

DEVELOPMENT OF A SULPHUR FREE LITCHI STORAGE PROTOCOL USING SEALED POLYPROPYLENE BAGS

By

Alison Joy Archibald

*Dissertation presented for the Degree of Master of Science (Agric) at the University
of KwaZulu – Natal*

December 2006

Supervisor: Prof. J. P. Bower
School of Agricultural Science and Agribusiness
Discipline of Horticultural Science
University of KwaZulu – Natal

Co – Supervisor: Dr. I. Bertling
School of Agricultural Science and Agribusiness
Discipline of Horticultural Science
University of KwaZulu – Natal

DECLARATION

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

Student: J Archibald

Date: 21/06/07

Supervisor: J Power

Date: 29-6-07

SUMMARY

The use of sulphur as a method of postharvest disease control and colour retention in litchis is soon to be restricted by the European Union. It is therefore essential that new postharvest treatments and packaging techniques be developed in order to retain internal and external fruit qualities and thus allow for export. Good litchi quality is not only important for the export market but also for use on the local market.

In this study, alternative methods for postharvest quality control were investigated with the aim of extending the litchi storage life to 40 days under modified storage. Packaging the fruit in polypropylene bags significantly decreased fruit water loss and resulted in an increase in shelf life, as determined by red colour and overall rind appearance. There was no distinct advantage of a modified atmosphere. The use of a punnet, lined with absorbent sheeting and placed within the sealed polypropylene bag, further improved the shelf life. The absorbent sheeting reduced the amount of free water and resulted in little pathogen infection, while the punnet was effective in protecting the fruit from damage. It was notable that most water loss occurred within the first 10 days of storage and that the majority would actually take place during the cooling phase. A hydrocooling technique was therefore investigated and was found to not significantly decrease water loss, possibly due to not hydrocooling the fruit for a long enough period of time.

Temperature management was extremely important for both colour retention and pathogen control. It was found that treatments stored at 5.5°C showed better colour retention after the 40 days storage than the 1°C storage treatment. The higher storage temperature, however, enhances the potential for postharvest diseases. Three compounds, namely ISR 2000, 'Biosave' and F10, were tested for pathogen

control. 'Biosave' showed the best results with the most effective concentration being 100 ml/l water and good pathogen control occurred when storage was at 1^o C. Polyphenol oxidase (PPO) activity in the litchi rind was evaluated as it is thought to be closely related to browning of litchi fruit, probably due to the degradation of phenolics by PPO. Brown fruit had a high PPO activity whilst red fruit had much lower activity. It was also shown that PPO activity decrease over storage time, possibly due to product inhibition of the enzyme.

The internal quality of the fruit was determined using the TSS: acid ratio of the pulp, as it is well correlated to mean eating quality. For fruit to have excellent taste, it must have a TSS: acid ratio of between 31:1 and 60:1. All the fruit had a ratio that met this criterion and would therefore ensure good eating quality.

Mom and Dad

In recognition of all the opportunities, love and support you have given me

ACKNOWLEDGEMENTS

I am grateful to the following people and institutions:

- My supervisor, Prof. John Bower, who has provided much support and understanding, especially during my time away. Without your commitment and scientific contribution this work would not be possible.
- My co-supervisor, Dr. Isa Bertling, for her technical insight and assistance with physiological laboratory procedures. Thank you for always being available when I was uncertain.
- Litchi Growers' Association of South Africa for their generous funding of this work.
- Producers in Salt Rock, Port Shepstone and Malelane areas for kindly donating fruit and providing trial sites.
- Joanie Kift for conducting and monitoring the Malelane trials when I could not be there.
- Terry Denison and Gail Papli for their help in packaging and evaluating fruit. Without your assistance I would have spent many more hours organizing these trials.
- Fellow students, Allan, Martin, Michelle and Phillip, who assisted when everyone was too busy and were of great help in getting laborious work done.
- To my brother, Paul, for giving up his time to assist with preharvest treatments.
- To Sakata MayFord and staff for giving me the time needed to complete this work and for always urging me to finish. I am grateful for all the motivation and help.
- My parents (Roy and Jill), siblings (Juliet, Paul and James) and friends for their continued support and encouragement when things were tough and time limited.

CONTENTS	PAGE
LITERATURE REVIEW	1
PAPER 1: Litchi postharvest preservation using packaging and modified atmosphere techniques.	20
PAPER 2: The effect of temperature management of the retention of litchi colour and quality.	46
PAPER 3: The effect of packaging and biological disease control on the shelf life of litchi fruit.	63
FINAL CONCLUSIONS	81
RECOMMENDATION	85

LITERATURE REVIEW

INTRODUCTION

The Litchi (also called Lychee or Leechee) belongs to the family Sapindaceae that includes several other exotic fruits. Pierre Sonnerat was the first to give a full description of this fruit in 1782, naming it *Litchi chinensis* (Oosthuizen, 1992). As its scientific name implies, Litchi has its origin in China, where it has been grown in the southern Kwangtung province for thousands of years (Cull & Lindsay, 1995). Although this crop has been grown and sold in China over some time, the culture of the crop has not advanced to the high level that we find in citrus and deciduous fruit.

With the increasing popularity of exotic fruits on the worldwide market, litchi production has steadily grown in importance. Over the past 5 litchi seasons, South Africa has exported an average of 4 million tones of which 95% are destined for the European Union (P.P.E.C.B. Export Directory, 2006).

As export marketing of litchi requires transportation times of at least two weeks, the short storage life of litchis under ambient conditions (from 2 to 7 days at 25°C) has been a significant impediment to this trade (Underhill, Coates & Saks, 1997). It was not until the late 1940s that reports of postharvest research aimed at establishing suitable storage conditions and packaging materials for litchis first appeared in scientific literature (Marloth, 1949). Initial research involved evaluation of various forms of plastic packaging (Mukerjee, 1957; Cambell, 1959) and optimization of storage temperatures (Thompson, 1956). Macfie (1955) and Dennison and Hall (1957) noted that litchi storage life was primarily limited by physiological browning and disease (Underhill, Coates & Saks, 1997). The litchi rind is relatively thin and lacks a thick, durable cuticle. Consequently, desiccation is a major factor during postharvest storage life. As a result of desiccation, litchi

fruit rapidly lose colour and unless treated immediately after harvest, will turn an unsightly brown colour (Kaiser, 1998). Much research has been undertaken with the hope of solving the problem; however, this continues to be the greatest challenge in the storage of litchis.

The first breakthrough in controlling both browning and disease involved chemical fungicide treatment, and fruit over – wrapped with a semi permeable film with storage at 5°C. This was a result of research by Scott *et al.* in 1982. This meant that fruit could now be commercially stored for up to four weeks (Tongdee *et al.*, 1982; Huang & Scott, 1985). Packaging, temperature control and chemical treatments still remain the most important methods of postharvest control. However, Tan & Li (1984) and Swarts (1989) found that fumigation of fruit with SO₂ could suppress browning of fruit. The problem of poor colour recovery was only really overcome with additional low pH treatments employed by Zauberman *et al.* (1989). Presently, the South African litchi industry uses sulphur fumigation to prevent browning, but sulphur is undesirable for several reasons. It causes off-tastes, is ineffective against some fungi and constitutes a potential health problem. Furthermore, overseas markets have lowered the tolerance level of sulphur to 10 mg/kg sulphur in the fruit flesh. Consequently, it was imperative that research aimed at eliminating sulphur fumigation must be undertaken. (Kaiser, 1998). The European Union has recently severely restricted the use of sulphur and it will probably eventually be banned.

PHYSIOLOGY

Litchi fruit, although of tropical origin, is non-climacteric and able to endure low storage temperatures. Respiration and ethylene production can be greatly reduced using low temperatures, but they increase rapidly when transferred to temperatures of 25°C (Jiang – Ping *et al.*, 1986).

Mature litchi fruit are characterised by a uniform red pericarp colour. Anthocyanin, the main red pigment, is synthesised in the pericarp at around 60 to 80 days after anthesis, coinciding with a degradation of chlorophyll (Paull *et al.*, 1984; Jaiswal *et al.*, 1987 ; Underhill & Critchley, 1994). The anthocyanin pigments are vacuole bound appearing in the upper mesocarp tissue immediately below the pericarp protuberance apices. Anthocyanin distribution is progressively extended, forming a continuous layer in the mesocarp and epicarp tissue (Underhill & Simons, 1993).

The internal quality of the litchi is measured by the amount of total soluble solids (TSS), with the main sugars in a mature fruit being that of sucrose, fructose and D – glucose. These sugars account for a TSS range of 13 – 20° Brix (Nagar, 1994). Generally the relationships between TSS, pericarp colour, acidity and eating quality have shown to be very variable. However, Underhill and Wong (1990) found that the Brix: acid ratio of the pulp is well correlated with mean eating quality. They reported that in order for the fruit to have excellent taste, it must have a TSS: acid ratio of between 31:1 and 60:1. This ratio is now used internationally as the commercial standard for litchi maturity.

PHYSIOLOGICAL DISORDERS

The main postharvest disorder associated with litchi is that of pericarp browning. This browning can be caused by a number of different stresses including: climatic conditions prior to fruit maturation, disease, desiccation, fruit senescence and heat injury. Rapid postharvest pericarp desiccation and disease are by far the most common causes of browning (Underhill, Coates & Saks, 1997).

It is thought that litchi pericarp browning is mainly as a result of rapid degradation of phenols by the activity of polyphenol oxidase (PPO). However, where polyphenol oxidase activity has been determined in storage, results are inconsistent. The significance of this compound's activity is complicated by the fact that peroxidase (POD), a similar oxidative enzyme, is also present in the pericarp of litchi (Zauberman *et al.*, 1991). The activity of this enzyme increases during the storage of fruit.

The success of commercial sulphite treatments in controlling pericarp browning (Zauberman *et al.*, 1991 ; Duvenhage *et al.*, 1995) is evidence for the hypothesis that browning is due to some type of oxidative enzyme. It is most likely that a combination of both PPO and POD are involved.

Several researchers have tried to link the browning of fruit to the degradation of anthocyanins and thus a reduction in red colouration. Although anthocyanin degradation has been observed, it seems to occur at a slower rate than that of actual pericarp browning and therefore may not be linked to the process. The current success of commercial SO₂ – low pH treatments means that further work is required to clarify the role of anthocyanin in browning.

POSTHARVEST HANDLING AND STORAGE

The postharvest handling and storage of litchis has proved to be a difficult process which to date is still inadequate. There are two main methods for the preservation of the litchi fruit: decreasing the loss of water from the fruit by various methods, and suppressing browning by chemical or physical means.

REDUCTION OF WATER LOSS

Refrigerated storage

Litchis are able to be stored at low temperatures that in turn enhance the storage life of the fruit. Generally the litchis are stored at temperatures of 5°C although some research has shown that fruit can be held as low as 0°C for up to three weeks (Sandhu and Randhawa, 1992). Fruit are usually stored at a relative humidity of 95%, but this has not really been researched (Underhill, Coates & Saks, 1997).

Precooling

This method is used to quickly cool fruit to about 3°C once it has been harvested. When fruit is merely placed into cool storage after picking it takes some time to cool. Cooling the fruit will lead to a decrease in water loss and reduce fruit susceptibility to disease. Precooling is most effective if done before the fruits are packed into plastic bags, since cooling time is considerably shorter and the likelihood of condensation inside the plastic bag is reduced (Underhill, Coates & Saks, 1997). This is either done by forced – air cooling or hydrocooling. Hydrocooling is faster and less fruit is lost through desiccation than in forced – air cooling.

Chemical Treatments

Various treatments aimed at increasing cell wall strength or delaying fruit senescence have been reported in the literature. Much of the work is the result of previous studies done on avocado and mango, where calcium has been shown to reduce the incidence of physiological disorders. Postharvest calcium nitrate application was reported to extend litchi storage life and reduce the rate of whole fruit weight loss. It had little effect on the quality of the fruit (Underhill, Coates & Saks, 1997).

Surface Coatings

It was first thought that litchi would be an excellent candidate for surface coatings in the control of water loss. However, wax emulsions proved to have little benefit, as there was either continued dehydration or pericarp discolouration. Underhill and Simons (1993) reported the development of pericarp micro cracking shortly after harvest. Similar cracking was also observed in wax – treated fruit after 24 hours and was thought to enhance desiccation. This can be used to explain the poor results obtained from current commercial coatings aimed at reducing water loss and so inhibiting pericarp browning. Discolouration usually occurs soon after the wax is applied and appears to be restricted to high pH wax coatings. Polysaccharide and similar sucrose ester – based coatings show a positive effect (Underhill, Coates & Saks, 1997).

Packaging

One of the most commonly used means to control water loss is through the use of specialised packaging. Early packaging included the use of paper and cloth bags, plastic films, PVC bags, and perforated plastic bags. The results were very variable. It was found that most techniques had inadequate moisture control or condensation was high resulting in disease problems. It was shown that packing fruit in plastic containers and overwrapping with a semi – permeable membrane reduced fruit desiccation and showed minimum condensation (Scott *et al.*, 1982; Huang and Scott, 1985; Wara – Aswapati *et al.*, 1990; and Wong *et al.*, 1991). This method followed by refrigerated storage remains a very effective non – chemical means of controlling pericarp browning and water loss. Presently, the standard ventilated cartons used in the litchi industry result in excessive desiccation, which decreases net carton fruit weight and fruit unacceptably (Roe, 1996).

REDUCING TISSUE BROWNING

Sulphur dioxide

Sulphur dioxide is used world – wide and is currently the major treatment to control pericarp browning. The fruit is usually treated at the farm with SO₂ fumigation. Sulphur is thought to prevent pericarp browning by inhibiting the action of polyphenol oxidase. It is also useful as it has fungicidal properties (Underhill, Coates & Saks, 1997). One of the main problems with this treatment is that it tends to lead to bleaching on the pericarp surface due to the formation of a colourless anthocyanin – SO₃H complex. The colour will partially return depending on the rate of SO₂ release, a function of storage temperature and air-flow around the fruit (Zauberman *et al.*, 1991).

The method of sulphur application varies and seems to be critical to its success. Numerous methods from metabisulphite dipping, slow release ‘grape – guard’ pads, fast releasing SO₂ paper sheets, burning sulphur powder and SO₂ fumigation have been used, with results being variable. The standard approach for preserving the quality of litchi fruit in South Africa involves the use of sulphur dioxide fumigation (De Jager & Korsten, 2003).

It has been found that SO₂ / low pH treatments lead to prolonged red colour of the pericarp and is very effective in postharvest fungal disease control (Duvenhage, 1993). However, this treatment is associated with the development of off – flavours and reduced pericarp colouration after extended cold storage. Also, its application is meeting with consumer resistance (Oosthuysen, 2002). The previous maximum SO₂ residue level acceptable on the European market was 10 ppm in the fruit pulp (Swarts, 1993). The recent dislike of sulphur use by the European Union now leads to urgency in finding alternative methods to SO₂ fumigation that will prove to be as effective.

Hot water and acid treatment

Physical preservation methods, such as heat treatments, were investigated once it was found that chemical methods would not be sustainable. Kaiser (1994, 1995) reported that immersion of fruit in hot water for 30 seconds followed by a low pH treatment could significantly improve colour retention of the pericarp. It does appear to reduce internal quality to an extent. Boiling water tends to be too harsh and results in discolouration of the pulp (Kaiser, Levin & Wolstenholme, 1996). It is thought that high temperature degrades PPO while the low pH has an effect on non – degraded anthocyanins. The treatment therefore inhibits oxidative activity while at the same time stabilises or increases anthocyanin content. HCl in conjunction with steam treatments have also been investigated and are found to result in good colour, but decreased firmness and taste (Roe, 1996). Acids have often been investigated in conjunction with physical treatments, with HCl being the most commonly used.

POSTHARVEST DISEASE

Incidence of postharvest disease can contribute to a high loss of litchi fruit in the market. This is due to a wide range of fungal pathogens. Many of the pathogens are also a problem in other tropical and subtropical fruits. Some bacteria have been isolated from decaying litchi but these are seldom the cause of disease. Little is specifically known about the mode of infection in litchi. In other fruits infection occurs either through wound – invading fungi that infect during or after harvest, or pathogens that infect fruit in the field before harvest. Time of infection will greatly influence the effectiveness of disease control measures, and should therefore be carefully considered when developing postharvest treatments (Underhill, Coates & Saks, 1997).

Chemical disease control

Strategies for the control of litchi postharvest diseases include the use of postharvest fungicides, refrigeration, heat treatments, packing shed and orchard hygiene, correct packing materials and preharvest insect control (Lonsdale, 1988). Refrigeration is an essential component in the control of postharvest disease in litchis as disease development is greatly reduced by maintaining the cold – chain through all stages of postharvest handling.

A number of fungicides have been tested for the control of postharvest disease. Many of these fungicides often prove to be quite effective but none have been adequate enough to replace SO₂ fumigation. Treatments, however, must be done with caution as higher dip temperatures or longer dip times may result in pericarp browning. In Australia, the recommended postharvest fungicide treatment for litchis is a 2-minute dip in 500 ppm benomyl at 50°C (Coates *et al.*, 1993). Sulphur dioxide is seen to have fungicidal properties and aids in the prevention of postharvest decay. Due to the concerns of world – wide consumers, it is essential that biological fungicides be investigated. It is almost certain that the future use of chemical fungicides will be severely regulated. The use of naturally occurring phylloplane micro organisms to combat postharvest diseases of litchi has been investigated in South Africa and shows considerable potential (Korsten *et al.*, 1993).

Heat treatments such as hot – air heating and hot water have been shown to have some potential but are limited by the susceptibility of the litchi to heat damage and consequent pericarp browning. The results obtained from studies of the use of gamma – irradiation on litchis were not promising and showed little disease control (Lonsdale, 1993). There is very definitely a need to further research into any possible treatments that may prove to be valuable in the control of postharvest disease in litchi fruit.

Biological disease control

There are several biological control agents that may be useful in controlling postharvest pathogens. The most interesting include fungi, yeasts and bacteria. The identification and commercial evaluation of such compounds is still relatively new and therefore much research is needed. There are a few important methods of biological control that need to be researched and developed before they may replace current chemical controls.

The biological control agent may produce antifungal substances that prevent pathogen development and in doing so control decay of fruit. This reaction is known as induced systemic resistance. Another method is whereby the disease is controlled by organisms that feed on the pathogen and thus limit its spread (Wills *et al.*, 1998). Competition between organisms is also a promising area of biological control, with the growth of non – pathogenic organisms preventing the growth of a pathogenic organism. The biological control organism competes with the pathogen for space and nutrients thus inhibit its development (Wills *et al.*, 1998). It is, however, important that such an organism does not produce an antibiotic that may be toxic to consumers. Although antifungal substance and the organisms themselves may occur naturally, they will need to be screened for human toxicity and especially allergic reactions.

DISCUSSION AND CONCLUSION

Litchi postharvest research has been ongoing since the 1940s and has significantly escalated in recent years. Most of the current literature has focussed on aspects of SO₂ / low pH treatment and the physiology of the pericarp. Pericarp physiology has had a major influence on the research undertaken in recent years, as it is the key to diminishing pericarp browning. However, there is still little knowledge of the physiological processes taking place, leading to much trial and error. It is thought that litchi pericarp browning is mainly as a result of rapid degradation of phenols by the activity of polyphenol oxidase and peroxidase. The success of sulphur fumigation is evidence for the hypothesis that browning is due to some type of oxidative enzyme. However, with growing consumer resistance to chemical controls more emphasis needs to be placed on potential sustainable methods.

