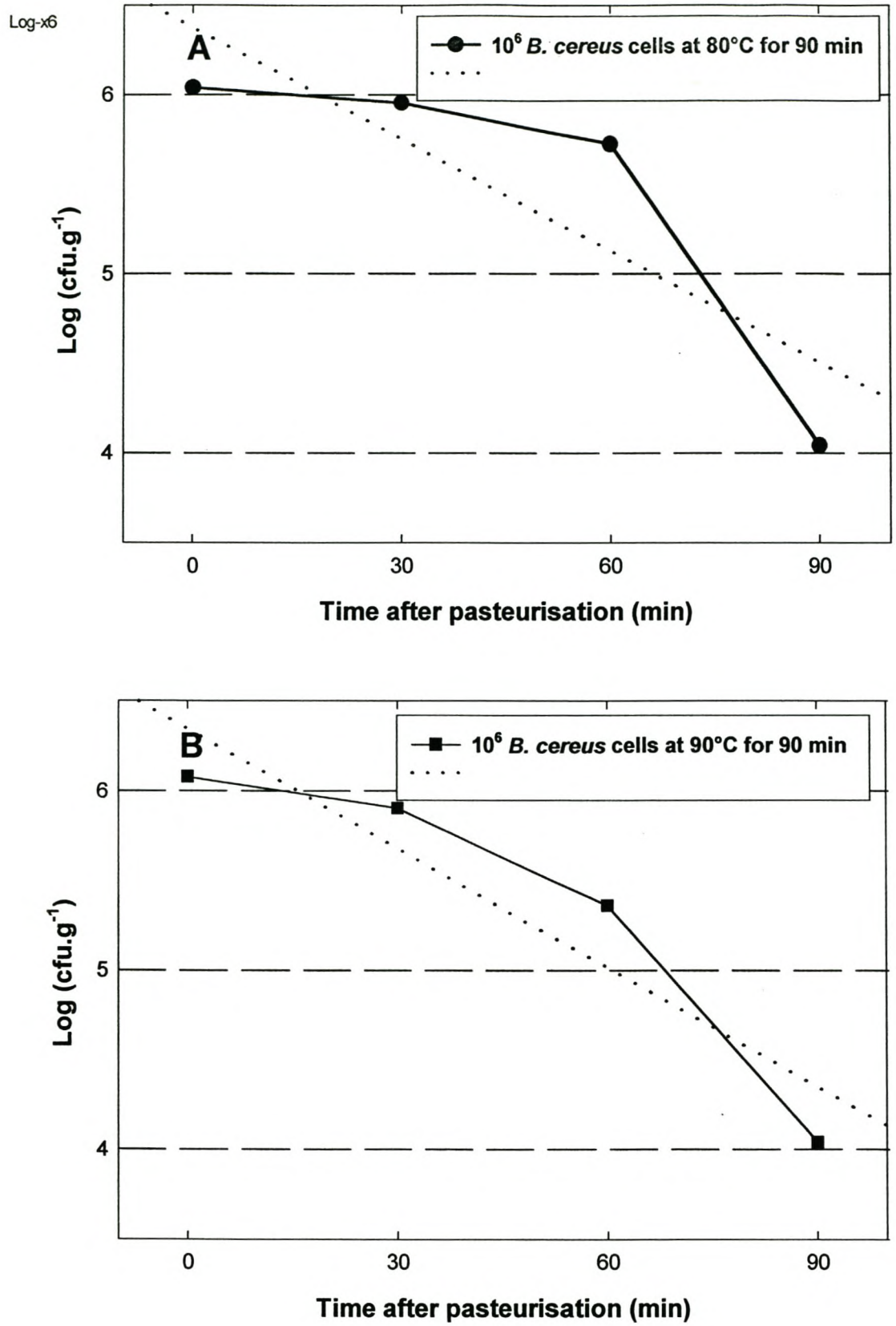

Figure 2. Influence of 80° (A) and 90°C (B) pasteurisation for 90 min on the survival of *B. cereus* (10^6 cfu.g^{-1} inoculum) in HMD nectarines (The dotted line represents the regression of the values).

The wide variation in DRT values for the 10^6 and 10^4 inoculums can probably be ascribed to the poor heat penetration, which was clearly not uniform throughout the HMD nectarine-containing pouches. Thus, DRT variation could also probably be ascribed to the survival and subsequent germination of endospores, as well as to the protection due to the physical form and composition of the fruit. The results indicated that the specific time/temperature steam pasteurisation combinations used in this experimental study were not effective enough to ensure a shelf-stable product.

