


**THIN MONOLITHIC SLOW-RELEASE DEVICES FOR
OPTIMUM IN-PACKAGE PRESERVATION OF EXPORT
TABLE GRAPE VARIETIES**

WILLEM JACOBUS OPPERMAN

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The crest of the University of Stellenbosch is centered behind the text. It features a shield with a blue and white design, topped by a red and white crown. The shield is flanked by two red and white figures, possibly lions or griffins, and a red banner with white text is draped across the top.

Promoter: Prof. Dr. R.D. Sanderson

Co-Promoter: Prof. Dr. T.J. Britz

March 2002

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.

Willem Jacobus Opperman

March 2002

Abstract

Prototypes of a new polymer SO₂ gas-generating sheet for the control of *Botrytis cinerea* during the post-harvest storage of table grapes, were developed and manufactured for evaluation using a pilot scale production plant. Attention was paid to the appearance of the sheet, in order to make it technologically efficient as well as aesthetically acceptable to both industry and consumers.

The storage quality of semi-commercial export consignments of various cultivars table grapes packed with the monolithic thin-film polymer SO₂ slow release sheet, was evaluated and compared to results obtained using the locally manufactured Uvasys SO₂ sheet. The following were investigated: the efficacy of the new polymer sheets in controlling storage decay, the stage at which SO₂ damage is manifested on table grapes, the level of SO₂ damage associated with different SO₂ concentrations, whether SO₂ damage is manifested more readily at a particular position on the bunch, and the possible effect of an increase in storage temperature, from an initial storage at -0.5°C to 10°C, on the levels of SO₂ bleaching.

Results showed that the new polymer SO₂ sheet compared favourably with the existing, commercially available Uvasys SO₂ sheets. The exact SO₂ concentration required for effective decay control varied for different cultivars, as well as for the different types of grape packages. The SO₂ concentration incorporated within the sheet was shown to be lower for grapes packed in non-perforated bags, and slightly higher for those in perforated bags. Differences between cultivars occurred with regard to the level of control and the levels of SO₂ damage. Levels of SO₂ damage were also significantly affected by the storage period and temperature fluctuations. No significant differences in the levels of decay development and SO₂ damage were observed in relation to the orientation of the bunches in the carton.

The extent of damage incurred to grape tissue by the absorption of SO₂ gas was determined by low-temperature scanning (LTSEM) and

transmission electron microscopy (TEM) techniques. LTSEM and TEM micrographs of areas damaged by SO₂ gas revealed that exposure to SO₂ gas may lead to plasmolysis and the loss of cellular fluids. Although damage to the cell walls, cell wall structures and cell membranes, caused by SO₂ gas, was more prominent in the tissue layers nearer to the fruit surface, damage also occurred to a lesser extent in deeper tissue layers.

SO₂ gas release-rate studies of polymer SO₂ sheets containing various concentrations Na₂S₂O₅ revealed that levels of SO₂ gas emitted depended largely on the levels of Na₂S₂O₅ incorporated into the sheets. Higher levels of SO₂ gas were released with the polymer sheets of higher concentrations Na₂S₂O₅. The release curve for the commercial Uvasys SO₂ sheet was very different to that of the polymer sheets, with much higher levels of SO₂ gas emitted initially by the Uvasys SO₂ sheet compared to the polymer sheets, while the polymer sheets emitted low levels of SO₂ gas for longer periods compared to the Uvasys SO₂ sheet.

The manufacturing process and the pilot scale production plant that was developed and constructed was successfully used to manufacture polymer SO₂ generating sheets that are technically sound and efficient, and aesthetically acceptable to industry. The efficacy of such sheets, regarding levels of decay control and SO₂ damage, was similar to that obtained with the presently available, commercially used Uvasys SO₂ sheet.

Opsomming

'n Nuwe polimeriese SO₂-gasvystellingsvel vir die beheer van *Botritis cinerea* gedurende die na-oes opberging van tafeldruive is ontwikkel en vervaardig. 'n Nuwe loodsaanleg is spesiaal vir hierdie doel ontwerp en gebou. Aandag is geskenk aan die voorkoms van die velle aangesien dit belangrik is dat die nuwe velle beide tegnologies effektief en esteties aanvaarbaar moet wees vir die sagtevrugtebedryf en verbruikers.

Die opbergingskwaliteit van semi-kommersiële uitvoerbesendings van verskeie kultivars tafeldruive, verpak met die nuwe monolitiese SO₂-gasvystellingsvelle, is bepaal. Die volgende is ook bepaal: die effektiwiteit van die nuwe polimeriese velle, die stadium waarby SO₂-skade op die druive duidelik word, die vlak van SO₂-skade wat met verskillende konsentrasies SO₂-gas geassosieer is, die moontlike invloed wat 'n toename in temperatuur (vanaf -0.5° tot 10°C) op die verbleiking deur SO₂ sal hê, en of die SO₂-skade by voorkeur in 'n sekere posisie op die druive sal plaasvind.

Die nuwe SO₂-vel het baie goed vergelyk met die kommersieël beskikbare Uvasys SO₂-vel. Die SO₂-konsentrasie benodig vir die effektiewe beheer van *Botritis cinerea* beskadiging het egter van kultivar tot kultivar verskil. Die keuse van die tipe verpakking, geperforeerd of ongeperforeerd, het ook 'n rol gespeel. Die konsentrasie SO₂-gas benodig vir effektiewe beheer was laer wanneer die druive in die nie-geperforeerde sakke verpak was. Vlakke van SO₂-skade is ook noemenswaardig beïnvloed deur die opbergingsperiode en variasies in temperatuur. Daar was geen duidelike verskil in die ontwikkeling van bederf en SO₂-skade ten opsigte van die posisie van die trosse in die karton nie.

Die mate van SO₂-skade aan vrugweefsel is deur middel van lae-temperatuurskandeerelektronmikroskopie (LTSEM) en transmissie-elektronmikroskopie (TEM) bepaal. Daar is bevind dat die blootstelling aan SO₂ moontlik tot plasmolise en die uitlek van sellulêre vloeistof kon lei. Alhoewel SO₂-skade aan die selwande en membrane meer prominent in die

weefsel naby die oppervlak van die vrug was, het skade ook in die onderliggende lae plaasgevind.

Die vlakke van vrygestelde SO_2 -gas het grootendeels afgehang van die konsentrasie natriummetabisulfaat in die velle. Die SO_2 -vrystellingskurwe van die nuut ontwikkelde polimeriese SO_2 -velle het baie verskil van dié van die Uvasys vel. Laasgenoemde lewer aanvanklik 'n hoë konsentrasie vrygestelde SO_2 -gas vir 'n kort periode, gevolg deur baie lae SO_2 vlakke daarna, terwyl eersgenoemde 'n laer aanvanklike SO_2 vrystelling het, gevolg deur vergelykederwys hoër SO_2 konsentrasies daarna.

Die ontwikkelde vervaardigingsproses en die loodsaanleg wat daaruit voortvloei het is dus suksesvol aangewend om goeie polimeriese SO_2 -vrystellingsvelle te vervaardig. Hierdie velle is tegnies effektief vir die beheer van *Botrytis cinerea* gedurende die na-oes verpakking van tafeldruive en is esteties aanvaarbaar vir die Suid Afrikaanse sagtevrugtebedryf.

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

Chapter 1

Introduction and objectives

Introduction

Table grapes are one of the world's largest fruit crops; more than 500 million cartons are exported each year (Nelson, 1979). The major table-grape producing regions on the world market are South America, Europe, North America and Southern Africa. According to statistics for the 2000 season from the Deciduous Fruit Producers Trust, South Africa contributes approximately 50 million cartons to the world market. Although table grapes are, physiologically speaking, very durable fruit, there are several deterioration factors that, if not well controlled, will shorten the potentially long post-harvest life of table grapes drastically. These factors include susceptibility to decay caused by *Botrytis cinerea*, injury and water loss. Modern technology has reduced these problems to the extent that table grapes can now be marketed for long periods of time on most of the world's major markets (Nelson, 1979). Continued research is nonetheless required, to improve current systems and develop new ones for preservation and packaging that will assure that the full post-harvest life of the fruit is utilized (Nelson, 1979).

The use of sulphur as preservative has long been known; it has been used since as early as 2 000 BC. In ancient times it was used for preparing gunpowder and warfare materials. Its unique flame and pungent odor also gave it an important role in temple sacrifices and purification rites. Today sulphur is extensively used by industry and agriculture, and is important in food production and preservation (Kilmer, 1978).

In the table grape industry the importance of sulphur was first identified during the early 1920s. During those days, in California, the rapidly increasing volume of fresh grapes being shipped taxed the resources of the

railroads severely, often resulting in congestion. Delays in deliveries increased the deterioration of fruit quality in transit or storage, mainly due to the evaporation of moisture from the grapes, resulting in shriveling and the activity of various decay-causing micro-organisms (Winkler, 1925).

In consequence to these losses, many inquiries were sent to the Department of Agriculture as to methods of avoiding these losses - either by improving the efficiency of the refrigerator cars or by means of chemical preservation. More efficient cold storage and sporadic fumigation with sulphur dioxide (SO₂) gas were identified as the most efficient and cost effective means by which to improve the quality of grapes during post-harvest storage (Winkler, 1925). However, cold storage was limited to ice banks in the railway cars, without any fans, and SO₂ was obtained by burning sulphur in the cars before closing the doors or at sporadic intervals during storage (Winkler, 1925; Nelson, 1979). These grapes generally reached the market in no better condition than the untreated grapes and in many instances their quality was even poorer (Winkler, 1925). The loss in quality was mainly due to shrinkage, decay and SO₂ bleaching injury. Water loss, mainly through the vents of containers, was one of the biggest problems. These vents were necessary for the introduction of cold air and SO₂ gas for fumigation from an external source (Nelson & Ahmedullah, 1970).

Subsequently, in the late sixties and early seventies, the in-package generation of SO₂ was developed. Specially manufactured sheets containing NaHSO₃ were placed inside the grape package and SO₂ gas was released during two stages, a quick and a slow release stage (Nelson, 1983).

Research conducted by the Deciduous Fruit Board in South Africa resulted in the introduction of a polyethylene bag used in combination with the SO₂ gas sheets (Jooste, 1987). The primary objectives of the use of SO₂ gas in combination with polyethylene bags was to kill off superficial spores of *Botrytis cinerea* and to minimize water loss. These effects were maximized when used in combination with effective and fast cold storage (Jooste, 1987). However, the concentration of SO₂ gas released inside the package had to be very carefully controlled so as not to lead to excessive bleaching damage on

the grape berries, caused by too high concentrations of SO₂ gas inside the package (Nelson, 1983; Jooste, 1987).