Some success in decreasing pericarp browning was obtained with the use of hot water and low pH treatments but this lead to a decrease in the internal quality of the fruit. Other research has involved the use of specialised packaging, surface coatings, biological treatments, precooling and refrigerated storage. Most of the packaging methods using bags have to date resulted in a high level of condensation within the bag. This leads to a greater level of disease and increased fruit decay. Plastic containers with semi permeable over wrapping have been quite successful and have resulted in lower fruit desiccation. This practice in conjunction with refrigeration is one of the most effective non – chemical methods of controlling pericarp browning. Refrigeration has also proved to be a very important aspect in the postharvest handling of litchi fruit as it can increase storage life.

Polypropylene bags are to be used in the packaging of the litchis in this research. These bags are micro-perforated and therefore allow some moisture to move out of the bag when condensation occurs during refrigeration. This means that less moisture will remain in the bag, which would have created an ideal situation for fungal infection. The bag also has an anti – mist coating. The polypropylene bag maintains a controlled atmosphere that can be altered by the addition of various gases. The relative humidity also remains very high within the bag and will reduce the amount of water lost from the fruit.

Postharvest pathology to date has concentrated mainly on the use of fungicides as well as SO₂ fumigation. However, these methods again are not sustainable as the public is adverse to the use of chemicals. Biological dips have been formulated with the aim of controlling pathogens that cause fruit decay. The literature shows that no alternative controls have proved to be nearly as effective as SO₂ in the prevention of disease and pericarp browning. Therefore new products need to be continually researched and their potential evaluated.

SO₂ fumigation has proved to be an exceptional treatment in the preservation of litchi fruit. However, it has always been problematic. Aftertastes and residues have caused increasing health concerns and as a result this method was recently restricted by the European Union. This method is to be discontinued in time, and so there is now a need for alternatives for the preservation of litchis. It is essential that a method of even better postharvest preservation be found or the future of the South African litchi is uncertain. Without the potential for export to European markets, the litchi industry will be severely restricted and the long term prospects are questionable. The objective of this study was therefore to investigate methods of restoring the red colour of litchi fruit for as long as possible (to particularly allow for sea freight), and at the same time, prevent fungal decay, without the use of sulphur.

LITERATURE CITED

Cambell, C.W. (1959) Storage behaviour of fresh Brewster and Bengal lychees. *Proceedings of the Florida State Horticultural Society, Vol 72, 356 – 360.*

Coates, L.M., Doogan, V.J. and Gardiner, S. (1993) Postharvest disease control in lychees using sulphur dioxide treatment. *Proceedings of the Australasian Postharvest Conference, Gatton, Queensland, September 1993, 73 – 76.*

Cull, B. and Lindsay, C. (1995) Fruit growing in warm climates. *Reed Books Australia.*

De Jager, E.S. and Korsten, L. (2003) Effect of fungicides and disinfectants on the prevention of litchi pericarp browning and control of post-harvest diseases. *South African Litchi Growers' Association, Vol 15, 16-23.*

Dennison, R.A. and Hall, C.B. (1957) Studies on the handling of fresh lychees. *Florida Lychee Growers' Association Yearbook, Vol 3, 31 – 33.*

Duvenhage, J.A., Moster, M.M. and Marais, J.J. (1995) Postharvest sulphuring and low pH treatment for retention of red skin colour of litchi fruit. *South African Litchi Growers' Association Yearbook, Vol 7, 44 – 46.*

De Jager, E.S. and Korsten, L. (2003) Effect of fungicides and disinfectants on the prevention of litchi pericarp browning and control of post-harvest diseases. *South African Litchi Growers' Association, Vol 15, 16 - 23.*

Huang, H. and Scott, K.J. (1985) Control of rotting and browning of litchi fruit after harvest at ambient temperatures in China. *Tropical Agriculture*, Vol 62, 2 – 4.

Jaiswal, B.P., Sah, N.L. and Prasad, U.S. (1987) Regulation of colour break during *Litchi chinensis* Sonn. Ripening. *Indian Journal of Experimental Biology*, Vol 25, 66 – 72.

Jiang – Ping, J., Mei – xia, S. and Pei – man, L. (1986) The production and physiological effect of ethylene during ontogeny and after harvest of litchi fruit. *Acta Phytophysiologica Sinica*, Vol 72, 95 – 103.

Kaiser, C. (1994) Litchi (*Litchi chinensis* Sonn.) pericarp colour retention. *Journal of the South African Society for Horticultural Science*, Vol 4, 6 – 12.

Kaiser, C. (1995) Litchi (*Litchi chinensis* Sonn.) pericarp colour retention – alternatives to sulphur. *South African Litchi Growers' Association Yearbook*, Vol 7, 47 – 52.

Kaiser, C. (1998) An overview of post-harvest storage of Mauritius litchi fruit [summary]. *South African Litchi Growers' Association*, Vol 9, 57.

Kaiser, C., Levin, J. and Wolstenholme, B.N. (1996) Steam and low pH dips improve litchi (*Litchi chinensis* Sonn) pericarp colour retention. *South African Litchi Growers' Association*, Vol 8, 12-14.

Korsten, L., de Villiers, E.E., de Jager, E.S., van Harmelen, M.W.S. and Heitmann, A. (1993) *South African Litchi Growers' Association Yearbook*, Vol 5, 36 – 40.

Londsdale, J.H. (1988) Postharvest diseases of litchis (*Litchi chinensis* Sonn) and their control. *South African Litchi Growers' Association Yearbook, Vol 1, 16 – 18.*

Londsdale, J.H. (1993) Maintaining market quality of litchis overseas without SO₂. *South African Litchi Growers' Association Yearbook, Vol 5, 25 – 28.*

Macfie, G.B. (1955a) Wrapping and packaging of fresh lychees. *Florida Lychee Growers' Association Yearbook, Vol 1, 25 – 28.*

Macfie, G.B. (1955b) Packaging and storage of lychee fruits, preliminary experiments. *Florida Lychee Growers' Association Yearbook, Vol 1, 44 – 48.*

Marloth, R.H. (1949) The litchi in South Africa. *South African Citrus and Subtropical Research Station, Bulletin 286, 16 pp.*

Mukerjee, P.K. (1957) Preservation of natural colour in litchis under cold storage. *Science and Culture, Vol 23, 101 – 103.*

Nagar, P.K. (1994) Physiological and biochemical studies during fruit ripening in litchi (*Litchi chinensis* Sonn). *Postharvest Biology and Technology, Vol 4, 25 – 234.*

Oosthuizen, Institute for Tropical and Subtropical Crops. (1992) The cultivation of Litchis. *Agricultural Research Council, Bulletin 425.*

Oosthyse, S.A. (2002) Effect of reduced sulphur exposure and of controlled atmosphere storage on the post-storage quality of Madras litchi. *South African Litchi Growers' Association, Vol 13, 12-16.*

Paull, R.E., Chen, N.J., Deputy, J., Huang, H., Cheng, G. and Goa, F. (1984) Litchi growth and compositional changes during fruit development. *Journal of the American Society for Horticultural Science*, Vol 109, 817 – 821.

P.P.E.C.B. Export Directory, 2006.

Roe, D.J. (1996) A comparison of different postharvest treatments and packing materials for McLean's Red litchi fruit. *South African Litchi Growers' Association*, Vol 8, 44-47.

Sandhu, S.S. and Randhawa, J.S. (1992) Effect of postharvest application of methyl – 2 – benzimidazole carbamate and in pack fumigant on the cold storage life of litchi cultivars. *Acta Horticulturae*, Vol 269, 185 – 189.

Scott, K.J., Brown, B.I., Chaplin, G.R., Wilcox, M.E. and Bain, J.M. (1982) The control of rotting and browning of litchi fruit by hot benomyl and plastic film. *Scientia Horticulturae*, Vol 16, 253 – 262.

Swarts, D.H. (1989) Die effek van SO₂ – behandeling op na-oes bederf ontwikkeling by lietsjies. *South African Litchi Growers' Association Yearbook*, Vol 2, 22 – 24.

Swarts, D.H. (1993) SO₂ – residu's van beroekte Mauritius-lietsjies. *South African Litchi Growers' Association*, Vol 5, 17 - 18.

Tan, X. and Li, Y. (1984) The partial purification and properties of polyphenol oxidase from the pericarp of litchi (*Litchi chinensis* Sonn). *Acta Phytophysiologica Sinica*, Vol 10, 339 – 346.

Thompson, B.D. (1956) A progress report on handling and storage of fresh lychee. *Florida Lychee Growers' Association Yearbook, Vol 2, 27 – 28.*

Tongdee, S.C., Scott, K.J. and McGlasson, W.B. (1982) Packaging and cool storage of litchi fruit. *CSIRO Food Research Quarterly, Vol 42, 25 – 28.*

Underhill, S.J.R. and Critchley, C. (1994) Anthocyanin decolourisation and its role in lychee pericarp browning. *Australian Journal of Experimental Agriculture, Vol 34, 115 – 122.*

Underhill, S.J.R. and Wong, L.S. (1990) A maturity standard for lychee (*Litchi chinensis* Sonn). *Acta Horticulturae Vol 16, 245-251.*

Underhill, S.J.R. and Simons, D.H. (1993) The anatomy of lychee (*Litchi chinensis* Sonn.) pericarp and the importance of postharvest micro – cracking. *Scientia Horticulturae, Vol 54, 287 – 294.*

Underhill, S.J.R., Coates, L.M. and Saks, Y. (1997) Postharvest Physiology and Storage of Tropical and Subtropical Fruits. Ed. Mitra, S.K. *CAB International: Wallingford.*

Wara – Aswapati, O., Sornsrivichai, J., Uthaibutra, J. and Oogaki, C. (1990) Effect of seal packaging by different plastic films on storage life and quality of litchi (*Litchi chinensis* Sonn.) fruits stored and three different temperatures. *Japanese Journal of Tropical Agriculture, Vol 34, 68 – 77.*

Wong, L.S., Jacobi, K.K. and Giles, J.E. (1991) The influence of hot benomyl on the appearance of stored lychee (*Litchi chinensis* Sonn.). *Scientia Horticulturae*, Vol 46, 245 – 251.

Wills, R., McGlasson, B., Graham, D. and Joyce, D. (1998) Postharvest: An introduction to the physiology and handling of fruit, vegetables, and ornamentals. *CAB International: Wallingford*.

Zauberman, G., Ronen, R., Akerman, M. and Fuchs, Y. (1989) Protection of the colour of litchi after harvest. *Hasade*, Vol 70, 82 – 84.

Zauberman, G., Ronen, R., Akerman, M., Weksler, A., Rot, I. and Fuchs, Y. (1991) Postharvest retention of the red colour of litchi fruit pericarp. *Scientia Horticulturae*, Vol 47, 89 – 97.

PAPER 1: LITCHI POSTHARVEST PRESERVATION USING PACKAGING AND MODIFIED ATMOSPHERE TECHNIQUES

ABSTRACT

The majority of litchi fruit produced in South Africa is treated postharvest with sulphur to decrease fruit browning and control postharvest diseases. However, 95 % of exported fruit are sold to the European Union, which is beginning to deem sulphur as unacceptable. Alternative forms of fruit quality maintenance are thus necessary. The primary cause of fruit postharvest browning is believed to be desiccation. Previous work using PVC bag packaging has, however, resulted in enhanced postharvest fungal decay, due to excessive condensation. In this study, 2 sites of origin were used with a total of 3 harvest dates. Fruit were packaged in polypropylene bags, with specific anti-mist and gas exchange properties. Three modified atmospheres within the bag were tested, while non packaged (standard carton) fruit were used as controls. For postharvest decay control, a systemic resistance inducer applied pre- and postharvest, as well as a micro organism containing compound were tested. Fruits were stored at 1°C for 30 days, with evaluations at 10 day intervals. Packing in bags significantly reduced fruit water loss and enhanced retention of fruit colour for up to 20 days, while control fruit were brown within 10 days. There was no advantage of a modified atmosphere over standard atmosphere packaging. Most water loss occurred in the first 10 day period of storage. Total soluble solids (TSS) of packaged fruit decreased with storage but remained high (15 to 20° Brix) while acidity decreased to between 0.19 and 0.49%. The TSS: acid ratio showed good eating quality as it remained between 40:1 and 60:1. The anthocyanin content of packaged fruit increased during the first 10 days of storage and then gradually declined, but not below that of the initial fruit. No measurable decay was found within the context of the study.

INTRODUCTION

With the increasing popularity of exotic fruits on the world market, litchi production has steadily grown in importance. South Africa exports 4 million tonnes of litchis on average, of which 95 % are destined for the European Union (P.P.E.C.B. Export Directory, 2006). In order to maintain an attractive appearance in the market, litchi fruit need to be well coloured (high levels of anthocyanin) and be free of postharvest diseases.

As export marketing of litchi requires transportation times of at least two weeks, the short shelf life of litchis under ambient conditions (from 2 to 7 days at 25°C) has been a significant impediment to this trade (Underhill *et al.* 1997). This is largely due to the litchi rind being relatively thin and lacking a thick, durable cuticle. Consequently, desiccation is a major factor during postharvest storage. As a result of desiccation, litchi fruit rapidly lose colour and unless treated immediately after harvest, will turn in an unsightly brown colour (Kaiser, 1998). Colour cannot recover later, as the cells containing anthocyanin are in the mesocarp immediately below the pericarp protuberances (Underhill & Simons, 1993) which are likely to be damaged by dehydration. It is thought that litchi pericarp browning is mainly as a result of rapid degradation of phenols by the activity of polyphenol oxidase (PPO) and/or peroxidase (PO) (Zauberman *et al.*, 1991) and is irreversible. Coupled with postharvest disease, this forms the greatest impediment to fruit quality maintenance (Underhill *et al.*, 1997).

Tan & Li (1984) and Swarts (1989) found that fumigation of fruit with SO₂ could suppress browning of fruit. This also decreases postharvest pathogen induced damage. Presently, the South African litchi industry uses sulphur fumigation to prevent browning, but sulphur is undesirable as it causes aftertastes, is ineffective against some fungi and constitutes a potential health problem.

Recently the European Union announced its intention to decrease the use of sulphur for the postharvest treatment of litchis. Consequently, it was imperative that research aimed at eliminating sulphur fumigation must be undertaken (Kaiser, 1998).

In order to decrease water loss, various surface coatings have been tried, with little success (Underhill & Simons, 1993). Various forms of PVC packaging have shown good results (Scott *et al.*, 1982) and when condensation within the pack is minimised postharvest disease is controlled. Litchi fruit are relatively cold tolerant and thus an integrated system to control water loss while at the same time limiting the opportunity for postharvest disease, could be a viable means of extending shelf life without SO₂.

The objectives of this study were to investigate the use of polypropylene packaging with micro-perforations to allow for some gas (including water) transfer, together with the use of biological disease control methods.

METHODS AND MATERIALS

Fruit was collected from two sites in KwaZulu-Natal. These were Salt Rock, on the north coast, and Port Shepstone, on the south coast. Two consignments of fruit were obtained from the north coast and one from the south coast. Each consignment was subjected to different treatments.

Salt Rock Site Group a

Fruit was collected from the pack house, after standard pack house grading and packing, on the 22 December 2003 the morning of harvest. It was then taken directly to Pietermaritzburg for further treatment.

Two experimental dips, 'Biosave' and ISR 2000, were used in this research. Both are biological and aim at the control of postharvest disease in the litchi especially in respect to blue mould (*Penicillium italicum*).

Biosave

This biological dip is made up of the ESC 10 strain of the active bacteria *Pseudomonas syringae*. The mechanism of control is dependant on the establishment of the bacteria. This harmless bacterial colony will compete for all nutrients and space on the surface of the litchi pericarp. This restricts the growth of any fungal pathogens, as they do not have the reserves to become established. It is therefore important that the bacteria are present before the pathogen so that they can possess a competitive advantage and inhibit disease growth.

ISR 2000®

This mixture contains a Yucca extract as well as cytokinins, gibberellins, auxin, micronutrients, vitamins and a yeast cell wall extract. The yeast cell wall extract is thought to be the most important component of this biological dip. This extract contains a mannanoligosaccharide, and is perceived by the fruit rind cells as a fungal attack and stimulates the production of anti fungal substances (induced systemic resistance). This inhibits growth of the pathogen and therefore decreases disease incidence. The plant growth regulators found in ISR 2000® are all involved in the regeneration of cells and their resistance to decay. These plant growth regulators therefore are thought to delay senescence of the fruit and so further inhibit disease infection.

On arrival at the laboratory, fruit was divided into groups of 10 fruit per sample. Half the samples were treated with a postharvest antifungal compound, registered on citrus and deciduous fruit in the USA and sold as 'Biosave' and the other half untreated. Fruit were dipped in a 100 ml/l concentration of 'Biosave' as registered for citrus, for two minutes. Fruit was then allowed to dry before packaging. There were five replications of each treatment and this was reproduced four times to allow for fruit to be removed and evaluated throughout the storage period.

The fruit treatments and packaging were as follows:

T1- Control. No Biosave or packaging used.

T2- Treated with Biosave, but no packaging.

T3- No Biosave but packaged in micro-perforated (9 μ m) anti-mist coated polypropylene bags and sealed.

T4- Biosave treated, and packaged in micro-perforated (9 μ m) anti-mist coated polypropylene bags and sealed.

T5- No Biosave but packaged in micro-perforated (9 μ m) anti-mist coated polypropylene bags, purged with a gas mixture of 2% O₂: 15% CO₂: 83% N₂ and sealed.

T6- Biosave treated, and packaged in micro-perforated (9 μ m) anti-mist coated polypropylene bags, purged with a gas mixture of 2% O₂: 15% CO₂: 83% N₂ and sealed.

T7- No Biosave but packaged in micro-perforated (9 μ m) anti-mist coated polypropylene bags, purged with a gas mixture of 10% O₂: 30% CO₂: 60% N₂ and sealed.

T8- Biosave treated and packaged in micro-perforated (9 μ m) anti-mist coated polypropylene bags, purged with a gas mixture of 10% O₂: 30% CO₂: 60% N₂ and sealed.

Fruits were stored at 1°C and evaluated after 10, 20 and 30 days. Before packaging, fruit was weighed and colour determined with a Minolta colorimeter. At each post storage evaluation, fruit was weighed to determine water loss as it is believed to strongly affect colour. Colour was measured using a colorimeter, and general observations were noted.

Statistical analysis for all experiments was based on a factorial design of treatments with five replications. Components were tested for statistical difference using Analysis of Variance procedures of GenStat Ninth Edition (GenStat release 9.1, Lawes Agricultural Trust, 2006).

Salt Rock site group b

Fruit was collected from the packhouse on 8 January 2003.

All procedures were the same as group a, with the exception that the Biosave was replaced with ISR 2000 at a concentration of 1 ml/l water. This product is considered to stimulate systemically induced resistance. It is also possible that in doing so, anthocyanin production may be stimulated, which could enhance fruit colour. In addition, the treatment numbers were modified in terms of which received which gas during packaging. After packaging the fruit was treated as described in the first trial.

The treatments were thus:

T1- Control. No ISR 2000 or packaging used.

T2- Treated with ISR 2000, but no packaging.

T3- No ISR 2000 but packaged in micro-perforated (9 µm) anti-mist coated polypropylene bags and sealed.

T4- ISR 2000 treated, and packaged in micro-perforated (9 µm) anti-mist coated polypropylene bags and sealed.

T5- No ISR 2000 but packaged in micro-perforated (9 µm) anti-mist coated polypropylene bags, purged with a gas mixture of 2% O₂: 15% CO₂: 83% N₂ and sealed.

T6- ISR 2000 treated, and packaged in micro-perforated (9 µm) anti-mist coated polypropylene bags, purged with a gas mixture of 2% O₂: 15% CO₂: 83% N₂ and sealed.