This aspect is further reinforced when taking the F-value of the death curve into consideration. The F-value can be defined as the equivalent time, in minutes, of all heat considered at a specific temperature, with respect to its capacity to destroy spores or vegetative cells of a particular organism (Jay, 1996). The calculated F-value for the 10^4 inoculum, pasteurised at 80°C for 90 min was 410 min and that of the 10^6 inoculum was F = 595 min (Fig. 1). The F-values when a pasteurisation temperature of 90°C was applied, was F = 285 min for the 10^4 inoculum and F = 310 min for the 10^6 inoculum (Fig. 2). This clearly indicates the inability of the specific pasteurisation time/temperature combination to destroy the indicator organism. Thus, it is advised that the length of the heat treatment must be increased or that the pasteurisation temperature must be increased.

Experimental Study II - Effectiveness of steam pasteurisation at 90°C for 120 min on the survival of Bacillus cereus at inoculum concentrations of 10^3 or 10^4 cfu.g^{-1} in HMD nectarines

Based on the results from Experimental Study I that clearly indicated the inefficiency of the chosen time/temperature combination to eliminate the *B. cereus* strain at the inoculums applied, it was decided to increase the pasteurisation time and to lower the inoculums applied, making it more representable of natural contamination in HMDF. The data on the survival of the cells from the 10^3 and 10^4 inoculums at 90°C pasteurised for 120 min, are summarised in Tables 6 and 7. The enumeration data is also illustrated in the log form in Fig. 3.

Table 6. Microbial enumeration of HMD nectarines inoculated with 10^3 cfu per g of *B. cereus* and processed at 90°C for 120 min in a steam tunnel (average of triplicates).

Pasteurisation time (min)	***B. cereus* counts (cfu.g^{-1})**	**Minimum - maximum (cfu.g^{-1})**
0 (before pasteurisation)	1 200	1 000 000 - 1 200 000
30	970	750 000 - 910 000
60	630	520 000 - 550 000
90	410	110 000 - 112 000
120	200	190 - 210

Table 7. Microbial enumeration of HMD nectarines inoculated with 10^4 cfu per g of *B. cereus* and processed at 90°C for 120 min in a steam tunnel (average of triplicates).

Pasteurisation time (min)	***B. cereus* counts (cfu.g^{-1})**	**Minimum - maximum (cfu.g^{-1})**
0 (before pasteurisation)	16 000	15 300 - 17 200
30	14 500	13 900 - 15 600
60	5 500	4 300 - 5 700
90	2 600	2 300 - 2 700
120	510	480 - 560