During the past decade concern has increased regarding the effect of sulphurous compounds present in foods on certain individuals. The health effect of concern is sulfite sensitivity. The Food and Drug Administration (FDA) and Environmental Protection Agency (EPA) have established that the use of sulfites as preservatives on fresh fruits and vegetables poses a risk to a small segment of the population which is sulfite sensitive (Anonymous, 1986). Based upon this, the FDA and the EPA revoked the generally regarded as safe (GRAS) status of sulfites used as preservative on fresh fruits (Anonymous, 1986) and placed a 10 parts per million (ppm) tolerance level on sulfite residues found in grapes to be sold to the public (Anonymous, 1989). This posed a major problem for fruit exporters and SO₂ sheet manufacturers as the SO₂ residues found in fruit after some of the commercial packaging methods exceeded this 10 ppm tolerance level imposed by the FDA and EPA (Smilanick *et al.*, 1990).

The stricter food legislation and export requirements necessitated that thorough and new research with regard to the use of in-package SO₂ generators be performed to ensure that South Africa remains competitive in the world market in terms of grape exports. Another reason for the need to develop a new SO₂ sheet was that several problems had been experienced with the presently available, locally manufactured Uvasys SO₂ sheets regarding lack of availability, increased cost and unacceptably high SO₂ gas emission levels. New technology was required for the in-package production of SO₂ gas that is inexpensive yet offering a wider versatility in its gas-generating mechanism, in order that sheets can evolve, along with the ever-changing demands of the table grape industry.

In a recent research programme, initialized by industry and carried out by the author, an alternative SO₂ gas generating sheet was developed, based on technology widely used in the pharmaceutical industry (Opperman, 1995). These sheets were manufactured manually and successfully tested on laboratory scale (Opperman *et al.*, 1999).

Objectives

Each chapter in this dissertation is an individual entity with its own objectives. However, the overall objectives of the present study were:

- i) Determining whether it would be possible to upgrade the manufacturing process of the previously researched hand-made SO₂ sheets (Opperman, 1995) from a laboratory scale to a semi-commercial scale. Tasks were to include consideration of all the aspects of the design and construction of a suitable production facility and manufacturing of polymer SO₂ sheets in the production facility that are both efficient in terms of SO₂ gas release and aesthetically acceptable to industry;
- ii) Testing the efficacy of the new machine-manufactured devices, and comparing results to those obtained with the earlier hand-made devices, with regards to preventing post harvest *Botrytis cinerea* decay development;
- iii) Comparing the levels of *Botrytis cinerea* decay control and SO₂ damage of the new machine-manufactured polymer device with those obtained with other commercially available sheets (e.g. Uvasys);
- iv) Establishing the exact concentration of SO₂ to be incorporated into the new polymer devices to achieve the optimum balance between *Botrytis cinerea* decay control and reducing levels of SO₂ damage;
- v) Investigating the effect of various packaging materials, such as bag type, or use of moisture absorbers, on the efficacy of the new machine-manufactured polymer devices, with regards to decay control and SO₂ damage, during the post harvest storage of table grapes; and
- vi) Investigating the effect of variations in temperature and storage period on the efficacy of the new machine-manufactured polymer devices, with regards to decay control and SO₂ damage, during the post harvest storage of table grapes.

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Chapter 2

Literature Review

Polymers in controlled release technology

Controlled release may be defined as a technique or method by which active chemicals are made available to a specific target at a rate and for a duration designed to accomplish an intended effect. In chemical terms, controlled release is the permeation-moderated transfer of an active material from a reservoir to a target surface to maintain a predetermined concentration or emission level for a specified period of time (Kydonieus, 1980).

Polymer membranes have been used extensively as protective permeation barriers such as coatings or packaging films. During the last two decades, polymer membranes have been developed to serve as specific media for the separation of mixtures by, for example, reverse osmosis, dialysis and ion exchange (Paul, 1976), and as media for controlled release of active chemical or biological agents (Benedict & Strange, 1980; Donbrow & Friedman, 1975; Kydonieus, 1980; Paul, 1976). Applications include a reliable means of the controlled release of drugs and pharmacologically active agents, fertilisers, pesticides, and herbicides (Paul, 1976).

The principle advantage of using controlled release formulations in agriculture is that much less chemicals are required and they are used more effectively for a given time period. This means that the harsh environmental processes that are used to remove the conventionally applied chemicals are avoided. Though the advantages of controlled release are impressive, there are some negative effects to be taken into account. These include the generally higher costs associated with controlled release system preparation and processing, environmental impact of the polymer matrix, additives,

polymer degradation products, and the costs associated with government registration of the product, if required (Kydonieus, 1980).

Types of release mechanisms

The concept and practice of controlled release from membrane systems include many types and mechanisms of release kinetics and application specifications. The underlying basis of the behaviour of all membrane systems, regardless of the application, is common insofar as the solution, transport, and other properties of the membrane materials are governed by the physicochemical composition and structure of the components under given environmental conditions. The phenomena of controlled release are similar to those involved in plasticiser technology, environmental resistance of polymers, and related areas of polymer technology (Rogers, 1976).

Release rates can be manipulated to suit specific needs by modification of the polymer structure. The modification of polymer structures can be achieved by a number of methods and procedures. Three general considerations are composition, morphology, and geometry. This is especially evident where one can alter chemical compositions, proportions and spatial arrangements of the components (Rogers, 1976; Van de Witte *et al.*, 1993). A key problem in controlled release formulations is the combination of the active agent with the carrier in an economical manner such that a release profile that is suitable for the application is achieved. These two factors (combination and release profile) are often in opposition to one another, so compromises must be made (Paul, 1976).

Once an application has been investigated and found suitable, the controlled release technology that best fits the application must be selected. This includes the basic physical form of the release device, the rate-controlling polymer matrix and active agent to be used (Kydonieus, 1980). Polymeric systems that exist and which can be used for controlled release are categorised in Table 1.

Table 1. A summary of the most important categories of polymeric systems utilised for controlled release technology (Kydonieus, 1980).

Physical systems	
A	Reservoir systems with rate-controlling membranes
1	Microencapsulation
2	Macroencapsulation
3	Membrane systems
B	Reservoir systems without rate-controlling membranes
1	Hollow fibres
2	Poroplastic® and Sustrelle® Ultramicroporous Triacetate
3	Porous polymeric substrates and foams
C	Monolithic systems
1	Physically dissolved in non-porous, polymeric, or elastomeric matrix
a	Non erodible
b	Erodible
c	Environmental agent ingression
d	Degradable
2	Physically dispersed in non-porous, polymeric, or elastomeric matrix
a	Non erodible
b	Erodible
c	Environmental agent ingression
d	Degradable
D	Laminated structures
1	Reservoir layer chemically similar to outer control layers
2	Reservoir layer chemically dissimilar to outer control layers
E	Other physical methods
1	Osmotic pumps
2	Adsorption onto ion-exchange resins
Chemical systems	
A	Chemical erosion of polymer matrix
1	Heterogeneous
2	Homogeneous
B	Biological erosion of polymer matrix
1	Heterogeneous
2	Homogeneous

The controlled release mechanism selected for the specific application in this study viz., the slow release of SO₂ gas during post harvest storage of table grapes, is based on the monolithic release system. Therefore, the rest of this section will be devoted only to monolithic polymer devices.

Monolithic polymer devices

Use of monolithic polymer devices is probably the simplest and least expensive way to control the release of an active agent. A monolithic polymer device comprises an active agent incorporated into a polymeric matrix, and migration of the active agent to the surface occurs either by diffusion through pores within the matrix structure or by diffusion through the polymeric phase itself (Roseman & Cardarelli, 1980).

In order to establish a uniform and continuous release of agent molecules from the surface of a given plastic dispenser, the agent must be incorporable in that plastic and the agent molecules must in some manner be able to migrate through the matrix towards the surface. The release of an active agent from a plastic material can take place by one of the following processes: i) leaching, where the ingress of a liquid, such as water, activates the active agent which then migrates outward, following the pore structure; ii) volatilisation, where the active agent molecule, in a gaseous form, moves in a random motion following the free volume network to the dispenser surface; and iii) matrix degradation due to chemical, biological, and physical stresses. Generally, the pore size and diffusion coefficient of the polymer will be the most important emission rate-controlling factors. In some instances however, extraordinary methods may be required to provide long-term, continuous, and effective agent emission (Roseman & Cardarelli, 1980).

Monolithic polymer devices for controlled SO₂ gas release

Plasticised poly (vinyl chloride) (PVC) dispensers are commonly used in the

pharmaceutical and agricultural industries for the dispersion of insecticides and herbicides. Generally the dispensers are manufactured by incorporating an active agent into a PVC plastisol before gelation. PVC plastisol is a paste consisting of a mixture of fine particles of PVC resin (dispersion grade) and plasticiser. The PVC resin is prepared by polymerising vinyl chloride monomer in the presence of an emulsifying agent in an aqueous medium, which produces spherical particles (average size approximately 1 μ m or smaller) which are then spray dried and ground. During the spray drying, particles fuse together and their agglomerates form. The agglomerates are then ground to particles of 15-0.2 μ m or smaller, containing various levels of voidage which take up the plasticiser. Within the particles, distinct crystalline regions exist. The latter act as crosslinks and are of great importance in preserving the physical strength of PVC (Werner, 1977; Kwak, 1995).

PVC plastisols convert from a liquid to a solid and yet flexible state when heated to temperatures of 140 to 200°C. As the temperature of the PVC compound is increased from room temperature, plasticiser begins to penetrate each resin particle. When the resin has taken up all the plasticiser, the system loses its fluidity. This is referred to as the gelled state. At this stage, the gelled mass has no cohesive strength. As heat continues to penetrate the system the polymer starts dissolving in the plasticiser until all the polymer chains are uniformly dissolved in the plasticiser. This is the fused state which when cooled, provides a plastic material of considerable strength and utility (Werner, 1977; Kwak, 1995).