T7- No ISR 2000 but packaged in micro-perforated (9 µm) anti-mist coated polypropylene bags, purged with a gas mixture of 10% O₂: 30% CO₂: 60% N₂ and sealed.

T8- ISR 2000 treated and packaged in micro-perforated (9 µm) anti-mist coated polypropylene bags, purged with a gas mixture of 10% O₂: 30% CO₂: 60% N₂ and sealed.

Port Shepstone site

A preharvest spray application of ISR 2000 at 1 ml/l water two weeks before harvest was done at this site. At harvest, those fruit which had been sprayed were deemed to be group a and those which were controls (non sprayed) were group b.

Harvesting took place on 28 January 2004 and fruit were immediately brought back to Pietermaritzburg for further processing. All postharvest treatments were as for Salt Rock group b.

Fruit evaluation was identical to that of the Salt Rock groups with the addition that these samples were also tested for total soluble solids (TSS), acidity and rind anthocyanin content in order to establish quality.

Total soluble solids were determined using a Palette PR-101 electronic refractometer. Titratable acidity (%) was determined by the titration of 5 ml of litchi juice from each sample with 0.1 M NaOH. A phenolphthalein indicator was used to determine the end point of the titration.

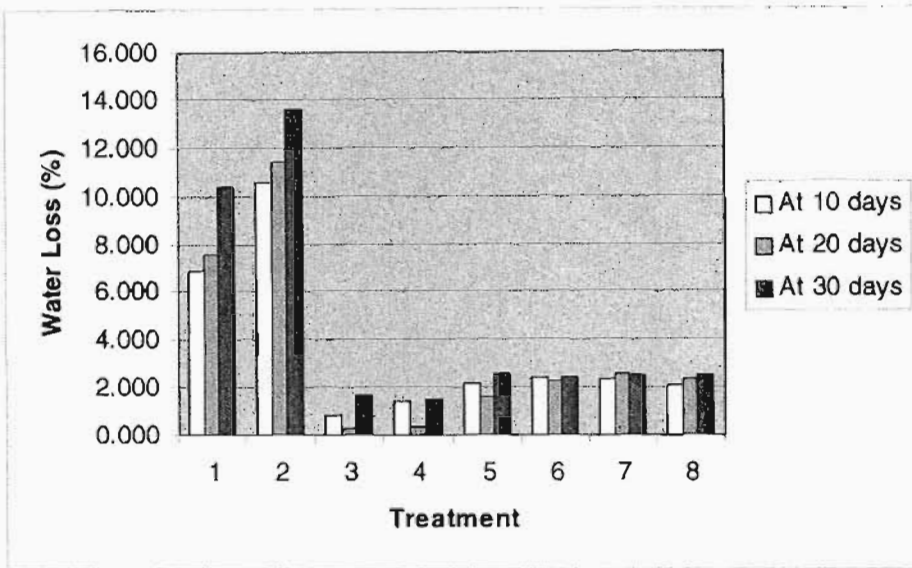
In order to extract anthocyanin, 10 rind discs from each sample were placed in 10 ml acidified methanol (HCl: methanol, 1: 99, v/v) and refrigerated at 4°C for 24 hours. Absorption spectra of the extracts were determined after filtration using the DU[®] 800 Spectrophotometer. The anthocyanin content was estimated from absorbance at 530 nm (Li *et al.*, 2004) and was recorded as a percentage of the anthocyanin content of the initial fruit. This was done for each treatment and replication.

RESULTS

Fruit water loss

Packaging had a clear effect on fruit mass. It is assumed that mass loss was primarily water loss. This was consistent at all sites and harvest dates. Non packaged fruit always lost significantly more weight ($p=0.01$) than fruit that was packaged. Fruit that was not packed had a water loss of approximately 10 to 14% over the 30 day storage period, while the packed fruit only lost 2 to 3% water. Small differences were noted between sites, however, the general trend in results was consistent over the sites. There were only marginal differences, which were insignificant and showed no clear trend, between the packaged treatments (Figure 1). The preharvest treatment of ISR 2000, as well as postharvest treatments of ISR 2000 and 'Biosave', had no significant effect on fruit water loss.

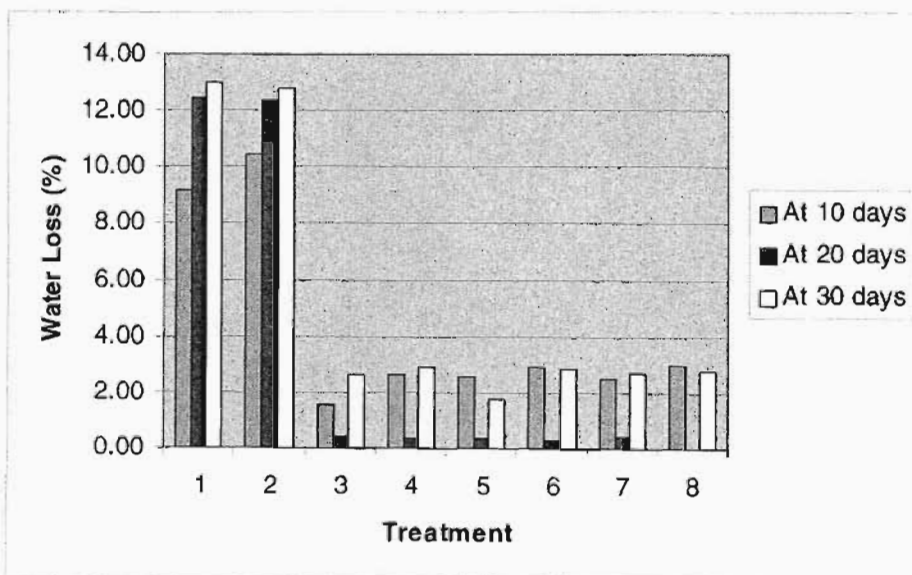
An important trend noted in the results is that the majority of water loss occurred in the first 10 days of storage. The actual loss of mass, and therefore water, may have occurred early during storage. This could not be determined as the first measurements were only recorded at 10 days after packaging. There appears to be a decrease in water loss between days 10 and 20 in the packaged fruit. This may be due to the uptake of water from the surrounding atmosphere in the bag. There was then again an increase in water loss from days 20 to 30 (Figures 1 – 4).



Treatment: p=0.01
 Day: ns
 Treatment.Day: ns
 I.s.d. 5% (Packaging): 4.642

Figure 1: Percentage water loss of stored litchi fruit from Salt Rock site, group a after 30 days storage at 1°C.

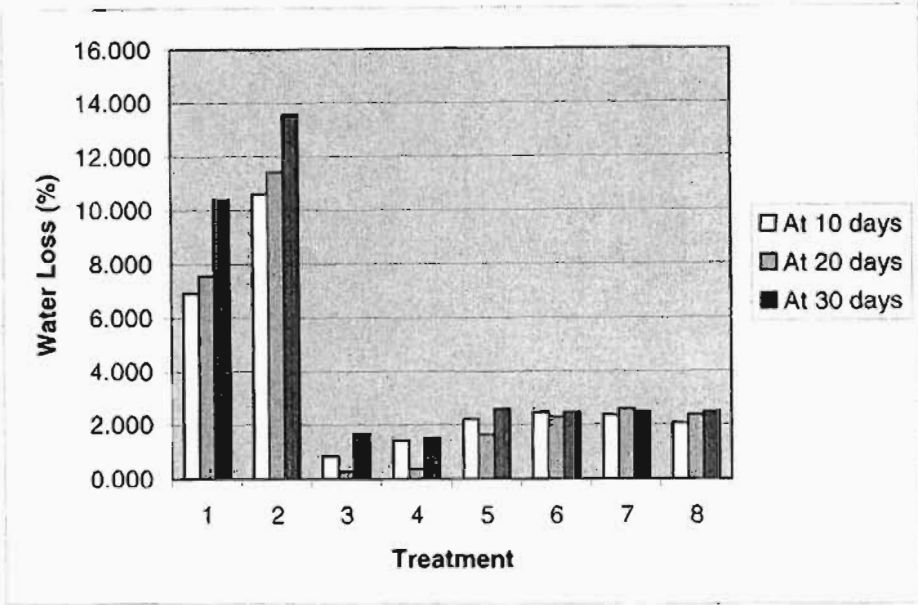
Treatments 1 and 2 were not packaged while 3 to 8 were packaged. No preharvest spray. Uneven treatment numbers had no Postharvest treatment, while even numbered treatments were treated with Biosave. Treatments 3 and 4 contained standard atmosphere, while 5 to 8 were modified atmospheres.



Treatment: p=0.01
 Day: p=0.01
 Treatment.Day: p=0.01
 I.s.d. 5% (Packaging): 1.287

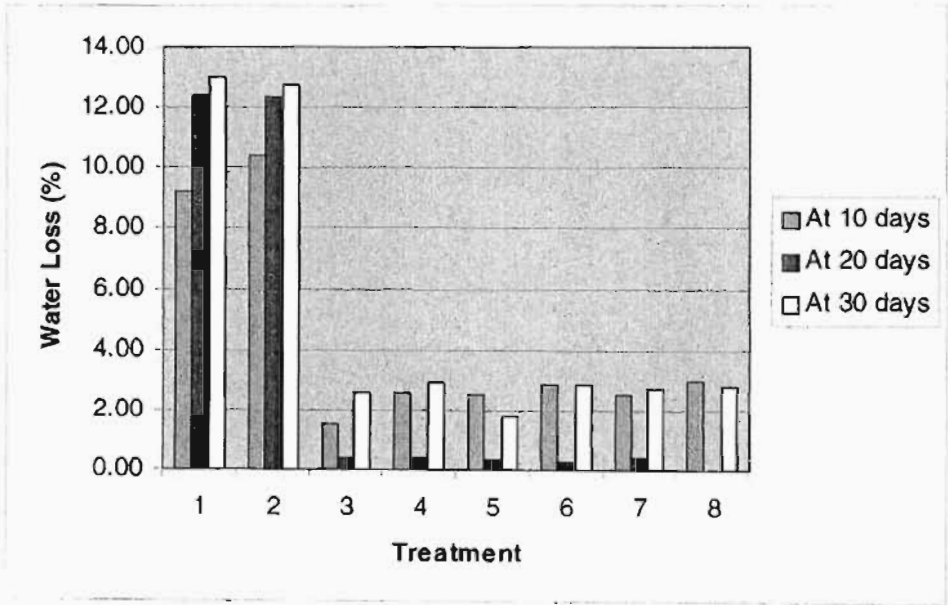
Figure 2: Percentage water loss of stored litchi fruit from Salt Rock site, group b after 30 days storage at 1°C.

Treatments 1 and 2 were not packaged while 3 to 8 were packaged. No preharvest spray. Uneven treatment numbers had no Postharvest treatment, while even numbered treatments were treated with Biosave. Treatments 3 and 4 contained standard atmosphere, while 5 to 8 were modified atmospheres.



Treatment: p =0.01
 Day: ns
 Treatment.Day: ns
 I.s.d. 5% (Packaging): 7.345

Figure 3: Percentage water loss of stored litchi fruit from Port Shepstone, group a after 30 days storage at 1°C. Treatments 1 and 2 were not packaged while 3 to 8 were packaged. Preharvest spray of ISR 2000. Uneven treatment numbers had no Postharvest treatment, while even numbered treatments were treated with ISR 2000. Treatments 3 and 4 contained standard atmosphere, while 5 to 8 were modified atmospheres.



Treatment: p =0.01
 Day: p =0.01
 Treatment.Day: p =0.01
 I.s.d. 5% (Packaging): 3.102

Figure 4: Percentage water loss of stored litchi fruit from Port Shepstone, group b after 30 days storage at 1°C. Treatments 1 and 2 were not packaged while 3 to 8 were packaged. No preharvest spray. Uneven treatment numbers had no Postharvest treatment, while even numbered treatments were treated with ISR 2000. Treatments 3 and 4 contained standard atmosphere, while 5 to 8 were modified atmospheres.

Fruit colour

Fruit colour deteriorated rapidly in the case of non packaged fruit (Figure 5a). After 10 days in storage, non packaged fruit from all sites regardless of pre- and postharvest treatments were brown. Packaged fruit, however, remained in excellent condition at this stage of storage. There were no clear trends relating to packing treatment (modified atmosphere), ISR 2000 or 'Biosave'. The ISR 2000 and 'Biosave' treatments did not noticeably affect colour retention. The slight differences noted were not consistent and should probably be discounted.

After 20 days of storage, fruit colour had started to deteriorate in all treatments, although it appeared somewhat better in treatment 4 (packaged with ambient air) (Figure 5b). The colour of fruit in all treatments deteriorated significantly after 30 days of storage.



Figure 5a: Fruit after 10 days storage in box pack.



Figure 5b: Treatment 4 after 10 days storage.



Figure 5c: Treatment 4 after 20 days storage.

Anthocyanin content

The results obtained from Port Shepstone group a show a significant ($p=0.01$) decline in anthocyanin content for all treatments from 10 to 30 days storage. It is, however, notable that there was an increase in the anthocyanin content from harvest to the first evaluation at 10 days storage. This occurred in all fruit, although at 20 days storage the non packaged fruit declined to below the initial anthocyanin content while packaged fruit decreased but retained a level above that of the initial fruit. A similar situation occurred in group b where the anthocyanin level increased at first and later decreased to a measure above that of the initial fruit (Figure 6). There was a significant difference ($p=0.01$) in anthocyanin content between group a and b. This is due to the high anthocyanin content of treatments 4, 5 and 6 of group b. It is thought that these values are probably outliers (although the reasons are unknown) and should therefore be excluded (Figure 8).

The trend of severe anthocyanin decrease in non packaged fruit is in agreement with the colour results that showed a complete browning and desiccation of the fruit. Packaged fruit appears to not lose anthocyanin but still becomes brown after 20 days storage (Figure 6). This is thought to be a result of the anthocyanin becoming masked as the fruit loses its colour and undergoes physiological browning. In the case of packaged fruit, anthocyanin in fact even increased in the first 10 days of storage. Anthocyanin is known to be stimulated in cold conditions as its structural and regulatory genes were previously found to be induced by low temperatures in various tissues of many different plants (Christie *et al.*, 1994) and therefore can be formed during the cold storage. The anthocyanin did not decline below the initial level, probably as the cells of the packaged fruit are not severely damaged as in the case of non packaged fruit. The rind of the non packaged, desiccated fruit appeared badly damaged and presumably resulted from the

destruction of cells. This is thought to possibly be the reason that anthocyanin is lost from the rind.

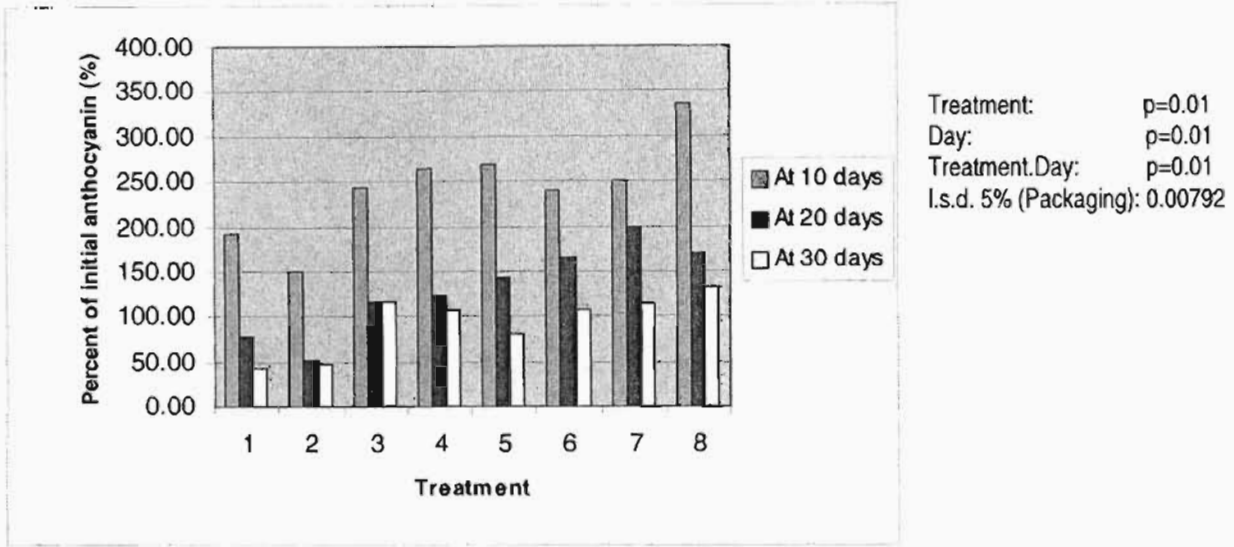


Figure 6: Anthocyanin content of stored litchi fruit from Port Shepstone site, group a, as percentage of initial content.

Treatments 1 and 2 were unpackaged while 3 to 8 were packaged. Preharvest spray of ISR 2000. Uneven treatment numbers had no Postharvest treatment, while even numbered treatments were treated with ISR 2000. Treatments 3 and 4 contained standard atmosphere, while 5 to 8 were modified atmospheres.

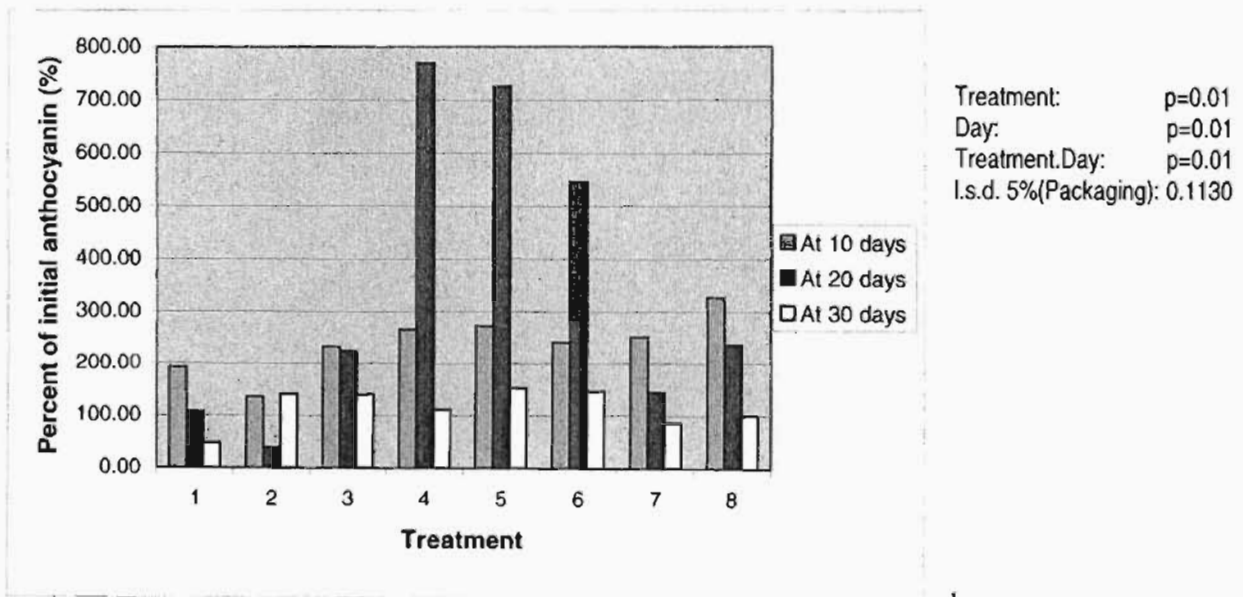


Figure 7: Anthocyanin content of stored litchi fruit from Port Shepstone site, group b, as percentage of initial content.

Treatments 1 and 2 were unpackaged while 3 to 8 were packaged. No preharvest spray. Uneven treatment numbers had no Postharvest treatment, while even numbered treatments were treated with ISR 2000. Treatments 3 and 4 contained standard atmosphere, while 5 to 8 were modified atmospheres.