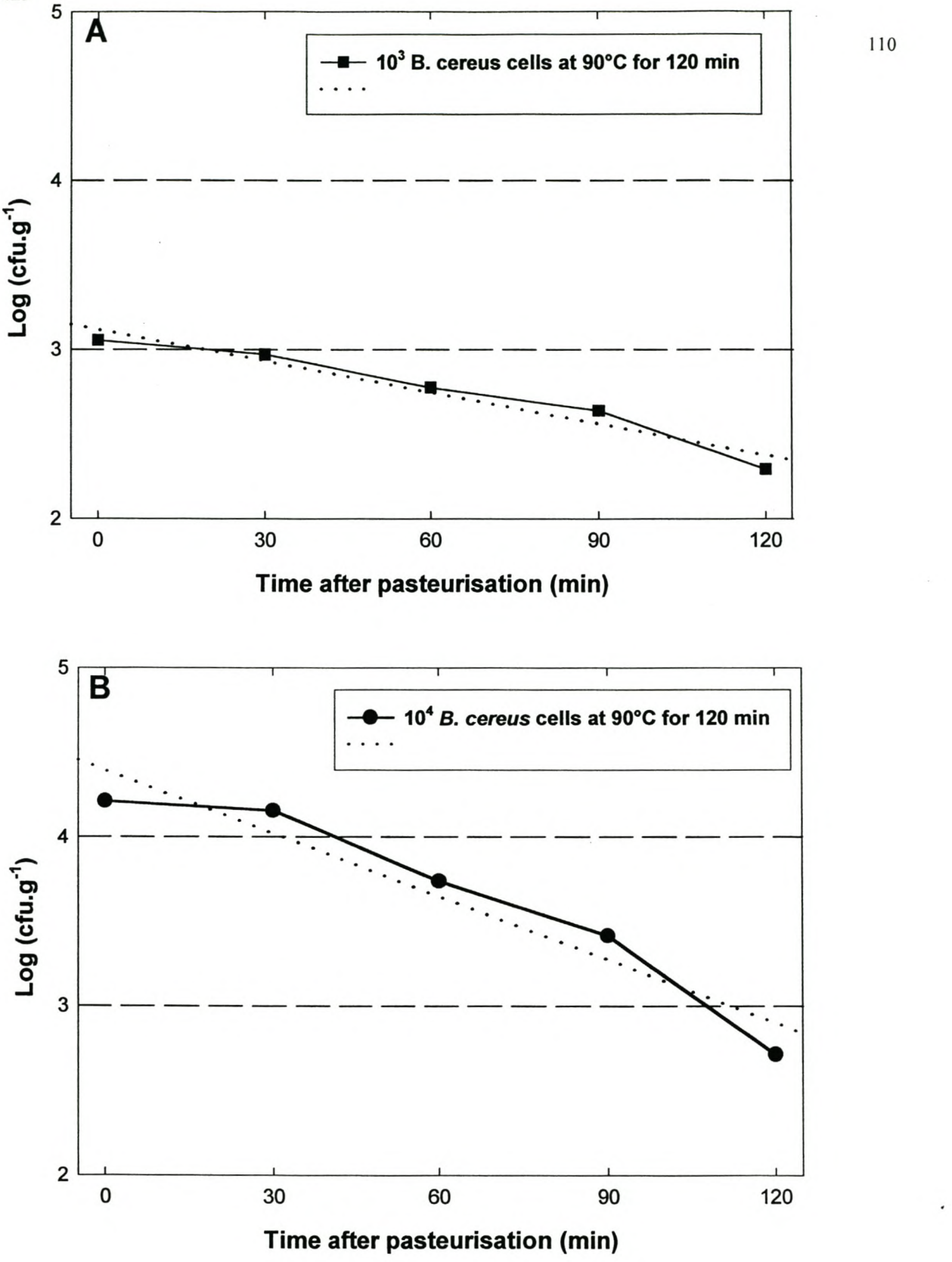

Figure 3. Influence of 10^3 (A) and 10^4 (B) cfu.g^{-1} inoculum sizes on the survival of *B. cereus* after pasteurisation at 90°C for 120 min in HMD nectarines (The dotted line represents the regression of the values) .

In this study, the data showed that there was no decimal reduction for the 10^3 inoculation size, but for the 10^4 inoculum a decimal reduction was found (Fig. 3). However, when the available data was used to determine the DRT it was found to be 141 min for the 10^3 inoculum and 99 min for the 10^4 inoculum. In both inoculum cases it was found that a steady decrease only started 30 min of heat treatment. It was also found that the decline was steeper in the case of the 10^4 inoculation. The data clearly showed that even after 120 min, not all the cells were eliminated and sufficient numbers remained to be a possible cause of spoilage. Although most spoilage microbes that have been associated with HMD product spoilage are not as thermostable as *B. cereus*, this organism is an excellent indication of the efficiency of the time/temperature combinations that can be applied during the pasteurisation method.

The data obtained for the 120 min pasteurisation at 90°C indicated that a further optimisation is necessary. It is possible that if the time period is increased with a further 30 min, to 150 min at 90°C, a better reduction of cells would be found. The study also showed that it is extremely important to make absolutely sure that the steam tunnel is at the required temperature, as this will influence the cell reduction. The calculated F-values (Jay, 1996) for these two inoculum concentrations at the 90°C pasteurisation temperature were, 210 min for the 10^3 inoculum and 350 min for the 10^4 inoculum. It was interesting to note that the F-value for the 10^4 inoculum of this study was very similar to the F value of 310 min as found in Experimental Study I. The difference between the F-values can be ascribed to the heterogeneous nature of the dried fruit and the fact that heat penetration is not the same throughout the pouch.

Experimental Study III - Microbial content of steam pasteurised (150 min at either 90° or 100°C) HMD nectarines packaged under modified atmospheric conditions

In Experimental Studies I and II, *Bacillus cereus* was used as an indicator to monitor the pasteurisation efficiency at different time/temperature combinations. From the data obtained in these studies it can be argued that the *Bacillus* inoculum concentration employed, is much higher than will ever be found in good quality HMD products, and furthermore, that *Bacillus* is an endospore former, which would also

influence the survival rate and thus the final microbial content in the HMD nectarines. It was also observed from the results in Chapter 4 of this thesis, that the HMD nectarines were heat sensitive, with Maillard browning being a serious problem. Thus, it would not be advisable to increase the temperature, but rather to evaluate a longer temperature period. It was, therefore, decided to also apply MAP to try and minimise the effect of Maillard browning, resulting from the excessive heat treatment and the oxygen present in the pouches. In this study, time/temperature combinations of 90° and 100°C for 150 min were evaluated, but specifically on HMD nectarines that had not been inoculated with an indicator organism. The reason for this was to determine the effect of MAP at this specific time/temperature combination on the natural occurring microbial population and colour stability of HMD nectarines.