The first known product based on the monolithic dispersal of a fungicide incorporated in a PVC matrix to control *Botrytis cinerea* during post harvest storage of table grapes was documented by the author, Opperman (1995) and Opperman *et al.* (1999). Sodium metabisulphite ($\text{Na}_2\text{S}_2\text{O}_5$) was used as the active agent in this device and is incorporated as a fine powder into the PVC matrix (Fig. 1). Once the $\text{Na}_2\text{S}_2\text{O}_5$ reacts with the moisture that is ever present inside the grape package, SO_2 gas, which is fungicidal, is released through a diffusion process. The device may contain levels of 10 to 50% $\text{Na}_2\text{S}_2\text{O}_5$, depending on the levels of SO_2 gas required, and the plasticiser content is approximately 50% (Opperman *et al.*, 1999).

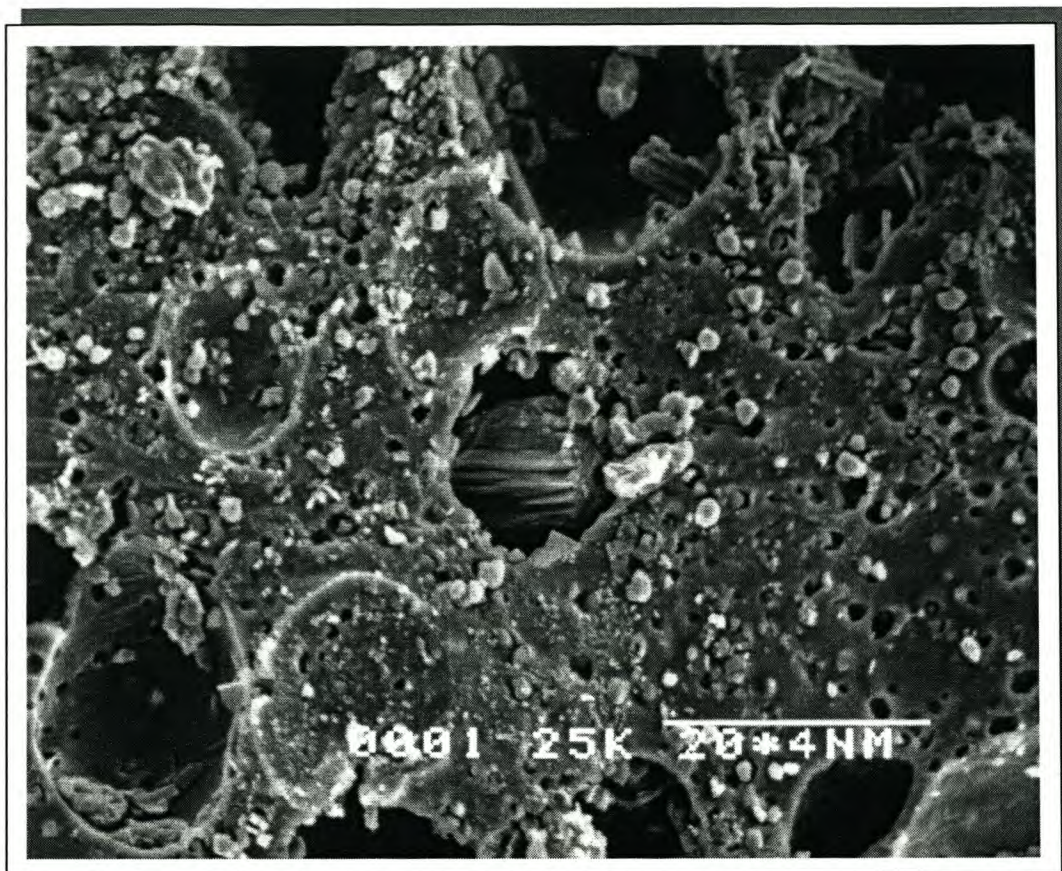


Figure 1 Scanning electron micrograph showing structural details of the PVC matrix designed to generate SO₂ gas by incorporating an active agent (Opperman, unpublished data).

Emission levels of SO₂ gas were not influenced by the plasticiser used but could be manipulated to some extent by adding blowing agents and/or humectants to the plastisol formulation. Generally higher levels of SO₂ gas, with a longer sustained release rate, were achieved by incorporating a blowing agent or humectant, or both, into the PVC matrix. The level of Na₂S₂O₅ incorporated into the matrix influenced the emission levels of SO₂ gas. The higher the Na₂S₂O₅ concentration, the higher the emission level of SO₂ gas (Opperman, 1995).

SO₂ gas and grapes

Inhibitory activity of sulphur dioxide

The toxicity of SO₂ is greatly influenced by two key factors, namely, i) the temperature of the fruit and ii) the acidity of the medium.

B. cinerea spores showed a two to four fold increase in SO₂ sensitivity for each 10°C temperature increase from 0 to 32°C at pH 4.0. The increase in SO₂ sensitivity may be attributed to the influence of temperature on SO₂ incorporation (Smilanick *et al.*, 1990a).

In fungi, SO₂ is transported by a specific permease enzyme at neutral acidities (Tweedie & Segel, 1970; Smilanick *et al.*, 1990a). Under more acidic conditions neutral “molecular” SO₂ passively diffuses very rapidly through the plasma membrane, where it is ionised in the near-neutral acidity of the spore or hyphal cytosol and hence trapped, since the sulphite ions cannot pass through the hydrophobic plasma membrane (Smilanick *et al.*, 1990a). Death of conidia and mycelium of *B. cinerea* occurs *in vitro* after about 20 to 30 minutes of exposure to 100 ppm SO₂ under the high humidity conditions at which grapes are stored. To inhibit decay during cold storage a constant atmosphere of only 5 to 10 ppm SO₂ is required (Smilanick *et al.*, 1990b).

Hocking and Hocking (1977) reported that the rate-limiting step in the fungicidal action of SO₂ may be the passage of SO₂ or sulphite across the cell membrane of fungi and that the passage of the non-ionic form, SO₂, is favoured over that of the ionic species. However, the possibility that the ionic species are the agents causing biochemical injury cannot be excluded (Peiser & Yang, 1985).

According to Smilanick *et al.* (1990a), the primary contributor to the toxicity of SO₂ is “molecular” SO₂ and that it is equally present at LD₉₉ concentrations for *B. cinerea* spores at pH 3 to 4. This view is not supported by Babich and Stotzky (1978), who reported that bisulphite was the primary

SO₂ species which was toxic to *B. cinerea* at pH 4.0 and that “molecular” SO₂ was not present in sufficient quantities above pH 3.5 to provide germicidal activity.

McBean *et al.* (1964) concluded that diffusion mechanisms related to the gaseous forms of SO₂ are more important to SO₂ absorption than those forms of SO₂ dissolved in the liquid phase. Alterations in the structure of the surface tissue of fruit will retard the rate of SO₂ absorption and will probably affect the total uptake of SO₂. Disorganisation of tissue by plasmolysis presumably leads to blocking or breakdown of intercellular pathways, adversely affecting the diffusion of SO₂ gas into the tissue (McBean *et al.*, 1964).

Sulphur dioxide residues present in grape tissue

Peiser and Yang (1985) proposed that there are free and bound forms of sulfite present in grape tissue. The free sulfite is lost rapidly, with a half-life of about 4 hours, whereas the bound sulfite is relatively stable, with a half-life of about 20 hours. The sulfite reacts with aldehydes, methyl ketones and cyclic ketones to form hydroxy sulfonic acids. This reaction is readily reversible. The irreversible oxidation of free sulfite to sulfate could cause a steady equilibrium shift from hydroxysulfonates to free sulfite plus organic products. Presumably the readily reversible bound sulfite can be regarded as a “storage form”, which will release sulfite when the concentration of free sulfite becomes low (Peiser & Yang, 1985).

Austin *et al.* (1997) reported a slower rate of residue loss than that predicted by Peiser and Yang (1985). The results suggested that the slower rate of residue loss, sulfites bound to aldehydes and ketones, predominated, and that most of the sulfite in the grapes was in the form of hydroxysulfonates. This is however, not supported by the results of Lagunas-Solar *et al.* (1992).

Lagunas-Solar *et al.* (1992) reported that two phases exist for SO₂ uptake during the fumigation process. There is a rapid SO₂ diffusion phase which increases, almost linearly, with time, and a SO₂ chemical (conversion)

phase where other SO₂ modifying mechanisms predominate within the system. During this phase SO₂ residues were increasingly modified to form sulfates, indicating that the oxidation of sulfite-to-sulfate takes place efficiently, although at a lower overall rate than the SO₂ diffusion phase (Lagunas-Solar *et al.*, 1992). The initial SO₂ uptake corresponded to values reported previously by Peiser & Yang (1985) and Smilanick *et al.* (1990a). Lagunas-Solar *et al.* (1992) further reported that no other chemical species other than sulfites and sulfates were identified in grape samples after short and long-term storage, indicating that the absorption and conversion processes in table grapes do not include the formation of irreversibly bound SO₂-derived organic compounds, as reported by Austin *et al.* (1997).

Several researchers (Peiser & Yang, 1985; Harvey, 1955; Smilanick *et al.*, 1990a) are of the impression that SO₂ only plays a role in disease control by periodic elimination of surface mycelial growth of infected grapes and not by eradication of the fungi in the grape tissue. Peiser and Yang (1985) suggested that high dosages of SO₂ are necessary during fumigation in order to leave sufficient residues to impart fungal inhibition. They furthermore indicated that the inhibition of spore germination correlated with the calculated concentration of SO₂, but not with that of bisulphite or sulphite.

However, Smilanick *et al.* (1990a) concluded that SO₂ residues approaching the lethal dosage to spores were only found in the surface tissue of the grapes, not in the pulp. Of greater significance was the fact that if the dose of SO₂ fumigant was increased there was a corresponding increase in the levels of residues found in the surface tissue, but only a very slight increase in residue levels found in the pulp. According to them the levels of residues remaining after fumigation were probably too low in concentration to play a role in disease control (Smilanick *et al.*, 1990a).

Smilanick *et al.* (1990a) concluded that the important inhibition of the pathogen lies in the air spaces between the grapes. This explains why very low dosages of SO₂ gas, shown to suppress mycelial growth and spore germination (Peiser & Yang, 1985, Smilanick *et al.*, 1990a), can also retard decay development (Marois *et al.*, 1986; Combrink & Ginsburg, 1972).

Legislation on sulphur dioxide residues

The vast majority of experts qualified by scientific training and experience to evaluate the safety of the use of sulphites on fresh fruits and vegetables agree that the use of SO₂ is unsafe. The Environmental Protection Agency (EPA) therefore recently concluded that the use of sulphites on fresh fruits and vegetables intended to be served raw or sold raw to consumers is no longer GRAS (generally regarded as safe), rather, it poses a health risk to that relatively small segment of the population which is sulphite sensitive (Anonymous, 1986a).