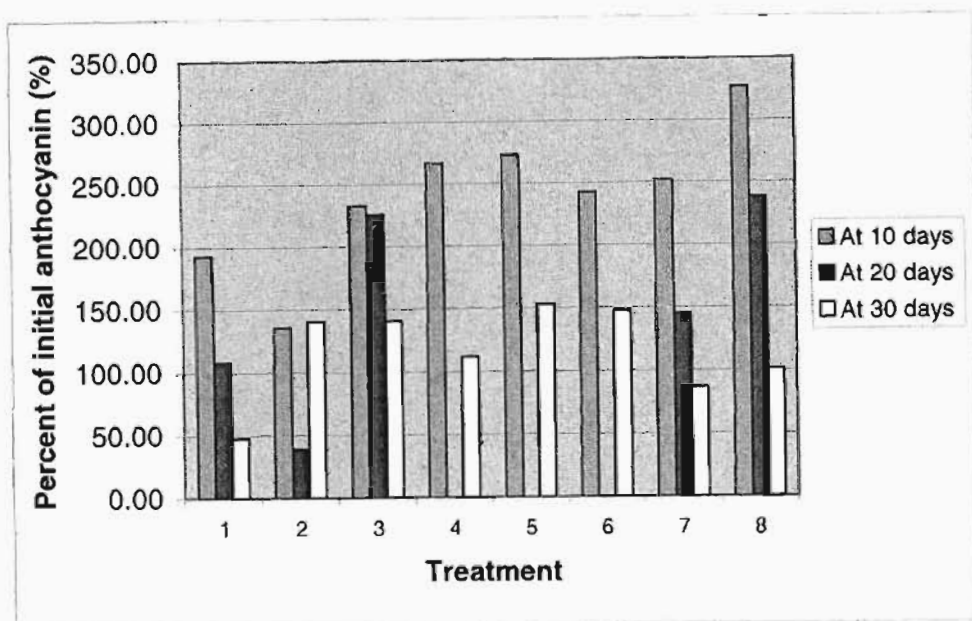


Figure 8: Anthocyanin content of stored litchi fruit from Port Shepstone site, group b, as related to initial content and eliminating possible outliers.

Treatments 1 and 2 were unpackaged while 3 to 8 were packaged. Graph (a) Port Shepstone group a. Preharvest spray of ISR 2000. Uneven treatment numbers had no Postharvest treatment, while even numbered treatments were treated with ISR 2000. Treatments 3 and 4 contained standard atmosphere, while 5 to 8 were modified atmospheres.

Total Soluble Solids

There was a clear difference between the total soluble solids in that of non packaged and packaged fruit. Non packaged fruit had significantly higher soluble solids ($p=0.01$) than that of the packaged fruit. This is largely due to the high amount of water loss presumably resulting in a concentration of the soluble solids. Although packaged fruit tended to have lower soluble solids, no clear treatment differences were noted. The results show that there was a clear trend in the decrease of soluble solids over the storage period for both Port Shepstone groups (Figure 9 & 10). This decrease was found to be significant ($p=0.01$) in group a. However, group b shows no significant decrease in soluble solids over the storage period. No significant difference in soluble solids was found between the two groups. The data obtained from group a, however, appears to have a more uniform trend than that of group b. This could be an effect of the preharvest ISR 2000 spray that was applied to group a. In addition, all fruit had total soluble solids above 15° Brix. This is important as the acceptable commercial level is between 13 and 20° Brix.

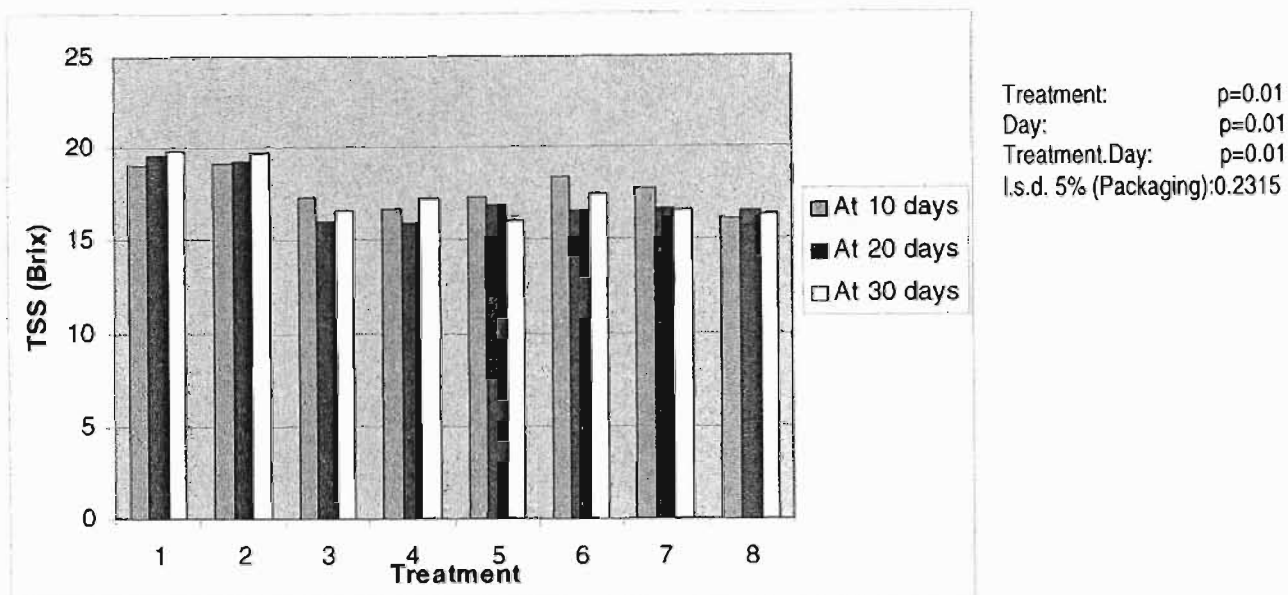


Figure 9: Total soluble solids (TSS) of stored litchi fruit from Port Shepstone site, group a

Treatments 1 and 2 were non packaged while 3 to 8 were packaged. Preharvest spray of ISR 2000. Uneven treatment numbers had no Postharvest treatment, while even numbered treatments were treated with ISR 2000. Treatments 3 and 4 contained standard atmosphere, while 5 to 8 were modified atmospheres.

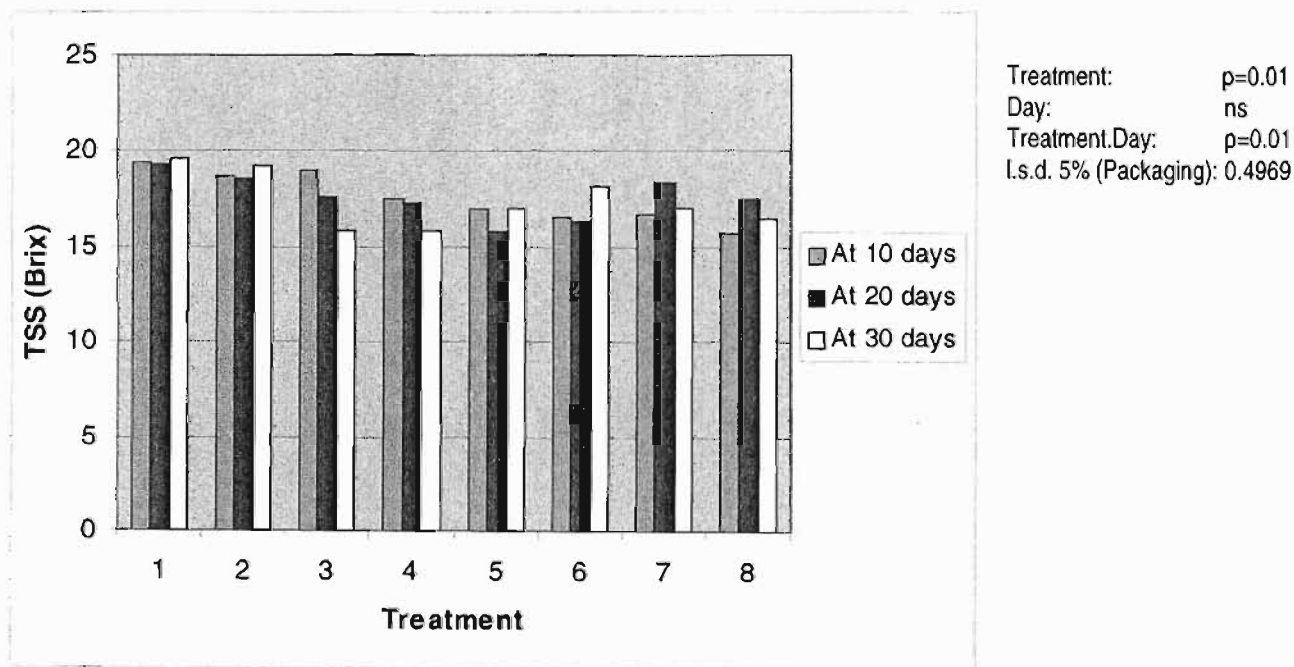
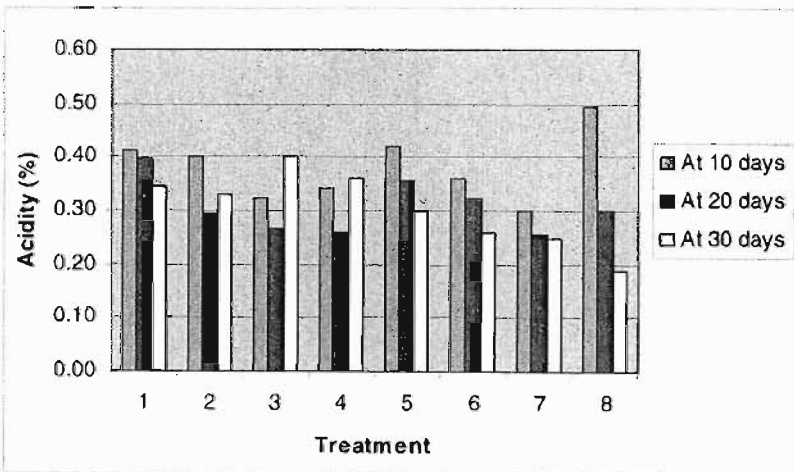


Figure 10: Total soluble solids (TSS) of stored litchi fruit from Port Shepstone site, group b.

Treatments 1 and 2 were non packaged while 3 to 8 were packaged. No preharvest spray. Uneven treatment numbers had no postharvest treatment, while even numbered treatments were treated with ISR 2000. Treatments 3 and 4 contained standard atmosphere, while 5 to 8 were modified atmospheres.

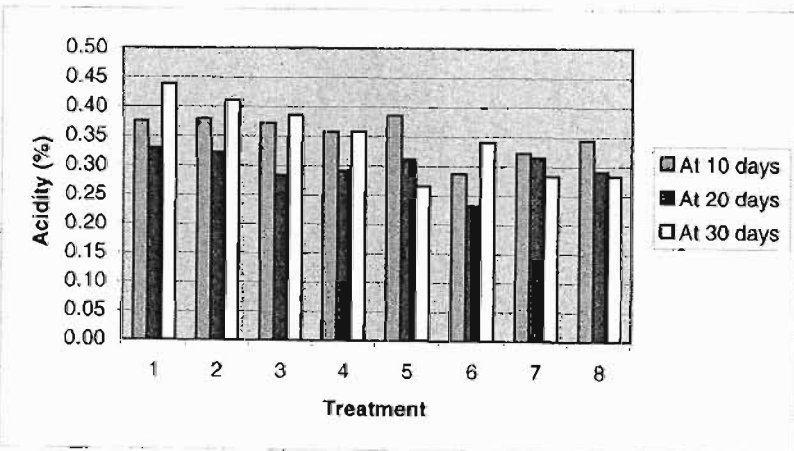
Acidity

The acidity of the fruit after storage was similar in that of packaged and non packaged fruit. The fruit showed a significant decline in acidity ($p=0.01$) over the 30 day storage period (Figure 11). This resulted in fairly low acidity of the fruit after 30 days of storage due to its continuing respiration. The acidity of all fruit decreased to below 0.45 % by day 10, which is considered to make the fruit taste bland (Swarts, 1992). There was no clear difference in acidity levels between treatments. It can again be noted that the decrease in acidity of Port Shepstone group a was more uniform than that of group b although there was no significant difference between the two groups.



Treatment: ns
 Day: $p=0.01$
 Treatment.Day: ns
 I.s.d. 5% (Packaging): 0.2369

a



Treatment: $p=0.01$
 Day: $p=0.01$
 Treatment.Day: ns
 I.s.d. 5% (Packaging): 0.2739

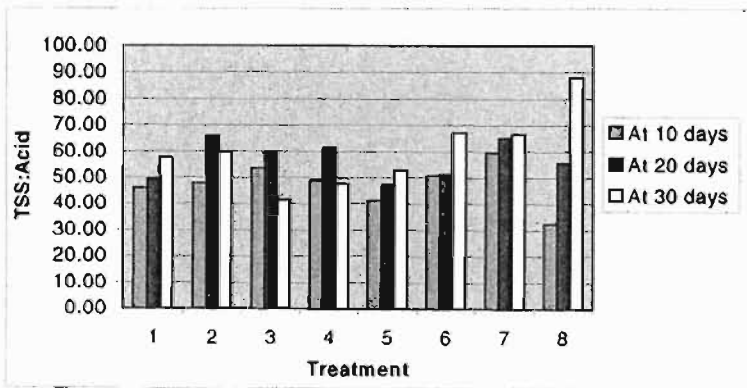
b

Figure 11: Acidity of stored litchi fruit

Treatments 1 and 2 were unpackaged while 3 to 8 were packaged. Graph (a) Port Shepstone group a. Preharvest spray of ISR 2000. Graph (b) Port Shepstone b. No preharvest spray. Uneven treatment numbers had no postharvest treatment, while even numbered treatments were treated with ISR 2000. Treatments 3 and 4 contained standard atmosphere, while 5 to 8 were modified atmospheres.

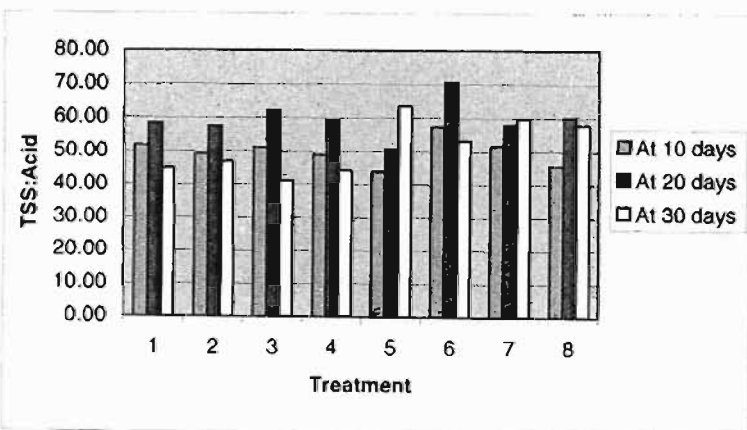
TSS : Acid ratio

There was a significant increase ($p=0.01$) in the TSS: acid ratio over the 30 day storage period (Figure 12). This was due to the increase in total soluble solids and the decrease of acidity after the fruit had been harvested. Underhill and Wong (1990) reported that the TSS: acid ratio of the pulp is well correlated to mean eating quality. In order for the fruit to have excellent taste, it must therefore have a TSS: acid ratio of between 31:1 and 60:1. In the case of group b all the fruit met this criteria and would be considered to be of good eating quality. However, in group a treatment 8 had a ratio that would be considered too high and would therefore have poor taste (Figure 6). There was no significant difference between the TSS: acid ratio of the two groups and no clear trend in the differences between treatments.



Treatment: ns
 Day: $p=0.01$
 Treatment.Day: $p=0.01$
 I.s.d. 5% (Packaging): 19.95

a



Treatment: ns
 Day: $p=0.01$
 Treatment.Day: ns
 I.s.d. 5% (Packaging): 15.78

b

Figure 12: TSS: Acid ratio of stored litchi fruit

Treatments 1 and 2 were non packaged while 3 to 8 were packaged. Graph (a) Port Shepstone group a. Preharvest spray of ISR 2000. Graph (b) Port Shepstone b. No preharvest spray. Uneven treatment numbers had no Postharvest treatment, while even numbered treatments were treated with ISR 2000. Treatments 3 and 4 contained standard atmosphere, while 5 to 8 were modified atmospheres.

Postharvest disease

The effects of pre- and postharvest ISR 2000 treatment as well as postharvest Biosave treatments on pathogens could not be evaluated. This was due to the absence of any measurable postharvest decay. Further work is needed in this regard.

DISCUSSION AND CONCLUSIONS

The polypropylene packaging system significantly decreased fruit water loss. There was also an increase in shelf life, as determined by red colour and overall rind appearance, when compared to standard packaging. This result is in accordance with previous work on packaging done by Scott *et al.*, (1982) and confirmed by the observations of Roe (1996) that the traditional cartons used for litchi are poor in relation to fruit quality management. Very little condensation occurred in the bag thus reducing the probability of pathogen infection. This is an improvement of previous packaging that resulted in much condensation and fungal infection.

It was notable that most water loss occurred within the first 10 days of storage and possibly early during this period. It is suggested that the majority would actually take place during the cooling phase (Wills *et al.*, 1998) as the hot fruit loses water to a cooler atmosphere. If this is the case, then ensuring that fruit is cooled rapidly after harvest can further extend shelf life. This may be possible particularly through the use of a hydrocooling system, as water is a good conductor of heat, and water would not be lost from the fruit to the atmosphere. This would also decrease the potential for condensation within the bag, especially if packaging is carried out in a cold room.

Ethylene plays a minor role in the senescence of litchi fruit due to the fact that it is non climacteric. Respiration, however, continues after harvest and ultimately affects internal and external quality. The storage temperature used was set low enough to sufficiently minimise respiration and is probably the reason that modified atmosphere showed little effect. At this low temperature (1°C), packaging in ambient air appears acceptable with no distinct advantage of modified atmosphere. This low temperature also reduces the action of PPO and/or PO thus decreasing the browning reaction. These enzymes may become

important when fruit is placed at ambient temperature, particularly if cellular damage has occurred.

The anthocyanin content of packaged fruit increased during the first 10 days of storage and then gradually declined. Importantly, the anthocyanin content did not drop to below that of fruit at harvest, although browning had occurred to an extent. The increase in anthocyanin is thought to be a result of synthesis stimulation in cold conditions and implies that the anthocyanin is not lost but is rather masked by the actions of PPO and/or PO as browning occurs. ISR 2000 is considered to stimulate systemically induced resistance and it is possible that in doing so anthocyanin production may also be stimulated, particularly under cold conditions. Both groups evaluated received a postharvest ISR 2000 dip that may have resulted in further stimulation of anthocyanin production. This would be particularly useful if fruit colour enhancement is required. Non packaged fruit at first showed an increase in anthocyanin content, but this then decreased to below that at harvest. This indicates that there was a loss of pigment probably due to cell leakage in tissue damaged by desiccation. The indication that there was an increase in anthocyanin of packaged fruit, particularly at early stages of storage, implies that some temperature manipulation to create conditions suitable for anthocyanin synthesis, but not allow for cell damage, would be useful.