The data obtained from the study are summarised in Tables 8 and 9 and the data from the PCA and CA enumerations are plotted in Fig. 4 and 5. The viable microbial numbers of the HMD nectarines pasteurised at 100°C were found to be lower than those from the 90°C treatment. It was also found that both the pasteurisation temperatures were effective in minimising the yeast and mould counts (CA-medium). The viable aerobic bacterial counts and the yeast and mould counts obtained in this Experimental Study (III) for the HMD nectarines, before and after pasteurisation, were not much higher than the numbers found during the evaluation of the microbial content of a range of commercially available HMD products (Table 2 in Chapter 3 of this thesis), and were also found to be lower than the standards set by SAD as given in Table 4 in Chapter 3 of this thesis.

The colony formation and morphological characteristics of the PCA colonies clearly showed that they were members of the aerobic endospore forming *B. cereus*. In the case of the colonies from the CA-medium, moulds were mostly found, with only a few yeast colonies. The initial count on the CA-medium was very high at 88 000 $cfu.g^{-1}$. This was expected, as yeasts and moulds are the most frequently isolated organisms from HMDF (El-Halouat *et al.*, 1998).

When the influence of storage temperature was evaluated, a further reduction in numbers was found after the 14-day storage at 5°C. In the case of storage at 40°C,

Table 8. Microbial content of HMD nectarines steam pasteurised for 150 min at 90°C and flushed with CO_2.

*Medium	Time			
	**t_0 (cfu.g^{-1})	***t_0^1 (cfu.g^{-1})	2 weeks storage at 5°C (cfu.g^{-1})	2 weeks storage at 40°C (cfu.g^{-1})
PCA	25 000 [21 000 - 28 000]	8 500 [7 400 - 8 800]	600 [510 - 650]	1 200 [980 - 1 350]
VRBA	0	0	0	0
CA	88 000 [86 000 - 91 000]	850 [680 - 890]	350 [320 - 370]	0
RCA	0	0	0	0

*Media: PCA = Plate Count Agar for viable aerobic count
VRBA = Violet Red Bile Agar for Enterobacteriaceae
CA = Yeasts and moulds (mycelial fungi)
RCA = Reinforced Clostridial Agar for detection of *Clostridium*

Time: **t_0 = before pasteurisation
***t_0^1 = directly after pasteurisation

Range: Values in square brackets represent the minimum - maximum (cfu.g^{-1}) counts found

Table 9. Microbial content of HMD nectarines steam pasteurised for 150 min at 100°C and flushed with CO_2.

Medium*	Time			
	t_0 (cfu.g^{-1})	***t_0^1 (cfu.g^{-1})**	**2 weeks storage at 5°C (cfu.g^{-1})**	**2 weeks storage at 40°C (cfu.g^{-})**
PCA	25 000 [21 000 - 26 000]	6 500 [6 100 - 6 700]	450 [380 - 470]	980 [900 - 1 100]
VRBA	0	0	0	0
CA	88 000 [82 000 - 90 000]	760 [710 - 780]	150 [120 - 170]	0
RCA	0	0	0	0

*Media: PCA = Plate Count Agar for viable aerobic count
VRBA = Violet Red Bile Agar for Enterobacteriaceae
CA = Yeasts and moulds (mycelial fungi)
RCA = Reinforced Clostridial Agar for detection of *Clostridium*

Time: **t_0 = before pasteurisation
***t_0^1 = directly after pasteurisation

Range: Values in square brackets represent the minimum - maximum (cfu.g^{-1}) counts found