In order to reduce risk associated with the occurrence of sulphite sensitive reactions, while still permitting the shipment of sulphite treated grapes into and within the United States, the EPA in consultation with the FDA developed an approach incorporating the following measures:

- i) Residues of sulphites (determined as sulphur dioxide) on grapes must be below the current level of detection, i.e. less than 10 ppm, when the grapes are offered for entry into the United States or are otherwise introduced into interstate commerce;
- ii) The shipping containers of both foreign and domestic grapes must be provisions of the Federal Food, Drug, and Cosmetic Act section 403(l) which requires shipping containers to be labelled when a raw agricultural commodity has received post-harvest pesticide treatment;
- iii) EPA requires domestic and foreign shippers to have a certification programme acceptable to FDA to assure that residue levels will be less than 10 ppm. In most circumstances shipments must be accompanied by a valid certificate of analysis documenting that the grapes do not contain detectable levels of sulphur dioxide. FDA will monitor this programme to assure compliance;
- iv) Any shipment found to have detectable levels of sulphur dioxide residues (10 ppm or higher) will be deemed to be adulterated and subject to seizure or detention by FDA; and

- v) Any shipment of sulphite treated grapes not covered by a certification programme will be deemed to be adulterated and subject to seizure or detention by FDA.

In establishing this policy, EPA has given consideration to the following: the importance of a year-round supply of grapes, the negative economic impact which would result from a curtailment of grape shipments, the many years that sulphites have been used to treat grapes without significant evidence of adverse effects, and the fact that only a discrete segment of the population is sulphite sensitive. Therefore, based on current information, the EPA has concluded that the benefits from the use of sulphiting agents on grapes (within certain limits) outweigh any risk associated with their use (Anonymous, 1986b).

Levels of sulphur dioxide residues

It was only after the GRAS (generally regarded as safe) status of SO₂ was revoked by the FDA (Anonymous, 1986a) that the importance of the levels of SO₂ residues found in grapes was recognised. Important matters that came to light were that the sulphite residues found in grapes may increase with periodic fumigation (Peiser & Yang, 1985; Smilanick *et al.*, 1990c) and that, after fumigation, the sulphite residues are rapidly oxidised to sulphate (Austin *et al.*, 1997; Lagunas-Solar *et al.*, 1992; Peiser & Yang, 1985; Smilanick *et al.*, 1990c). The residues have a relatively short half-life and there is still no clear evidence whether the mechanism for the increase in the levels of residues with repeated fumigations are of an enzymatic or chemical nature (Peiser & Yang, 1985).

Factors that influence the levels of SO₂ residues found in fresh table grapes

The maturity of the grapes has a significant influence on the levels of their sulphite residues; less mature grapes show higher residues after fumigation than more mature grapes do (Winkler & Jacob, 1925). Smilanick *et al.*

(1990c) confirmed this view for the varieties Thompson Seedless, Flame Seedless and Cardinal. Maturity did however not influence the sulphite residues in Black Monukka grapes. In fact, the less mature grapes showed lower residues than the more mature grapes (Smilanick *et al.*, 1990c). It would seem that the differences in the levels of residues associated with maturity after fumigation are probably related to penetrability of the grapes to SO₂. These differences in penetrability are caused by differences in anatomical features of the grape skin such as thickness, composition or porosity of the cuticle and epicuticular wax, and not due to titratable acidity, soluble solids, or other internal compositional changes associated with advancing maturity (Smilanick *et al.*, 1990c). Guelfat-Reich *et al.* (1975) also noted that the effect of the SO₂ treatment on the quality of the grapes varied greatly with the cultivar used and the method of SO₂ application.

It appears that factors determining access of SO₂ gas, such as berry density, distance from SO₂ generator and bunch shape (Nelson & Ahmedullah, 1972; Lagunas-Solar *et al.*, 1992) have a significant effect on the occurrence of SO₂ damage. Generally, a higher occurrence of *Botrytis cinerea* decay and little SO₂ damage are observed within densely packed bunches, suggesting that the berries on the inside of these bunches are exposed to lower levels of SO₂ gas than the berries towards the outside of the bunch (personal correspondence with J. Fourie, Capespan Technology Development, 1999). Vail & Marois (1991) made similar observations with regard to decay development.

According to some authors (Harvey, 1956; Nelson, 1979; Smilanick *et al.*, 1990c), injury associated with SO₂ fumigation also appeared to be associated more closely with physical damage to berries than with any particular SO₂ treatment. The SO₂ gas penetrates the fruit more readily where the skin is broken (Harvey, 1956; Nelson, 1979; Smilanick *et al.*, 1990c). Where the berry attachment (pedicel) area is affected by specific growing conditions, increased SO₂ damage can occur. Observations of SO₂ damage occurring mostly at the pedicel end of Thompson Seedless table grapes suggests the importance of these observations (personal correspondence with J. Fourie, Capespan Technology Development, 1999).

The fumigation period has no significant effect on the residues found in the grapes as long as the product of SO₂ dose and fumigation period is equivalent. Residue analysis showed that residues were generally proportional to the dose applied, with more than 90% of the total sulphite residues located in the grape “skin” and only about 7% of the total sulphite residues in the “pulp” (Smilanick *et al.*, 1990b).

With an increase in the temperature of the fruit there is an increase in the short-term SO₂ residues and the residues are less persistent at higher temperatures. This suggests that grapes fumigated at higher temperatures, then cooled, may contain higher residues than grapes fumigated and stored cold. Warming accelerated a loss in residue levels. This could probably be used to decrease levels of SO₂ residues in grapes that are above maximum residue levels. The increased accumulation of residues at higher temperatures indicated that the rate of SO₂ reaction with water, rather than the solubility, might be the predominant kinetic factor in SO₂ residue accumulation (Smilanick *et al.*, 1990c).

Harvey *et al.* (1988) reported that the storage period of the grapes has a significant influence on the quality of the fruit and found that higher levels of SO₂ damage occurred with longer storage periods. This view was supported by Smilanick *et al.* (1990c) who concluded that even though SO₂ residues have a short half-life there is a trend of accumulation after repeated fumigations during storage. Similarly, Nelson (1979) reported that, due to SO₂ bleaching, it became increasingly difficult to keep the damage to fruit within acceptable levels as the storage period was extended. Austin *et al.* (1997) reported that SO₂ residues tend to reach a maximum after repeated fumigation during post harvest storage and may in fact start to decline after periods of two months and longer in storage.

Packaging materials, such as boxes and poly(ethylene) bags, may act as secondary supply pools of SO₂ gas by a desorption process. This process is rapid, dependent on temperature and may, during long-term storage, provide additional SO₂ and increase the ambient SO₂ concentration. This may cause unacceptably high levels of SO₂ residues that may exceed the 10µl tolerance limit (Lagunas-Solar *et al.*, 1992)

The levels of SO₂ residues found in table grapes can be minimised by limiting lengthy storage and repeated SO₂ fumigation to mature grapes that are free from injury and decay and by maintaining temperatures at 0 to 1°C to inhibit decay (Smilanick *et al.*, 1990c).

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Chapter 3

Testing of machine-manufactured monolithic devices for in-package SO₂ gas generation to control post-harvest *Botrytis cinerea* decay on South African table grapes

Technology developed in this study is confidential and is protected by patent (South African Patent No. 96-2517, International Patents Pending, Provisional No. ZA 2001/1851).

Abstract

The use of SO₂ and, in particular, SO₂-generating sheets offers the protection needed against *Botrytis* infections during the post-harvest storage of table grapes. The availability and use of a locally manufactured, alternative SO₂ sheet, which is cheaper to produce, but as effective as the sheets used at present, is of major importance to the South African Table Grape industry. Prototypes of a new SO₂ sheet were developed and manufactured for evaluation on a pilot-scale production plant. Results showed that the new SO₂-generating sheet compared favourably with the existing, commercially available Uvasys SO₂ sheet. Results also indicated that the required, and exact, SO₂ concentration needed for effective control varied for different cultivars, as well as for the different grape packaging systems used. The SO₂ concentration incorporated within the sheet was shown to be lower for grapes packed in non-perforated bags, and slightly higher for those in perforated bags. Cultivar differences occur with regard to the level of control, as well as levels of SO₂ damage. The efficacy of the new SO₂ sheet to control decay over extended storage periods needs to be carefully compared to commercially available sheets.

Introduction

The use of SO₂ gas during the post-harvest cold storage of table grapes is considered essential for protection against *Botrytis cinerea* decay (Ballinger & Nesbitt, 1984; Harvey, 1956; Morris *et al.*, 1992; Mustonen, 1992; Nelson, 1979; Winkler & Jacob, 1925). The use of SO₂ generating sheets inside the grape package, to deliver constant levels of SO₂ gas during post-harvest storage, enables longer storage periods without significant losses in fruit quality (Ballinger & Nesbitt, 1982; Morris *et al.*, 1992; Nelson & Ahmedullah, 1976; Perkins-Veazie *et al.*, 1992). However, the application of SO₂ at the correct concentration to control *Botrytis* infections is essential, as the levels required for effective control of *Botrytis* decay are very close to the levels which may damage the fruit (Harvey, 1956; Nelson, 1979). Too high levels of SO₂ damage the berries by bleaching, and furthermore cause premature browning of the stems and increased water loss. These factors have a negative influence on the marketability of table grapes, especially of SO₂ sensitive cultivars (Marois *et al.*, 1986; Mustonen, 1992; Nelson, 1979).

Besides the loss of quality associated with too high levels of SO₂, fruit quality is also significantly affected by the packaging materials used (Ballinger & Nesbitt, 1982; Nelson, 1983; Nelson & Ahmedullah, 1976; Opperman *et al.*, 1995; Perkins-Veazie *et al.*, 1992) and the storage conditions viz., storage temperature and time (Nelson, 1983; Taylor *et al.*, 1990).