The low temperature of storage probably contributed to the insignificant degree of postharvest pathogen development, and thus the inability to evaluate the compounds used. These compounds may well be useful under conditions of higher temperature. Work in this regard should thus be continued. The use of ISR 2000 as a preharvest application did appear to affect the respiration of the fruit in some way. It is noted that fruit from Port Shepstone a, which was preharvest sprayed, showed greater stability in the trends relating to internal qualities than that of Port Shepstone b, although no significant differences

resulted. This leads to the proposal that some component of ISR 2000 contributes to the regulation of respiration and those chemical reactions continuing in stored fruit.

In a subsidiary observational experiment (data not shown) good colour retention was maintained at 8°C for 30 days in packaged fruit. Although litchi fruit can be considered chilling tolerant from the work of Sandu & Randhawa (1992), it is suggested that storage at 1°C for 30 days may have caused cell damage, contributing to colour loss (browning) after 20 days storage. Further work to evaluate storage temperature in the context of packaging techniques is therefore necessary. Higher storage temperature would enhance the potential for postharvest diseases. Therefore, this factor will also need clarification. There may be a case, however, for using different storage temperatures or temperature management, depending on market and need for storage period.

Total soluble solids (TSS) decreased over the storage period but remained high with all treatments having a value above 15° Brix. This is important as the TSS range for eating quality is 13 to 20° Brix (Nagar, 1994) and is used as an indication of commercial maturity. The acidity of the fruit decreased during the storage period to a fairly low level. The acidity dropped to below the minimum standard of 0.45 % (Swarts, 1992) in all fruit, which indicated that it was too low in respect to good eating. The non packaged fruit showed the least decline but this is misleading as the concentration of the acid is increased due to the large amount of water loss. Underhill and Wong (1990) reported that the TSS: acid ratio of the pulp is well correlated to mean eating quality. In order for the fruit to have excellent taste, it must therefore have a TSS: acid ratio of between 31:1 and 60:1. All the fruit had a ratio that met this criterion and would therefore ensure good eating quality. A preharvest ISR 2000 application did not affect the internal quality of the fruit as no significant difference was noted between the two Port

Shepstone groups. There was no clear difference between the treatments and their effect on internal quality. Further work into the specific effects of packaging on the internal quality of fruit is required.

In conclusion, results of the work have shown that considerable extension of shelf life (colour retention) can be achieved by packing the litchi fruit in sealed polypropylene bags with ambient air being a sufficient atmosphere. This is economically important as it is less costly than packaging in a modified atmosphere. It is suggested that the resultant shelf life extension is primarily due to the reduction of water loss and little condensation occurring in the bag. The internal quality of the fruit remained satisfactory after storage. It is important that further work is done to optimise storage temperatures depending upon the required storage period. The synthesis of anthocyanin at low temperature should also be exploited. Further work is required to develop an integrated biological postharvest pathogen control system of high quality. These postharvest treatments and packaging techniques developed, show promising results that with further research will enable retention of internal and external fruit qualities and thus allow for export to the European Union without the use of sulphur. The techniques are suitable in their present form to enhance quality on the local market.

LITERATURE CITED

Christie, P.J., Alfenito, M.R. and Walbot, V. (1994) Impact of low-temperature stress on general phenylpropanoid and anthocyanin pathways: Enhancement of transcript abundance and anthocyanin pigmentation in maize seedlings. *Planta*, Vol 194, 541-549.

Kaiser, C. (1998) An overview of post-harvest storage of Mauritius litchi fruit [summary]. *South African Litchi Growers' Association*, Vol 9, 57.

Li, X.J., Hou, J.H., Zhang, G.L., Liu, R.S., Yang, Y.G., Hu, Y.X. and Lin, J.X. (2004) Comparison of anthocyanin accumulation and morpho-anatomical features in apple skin during colour formation at two habitats. *Scientia Horticulturae*, Vol 99, 41-53.

Nagar, P.K. (1994) Physiological and biochemical studies during fruit ripening in litchi (*Litchi chinensis* Sonn). *Postharvest Biology and Technology*, Vol 4, 25–234.

P.P.E.C.B. Export Directory, 2006.

Roe, D.J. (1996) A comparison of different postharvest treatments and packing materials for McLean's Red litchi fruit. *South African Litchi Growers' Association*, Vol 8, 44-47.

Sandhu, S.S. and Randhawa, J.S. (1992) Effect of postharvest application of methyl – 2 – benzimidazole carbonate and in pack fumigant on the cold storage life of litchi cultivars. *Acta Horticulturae*, Vol 269, 185–189.

- Scott, K.J., Brown, B.I., Chaplin, G.R., Wilcox, M.E. and Bain, J.M. (1982) The control of rotting and browning of litchi fruit by hot benomyl and plastic film. *Scientia Horticulturae*, Vol 16, 253–262.
- Swarts, D.H. (1984) Die effek van SO₂ behandeling op naoesbederf ontwikkeling by litsjies. *South African Litchi Growers Association Year Book 2*, 22-24.
- Swarts, D.H. (1992) Harvesting, handling and packing. In The cultivation of litchis. *Institute for Tropical and Subtropical Crops, Bulletin 425. Agricultural Research Council.*
- Tan, X. and Li, Y. (1984) The partial purification and properties of polyphenol oxidase from the pericarp of litchi (*Litchi chinensis* Sonn). *Acta Phytophysiologica Sinica*, Vol 10, 339 – 346.
- Underhill, S.J.R. and Wong, L.S. (1990) A maturity standard for lychee (*Litchi chinensis* Sonn). *Acta Horticulturae*, Vol 16, 245-251.
- Underhill, S.J.R. and Simons, D.H. (1993) The anatomy of lychee (*Litchi chinensis* Sonn.) pericarp and the importance of postharvest micro – cracking. *Scientia Horticulturae*, Vol 54, 287 – 294.
- Underhill, S.J.R., Coates, L.M. and Saks, Y. (1997) Postharvest Physiology and Storage of Tropical and Subtropical Fruits. Ed. Mitra, S.K. *CAB International: Wallingford.*
- Wills, R., McGlasson, B., Graham, D. and Joyce, D. (1998) Postharvest: An introduction to the physiology and handling of fruit, vegetables, and ornamentals. *CAB International: Wallingford.*

Zauberman, G., Ronen, R., Akerman, M., Weksler, A., Rot, I. and Fuchs, Y.
(1991) Postharvest retention of the red colour of litchi fruit pericarp. *Scientia Horticulturae*, Vol 47, 89–97.

PAPER 2: THE EFFECT OF TEMPERATURE MANAGEMENT ON THE RETENTION OF LITCHI COLOUR AND QUALITY

ABSTRACT

South Africa generally exports a substantial amount of litchis of which the majority is sold into the European Union. The European Union has now restricted the use of SO₂, which is extensively used as a postharvest treatment, and in doing so is threatening South Africa's litchi export market. New methods to retain litchi colour and quality are thus needed. In this study fruit was packaged in polypropylene bags, with specific anti-mist and gas exchange properties. Static and hydrocooling methods were tested and for postharvest decay control, a bacterial compound was used. Fruits were stored at 1° C and 5.5° C for 40 days, with evaluations at 10 day intervals. Packing in bags significantly reduced fruit water loss and enhanced retention of fruit colour for up to 20 days. There was no advantage of hydrocooling over standard static cooling, however, most water loss occurred in the first 10 days of storage. Total soluble solids of packaged fruit decreased with storage but remained high (15 to 20° Brix) while acidity was poor at below 0.40%. The TSS: acid ratio showed good eating quality as it remained between 31:1 and 60:1. Fruit that was hydrocooled and stored at a temperature of 5.5°C without a Biosave treatment showed a statistically superior colour which remained good for up to 40 days of storage. The higher storage temperature showed better colour retention, however, there was greater incidence of disease.

INTRODUCTION

Litchi fruit has gained considerable popularity in the temperate countries of the world, where it is seen as being exotic and scarce. South Africa generally exports an average of 4 million tonnes of litchis of which 95% will be marketed in the European Union (P.P.E.C.B. Export Directory, 2006). It is essential that export fruit has good red colour and is free of disease and other imperfections.

Litchi fruit has a very poor shelf life of only 2 to 7 days under ambient conditions of 25°C (Underhill *et al.* 1997). This limits the distribution of fruit and results in excess fruit during the peak season with very little possibility of market manipulation. Desiccation of the litchi rind is a major factor during postharvest storage and causes the fruit to turn an unsightly brown (Kaiser, 1998). Coupled with postharvest disease, this forms the greatest impediment to fruit quality maintenance (Underhill *et al.*, 1997).

Tan & Li (1984) and Swarts (1989) found that fumigation of fruit with SO₂ could suppress browning of fruit and decrease postharvest pathogen induced damage. This treatment is used by the majority of South African growers but it has several disadvantages, including a bleaching effect. It also causes aftertastes, is ineffective against some fungi and constitutes a potential health hazard. Recently the European Union has begun to reduce the use of sulphur for the postharvest treatment of litchis. It is therefore essential that research is done on investigating alternatives to sulphur dioxide

The previous work done indicated that polypropylene packaging was very effective in reducing water loss and thus desiccation of the litchi rind. It was also found that water loss occurs mainly within the first 10 days of storage and possibly immediately postharvest. Therefore a hydrocooling technique may be

useful in reducing water loss in this early period, as fruit would be rapidly cooled just prior to packaging. Litchi fruit are relatively cold tolerant and thus an integrated system to control water loss while at the same time limiting the opportunity for postharvest disease, could be a viable means of extending shelf life without SO₂.

The objectives of this study were to investigate the use of polypropylene packaging with micro-perforations to allow for some gas transfer, together with the use of biological disease control methods, coupled with differing storage temperatures and cooling techniques.

METHODS AND MATERIALS

Fruit was collected from a Salt Rock pack house in KwaZulu-Natal, after standard packing and grading, on the 6 January 2005 (the morning of harvest). It was then taken directly to Pietermaritzburg for further treatment. On arrival at the laboratory, fruit was divided into groups of 10 fruit per sample. Half the sample treatments included the use of a postharvest antifungal compound, registered on citrus and deciduous fruit in the USA and sold as 'Biosave'. This biological dip is made up of the ESC 10 strain of the active bacteria *Pseudomonas syringae* and controls pathogen infection by competitive inhibition. Five replications were used for each treatment and this was repeated four times to allow for fruit to be removed for evaluation at 10 – day intervals.

After treatment all fruit was packed into micro-perforated (9 µm), anti-mist coated polypropylene bags for storage and subjected to the following treatments:

- T1- Static cooling by refrigeration and stored at 1°C, with a Biosave treatment.
- T2- Static cooling by refrigeration and stored at 1°C.
- T3- Static cooling by refrigeration and stored at 5.5°C, with a Biosave Treatment.
- T4- Static cooling by refrigeration and stored at 5.5°C.
- T5- Hydrocooling of fruit in a 4°C water bath containing Biosave, for 10 minutes. Fruit were stored at 1°C.
- T6- Hydrocooling of fruit in a 4°C water bath for 10 minutes without Biosave. Fruit were stored at 1°C.
- T7- Hydrocooling of fruit in a 4°C water bath containing Biosave, for 10 minutes. Fruit were stored at 5.5°C.
- T8- Hydrocooling of fruit in a 4°C water bath for 10 minutes without Biosave. Fruit were stored at 5.5°C.

Fruits were stored at the respective temperatures and evaluated after 10, 20, 30 and 40 days. Before storage fruits were weighed, and colour determined with a Minolta colorimeter.

At each post storage evaluation, fruit was weighed to determine water loss, as this is believed to strongly affect colour. Colour was measured using a colorimeter, and general observations were noted. Fruit was also tested for total soluble solids (TSS), acidity and rind anthocyanin content in order to establish quality. Total soluble solids were determined using a Palette PR-101 electronic refractometer. Acidity was determined by the titration of 5 ml of litchi juice from each sample with 0.1 M NaOH. A phenolphthalein indicator was used to determine the end point of the titration

Anthocyanin was extracted from 10 rind discs from each sample with 10 ml acetone and was refrigerated at 4°C for 24 hours. Absorption spectrum of the extracts was determined using a DU[®] 800 Spectrophotometer. The anthocyanin content was estimated from absorbance at 530 nm (Li *et al.*, 2004) and was recorded as a percentage of the anthocyanin content of the initial fruit. This was done for each treatment and replication.

Statistical analysis was based on a factorial design of treatments with five replications. Components were tested for statistical difference using Analysis of Variance procedures of GenStat Ninth Edition (GenStat release 9.1, Lawes Agricultural Trust, 2006).

RESULTS

Fruit water loss

It is essential that the water loss from fruit is minimised as desiccation results in increased browning of fruit. It is assumed that mass loss was primarily water loss. The results show that the packing of fruit in polypropylene bags dramatically reduces water loss. In this study water loss remained below 2 % over the entire storage period compared to high water loss recorded in unpackaged fruit during previous trials. There were only marginal differences, which were insignificant and showed no clear trend, between the packaging treatments (Figure 1). Both the cooling methods and the postharvest application of Biosave, had no significant effect on fruit water loss. However, an important trend noted in the results is that the majority of water loss occurred in the first 10 days of storage. The actual loss of mass, and therefore water, may have occurred early during storage. This could not be determined as the first measurements were only recorded at 10 days after packaging. Thereafter there is a significant ($p=0.01$) increase in water loss throughout the storage period (Figure 1).

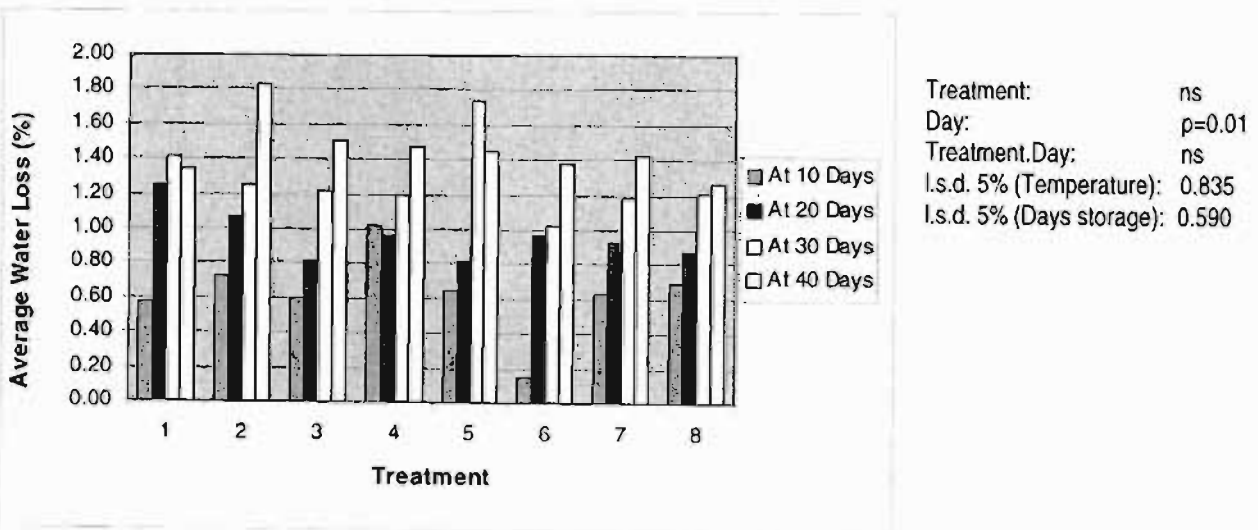


Figure 1: Percentage water loss of stored litchi fruit.

Even treatment numbers had no Postharvest treatment, while uneven numbered treatments were treated with 'Biosave'. Treatments 1 – 4 were static cooled while 5 – 8 were hydrocooled. Treatments 1, 2, 5 & 6 were stored at 1°C, while treatments 3, 4, 7, & 8 were stored at 5.5°C.

Fruit colour

Fruit colour was measured as a hue angle using a colorimeter. An angle of 35 – 40° indicates red fruit while an increasing hue angle is a result of the colour becoming brown. All fruit was found to be in good condition after 10 days of storage. However, colour appears to deteriorate at a fairly even rate after 10 days with significant ($p=0.01$) loss of colour over the 40 days storage. In general the fruit still had an acceptable colour at 20 days storage but thereafter tended to become brown. Treatment 8 produced a statistically ($p=0.01$) superior colour and retained good colour for the full 40 days of storage. This fruit was hydrocooled and stored at a temperature of 5.5°C without a 'Biosave' treatment. Treatments 3 and 4 also showed good colour results. These treatments were also stored at a higher temperature of 5.5°C, and were both statically cooled. It appears that storage at a higher temperature is favourable as it probably results in less damage to the rind of this subtropical fruit. Rind that is less stressed is then less likely to lose anthocyanins and/or become damaged followed by enzymatic browning. The 'Biosave' treatments did not noticeably affect colour retention.

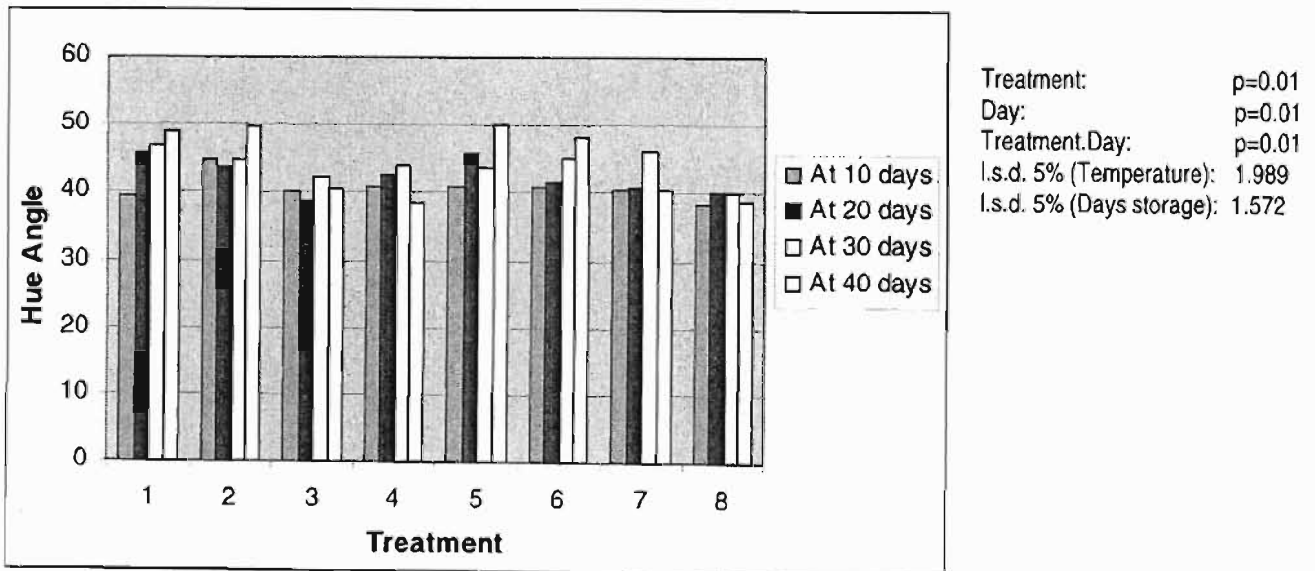


Figure 2: Hue Angle of stored litchi fruit.

Even treatment numbers had no Postharvest treatment, while uneven numbered treatments were treated with 'Biosave'. Treatments 1 – 4 were static cooled while 5 – 8 were hydrocooled. Treatments 1, 2, 5 & 6 were stored at 1°C, while treatments 3, 4, 7, & 8 were stored at 5.5°C.

Anthocyanin content

The results obtained generally showed a significant ($p=0.01$) decline in anthocyanin content for all treatments from 10 to 40 days storage. It is, however, notable that there was an increase in the anthocyanin content from harvest to the first evaluation at 10 days storage in some treatments. In these results treatment 4 showed significantly ($p=0.01$) better colour throughout the storage period. However, treatments 3 and 8 also show good colour with these results being in agreement with the hue angle measurements. These treatments were all stored at a higher temperature of 5.5°C which appears to result in better colour retention.