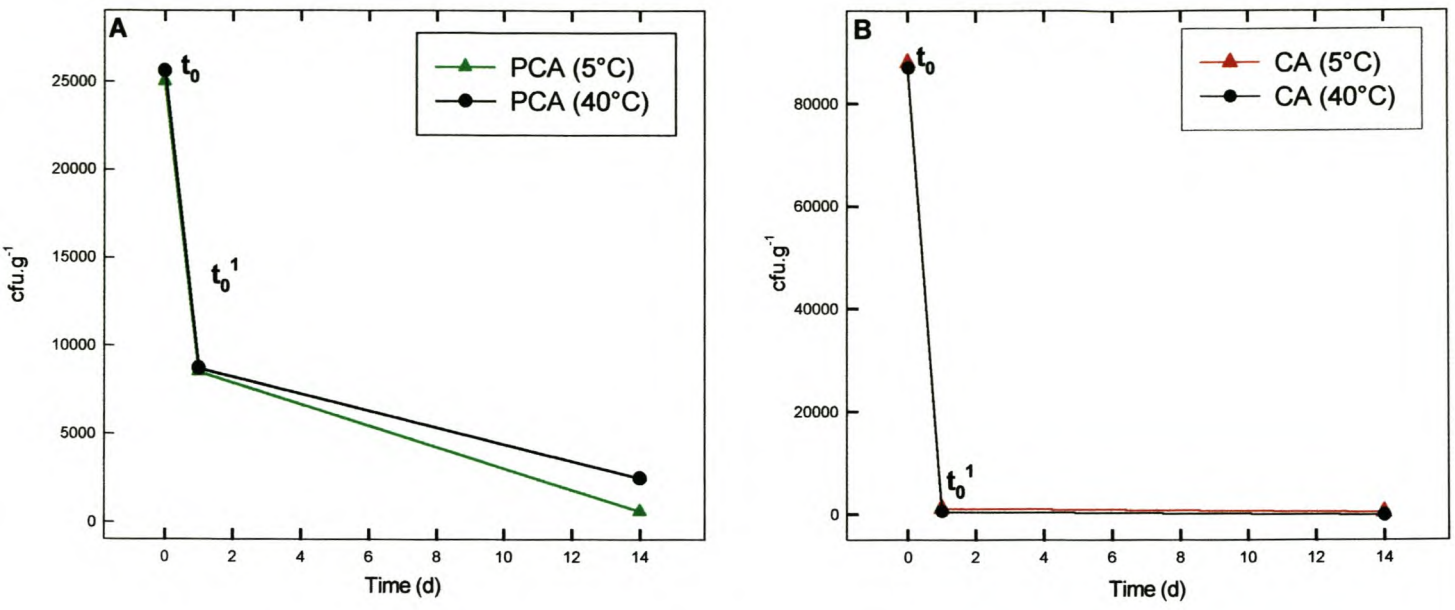

Figure 4. Microbial content of HMD nectarines pasteurised at 90°C for 150 min, packaged under MAP conditions and stored at 5° and 25°C, respectively for 14 d (A = PCA and B = CA) (t_0 = before pasteurisation; t_0^1 = directly after pasteurisation).