The continuous exposure of table grapes during post-harvest storage to SO₂ gas released by SO₂ gas-generating sheets, or fumigation with SO₂ gas, results in the formation of exogenous sulphur residues in the fruit. These residues, which include aqueous SO₂, sulphites (SO₃²⁻) and bisulphites (HSO₃⁻) (Anonymous, 1989; Wedzicha, 1986), have become the focus of increasing concern since it was discovered that food containing sulphite residues pose a health risk to sulphite-sensitive consumers. Consequently, the United States Food and Drug Administration (USFDA) revoked the "generally regarded as safe" status (GRAS) status of SO₂ when used in conjunction with fresh fruit products (Anonymous, 1986). A residue tolerance level of 10µg SO₂ per gram of fresh grapes was later established by the Environmental Protection Agency (EPA) (Anonymous, 1989).

The development and manufacture of a locally manufactured, alternative SO₂ sheet, which is readily available and cheaper, but as effective or better than the SO₂ sheets used at present, is of major importance for the South African Table Grape industry. A research programme was initiated by the industry to develop, manufacture and test a new SO₂ generating sheet (Opperman, 1995; Opperman *et al.*, 1999). Initially the prototype sheets were hand-made according to a process documented by Opperman (1995). To successfully investigate the true potential of these new sheets a production process was developed and a pilot scale production unit assembled in order to manufacture the sheets required for testing.

The objective of this chapter was to determine the efficacy of the machine manufactured polymer SO₂ sheet in terms of; (a) controlling the spread of *Botrytis cinerea* decay from infected grape berries to adjacent sound berries; and/or (b) inhibition of the establishment of new *B. cinerea* infections on the berry surface; and (c) determining the effect on the general quality of the grapes during and after storage.

Materials and methods

Manufacturing of SO₂ generating sheets

Hand-made sheets

SO₂ generating sheets were manually constructed in the laboratory according to a procedure documented by Opperman (1995) and Opperman *et al.* (1999).

Machine-manufactured sheets

The machine manufactured SO₂ gas-generating sheets were made on a pilot scale production plant purposely built for this project. Manufacturing of the SO₂ gas-generating sheets commences with the preparation of the PVC plastisol mixture.

Various levels of SO₂ gas can be released from the PVC matrix by incorporating different concentrations of sodium metabisulphite (Na₂S₂O₅) particles into the plastisol mixture. Once the desired plastisol mixture is obtained it is applied onto a carrier material by means of a nozzle. The carrier material with the plastisol deposit is sent through a series of curing ovens where the plastisol is cured to a solid state. The temperature inside the ovens is maintained at 145°C. Once cured, a second layer of non-woven material is laminated onto the carrier layer to enclose the PVC matrix between two sheets of material. A cooling section follows where the product is rapidly cooled down before it is cut to the desired specifications. The finished product is inspected visually for deviations in quality before it is packaged into poly(ethylene) bags ready for use.

Sheets releasing various concentrations of SO₂ gas were manufactured in order to determine which concentration delivers the optimum levels of SO₂ gas needed for effective decay control. Too high levels of SO₂ gas cause various quality defects, hence a balance must be obtained between levels needed for effective decay control and those causing quality defects. The various SO₂ gas concentrations were obtained by varying the concentration of Na₂S₂O₅ in the PVC plastisol mixture.

Packaging and SO₂ treatment

Fresh grapes of four cultivars, viz. Thompson Seedless, Waltham Cross, Red Globe and Dan-ben-Hannah table grapes, were packed at different pack-houses from three locations and transported to Stellenbosch for inoculation and placement of the different SO₂ sheets in the cartons. The grapes were packaged in cartons as for export, in either perforated or non-perforated bags. Individual bunches were packaged in carrybags or polycote bags, depending on the cultivar. The various SO₂ generators that were tested and compared during this trial were:

- i) Machine manufactured polymer sheets (MMPSs) with Na₂S₂O₅ concentrations varying between 8 - 26% (abbreviated, henceforth, as 8 – 26% SO₂ sheet);
- ii) Hand-made polymer sheet containing 20% Na₂S₂O₅ i.e. 20 % SO₂ sheet; and

- iii) Uvasys 70% (standard sheet used by South African table grape industry).

Note: All concentrations were not tested on all three cultivars. The choice of a particular treatment sequence varied between cultivars, based on the SO₂ sensitivity and the decay susceptibility.

The various cultivars packed were kept in cold storage for the following periods:

- i) Thompson Seedless; 5 w and 8 w at -0.5°C + 5 d at 7.5°C for each of the cooling periods;
- ii) Waltham Cross; 5 w and 8 w at -0.5°C + 5 d at 7.5°C for each of the cooling periods;
- iii) Red Globe; 5 w and 8 w at -0.5°C + 5 d at 7.5°C for each of the cooling periods; and
- iv) Dan Ben Hannah; 5 w and 8 w at -0.5°C + 5 d at 7.5°C for each of the cooling periods.

Inoculation Method

To ensure the presence of a sufficient dosage of *Botrytis cinerea* in the cartons the grapes were inoculated. Inoculation of the grapes was done either by placement of infected berries within three bunches and/ or by the spore settlement method.

A settling method with dry conidia was used in order for natural infection and sporulation to take place under the prevailing conditions. Conidia from ten day old *B. cinerea* cultures, grown on Potato Dextrose Agar (PDA) medium, were used to inoculate the grapes. The bottom halves of the inverted petri dish cultures were held in position approximately 5 to 10 cm above the exposed fruit and tapped gently to dislodge individual spores. Water was not used during this inoculation method (Fourie, 1994).

For the placement of infected berries, grapes were inoculated with conidia of *B. cinerea* cultures seven days prior to the day of inoculation. *B. cinerea* cultures were grown under diurnal conditions for ten days on a PDA medium. A spore suspension of 1×10^5 spores per mL was made, by dilution with sterilised water. Sound berries were randomly selected and removed from a number of bunches and the berry surface sterilised with a 70% ethanol and water solution. The berries were allowed to dry naturally for approximately 30 minutes prior to inoculation. A wound, 1mm deep by 1mm wide, was inflicted on the berry surface and a droplet (25 μ l) of the spore suspension placed onto the wound site. Inoculated berries were placed in an incubator/moist chamber at 22°C with a relative humidity of approximately 95% for one week prior to use. The seven day-old inoculated berries were placed amongst the bunches in each of the four corners to obtain a natural spread of decay to adjacent berries (Fourie, 1994).

Experimental layout and statistical detail

Completely randomised two-way ANOVA for all cultivars, with SO₂ sheet and bag type as Factors A & B, respectively. Data for the different inoculation types were analysed separately. Each treatment consisted of 5 replicate cartons of grapes, with the cartons completely randomised on a pallet. The grapes were examined after storage and factorial analyses of variance performed on the data. Students-T LSD was calculated to compare treatment means at a 5% significance level.

Examination parameters

Examination of the grapes took place according to Capespan protocols. Decay, SO₂ damage, berry split and browning were expressed by weight, as a percentage (%) of the sample mass, while stem condition was rated by visual assessment according to a five-point scale, 1 = green and 5 = brown and desiccated.

Results and discussion

The potential control of post-harvest *Botrytis* storage decay, and other quality defects, with use of different SO₂ sheet treatments, were determined. Trials were conducted with Thompson Seedless, Red Globe, Dan Ben Hannah and Waltham Cross table grapes. Different inoculation techniques and storage periods were used for the different cultivars. Decay, SO₂ damage, berry split and stem condition were assessed after storage for 5 w and/ or 8 w at –0.5°C, followed by 5 d at 7.5°C.

Statistical analysis of the above quality parameters resulted in a large volume of data. Thus, for clarity purposes and to simplify the discussion of the results, a comprehensive list of tables (Tables 1 to 8) containing the statistical analysis has been included as an “Appendix” at the end of this chapter.

A Thompson Seedless

Decay development

Inhibition of the establishment of new infections (inoculation by spore settlement)

A significantly higher level of decay occurred on Thompson Seedless, kept for 5 w at –0.5°C + 5 d at 7.5°C, with application of a machine manufactured 14% SO₂ polymer sheet (MMPS), compared to MMPSs applications of 17 and 20% SO₂ concentration, as well as the Uvasys sheet and hand made sheet (Table 1a). No further differences in the level of decay were observed between any of the treatments. This result implied that the 14% SO₂ MMPS can not effectively control the onset of new infections on the berry surface. Statistically, there was no differences between the grapes packed in perforated or non-perforated bags.

On the grapes kept for 8 w at –0.5°C + 5 d at 7.5°C (Table 2a), decay was significantly higher when packed in perforated bags with inclusion of the 14 and 17%

MMPSs, compared to the MMPS containing 20% SO₂, or the Uvasys SO₂ sheet, or the hand-made sheet (HMS). No significant differences in decay occurred for the grapes packed in non-perforated bags. There was however, a slightly higher level of decay, above 1%, for the 14% and 17% MMPSs, compared to the Uvasys and HMS. Both the 14 and 17% MMPSs resulted in significantly more decay when included in the perforated bag, compared to the non-perforated bag. The results indicated that 14% and 17% SO₂ is too low to inhibit the establishment of new infections on the surface of Thompson Seedless grapes, if packed in perforated bags and kept for 8 w at -0.5°C + 5 d at 7.5°C. The use of 20% SO₂ resulted in no significant difference in decay compared to the Uvasys sheet, however, the decay level was nearly double that of the Uvasys treatment. The use of slightly higher concentrations than 20% SO₂ in the MMPS for a perforated bag system needs to be considered for storage beyond 5 w.

Inhibition of the spread of decay (inoculation by placement of infected berry)

A significantly higher level of decay, by spread from an infected berry to adjacent sound berries, occurred on Thompson Seedless grapes packed in non-perforated bags and kept for 5 w at -0.5°C + 5 d at 7.5°C, with application of a 14 and 17% SO₂ concentration in the MMPS, compared to a MMPS of 20% SO₂ concentration, Uvasys sheet, or the HMS (Table 2c). Significantly less decay also occurred with use of the 20% MMPS and the HMS, than the Uvasys sheet. For grapes packed in perforated bags, no differences occurred between any of the MMPSs and the Uvasys sheet, however, a lower decay level occurred with the HMS than all other SO₂ treatments. For most of the treatments, no differences in decay occurred on grapes packed in either perforated or non-perforated bags, except for the 20% MMPS, where significantly less decay occurred in the non-perforated bag than the perforated bag. The results indicated that only the 20% MMPS effectively controlled the spread of decay from infected berries to adjacent, sound berries, but only for grapes packed in non-perforated bags. The use of a specific SO₂ sheet did not ensure a vast improvement in confining the spread of decay on grapes packed in perforated bags and kept for 5 w at -0.5°C + 5 d at 7.5°C.