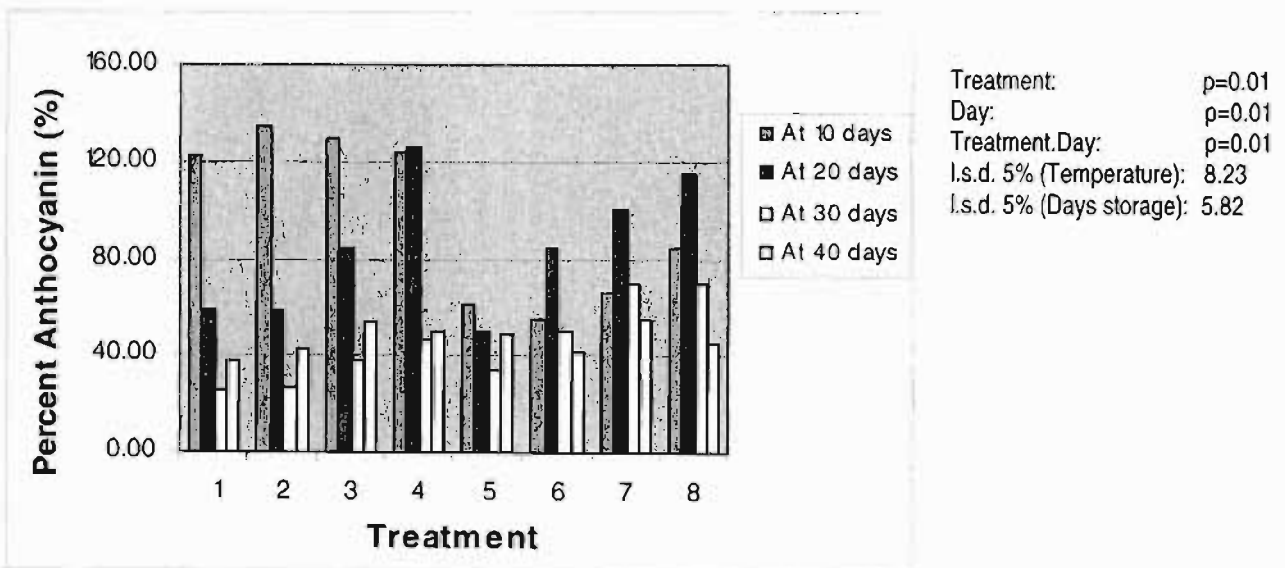


Figure 3: Anthocyanin content of stored litchi fruit as related to initial content.

Even treatment numbers had no Postharvest treatment, while uneven numbered treatments were treated with 'Biosave'. Treatments 1 – 4 were static cooled while 5 – 8 were hydrocooled. Treatments 1, 2, 5 & 6 were stored at 1°C , while treatments 3, 4, 7, & 8 were stored at 5.5°C .

Pathogen Control

The results show that there was significantly ($p=0.01$) less disease when fruit was stored at 1°C with greater disease incidence at 5.5°C . This result is expected as reduced temperature inhibits fungal growth and development. The fruit treated with 'Biosave' had slightly better pathogen control although results were not significantly different. Hydrocooling of fruit resulted in a slightly higher disease incidence and is probably due to the fruit being slightly wetter when packaged. The hydrocooling procedure was an additional handling step, and may also have led to some damage to the fruit rind and thus enabled disease infection to occur more easily through wound sites. Wound sites also cause the fruit to exude juice which then provides carbohydrates for pathogens to grow and develop. The use of protective packaging may decrease infection by ensuring that the fruit is not damaged and thus has no wound sites.

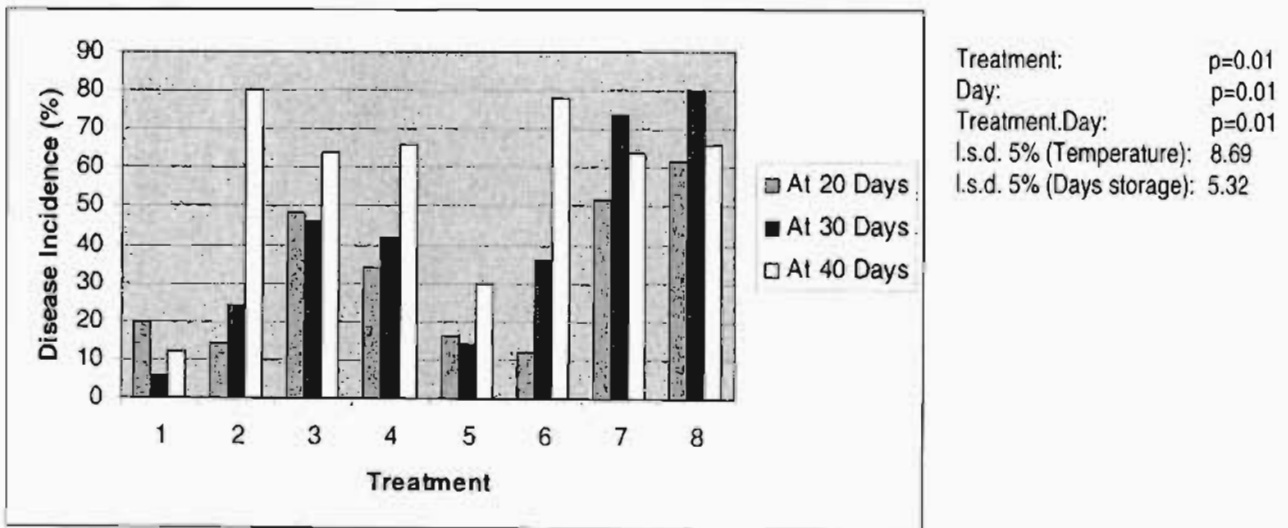


Figure 4: Disease incidence of stored litchi fruit.

Even treatment numbers had no Postharvest treatment, while uneven numbered treatments were treated with 'Biosave'. Treatments 1 – 4 were static cooled while 5 – 8 were hydrocooled. Treatments 1, 2, 5 & 6 were stored at 1°C , while treatments 3, 4, 7, & 8 were stored at 5.5°C .

Total Soluble Solids

The results show that there is a significant decrease ($p=0.01$) in the total soluble solids over the storage period (Figure 5) with the starting value of the fruit being 18.4° Brix. There was also a significant ($p=0.01$) difference between the total soluble solids of the various treatments. Treatment 5 had the highest TSS value with treatment 3 having the lowest value. However, all fruit had total soluble solids above 15° Brix. This is important as the level for commercial acceptability is between 13 and 20° Brix (Swarts, 1992).

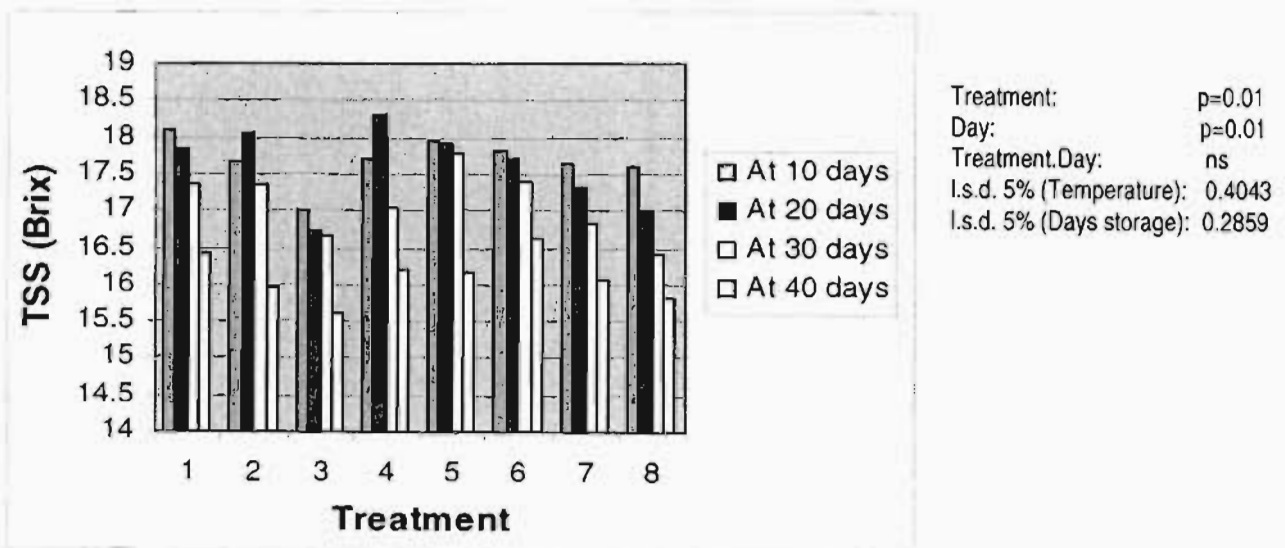


Figure 5: Total soluble solids (TSS) of stored litchi fruit

Even treatment numbers had no Postharvest treatment, while uneven numbered treatments were treated with 'Biosave'. Treatments 1 – 4 were static cooled while 5 – 8 were hydrocooled. Treatments 1, 2, 5 & 6 were stored at 1° C, while treatments 3, 4, 7, & 8 were stored at 5.5° C.

Acidity

While treatment did not appear to affect acidity, there was a significant ($p=0.01$) influence of the time of storage (figure 6) with a trend towards higher acidity with increasing storage time. However, the acidity of all fruit remained below 0.45% throughout the storage period. This is notable as an acidity of above 0.45% is considered to make the fruit taste less bland (Swarts, 1992).

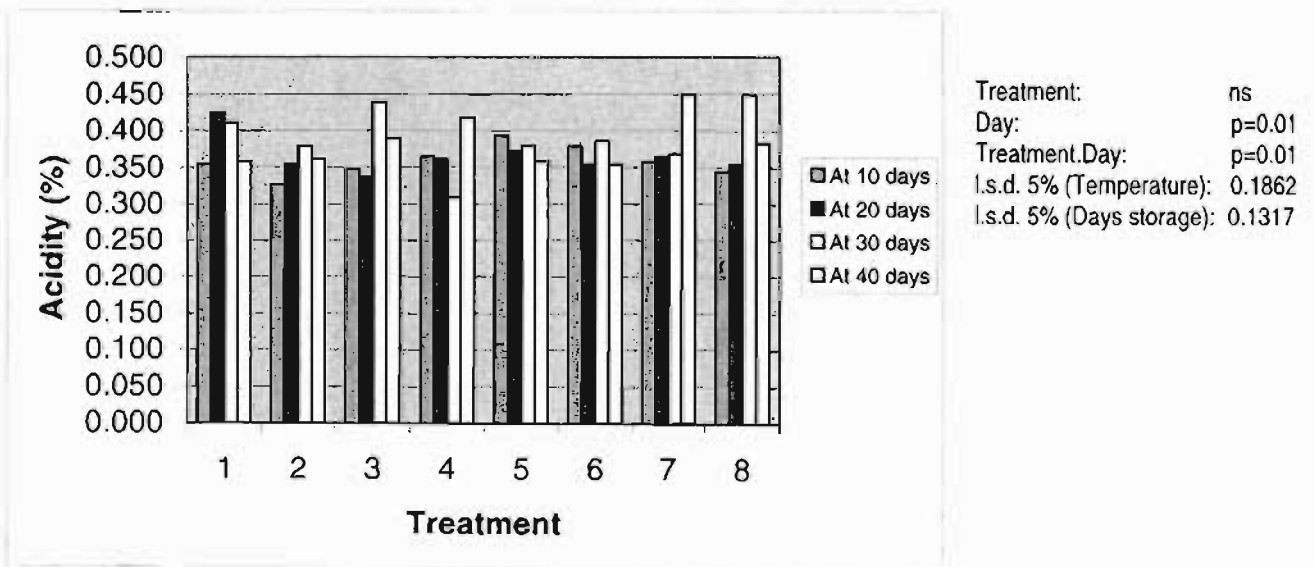


Figure 6: Acidity of stored litchi fruit

Even treatment numbers had no Postharvest treatment, while uneven numbered treatments were treated with Biosave. Treatments 1 – 4 were static cooled while 5 – 8 were hydrocooled. Treatments 1, 2, 5 & 6 were stored at 1°C , while treatments 3, 4, 7, & 8 were stored at 5.5°C .

TSS : Acid ratio

There was a significant ($p=0.01$) decrease in the TSS: acid ratio over the 40 day storage period (Figure 7). Underhill and Wong (1990) reported that the TSS: acid ratio of the pulp is well correlated to mean eating quality. In order for the fruit to have excellent taste, it must therefore have a TSS: acid ratio of between 31:1 and 60:1. In this case all the fruit met this criterion and would be considered to be of good eating quality.

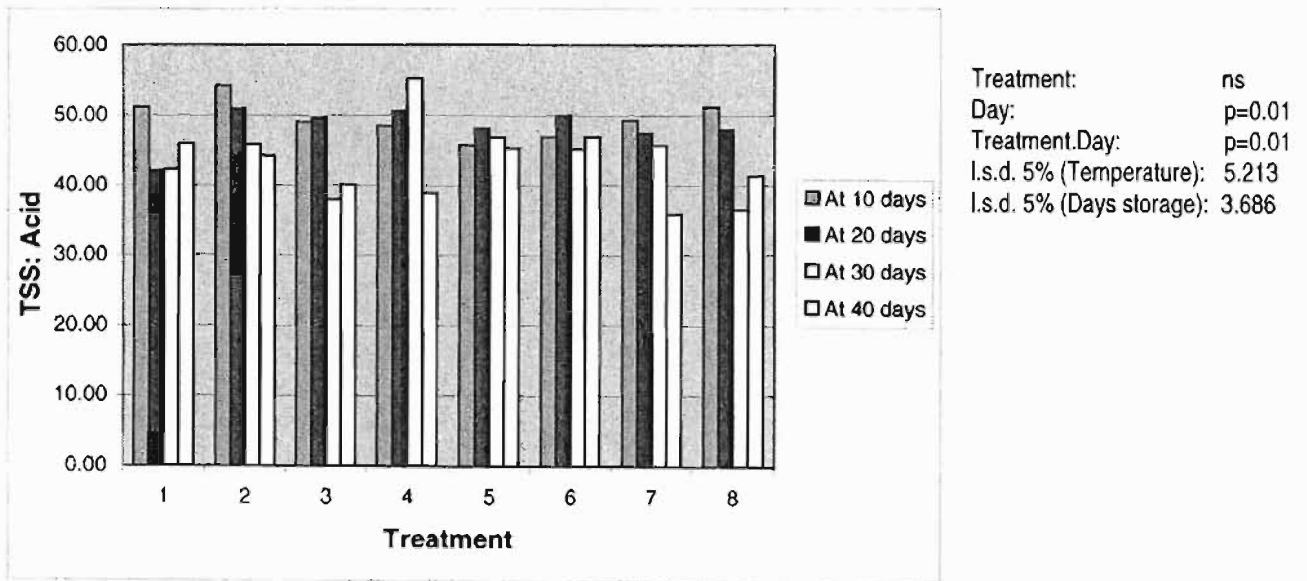


Figure 7: TSS: Acid ratio of stored litchi fruit

Even treatment numbers had no Postharvest treatment, while uneven numbered treatments were treated with Biosave. Treatments 1 – 4 were static cooled while 5 – 8 were hydrocooled. Treatments 1, 2, 5 & 6 were stored at 1^o C, while treatments 3, 4, 7, & 8 were stored at 5.5^o C.

DISCUSSION AND CONCLUSIONS

The polypropylene packaging system was previously found to significantly decrease fruit water loss, and therefore this system was used in all treatments. This packaging resulted in an increased shelf life with good colour retention and less postharvest decay. This result again confirms that the traditional litchi cartons are ineffective for postharvest management.

It was again noted that much of the water loss occurred within the first 10 days of storage and possibly very soon after harvest. It is suggested that the majority would actually take place during the cooling phase (Wills *et al.*, 1998) as the hot fruit loses water to a cooler atmosphere. If this is the case, then ensuring that fruit is cooled rapidly after harvest can further extend shelf life. However, the hydrocooling system did not significantly reduce the water loss and showed no clear advantage over fruit that was statically cooled. The hydrocooling method would probably have been more effective had it have been done on site immediately after the fruit was harvested. Hydrocooling of the fruit may also have had better results if it was done for a longer period of time. The negative effect of the hydrocooling was that disease incidence increased and is probably as a result of the fruit being packed before the rind was completely dry. In a commercial situation it would be imperative that the fruit is dried as it moves through the packing line. Packaging in polypropylene bags did result in some accumulation of water with fruit having a water soaked appearance. Thus a modification in the packaging technique is required to reduce the effect of free water.

The anthocyanin content of the fruit increased during the first 10 days of storage and then gradually declined. Although litchi fruit can be considered chilling tolerant from the work of Sandu & Randhawa (1992), it is suggested that storage at 1°C for 40 days may have caused cell damage, contributing to colour loss

(browning) after 20 days storage. This was reinforced when it was found that the treatments stored at 5.5°C showed better colour retention after the 40 days storage. The higher storage temperature, however, enhances the potential for postharvest diseases. Further work is therefore required to develop an integrated biological postharvest pathogen control system of high quality.

Total soluble solids (TSS) decreased over the storage period but remained high with all treatments having a value above 15° Brix. This is important as the TSS range for eating quality is 13 to 20° Brix (Nagar, 1994) and is used as an indication of commercial maturity. Underhill and Wong (1990) reported that the TSS: acid ratio of the pulp is well correlated to mean eating quality. In order for the fruit to have excellent taste, it must therefore have a TSS: acid ratio of between 31:1 and 60:1. All the fruit had a ratio that met this criterion and would therefore ensure good eating quality.

In conclusion, this study has shown that there was a noticeable extension of litchi shelf life with good colour retention. As the previous work indicated, shelf life extension is thought to be mainly as a result of decreased water loss from the fruit due to the packaging in polypropylene bags, as well as a slightly higher storage temperature of 5.5°C. These postharvest treatments combined with good temperature management are suitable for use on the local market and allow for better market manipulation. The increase shelf life allows the grower to release less fruit at one time as it can be easily stored for longer periods of time. This is also important for logistic management as transport can be limited to weekly deliveries instead of very regular deliveries. However, this system is only effective when there is good temperature management throughout the product chain from harvest to sale to the consumer. Further research will be required for the refinement of these techniques and possibly enable longer retention of

internal and external fruit qualities. This would then allow for export to the European Union without the use of sulphur.

LITERATURE CITED

Kaiser, C. (1998) An overview of post-harvest storage of Mauritius litchi fruit [summary]. *South African Litchi Growers' Association, Vol 9, 57.*

Li, X.J., Hou, J.H., Zhang, G.L., Liu, R.S., Yang, Y.G., Hu, Y.X. and Lin, J.X. (2004) Comparison of anthocyanin accumulation and morpho-anatomical features in apple skin during colour formation at two habitats. *Scientia Horticulturae, Vol 99, 41-53.*

Nagar, P.K. (1994) Physiological and biochemical studies during fruit ripening in litchi (*Litchi chinensis* Sonn). *Postharvest Biology and Technology, Vol 4, 25–234.*

P.P.E.C.B. Export Directory (2006).

Sandhu, S.S. and Randhawa, J.S. (1992) Effect of postharvest application of methyl – 2 – benzimidazole carbonate and in pack fumigant on the cold storage life of litchi cultivars. *Acta Horticulturae, Vol 269, 185–189.*

Swarts, D.H. (1989) Die effek van SO₂ behandeling op naoesbederf ontwikkeling by litsjies. *South African Litchi Growers Association Year Book 2, 22-24.*

Swarts, D.H. (1992) Harvesting, handling and packing. In The cultivation of litchis. *Institute for Tropical and Subtropical Crops, Bulletin 425. Agricultural Research Council.*

Tan, X. and Li, Y. (1984) The partial purification and properties of polyphenol oxidase from the pericarp of litchi (*Litchi chinensis* Sonn). *Acta Phytobiologica Sinica*, Vol 10, 339 – 346.