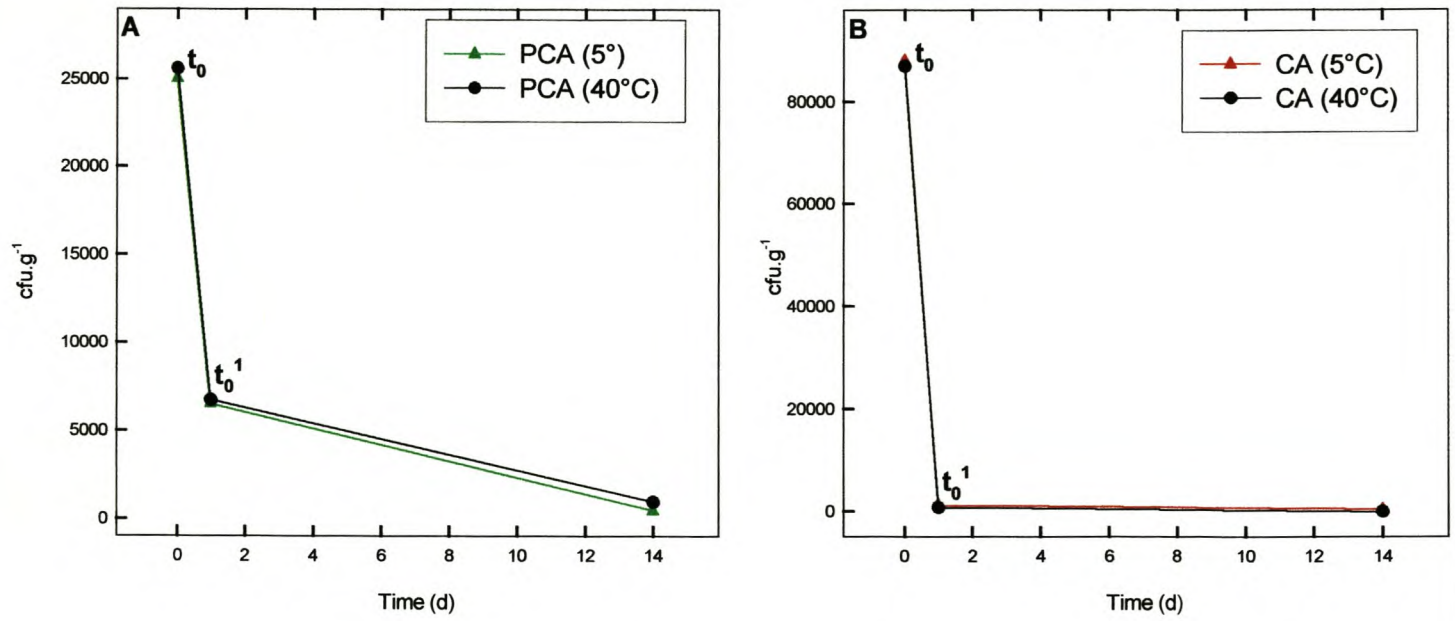

Figure 5. Microbial content of HMD nectarines pasteurised at 100°C for 150 min, packaged under MAP conditions and stored at 5° and 25°C, respectively for 14 d (A = PCA and B = CA) (t_0 = before pasteurisation; t_0^1 = directly after pasteurisation).

the moulds were completely eliminated but at this storage temperature an increase in the PCA numbers was found after 14 days. This was expected as the higher storage temperature favours the growth and survival of aerobic endospore formers. It was found that the CA counts were slightly higher for the HMD nectarines stored at 5°C than those stored at 40°C. This was expected because yeast and moulds are mostly psychrophiles and their growth is favoured at lower temperatures.

No members of the Enterobacteriaceae (VRBA) were detected and this was taken as an indication of good hygienic processing conditions. These results compared well with the data found in Chapter 4 of this thesis where HMD nectarines and prunes were inoculated with *Escherichia coli* as indicator of the Enterobacteriaceae.

In this study, CO_2 was used to flush the product to ensure a better colour retention of the end-product and to determine if members of the genus *Clostridium* were present in the semi-anaerobic atmosphere of the HMD nectarine pouches. The RCA-medium was thus included as a selective medium, as it specifically selects for members of the genus *Clostridium*. However, no members of the genus *Clostridium* were found in the HMD nectarines.

The SO_2 concentration of the HMD nectarines stored at 40°C were lower (884 $mg.kg^{-1}$ for the 100°C pasteurisation and 987 $mg.kg^{-1}$ for the 90°C pasteurisation) than those stored at 5°C (987 $mg.kg^{-1}$ for the 100°C pasteurisation and 1 023 $mg.kg^{-1}$ for the 90 min pasteurisation). This can be explained because Maillard browning occurs more rapidly at the higher pasteurisation and storage temperatures. The colour of the samples pasteurised at 90°C for 150 min and stored at 5°C was the best and it produced a microbial stable HMD nectarine end-product.

Conclusion

In this study the optimum steam pasteurisation time/temperature combination necessary to eliminate the endospore former *B. cereus*, was evaluated. The impact that MAP would have on HMD nectarines that were not inoculated with an indicator organism was also evaluated. The first time/temperature combinations that were

evaluated were 90 min steam pasteurisation at 80° or 90°C, with an inoculum concentration of 10^4 and 10^6 cfu.g^{-1} *B. cereus*. As expected the 90°C pasteurisation temperature gave a better elimination of *B. cereus* cells, but it was still not effective in achieving total elimination. It was thus decided to lengthen the pasteurisation time to 120 min and reduce the inoculum to 10^3 and 10^4 cfu.g^{-1}, making it more representative of the normal microbial content of HMDF products. As the indicator organisms were still not totally eliminated and counts between 190 and 560 cfu.g^{-1} were still found, the pasteurisation time was increased to 150 min.

At this stage it was argued that *B. cereus* is very difficult to eliminate totally, because of its capability to form endospores that are thermostable. Therefore, it was decided to exclude the indicator organisms and to increase the pasteurisation time to 150 min. The HMD nectarines were steam pasteurised at 90° or 100°C for 150 min and after pasteurisation the nectarines were stored at 5° and 40°C for 2 weeks. Nectarines are also extremely colour unstable and, therefore, MAP was applied to minimise the effect of Maillard browning. From these results, it was concluded that the 100°C pasteurisation temperature was more efficient in terms of microbial reduction than the 90°C temperature, but the colour stability of the samples pasteurised at 90°C pasteurisation was more stable.

From these results, it was concluded that a steam pasteurisation of at least 150 min at 90°C should be applied, because of the better colour stability. It is also important that the pasteurisation process is at the correct temperature, to ensure that all the HMD nectarines are subjected to a constant temperature as nectarines are heterogeneous and constant heat penetration throughout the product is very difficult to obtain. It is thus advisable to use other preservatives in combination with the pasteurisation and SO_2 treatment in the product to form hurdles that will prevent microbial spoilage and colour degradation, ensuring a shelf-life of at least six to nine months.