On grapes kept for 8 w at -0.5°C + 5 d at 7.5°C (Table 2c), decay was significantly higher when packed with a 14% MMPS, compared to a MMPS containing 20% SO_2 , or the hand made sheet (HMS). The grapes packed in perforated bags showed a significantly higher level of decay than grapes packed in the non-perforated bags. The results confirmed the inability of the sheets of lower SO_2 concentrations to confine the spread of decay, especially when extended storage was involved, and a perforated bag was used. Non-pooled data indicated that the Uvasys performed best in a non-perforated bag with extended storage.

Inhibition of the decay development on non-inoculated bunches

A significantly higher level of decay, resulting from natural infections, occurred on Thompson Seedless grapes kept for 5 w at -0.5°C + 5 d at 7.5°C with use of the 14% SO_2 concentration MMPS, compared to a MMPS of 20% SO_2 , as well as the hand made sheet (Table 1b). There was, however, no difference in decay between any of the MMPSs and the Uvasys SO_2 sheet. Although results indicated that any of the MMPSs could control decay as effectively as the Uvasys SO_2 sheet, in both bag types, the levels attained with the 14% MMPS would, in practice, be too high for both the perforated and non-perforated bags (see inset, Table 1b), especially under conditions of a potentially high inoculum load. The results again emphasized the need for a sheet of a particular concentration for grapes packed in perforated bags, as a higher level of decay could be expected.

On the grapes kept for 8 w at -0.5°C + 5 d at 7.5°C (Table 2b), decay was again significantly higher when packed with a 14% MMPS, compared to all other treatments. Decay levels in the perforated bags were generally much higher than in the non-perforated bags. This result indicated improved decay control with the use of non-perforated bags for the long-term storage of Thompson Seedless.

SO₂ damage

No significant difference in SO₂ damage occurred on the berry surface of Thompson Seedless grapes kept for 5 w at -0.5°C + 5 d at 7.5°C between any of the MMPS treatments and the Uvasys sheet (Table 1a & b). Almost similar results, except for the 14% MMPS treatment, showing less SO₂ damage than the 20% concentration for grapes in non-perforated bags (Table 1b), were achieved with regard to SO₂ damage at the pedicel end. Significantly higher levels of pedicel, as well as surface, SO₂ damage, however, occurred with the HMS compared to the other treatments when the grapes were packed in a non-perforated bag. Higher levels of SO₂ damage generally occurred on grapes packed in non-perforated bags compared to perforated bags, however, not significantly for each of the individual concentrations. Although not of statistical significance, the MMPS of 14% SO₂ concentration generally led to much lower levels of SO₂ damage than the other treatments, explaining the reason for experiencing less effective decay control with use of this particular concentration.

Almost similar results with regard to pedicel SO₂ damage were obtained for grapes kept for 8 w at -0.5°C + 5 d at 7.5°C (Table 2a & b), with only the HMS showing significantly higher levels of SO₂ damage than the other treatments. A higher level of surface SO₂ damage also occurred in the case of the HMS treatment, but only on grapes packed in non-perforated bags.

Berry split

No significant difference in berry split occurred between any of the MMPS treatments on Thompson Seedless grapes kept for 5 w at -0.5°C + 5 d at 7.5°C and which were inoculated by spore settlement (Table 1a). The HMS showed significantly more berry split than both the 14 and 17% MMPSs, as well as the Uvasys sheet. Berry split generally increased on grapes packed in non-perforated bags, compared to the perforated bag pack. The data for grapes inoculated by placement of infected berries showed slightly different results. In this instance, berry split was significantly reduced with the 14% MMPS compared to the other treatments (Table 1b).

On grapes kept for 8 w at $-0.5^{\circ}\text{C} + 5 \text{ d}$ at 7.5°C (Table 1b), no differences in berry split occurred between any of the treatments. Grapes packed in non-perforated bags showed significantly more berry split than those packed in perforated bags. Berry split generally increased with extended storage.

Stem condition

No difference in stem condition occurred between any of the SO_2 sheets, nor bag treatments on grapes kept for 5 w at $-0.5^{\circ}\text{C} + 5 \text{ d}$ at 7.5°C (Table 1a & b). There was however a difference in stem condition on Thompson Seedless grapes kept for 8 w at $-0.5^{\circ}\text{C} + 5 \text{ d}$ at 7.5°C (Table 2a & b). A MMPS of 14% showed greener stems than all other treatments. The stems of grapes packed in perforated bags were more desiccated than those in non-perforated bags after 8 w at $-0.5^{\circ}\text{C} + 5 \text{ d}$ at 7.5°C .

B Waltham Cross

Decay development

Inhibition of the decay development on non-inoculated bunches

No differences in decay development occurred on Waltham Cross kept for 5 w or 8 w at $-0.5^{\circ}\text{C} + 5 \text{ d}$ at 7.5°C between any of the SO_2 treatments. The bag type used also had no significant effect on decay development (Tables 3 & 4). Although not statistically significant, the levels of decay were generally slightly higher for Waltham Cross packed in perforated bags when kept for 8 w at $-0.5^{\circ}\text{C} + 5 \text{ d}$ at 7.5°C .

SO₂ damage

No difference in SO₂ damage occurred, either at the pedicel end or on the surface of Waltham Cross kept for 5 w or 8 w at $-0.5^{\circ}\text{C} + 5 \text{ d}$ at 7.5°C between any of the SO₂ treatments (Tables 3 & 4). The results indicated that SO₂ damage with use of any MMPs should most probably not affect the quality Waltham Cross grapes under normal handling and storage conditions.

Berry split

No difference in berry split occurred between any of the treatments on Waltham Cross grapes kept for 5 w or 8 w at $-0.5^{\circ}\text{C} + 5 \text{ d}$ at 7.5°C (Tables 3 & 4), however, berry split was higher for grapes packed in non-perforated bags for the 8w storage period (Table 4).

Stem condition

No difference in the stem condition occurred on Waltham Cross kept for either 5 w or 8 w at $-0.5^{\circ}\text{C} + 5 \text{ d}$ at 7.5°C between any of the treatments, for grapes packed in a specific bag type (Tables 3 & 4). The stem condition of most treatments was, however, more green in the non-perforated bags than in the perforated bags.

Browning

No difference occurred in browning on Waltham Cross as result of a particular treatment for grapes kept at -0.5°C for 5 w or 8 w + 5 d at 7.5°C (Tables 3 & 4). Waltham Cross packed in perforated bags, however, showed significantly more browning than in non-perforated bags. Although not statistically determined, browning increased with prolonged storage.

C Red Globe

Decay development

Inhibition of the spread of decay (inoculation by placement of infected berry)

No significant difference in the spread of decay occurred on Red Globe grapes kept for 5 w at $-0.5^{\circ}\text{C} + 5 \text{ d}$ at 7.5°C between any of the of SO_2 treatments (Table 5b). Grapes packed in perforated bags showed higher levels of decay than those packed in non-perforated bags for each of the individual treatments. No specific pattern was established with regard to the concentration of SO_2 incorporated in the different MMPSs. Similar results occurred for Red Globe kept for 8 w at $-0.5^{\circ}\text{C} + 5 \text{ d}$ at 7.5°C (Table 6b). No differences were detected for the different treatments, and grapes packed in perforated bags generally showed higher levels of decay. The results generally indicated the inability of most SO_2 sheets to effectively confine the spread of decay, irrespective of the storage period, when packed in perforated bags. A conclusion could be made that control of decay, similar to that obtained with the Uvasys sheet, could be achieved by using most MMPSs. However, decay control with the Uvasys sheet seemed to be slightly better than with a MMPS of a low concentration.

Inhibition of the decay development on non-inoculated bunches

A significantly higher level of decay, resulting from natural infections, occurred on Red Globe grapes kept for 5 w at $-0.5^{\circ}\text{C} + 5 \text{ d}$ at 7.5°C with use of the 17% SO_2 concentration MMPS, compared to a MMPS ranging between 20 - 26% SO_2 , as well as the Uvasys SO_2 sheet (Table 5a). No further differences in decay control occurred between the remainder of the treatments. Decay on Red Globe was generally significantly higher when packed in perforated bags than when packed in non-perforated bags. The results indicated that most of the MMPSs could control decay as effectively as the Uvasys SO_2 sheet, however, when examining the data for the specific bag types (inset, Table 5a), the efficacy of the 17% MMPS was at a

lower level than that of the Uvasys SO₂ sheet. These results drew attention to the likelihood that sheets of different concentrations of SO₂ might be required for use with different bag types.

No differences in decay development from natural infections occurred on grapes kept for 8 w at -0.5°C + 5 d at 7.5°C between any of the SO₂ treatments, neither in relation to the bag types used (Table 6a). Although not of statistical significance, decay for the 26% MMPS in non-perforated bags was much lower than with the other treatments, and likewise, the best result was achieved for the Uvasys sheet in a perforated bag (inset, Table 6a).

SO₂ damage

No difference in SO₂ damage at the pedicel end of Red Globe occurred between any of the MMPS treatments and the Uvasys sheet when kept for either 5 w or 8 w at -0.5°C, followed by 5 d at 7.5°C (Tables 5a & 6a). SO₂ damage manifesting on the surface of the grapes did also not differ between any of the treatments on grapes packed in perforated bags, for both storage periods. Surface SO₂ damage was significantly higher on grapes packed in non-perforated bags with inclusion of a MMPS of 26% SO₂, compared to all other treatments, when kept for 5 w at -0.5°C, followed by 5 d at 7.5°C. Almost similar results occurred on Red Globe packed in non-perforated bags, and kept for 8 w at -0.5°C + 5 d at 7.5°C. In this case the 26% MMPS showed significantly more surface SO₂ damage than all other treatments, and the Uvasys resulted in significantly less SO₂ damage to the surface of the berry than the MMPSs of 17% - 23% SO₂ concentration. Dissimilar to other cultivars, SO₂ damage also occurred at the calyx end of Red Globe grapes. No calyx-end SO₂ damage occurred on Red Globe which were packed in perforated bags and kept for 5 w at -0.5°C + 5 d at 7.5°C, and virtually none occurred after 8 w storage (Tables 5a & 6a). SO₂ damage at the calyx-end of the grapes was shown to be problematic on Red Globe packed in non-perforated bags. However, significantly higher levels of SO₂ damage only occurred with use of the 26% MMPS, compared to all other treatments.