Underhill, S.J.R. and Wong, L.S. (1990) A maturity standard for lychee (*Litchi chinensis* Sonn). *Acta Horticulturae*, Vol 16, 245-251.

Underhill, S.J.R., Coates, L.M. and Saks, Y. 1997. Postharvest Physiology and Storage of Tropical and Subtropical Fruits. Ed. Mitra, S.K. *CAB International: Wallingford*.

Wills, R., McGlasson, B., Graham, D. and Joyce, D. (1998) Postharvest: An introduction to the physiology and handling of fruit, vegetables, and ornamentals. *CAB International: Wallingford*.

PAPER 3: THE EFFECT OF PACKAGING AND BIOLOGICAL DISEASE CONTROL ON THE SHELF LIFE OF LITCHI FRUIT

ABSTRACT

Litchis produced in South Africa are commonly treated postharvest with sulphur dioxide in order to suppress browning and pathogen infection. However, the European Union has considerably reduced accepted sulphur residue levels. Since 95 % of exported fruit is sold into the European Union, alternative forms of fruit quality maintenance are thus necessary. The cause of fruit postharvest browning is believed to be desiccation and the oxidation of phenolics by polyphenol oxidase (PPO). Previous work using micro – perforated polypropylene bags was very successful in reducing water loss but resulted in some water soaking of fruit. In this study fruit was packaged in polypropylene bags, with specific anti-mist and gas exchange properties, as well as in punnets lined with absorbent sheeting. 'Biosave', and F10 were tested for postharvest decay control. Fruit were stored at 1^o C and 5.5^o C for 40 days, with evaluations at 10-day intervals. Packing in bags significantly reduced fruit water loss and enhanced retention of fruit colour for up to 30 days. Packaging of fruit in a punnet, within the polypropylene bag, reduced both postharvest decay and surface damage. Fruit treated with 100 ml/l of 'Biosave', packaged in a punnet and stored at 1^oC was the best treatment resulting in the least incidence of disease. The higher storage temperature showed better colour retention, however, there was greater incidence of disease. PPO activity was higher in the rind of fruit that had turned brown and lower in fruit with good colour retention. These postharvest treatments, packaging techniques and storage temperatures, showed promising results that will greatly increase the internal and external fruit qualities on the local market and may with further work allow for export.

INTRODUCTION

Litchi fruit has gained much popularity on the international market and therefore has become an important export crop for South African growers. South Africa exports the majority of its litchi fruit to the European Union where good prices can be attained. The fruit therefore must be of high quality and have an attractive appearance, being red in colour and free of postharvest disease.

Litchi fruit has a very short shelf life under ambient conditions and it is therefore very difficult to maintain good external and internal quality (Underhill *et al.*, 1997). Desiccation of the litchi rind is a major factor in the rapid loss of rind colour and the development of an unsightly brown colour (Kaiser, 1998). It is thought that following dehydration damage, litchi pericarp browning is mainly due to rapid degradation of phenols by the activity of polyphenol oxidase (PPO) (Zauberman *et al.*, 1991) and is irreversible. Coupled with postharvest disease, this forms the greatest impediment to fruit quality maintenance (Underhill *et al.*, 1997).

Tan & Li (1984) and Swarts (1989) found that fumigation of fruit with SO₂ could suppress browning of fruit and decrease postharvest pathogen induced damage. This method has been extensively used by South African growers but resistance to chemical treatments has forced the industry to investigate alternative methods. Recently the European Union announced its intention to disallow the use of sulphur for the postharvest treatment of litchis, and has considerably reduced the maximum residue level presently allowed. Canada does not allow sulphur. Consequently, it is imperative that research aimed at eliminating sulphur fumigation, be undertaken (Kaiser, 1998).

Previous work has shown that packaging in polypropylene bags extensively reduces water loss and therefore can extend shelf life. However, the packaging

did result in some accumulation of water, with a water soaked appearance of fruit where it came in contact with free water. Litchi fruit are relatively cold tolerant and thus an integrated system limiting the opportunity for postharvest disease, could be a viable means of extending shelf life without SO₂.

The objectives of this study were to investigate the use of polypropylene packaging with micro-perforations to allow for some gas transfer (including water), together with the use of punnets lined with absorbent paper. This is to decrease potential damage to fruit during transport, as well as absorb any free water which may accumulate in the package, thereby decreasing the incidence of water soaked fruit and minimising postharvest disease risk. Biological disease control methods, coupled with differing storage temperatures were also evaluated, within the outlined systems.

METHODS AND MATERIALS

Fruit was collected from a Malelane site in Mpumalanga on 17 December 2005 on the morning of harvest. It was then taken directly to a nearby commercial pack house for further treatment. On arrival, fruit was divided into samples of 10 litchis. Two thirds of the treatments included the use of a postharvest antifungal compound at a concentration of 100 ml/l and 160 ml/l. The compound is registered on citrus and deciduous fruit in the USA and sold as 'Biosave'. This biological dip is made up of the ESC 10 strain of the active bacteria *Pseudomonas syringae* and controls pathogen infection by competitive inhibition. The final group was used as a control. Each treatment had five replications and was done four times to allow for fruit to be removed at the evaluation intervals.

After treatment (fruit dipped in the respective products for 2 minutes) half the fruit was packaged after being allowed to dry, in a sealed polypropylene bag with 9 micron perforations and anti mist coating, while the other half was packed into a punnet lined with absorbent paper and then within the same type of sealed polypropylene bag. Fruit was stored at 1°C and subjected to the following treatments:

T1- Packaged in a polypropylene bag with no other treatment.

T2- Packaged in a polypropylene bag with Biosave treatment at 100ml/l.

T3- Packaged in a polypropylene bag with Biosave treatment at 160 ml/l.

T4- Packaged in a punnet within a polypropylene bag. No other treatment.

T5- Packaged in a punnet within a polypropylene bag. Biosave treatment at a concentration of 100ml/l water.

T6- Packaged in a punnet within a polypropylene bag. Biosave treatment at a concentration of 160 ml/l water.

Fruits were stored at 1°C and evaluated after 10, 20, 30 and 40 days. Before storage fruits were weighed.

At each post storage evaluation, fruit was weighed to determine water loss, as this is believed to strongly affect colour. Disease incidence was recorded, and general observations were noted.

Another consignment of fruit was collected from a Salt Rock site in KwaZulu-Natal on 20 December 2005 on the morning of harvest. The fruit was then taken directly to Pietermaritzburg for further treatment. On arrival, fruit was divided into samples of 10 litchis. A third of the treatments included the use of 'Biosave'. Another third was used to test a Quarternary ammonium product known as F10 and the final group was used as a control. The treatments were replicated five times. This was repeated three times to allow for the removal of fruit at the evaluation times.

After treatment (fruit dipped in the respective products for 2 minutes and then allowed to dry) all fruit was packed into punnets lined with absorbent paper within polypropylene bags for storage and subjected to the following treatments:

T1- Stored at 1°C with no other treatment.

T2- Stored at 1°C with F10 treatment at a concentration of 3.5 ml/l water.

T3- Stored at 1°C with 'Biosave' treatment at a concentration of 100 ml/l water.

T4- Stored at 5.5°C with no other treatment.

T5- Stored at 5.5°C with F10 treatment at a concentration of 3.5 ml/l water.

T6- Stored at 5.5°C with 'Biosave' treatment at a concentration of 100 ml/l water.

Fruits were stored at the respective temperatures and evaluated after 15, 20 and 30 days. Before storage, fruits were weighed, and colour determined with a Minolta colorimeter.

At each post storage evaluation, fruit was weighed to determine water loss, and colour was measured using a colorimeter while disease incidence was recorded, and general observations noted.

Litchi rind from a previous season (2004 – 2005) that had been freeze dried was used to determine polyphenol oxidase activity. PPO activity was determined using a standard PPO assay (Van Rooyen & Bower, 2006). The crude enzyme extraction for soluble PPO was made by grinding 1 g litchi rind with 10 ml of cold 10 mM acetate buffer, pH 6.8, in an Ultra Turrax for 30 seconds. The homogenate was then centrifuged at 12 000 x g for 15 minutes at 4°C. The supernatant was used immediately as the crude enzyme for the soluble PPO assay. One spatula of 0.1% SDS was added to the acetate buffer to prepare the crude enzyme extraction for the insoluble PPO assay. For the PPO assay, 0.25 ml crude enzyme was added to 0.5 ml of 100 mmol⁻¹ 4 – methylcatechol and 1.0 ml acetate buffer. The increase in absorbance was measured at 420 nm for 2 minutes. The final results recorded were for total PPO (soluble + insoluble) and expressed as change in OD/min/g protein. The protein content of the extracts was determined following precipitation of protein by 5% trichloroacetic acid, by the method of Lowry *et al* (1951) as modified by Leggett – Bailey (1962). Three treatments were tested for PPO activity. These were the treatments with worst, best and intermediate colour retention over the entire storage period. PPO was tested after 10, 20, 30 and 40 days storage at specific temperatures. Fruit was packaged in a sealed polypropylene bag with 9 micron perforations. Five replications were used for each treatment.

The fruit was subjected to the following treatments:

T2 (worst colour retention) - Stored at 1°C with no other treatment.

T7 (intermediate colour retention) - Stored at 5.5°C with 'Biosave' treatment at a concentration of 100 ml/l water.

T8 (best colour retention) - Stored at 5.5°C with no other treatment.

Statistical analysis for all experiments was based on a factorial design of treatments with five replications. Components were tested for statistical difference using Analysis of Variance procedures of GenStat Ninth Edition (GenStat release 9.1, Lawes Agricultural Trust, 2006).

RESULTS

The colour of fruit obtained from Malelane lacked redness, presumably due to climatic conditions. Thus, this fruit could not be evaluated in a meaningful manner, and will only be discussed in relation to disease incidence.

Fruit water loss

It is essential that the water loss from fruit is minimised as desiccation results in increased browning of fruit. Mass loss is also extremely important as litchi fruit is sold by mass and one would want to prevent loss of profit. It is assumed that mass loss was primarily water loss. The results again show that the packing of the Salt Rock fruit in polypropylene bags dramatically reduced water loss. In this study water loss remained at below 5 % over the entire storage period, compared to high water loss of 10 to 14 % recorded in unpackaged fruit during previous trials. There were only marginal differences, which showed no clear trend, between the packaging treatments (Figure 1).

There appears to be a general increase in loss of mass, and therefore water loss, throughout the storage period. However, at times there is also a gain in mass which may be due to the fruit re – absorbing free water during the storage period. (Figure 1).

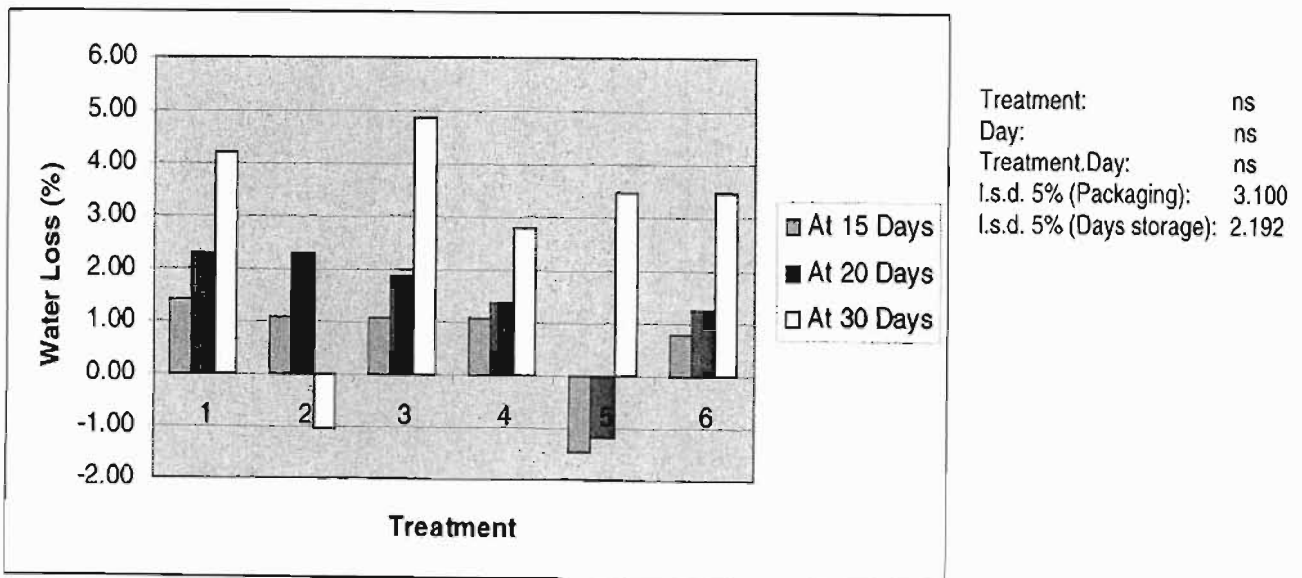


Figure 1: Water loss of stored litchi fruit.

Treatments 1 & 4 had no Postharvest treatment, while treatments 2 & 5 were treated with F10 and treatments 3 & 6 with 'Biosave'. Treatments 1 - 3 were stored at 1^o C, while treatments 4 - 6 were stored at 5.5^o C.

Fruit colour

Fruit colour of the Salt Rock litchis was measured as a hue angle using a colorimeter. An angle of 35 – 40° indicates red fruit while an increasing hue angle is an indicator of browning fruit. Fruit arriving from the Salt Rock site had colour with an average hue angle of 47°. Initially, some of the treatments appeared to increase in terms of red colour, and in general, good colour retention occurred throughout the storage period. Treatment 4 produced a significantly ($p=0.01$) superior colour and retained good colour for 30 days of storage. This fruit was not treated for pathogen control and was stored at a temperature of 5.5°C. The treatments stored at a higher temperature of 5.5°C generally had superior colour to those stored at 1°C. It is suggested that storage at a higher temperature is favourable as it probably results in less low temperature damage to the rind of this subtropical fruit. Rind that is less stressed is then less likely to lose anthocyanins and become brown. The 'Biosave' and F10 treatments had significantly ($p=0.01$) poorer colour retention than fruit that did not receive a postharvest treatment. Figure 3 indicates the generally good appearance of the treatments stored in punnets with an absorbent base lining.

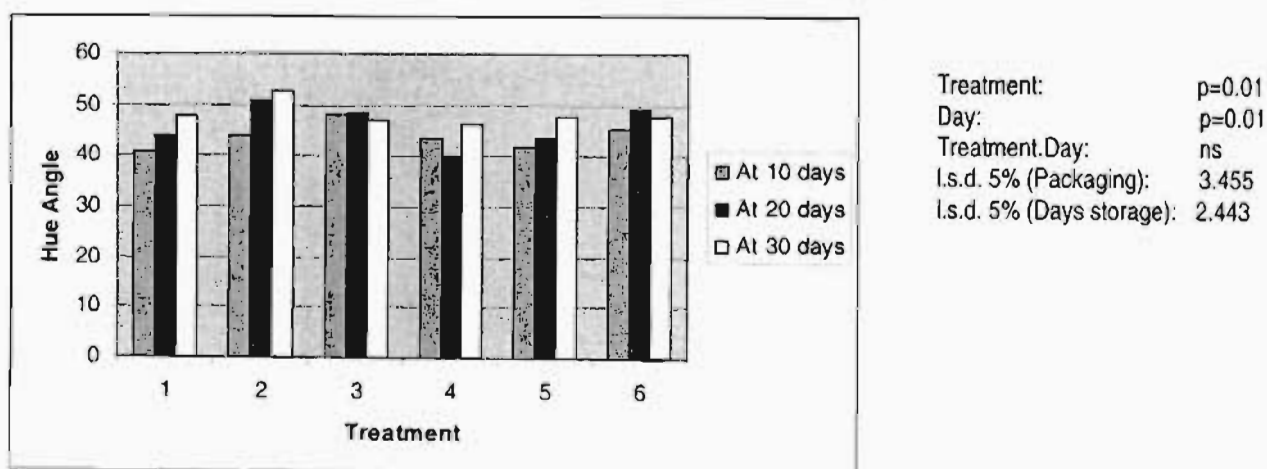


Figure 2: Hue Angle of stored litchi fruit

Treatments 1 & 4 had no Postharvest treatment, while treatments 2 & 5 were treated with F10 and treatments 3 & 6 with 'Biosave'. Treatments 1 - 3 were stored at 1°C, while treatments 4 - 6 were stored at 5.5°C.



Figure 3: Litchi colour after 20 days storage at 5.5°C and packaged in punnets lined with water absorbent paper and sealed in micro-perforated polypropylene packaging with anti-mist coating.

Polyphenol Oxidase (PPO) Activity

Polyphenol oxidase (PPO) activity of the rind was determined in three treatments over the 40 day storage period. These treatments were packed in a polypropylene bag but were stored at different temperatures. Treatment 2 was stored at 1°C and had the worst colour retention over the storage period. Treatment 8 had very good red colour and was stored at 5.5°C whilst treatment 7, which had intermediate colour retention, was stored at 5.5°C and was treated postharvest with Biosave. The results show that treatment 2 had a significantly higher ($p=0.01$) PPO activity during the initial period of storage, while treatment 8 had very low PPO activity with treatment 7 showing intermediate results. This correlates closely to the colour retention of the fruit, with the brown fruit of treatment 2 having a higher PPO activity than that of the red fruit in treatment 8. This indicates that PPO is closely involved the degradation of phenolics and the loss of colour. Towards the end of the storage period, the PPO activity generally decreased, while the colour of the fruit continues to become browner. This is maybe due to product inhibition of the PPO enzyme, as the brown pigments are formed. Cell damage and death may finally render the enzyme inactive.

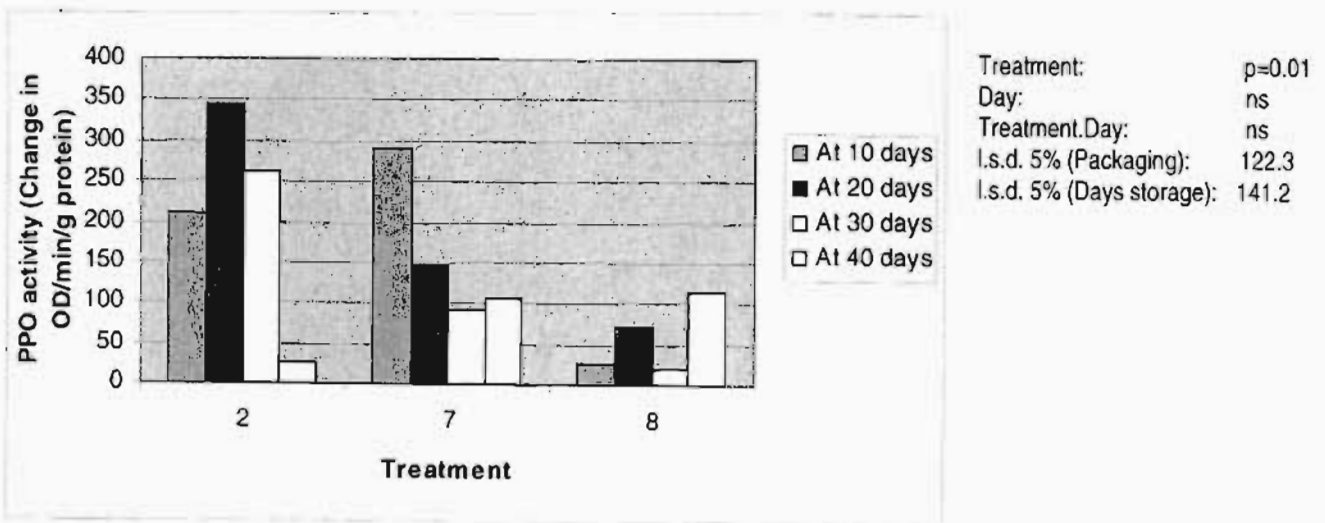


Figure 3: Total Polyphenol Oxidase activity (change in OD/min/g protein) of stored litchi fruit

Treatment 2 was stored at 1°C while treatments 7 & 8 were stored at 5.5°C. Treatment 7 was treated postharvest with Biosave.