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CHAPTER 7

GENERAL DISCUSSION AND CONCLUSIONS

Background

Sun drying of fruit is one of the oldest processing techniques applied in the preservation of food. Traditional dried fruit remains very popular, but there is a world-wide trend towards dried fruit with a higher moisture content that is softer and more palatable (Salunkhe *et al.*, 1991). The SAD (SA Dried Fruit Co-operation Ltd.) followed this trend and produced a "soft-eating", high moisture dried fruit (HMDF) product range that includes cling stone peaches, apricots, nectarines, prunes and raisins. The HMDF are produced by rehydrating conventional dried fruit to a moisture content of *ca* 36% (w/w) and a water activity (a_w) of *ca* 0.85, where after the products are pasteurised for 7 h at 80°C. It is this increase in the moisture content that leads to a serious microbial spoilage problem and a shortened product shelf-life. Although the fruit are pasteurised, the process is not totally effective in removing all the microbes and spoilage remains a problem. An additional defect is the colour instability, especially with the HMD nectarines, resulting from Maillard browning that is enhanced at the higher pasteurisation temperatures. SAD exports HMDF products and to be successful in the competitive market abroad, they have to ensure a quality product with a shelf-life of at least nine months. This study was thus undertaken to determine the microbial content and composition of commercially available HMDF products and to challenge HMDF with indicator organisms chosen to represent the microbial population mostly found in the commercially available HMDF. The priority was to minimise the presence of spoilage microbes, so as to produce a quality HMDF product by changing the processing conditions.

Microbial content of commercially available HMDF

The higher a_w and moisture content of HMDF makes these products more prone to spoilage and it is thus important to determine which microbes are generally present in commercially available South African HMDF products.

From the data obtained in Chapter 3, it was found that HMD prunes and raisins had the highest microbial counts. The most numerous bacteria found were members of the aerobic endospore forming genus *Bacillus*, while the yeast and mould (mycelial fungi) counts were very high especially in the HMD apricots (1 144 cfu.g^{-1}). Lactobacilli were also found, but the numbers were fairly low (< 300 cfu.g^{-1}) and it was thus concluded that Lactobacilli should under normal processing conditions not be a problem in HMDF products. Members of the genus *Staphylococcus* were found in the HMD raisins, as well as *Salmonella* in the HMD prunes, indicating processing limitations and possible food safety implications. Thermoduric organisms were also detected in the HMD prunes and the presence of these organisms are a clear indication that the current pasteurisation process of 7 h at 80°C is not totally adequate in ensuring microbial stable HMDF products. It was, therefore, essential that the efficiency of the current pasteurisation process be evaluated and that modifications of the process be considered.

Challenging of HMD nectarines and prunes with indicator organisms

Based on the data obtained in the previous section, the efficiency of the pasteurisation method currently used in the processing of HMDF products (7 h at 80°C) was evaluated in this study (Chapter 4) by selecting representative organisms of the microbial population found in the commercially available products (Chapter 3). The indicator organisms used included strains of the endospore former *Bacillus cereus*, the osmotolerant yeast *Zygosaccharomyces rouxii*, the mycelial fungus *Penicillium chrysogenum* and *Escherichia coli* as representative of the Enterobacteriaceae. From the data obtained in Chapter 3, HMD nectarines and prunes were chosen as study material, as nectarines are colour unstable and prunes generally have a higher product pH of 4.0 and, therefore, spoil fairly easily. The microbial challenging studies were done over a 6 month storage period at either 5° or 25°C to evaluate survival and growth of the indicator organisms.

The challenging data (Chapter 4) clearly showed that despite the 80°C for 7 h pasteurisation step, a large number of three of the four indicator organisms survived the HMDF pasteurisation step. Even though a further

decline took place over the 6 month storage period, enough microbes survived the process to indicate that their presence, when found in high numbers in the raw product, must be considered a serious spoilage risk. Therefore, it is advisable that the current pasteurisation process be optimised in terms of the time/temperature combinations or that natural preservatives be included in the HMDF products to ensure a safe quality product. Furthermore, the data obtained clearly showed that it is advantageous to store HMDF products at a lower temperature (5°C) so as to minimise microbial growth and Maillard reactions, ensuring a quality product.

Impact of preservatives on the survival and growth of *B. cereus* and *Z. rouxii* in nectarine puree

In this study (Chapter 5) nectarine puree, prepared from conventional dried nectarines, was used as a model to simulate the survival of selected indicator organisms in HMD nectarines. The puree samples were inoculated with either *B. cereus* or *Z. rouxii* as representative indicator organisms, and their survival and growth were evaluated after the addition of five different commercially available preservatives, namely lysozyme, pimaricin, potassium sorbate (K-sorbate), sodium benzoate (Na-benzoate) and an imidazole compound. The best inhibition results for *B. cereus* was obtained with lysozyme (70% inhibition after 12 h). This was expected, as lysozyme is known to be effective against Gram-positive bacteria. Overall, it was found that it is extremely difficult to eliminate *B. cereus*, because of its ability to form environmentally resistant endospores.