Stem condition

No difference in the stem condition of Red Globe occurred between any of the SO₂ sheets, when kept for 5 w at -0.5°C + 5 d at 7.5°C (Table 5a). Differences in stem condition did, however, occur on grapes kept for 8 w at -0.5°C + 5 d at 7.5°C (Table 6a). The latter can not be ascribed to a specific SO₂ treatment effect, but rather to the long storage period. For both storage durations the stems of the grapes in perforated bags were slightly more desiccated than those packed in non-perforated bags.

D Dan Ben Hannah

Decay development

Inhibition of the decay development on non-inoculated bunches

Decay development was significantly reduced on Dan Ben Hannah grapes kept for 5 w at -0.5°C + 5 d at 7.5°C by the 23 and 26% MMPSs, as well as by the Uvasys SO₂ sheet, compared to the 17% MMPS (Table 7). An almost similar result was achieved for the 8 w at -0.5°C + 5 d at 7.5°C storage period, with significantly more decay occurring with use of the MMPS of 20% SO₂ than the two aforementioned treatments (Table 8). In both storage periods, grapes packed in perforated bags exhibited significantly higher levels of decay, especially when stored for 8 w. The results indicated that the use of a MMPS of too low SO₂ concentration might result in higher decay levels than anticipated, especially under conditions of extended storage.

SO₂ damage

No difference in SO₂ damage at the pedicel end, as well as on the surface, of Dan Ben Hannah occurred between any of the MMPS treatments and the Uvasys sheet for the grapes packed in perforated bags and kept for 5 w at -0.5°C, followed by 5 d

at 7.5°C (Table 7). However, when packed in non-perforated bags, the MMPS of 26% SO₂ concentration showed significantly higher levels of SO₂ damage (of both types) compared to all other treatments. A significantly higher level of both pedicel, and surface SO₂ damage occurred with use of the 26% MMPS on grapes packed in non-perforated bags compared to those in perforated bags. In addition to the 26% MMPS, a significantly higher level of pedicel SO₂ damage occurred on Dan Ben Hannah grapes kept for 8 w at -0.5°C + 5 d at 7.5°C (Table 8), with use of the Uvasys SO₂ sheet, compared to the MMPSs of 17 – 23% SO₂ concentration. Only the 26% MMPS showed significantly higher levels of surface SO₂ damage for grapes kept in storage for 8 w at -0.5°C + 5 d at 7.5°C. A higher level of SO₂ damage generally occurred when grapes were packed in non-perforated bags, compared to those in perforated bags.

Berry split

No difference in berry split occurred between any of the treatments on Dan Ben Hannah grapes kept for 5 w at -0.5°C + 5 d at 7.5°C, however, berry split was generally higher for grapes packed in non-perforated bags (Table 7). On Dan Ben Hannah grapes kept for 8 w at -0.5°C + 5 d at 7.5°C, however, significantly higher levels of berry split occurred for the 23 and 26% MMPS treatments compared to the 20% MMPS.

Stem condition

Differences in stem condition occurred on Dan Ben Hannah kept for 5 w at -0.5°C + 5 d at 7.5°C (Table 7), but not for grapes kept for 8 w at a similar temperature (Table 8).

Conclusions

It appears that control of decay, similar to that obtained with the Uvasys sheet, can be achieved with the use of most MMPSs that were tested. However, decay control with the Uvasys sheet seemed to be slightly better than with a MMPS of a too low SO₂ concentration. The packaging materials, in this case perforated and non-perforated bags, had a significant impact on decay development irrespective of the inoculation method used for all the cultivars tested. Results generally indicated the inability of most SO₂ sheets to effectively confine the spread of decay, irrespective of the storage period, when packed in perforated bags. The results drew attention to the likelihood that sheets of different concentrations of SO₂ might be required with the use of different bag types.

Similar results were found for levels of SO₂ damage. Generally, higher levels of SO₂ damage occurred on grapes packed in non-perforated bags compared to perforated bags for each of the individual concentrations. The 26% SO₂ treatment showed significantly higher levels of surface and pedicel SO₂ damage with the more sensitive cultivars, especially during the longer storage periods. Generally, higher levels of surface SO₂ damage also occurred with the more sensitive cultivars for the HMS treatment, but only on grapes packed in non-perforated bags.

With regards to the other quality parameters examined, viz. berry split and stem condition, generally no differences occurred between any of the treatments on all of the cultivars tested. However, berry split was generally higher for grapes packed in non-perforated bags for long storage periods with the high concentration SO₂ treatments. The stem condition of most treatments was, however, more green in the non-perforated bags than those in the perforated bags.

It was shown that the original hand-made sheet could be machine manufactured without affecting its efficacy, and it is a potentially viable option for use in future, comparing favourably with the Uvasys SO₂ sheet. The exact SO₂ concentration could vary for different cultivars, as well as for different grape packages. The SO₂ concentration to be incorporated within the polymer sheet was shown to be in the order of approximately 20% for grapes packed in non perforated bags, and 23% for those in perforated bags. Cultivar differences occur with regard

to level of control, as well as levels of SO₂ damage. The efficacy of the new sheet to control decay over extended storage periods, 6 to 8 w, needs to be carefully compared to the Uvasys SO₂ sheet.

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Appendix to Chapter 3

To simplify discussion of the results of the statistical analysis, a comprehensive list of tables (Tables 1 to 8) containing the statistical analysis of the quality parameters have been included as an “Appendix” here.

Table 1a. Effect of bag type and SO₂ treatment on the quality of Thompson Seedless table grapes, inoculated artificially by settlement of *Botrytis cinerea* conidia onto the berry surface, after storage for 5 w at -0.5°C, followed by 5 d at 7.5°C.

Examination parameter ⁵	Interaction ² Bag / SO ₂ sheet	Bag type ³ (Factor A)		SO ₂ treatment ⁴ (Factor B)					Prob. > F ¹		
		Non-perf	Perf	Pol 14	Pol 17	Pol 20	Uvasys	Hand-made Pol	A	B	AB
Decay		1.12	1.66	4.65a	0.86b	0.88b	0.23b	0.45b	ns	**	ns
Pedicle SO ₂		3.86a	1.94b	1.53b	2.56b	2.00b	2.24b	6.17a	*	*	ns
Surface SO ₂		1.98a	0.60b	0.52b	0.51b	1.96ab	0.24b	3.20a	*	*	ns
Berry split		6.27a	0.95b	2.47b	3.28b	3.61ab	2.26b	6.42a	**	*	ns
Stem condition		2.61	2.69	2.76	2.51	2.61	2.74	2.62	ns	ns	ns

Examination parameter	SO ₂ Sheet	Bag type	
		Non-perf	Perf
Decay ⁶	Pol 14	5.10	4.20
	Pol 17	0.39	1.34
	Pol 20	0.15	1.62
	Uvasys	0.17	0.33
	Hand-made Pol	0.08	0.80

- 1 Two-way ANOVA table for Factor A (Bag type) and Factor B (SO₂ sheet), with ns, *, ** and *** indicating non-significant and significant differences at the 5%, 1% and 0.1% levels, respectively
- 2 Non-pooled data for significant interaction between factors A and B.
- 3 Data pooled across SO₂ sheet for non-significant interactions and non-pooled for significant interactions between Factor A (Bag type = perforated and non-perforated bags) & for Factor B (SO₂ sheet = Polymer SO₂ sheets with varying Na₂S₂O₅ concentrations of 14 – 20%, Uvasys and hand-made polymer sheet with 20% SO₂). Values in the same row, followed by different letters indicate significant differences for pooled data (P<0.05) according to LSD test, whereas for non-pooled data different letters indicate significant differences across rows and columns.
- 4 Data pooled across bag type for non-significant interactions. Values in the same row, followed by different letters indicate significant differences.
- 5 Examination parameters: Decay, SO₂ damage and berry split are expressed as percentages of the total mass, with stem condition rated according to a 5-point scale (1 = green stems and 5 = brown and desiccated).
- 6 Inset table: Decay for each of the SO₂ treatments for both bag types.

Table 1b. Effect of bag type and SO₂ treatment on the quality of Thompson Seedless table grapes after storage for 5 w at -0.5°C, followed by 5 d at 7.5°C. The grapes were inoculated artificially by placement of decayed *Botrytis cinerea* berries into the four corner bunches, however, decay and the other defects were recorded on the remaining non-inoculated bunches.

Examination parameter ⁵	Interaction ² Bag / SO ₂ sheet	Bag type ³ (Factor A)		SO ₂ treatment ⁴ (Factor B)					Prob. > F ¹		
		Non-perf	Perf	Pol 14	Pol 17	Pol 20	Uvasys	Hand-made Pol	A	B	AB
Decay		0.27a	0.59b	0.98a	0.49ab	0.06b	0.52ab	0.11b	*	*	ns
Pedicle SO ₂	Pol 14	2.08c	1.01c						**	**	**
	Pol 17	4.07bc	1.16c								
	Pol 20	7.13b	0.84c								
	Uvasys	3.34bc	1.75c								
	Hand-made Pol	20.89a	4.42bc								
Surface SO ₂		4.34a	0.33b	0.27b	1.50b	3.05ab	1.08b	5.77a	*	*	ns
Berry split		8.71a	0.83b	1.30a	5.55b	6.34b	5.65b	4.99b	**	*	ns
Stem condition		2.57	2.71	2.55	2.62	2.73	2.54	2.77	ns	ns	ns

Examination parameter	SO ₂ Sheet	Bag type	
		Non-perf	Perf
Decay ⁶	Pol 14	0.81	1.15
	Pol 17	0.35	0.63
	Pol 20	0.00	0.11
	Uvasys	0.04	0.99
	Hand-made Pol	0.14	0.09

1 - 6 See Table 1a for explanations.

Table 1c. Decay development on Thompson Seedless table grapes inoculated by placement of *Botrytis cinerea* infected berries in the four corner bunches, after storage for 5 w at -0.5°C , followed by 5 d at 7.5°C .

Examination parameter ⁵	Interaction ² Bag / SO ₂ sheet	Bag type ³ (Factor A)		SO ₂ treatment ⁴ (Factor B)					Prob. > F ¹		
		Non-perf	Perf	Pol 14	Pol 17	Pol 20	Uvasys	Hand-made Pol	A	B	AB
Decay	Pol 14	10.76a	8.43ab						ns	**	*
	Pol 17	11.55a	8.52ab								
	Pol 20	1.97d	7.58ab								
	Uvasys	6.26bc	7.63ab								
	Hand-made Pol	1.13d	2.38cd								

1 - 5 See Table 1a for explanations.