Pathogen control

Pathogen control is extremely important when an increased shelf life is required. The two packaging methods used in this study were tested in order to establish which method would result in the least pathogen infection. It was also important that the two concentrations of the *Pseudomonas syringae* compound 'Biosave' and the Quarternary ammonium product F10 were tested for pathogen control. The litchis collected from Malelane in Mpumalanga were either packed in a polypropylene bag or in a punnet lined with absorbent paper within the polypropylene bag and were treated with 100ml/l or 160 ml/l of 'Biosave'. The results after 40 days storage at 1°C show the treatments 4 – 6, which were packaged in the punnet, had less disease incidence than those only packaged in the polypropylene bag (figure 5). This indicated that the absorbent paper is likely to be reducing the amount of free water in the packaging and therefore decreases water soaking of the litchis which would in turn result in less disease incidence. The fruit packed in the punnet is also protected from damage and therefore has fewer wounds that would be ideal infection points. The results show that treatment 5 had the best pathogen control with a disease incidence of only 17% infected fruit after 40 days storage. This fruit was packaged in a punnet lined with absorbent paper and was treated with 100 ml/l of 'Biosave'. Treatments 3 and 6 were both treated with the higher concentration of 'Biosave' and resulted in higher pathogen infection. However, generally the disease incidence was low throughout the trial with the highest incidence of disease only being 32% which is suggested to be a good result after 40 days of storage. This study showed that the ideal treatment would be that of 100 ml/l 'Biosave' packaged in the punnet within the polypropylene bag.

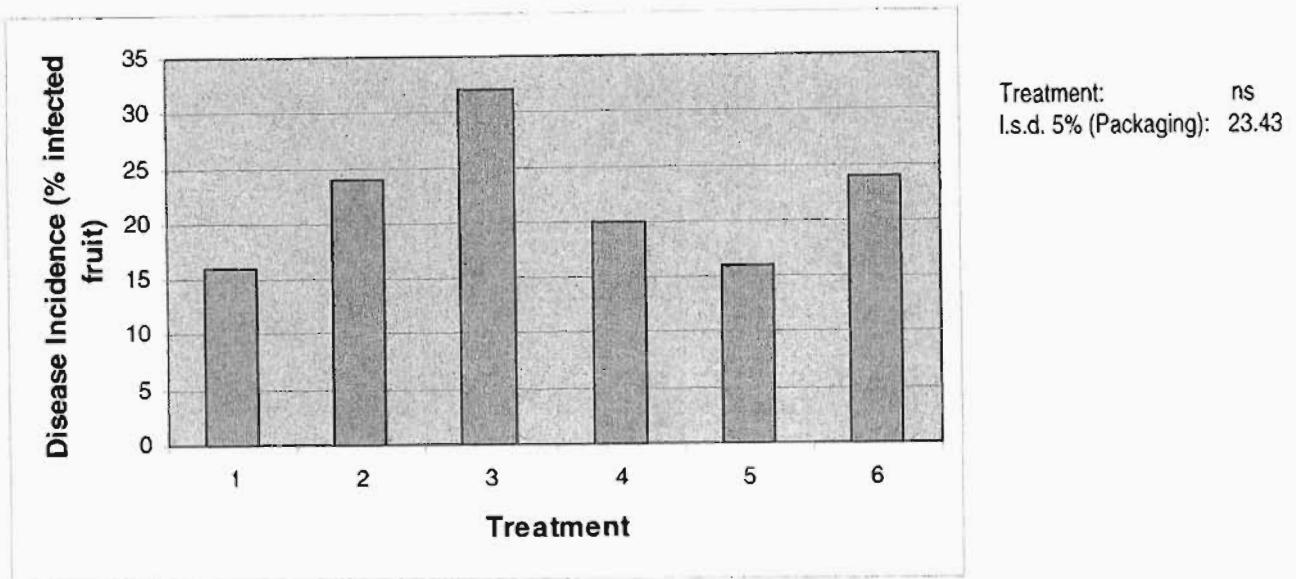
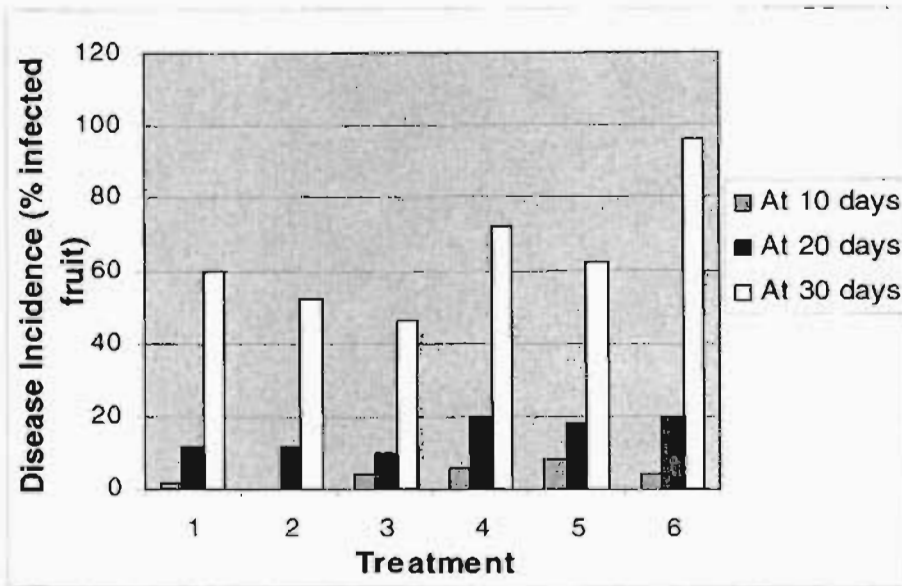


Figure 5: Disease incidence of stored fruit from Malelane after 40 days of storage at 1°C.

Treatments 1 – 3 were packed in polypropylene bags while treatments 4 – 6 were packed in punnets lined with absorbent paper within the polypropylene bag. Treatments 1 & 4 had no postharvest treatment, while treatments 2 & 5 were treated with 100 ml/l 'Biosave' and treatments 3 & 6 with 160 ml/l 'Biosave'.

The litchis collected from Salt Rock in KwaZulu – Natal were all packed into punnets and were used to again test 'Biosave' as well as the Quarternary ammonium product F10. The added complexity of temperature management was also investigated. Results showed that treatments 1 – 3 stored at 1°C had a significantly ($p=0.01$) lower disease incidence than the other treatments that were stored at the higher temperature of 5.5°C. This is expected as pathogen growth is stunted at lower temperatures and it would take longer for the pathogen to invade the fruit. Treatment 3 produced the best results and was a treatment of 100ml/l of 'Biosave' stored at 1°C. This result was in agreement with the results obtained from Malelane fruit and would be the recommended method for pathogen control. F10 produced poor results and was not as effective as 'Biosave' at the 1°C storage temperature.



Treatment: p=0.01
 Day: p=0.01
 Treatment.Day: p=0.01
 I.s.d. 5% (Packaging): 6.889
 I.s.d. 5% (Days storage): 4.871

Figure 6: Disease incidence of stored fruit from Salt Rock

Treatments 1 & 4 had no Postharvest treatment, while treatments 2 & 5 were treated with F10 and treatments 3 & 6 with 'Biosave'. Treatments 1 - 3 were stored at 1^o C, while treatments 4 - 6 were stored at 5.5^o C.

DISCUSSION AND CONCLUSIONS

The polypropylene packaging system was previously found to significantly decrease fruit water loss, and therefore this system was used in all treatments. There was again an increase in shelf life as determined by colour and decreased disease incidence. Little condensation occurred in the polypropylene bag thus reducing the probability of pathogen infection. Packaging in a punnet lined with absorbent paper within the polypropylene bag was successful in further decreasing pathogen infection and reducing water soaking of fruit. The punnet is also likely to be effective in protecting the fruit from damage and wounding. This is an improvement of previous packaging that resulted in too much condensation and therefore fungal infection.

Fruit water loss was reduced to 5%, which is well below that of litchis packed in commercial boxes without protective treatment. This is essential in the retention of litchi colour as desiccation is one of the primary causes of litchi browning. It is also noted that water loss is a measurement of weight loss and therefore has an impact on commercial litchi sales which are based on weight. Therefore, such packaging will decrease the loss of weight and will in turn increase the marketable value of a crop.

Although litchi fruit can be considered chilling tolerant from the work of Sandu & Randhawa (1992), results suggested that storage at 1°C for 30 days may have caused cell damage, contributing to colour loss (browning) during storage. This was reinforced when it was found that the treatments stored at 5.5°C showed better colour retention after the 30 days storage. The higher storage temperature, however, enhances the potential for postharvest diseases. It is important to note that fruit colour was fairly poor on arrival (lack of intense red colour, especially Malelane fruit) and therefore colour retention should be

measured based on the average colour of fruit on arrival. Results show that polyphenol oxidase (PPO) activity in the litchi rind is closely related to browning of litchi fruit and is probably due to the degradation of phenolics by PPO. It is suggested that this process is driven by cell damage caused by progressive desiccation. Brown fruit has a high PPO activity whilst red fruit has much lower activity. It is also shown that PPO activity decreases over storage time, possibly due to product inhibition of the enzyme.

Pathogen control was most effective when fruit was packed in a lined punnet within the polypropylene bag, and stored at 1°C with a postharvest treatment of 100 ml/l 'Biosave'. The punnet is effective in reducing fruit damage and the liner reduces water soaking and therefore decreases disease incidence.

These results have thus shown that the shelf life of litchis can be extended by packing the litchi fruit in punnets lined with absorbent paper within sealed polypropylene bags. The increased shelf life can mainly be attributed to the reduction of water loss and decreased water soaking. Disease incidence can be reduced with a postharvest treatment of 100 ml/l of 'Biosave' and storage at 1°C. These postharvest treatments have produced good results that have enabled good retention of internal and external fruit qualities. The techniques are suitable to enhance quality on the local market in their present form. While colour retention was good for 30 days, further work will be necessary to ensure a 40 day storage life to ensure adequate time for distribution within the European Union.

LITERATURE CITED

Kaiser, C. 1998. An overview of post-harvest storage of Mauritius litchi fruit [summary]. *South African Litchi Growers' Yearbook* 9, 57.

Leggett – Bailey, J. (1962). Techniques in protein chemistry. *Elsevier Publishing Co. Amsterdam*.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the Folin Phenol reagent. *Journal of Biological Chemistry*. 193, 265 – 75.

Sandhu, S.S. and Randhawa, J.S. 1992. Effect of postharvest application of methyl – 2 – benzimidazole carbonate and in pack fumigant on the cold storage life of litchi cultivars. *Acta Horticulturae*. 269, 185–189.

Swarts, D.H. (1989) Die effek van SO₂ behandeling op naoesbederf ontwikkeling by litsjies. *South African Litchi Growers Association Year Book* 2, 22-24.

Swarts, D.H. (1992) Harvesting, handling and packing. In The cultivation of litchis. *Institute for Tropical and Subtropical Crops, Bulletin 425. Agricultural Research Council*.

Tan, X. and Li, Y. 1984. The partial purification and properties of polyphenol oxidase from the pericarp of litchi (*Litchi chinensis* Sonn). *Acta Phytobiologica Sinica*. 10, 339 – 346.

Underhill, S.J.R., Coates, L.M. and Saks, Y. (1997) Postharvest Physiology and Storage of Tropical and Subtropical Fruits. Ed. Mitra, S.K. *CAB International: Wallingford*.

Van Rooyen, Z. and Bower, J.P. 2006. Effects of storage temperature, harvest date and fruit origin on post-harvest physiology and severity of mesocarp discoloration in 'Pinkerton' avocado (*Persea Americana Mill.*). *Journal of Horticultural Science and Biotechnology*. 81, 89-98.

Zauberman, G., Ronen, R., Akerman, M., Weksler, A., Rot, I. and Fuchs, Y. (1991) Postharvest retention of the red colour of litchi fruit pericarp. *Scientia Horticulturae*, 89-47, 97.

FINAL CONCLUSIONS

This study has provided much insight into postharvest management of litchi fruit. It has also confirmed the most important factors that contribute to retention of both external and internal fruit quality. This has in turn enabled the manipulation of such factors and thus improved postharvest techniques.

Packaging Technique

The polypropylene packaging system significantly decreased fruit water loss. There was also an increase in shelf life, as determined by red colour and overall rind appearance. This result is in accordance with previous work on packaging done by Scott *et al.*, (1982) and confirmed by the observations of Roe (1996) that the traditional cartons used for litchi are poor in relation to fruit quality management. Some condensation occurred in the bag and resulted in fruit becoming water soaked at times with increased fungal infection. This problem was overcome with the use of punnets lined with absorbent sheeting and placed within the sealed polypropylene bag. The absorbent sheeting reduced the amount of free water and resulted in little pathogen infection. The punnet is also effective in protecting the fruit from damage during storage and transport, thus reducing the availability of wound sites for disease entry.

It was notable that most water loss occurred within the first 10 days of storage and possibly early during this period. It is suggested that the majority of water loss would actually take place during the cooling phase as the hot fruit loses water to a cooler atmosphere. If this is the case, then ensuring that fruit is cooled rapidly after harvest can further extend shelf life. Rapid cooling can be attained through the use of a hydrocooling system as water is a good conductor of heat, and water would not be lost from the fruit to the atmosphere. Although a hydrocooling technique was investigated and was found to not significantly

decrease water loss, this was possibly due to not hydrocooling for a long enough period or the fact that it was not done immediately postharvest, when it may have been more effective.

Ethylene plays a minor role in the senescence of litchi fruit due to the fact that litchi is non climacteric. Respiration, however, continues after harvest and ultimately affects internal and external quality. The storage temperature used was set low enough to sufficiently minimise respiration and is probably the reason that a modified atmosphere showed little effect. At this low temperature, packaging in ambient air appears acceptable with no distinct advantage of modified atmosphere.

Temperature Management

The anthocyanin content of the fruit increased during the first 10 days of storage and then gradually declined. Although litchi fruit can be considered chilling tolerant from the work of Sandu & Randhawa (1992), it is suggested that storage at 1°C for 40 days may have caused cell damage, contributing to colour loss (browning) after more than 20 days storage. This suggestion is reinforced when it is considered that the treatments stored at 5.5°C showed better colour retention after the 40 days storage than the 1°C storage treatment. The higher storage temperature, however, enhances the potential for postharvest diseases.

Polyphenol Oxidase Activity

Results showed that polyphenol oxidase (PPO) activity in the litchi rind is closely related to browning of litchi fruit and is probably due to the degradation of phenolics by PPO. It is suggested that this process is driven by cell damage caused by progressive desiccation. Brown fruit has a high PPO activity whilst red fruit has much lower activity. It was also shown that PPO activity decreases over storage time, possibly due to product inhibition of the enzyme.

Pathogen Control

Three compounds, namely ISR 2000, 'Biosave' and F10, were tested for pathogen control. The *Pseudomonas syringae* compound 'Biosave' showed the best results of the three compounds. The most effective concentration was found to be 100 ml/l and good pathogen control occurred when storage was at 1° C. In this study pathogen control has been the limiting factor in shelf life extension and further work is therefore required to develop an integrated biological postharvest pathogen control system of high quality.

Internal Quality

Total soluble solids (TSS) decreased over the storage period but remained high with all treatments having a value above 15° Brix. This is important as the TSS range for eating quality is 13 to 20° Brix (Nagar, 1994) and is used as an indication of commercial maturity. The acidity of the fruit decreased during the storage period to a fairly low level. The acidity dropped to below the minimum standard of 0.45 % in all fruit which indicated that it is too low in respect to good eating quality. This was, however, true for all treatments, and was thus a characteristic of the fruit. The TSS: acid ratio of the pulp is well correlated to mean eating quality. In order for the fruit to have excellent taste, it must therefore have a TSS: acid ratio of between 31:1 and 60:1. All the fruit had a ratio that met this criterion and would therefore ensure good eating quality.

It is therefore considered that the work has shown it is possible to ship litchi fruit without the use of sulphur treatment, provided that water loss control is in place with the use of correct packaging. Acceptable cold chain management coupled with postharvest pathogen control is also necessary. Further work will be required to optimise cooling systems and storage temperature to extend shelf life to the targeted 40 days. More efficient pathogen control may also be possible.

LITERATURE CITED

Nagar, P.K. (1994) Physiological and biochemical studies during fruit ripening in litchi (*Litchi chinensis* Sonn). *Postharvest Biology and Technology*, Vol 4, 25 – 234.

Roe, D.J. (1996) A comparison of different postharvest treatments and packing materials for McLean's Red litchi fruit. *South African Litchi Growers' Association*, Vol 8, 44-47.

Sandhu, S.S. and Randhawa, J.S. (1992) Effect of postharvest application of methyl – 2 – benzimidazole carbamate and in pack fumigant on the cold storage life of litchi cultivars. *Acta Horticulturae*, Vol 269, 185 – 189.

Scott, K.J., Brown, B.I., Chaplin, G.R., Wilcox, M.E. and Bain, J.M. (1982) The control of rotting and browning of litchi fruit by hot benomyl and plastic film. *Scientia Horticulturae*, Vol 16, 253 – 262.

RECOMMENDATION

It is recommended that growers should follow an integrated management system to maximize the shelf life of litchi fruit. Both packaging technique and cold chain management are key factors in the postharvest management of litchis. Once the fruit has been harvested, it is suggested that the fruit should be hydrocooled immediately in water of about 4^o C for at least 20 minutes. Although this research did not show a significant difference in fruit that was hydrocooled, it is very likely that it will have a positive effect if done immediately after harvesting. The fruit should also be treated with 100 ml/l 'Biosave' which can be incorporated into the hydrocooling water. This would reduce the time required as two treatments can be completed at one time. The fruit should then be dried using a fan system to ensure that there is no excess free water. The packaging of fruit in sealed micro perforated polypropylene bags is essential in retaining the postharvest quality. This packaging has a significant effect on colour and weight retention of the fruit. It is suggested that one should pack the fruit into a punnet that is lined with absorbent sheeting and is placed inside the sealed polypropylene bag. Following this it is imperative that there is good cold chain management. Fruit should be stored immediately at 1^o C until transportation is required. During transport it is essential that the cold chain is well managed and the fruit does not increase in temperature. If this were to occur there would be some water loss and greater incidence of disease. It is also very important that the fruit is stored at low temperature in the retail outlet as this is often where pathogen infection becomes excessive. Growers should educate their retail outlet to store and display the litchi fruit in a refrigerated area.

These postharvest techniques are able to extend the shelf life of litchi fruit to 30 days if well managed. The increased shelf life allows the grower to release less fruit at one time as it can be easily stored for longer periods, thus optimising

supply to the market and maximising returns. This is also important for logistic management as transport can be limited to weekly deliveries instead of regular deliveries. Further research will be required for the refinement of these techniques and to possibly enable shelf life extension to 40 days as required for shipment to the European Union.