Zygosaccharomyces rouxii was much more susceptible to the addition of the selected antifungal preservatives. Na-benzoate and K-sorbate were both very effective (100% inhibition after 24 h) and the fact that the added levels were not higher than the recommended maximum levels set by South African legislation (Anon., 1972), is of value to the SA dried fruit industry. The possibility that the *Z. rouxii* strain could probably metabolize lysozyme was of interest, and it is important to take this into consideration when intending to use a combination of preservatives to control the yeast and bacterial levels in HMDF.

It is also important to note that nectarine puree was used as model to simulate processing conditions of HMD nectarines. This was done because microbial inhibition profiles are much easier to monitor in a liquid medium than on solid heterogeneous medium such as found with HMD nectarines. It is of importance to take into consideration that the puree had a higher a_w than that found for HMD nectarines. Thus, indicator organisms would much easier be eliminated in HMDF, as the optimum a_w for growth of *B. cereus* and *Z rouxii* is closer to the a_w of the puree than is found in the HMDF.

Evaluation of different time/temperature pasteurisation combinations

In this study (Chapter 6), different pasteurisation time/temperature combinations were evaluated to determine the survival rate of the endospore former *B. cereus*, as well as the effect that modified atmosphere packaging (MAP) will have on the natural microbial composition of HMD nectarines. The first time/temperature combinations that were evaluated was a 90 min pasteurisation at either 80° or 90°C, with an inoculation concentration of either 10^4 and 10^6 cfu.g^{-1} *B. cereus* cells per gram of the product. As expected, the 90°C pasteurisation temperature gave a better elimination of *B. cereus* cells, but total removal was still not obtained. Therefore, it was decided to increase the pasteurisation time to 120 min and to decrease the inoculum to 10^3 and 10^4 cfu.g^{-1}, making the microbial content more representative of the normal microbial content of HMDF products. However, the *Bacillus* strain was still not totally eliminated and counts between 190 and 560 cfu.g^{-1} were still found. Therefore, it was decided to increase the pasteurisation time to 150 min.

At this stage it was argued that *B. cereus* is very difficult to eliminate totally because of the organisms apability to form endospores that are thermostable. Therefore, it was decided not to add an indicator organism, but to determine what effect an extended pasteurisation time of 150 min would have on/in the normal microbial population present on HMD nectarines. High moisture dried nectarines are also colour unstable and, therefore, MAP was applied to try and minimise the effect of Maillard browning. One aspect that must be taken into consideration when using MAP, is the possibility of members of the anaerobic genus *Clostridium* growing in these low oxygen

environments. The study parameters were also extended to evaluate the microbial stability of MAP packaged HMD nectarines after pasteurisation at either 90° or 100°C for 150 min. After pasteurisation the nectarines were stored at 5° and 40°C for 2 weeks.

From these results, it was concluded that the 100°C pasteurisation temperature was more efficient in terms of microbial elimination than the 90°C temperature. However, the colour of the fruit pasteurised at 100°C was not stable. It was, therefore, recommended that a pasteurisation time/temperature combination of 150 min at 90°C be applied and that it would be advantageous for the product to be stored at 5°C. It was also observed that it is important that the pasteurisation tunnel is at the optimum temperature, as nectarines are heterogeneous and a constant heat penetration throughout the product is very difficult to obtain. It is further recommended that other preservatives together with the pasteurisation and SO_2 are used in the product so as to form hurdles that will prevent microbial spoilage and colour degradation, ensuring a shelf-life of at least six to nine months.

Concluding remarks

This research lead to a new outlook on the microbial composition of HMDF, as there has been very little research done in the past on the microbial content of HMDF products. From the microbial content of the South African commercial HMDF products it could be concluded that the current pasteurisation method used by the dried fruit industry should be modified to ensure the elimination of spoilage microbes.

It is also important that future research should be done to evaluate combinations of natural preservatives together with a lower concentration sulphur dioxide to make South African HMDF products more acceptable to the European market. However, with the lowering of the SO_2, the colour stability of the HMDF will be negatively affected and it is, therefore, advisable that MAP is applied as part of the processing method.

Further studies should also be done to evaluate the best method for addition of alternative preservatives to the HMDF products, so that the

preservatives are effective in eliminating spoilage microbes. It is also important to do further research on the microbes that are frequently isolated from the HMDF and to determine the main contamination sources.

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