Table 2a. Effect of bag type and SO₂ treatment on the quality of Thompson Seedless table grapes, inoculated artificially by settlement of *Botrytis cinerea* conidia onto the berry surface, after storage for 8 w at -0.5°C, followed by 5 d at 7.5°C.

Examination parameter ⁵	Interaction ² Bag / SO ₂ sheet	Bag type ³ (Factor A)		SO ₂ treatment ⁴ (Factor B)					Prob. > F ¹		
		Non-perf	Perf	Pol 14	Pol 17	Pol 20	Uvasys	Hand-made Pol	A	B	AB
Decay	Pol 14	3.54bc	12.74a						**	*	*
	Pol 17	1.07bc	14.87a								
	Pol 20	0.93bc	6.04b								
	Uvasys	0.30bc	3.33bc								
	Hand-made Pol	0.19c	2.20bc								
Pedicel SO ₂ Surface SO ₂		13.26a	7.23b	5.37b	7.28b	8.76b	9.00b	20.83a	*	**	ns
	Pol 14	0.53bc	0.43bc						**	**	**
	Pol 17	2.61b	0.53bc								
	Pol 20	2.24bc	0.10c								
	Uvasys	0.64bc	0.31c								
	Hand-made Pol	11.05a	0.39bc								
Berry split		9.33a	0.92b	3.98	6.62	5.73	3.49	5.80	**	ns	ns
Stem condition		2.57a	3.37b	2.40c	3.16ab	3.00ab	2.90b	3.40a	**	*	ns

1 - 5 See Table 1a explanations.

Table 2b. Effect of bag type and SO₂ treatment on the quality of Thompson Seedless table grapes after storage for 8 w at -0.5°C, followed by 5 d at 7.5°C. The grapes were inoculated artificially by placement of decayed *Botrytis cinerea* berries into the four corner bunches, however, decay and the other defects were recorded on the remaining non-inoculated bunches.

Examination parameter ⁵	Interaction ² Bag / SO ₂ sheet	Bag type ³ (Factor A)		SO ₂ treatment ⁴ (Factor B)					Prob. > F ¹		
		Non-perf	Perf	Pol 14	Pol 17	Pol 20	Uvasys	Hand-made Pol	A	B	AB
Decay		1.63a	3.02b	5.04a	1.46b	1.41b	1.65b	2.06b	*	*	ns
Pedicle SO ₂		8.48	6.33	2.12b	4.38b	5.07b	6.54b	18.89a	ns	**	ns
Surface SO ₂		2.90a	0.71b	2.22	1.00	2.42	1.32	2.06	*	ns	ns
Berry split	Pol 14	3.27bc	1.71c						**	ns	*
	Pol 17	9.98a	1.69c								
	Pol 20	9.61a	0.75c								
	Uvasys	8.96ab	0.69c								
	Hand-made Pol	8.10ab	0.66c								
Stem condition		3.10	3.25	3.18	3.28	3.06	3.21	3.15	ns	ns	ns

Examination parameter	SO ₂ Sheet	Bag type	
		Non-perf	Perf
Decay ⁶	Pol 14	2.85	7.23
	Pol 17	0.71	2.21
	Pol 20	0.47	2.35
	Uvasys	0.43	2.86
	Hand-made Pol	0.44	3.68

1 - 6 See Table 1a for explanations.

Table 2c. Decay development on Thompson Seedless table grapes inoculated by placement of *Botrytis cinerea* infected berries in the four corner bunches, after storage for 8 w at -0.5°C , followed by 5 d at 7.5°C .

Examination parameter ⁵	Interaction ² Bag / SO ₂ sheet	Bag type ³ (Factor A)		SO ₂ treatment ⁴ (Factor B)					Prob. > F ¹		
		Non-perf	Perf	Pol 14	Pol 17	Pol 20	Uvasys	Hand-made Pol	A	B	AB
Decay		9.79a	15.37b	17.18a	13.95ab	11.28bc	12.55abc	7.95c	*	*	ns

Examination parameter	SO ₂ Sheet	Bag type	
		Non-perf	Perf
Decay ⁶	Pol 14	17.01	17.35
	Pol 17	11.32	16.59
	Pol 20	11.36	11.21
	Uvasys	6.21	18.89
	Hand-made Pol	3.07	12.83

1 - 6 See Table 1a for explanations.

Table 3. Effect of bag type and SO₂ treatment on the quality of Waltham Cross grapes after storage for 5 w at –0.5°C, followed by 5 d at 7.5°C. The grapes were not inoculated.

Examination parameter ⁵	Interaction ² Bag / SO ₂ sheet	Bag type ³ (Factor A)		SO ₂ treatment ⁴ (Factor B)				Prob. > F ¹		
		Non-perf	Perf	Pol 14	Pol 17	Pol 20	Uvasys	A	B	AB
Decay		0.42	0.26	0.07	0.00	0.20	0.00	ns	ns	ns
Pedicle SO ₂		0.02	0.02	0.02	0.00	0.00	0.05	ns	ns	ns
Surface SO ₂		0.19	0.04	0.13	0.08	0.11	0.14	ns	ns	ns
Berry split		0.33	0.07	0.53	0.07	0.03	0.13	ns	ns	ns
Browning		0.89a	2.27b	1.41	1.35	1.82	1.22	***	ns	ns
Stem condition	Pol 14	2.38d	3.20ab					***	ns	***
	Pol 17	2.44d	3.20ab							
	Pol 20	2.34d	3.28a							
	Uvasys	2.68cd	3.06abc							

1 Two-way ANOVA table for Factor A (Bag type) and Factor B (SO₂ sheet), with ns, *, ** and *** indicating non-significant and significant differences at the 5%, 1% and 0.1% levels

2 Non-pooled data for significant interaction between factors A and B.

3 Data pooled across SO₂ sheet for non-significant interactions and non-pooled for significant interactions between Factor A (Bag type = perforated and non-perforated bags) & for Factor B (SO₂ sheet = Polymer SO₂ sheets with varying Na₂S₂O₅ concentrations of 14 – 20% and Uvasys SO₂ sheet). Values in the same row, followed by different letters indicate significant differences for pooled data (P<0.05) according to LSD test, whereas for non-pooled data different letters indicate significant differences across rows and columns.

4 Data pooled across bag type for non-significant interactions. Values in the same row, followed by different letters indicate significant differences.

5 Examination parameters: Decay, SO₂ damage, berry split and browning are expressed as a percentage of the total mass, with stem condition rated according to a 5-point scale (1 = green stems and 5 = brown and desiccated).

Table 4. Effect of bag type and SO₂ treatment on the quality of Waltham Cross grapes after storage for 8 w at –0.5°C, followed by 5 d at 7.5°C. The grapes were not inoculated.

Examination parameter ⁵	Interaction ² Bag / SO ₂ sheet	Bag type ³ (Factor A)		SO ₂ treatment ⁴ (Factor B)				Prob. > F ¹		
		Non-perf	Perf	Pol 14	Pol 17	Pol 20	Uvasys	A	B	AB
Decay		0.50	0.78	0.31	0.13	0.31	0.03	ns	ns	ns
Pedice SO ₂		0.02	0.01	0.02	0.02	0.00	0.03	ns	ns	ns
Surface SO ₂		0.14	0.08	0.12	0.07	0.08	0.23	ns	ns	ns
Berry split		0.13a	0.00b	0.07	0.00	0.17	0.00	*	ns	ns
Browning		8.14a	12.70b	13.07	8.21	9.17	10.03	**	ns	ns
Stem condition	Pol 14	2.76d	3.46ab					**	***	***
	Pol 17	2.90cd	3.20bc							
	Pol 20	2.66d	2.92cd							
	Uvasys	2.82d	2.76a							

1 - 5 See Table 3 for explanations.

Table 5a. Effect of bag type and SO₂ treatment on the quality of Red Globe table grapes after storage for 5 w at –0.5°C, followed by 5 d at 7.5°C. The grapes were inoculated artificially by placement of decayed *Botrytis cinerea* berries into the four corner bunches, however, decay and the other quality parameters were recorded on the remaining non-inoculated bunches.

Examination parameter ⁵	Interaction ² Bag / SO ₂ sheet	Bag type ³ (Factor A)		SO ₂ treatment ⁴ (Factor B)					Prob. > F ¹		
		Non-perf	Perf	Pol 17	Pol 20	Pol 23	Pol 26	Uvasys	A	B	AB
Decay		0.16a	0.41b	0.68a	0.22b	0.19b	0.14b	0.19b	*	*	ns
Pedice SO ₂		0.39	0.18	0.13	0.30	0.23	0.40	0.35	ns	ns	ns
Surface SO ₂	Pol 17	4.68cd	0.20d						**	**	**
	Pol 20	11.93b	1.03d								
	Pol 23	8.76bc	1.48d								
	Pol 26	24.74a	0.50d								
	Uvasys	2.26cd	1.19d								
Calyx SO ₂	Pol 17	0.47b	0.00b						*	*	*
	Pol 20	0.39b	0.00b								
	Pol 23	0.42b	0.00b								
	Pol 26	3.10a	0.00b								
	Uvasys	0.11b	0.00b								
Stem condition		2.65a	3.27b	2.90	2.84	2.90	3.08	3.06	**	ns	ns

Examination parameter	SO ₂ Sheet	Bag type	
		Non-perf	Perf
Decay ⁶	Pol 17	0.31	1.05
	Pol 20	0.14	0.30
	Pol 23	0.00	0.33
	Pol 26	0.07	0.21
	Uvasys	0.27	0.10

- 1 Two-way ANOVA table for Factor A (Bag type) and Factor B (SO₂ sheet), with ns, *, ** and *** indicating non-significant and significant differences at the 5%, 1% and 0.1% levels
- 2 Non-pooled data for significant interaction between factors A and B.
- 3 Data pooled across SO₂ sheet for non-significant interactions and non-pooled for significant interactions between Factor A (Bag type = perforated and non-perforated bags) & for Factor B (SO₂ sheet = Polymer SO₂ sheets with varying Na₂S₂O₅ concentrations of 17 – 20% and Uvasys SO₂ sheet). Values in the same row, followed by different letters indicate significant differences for pooled data (P<0.05) according to LSD test, whereas for non-pooled data different letters indicate significant differences across rows and columns.
- 4 Data pooled across bag type for non-significant interactions. Values in the same row, followed by different letters indicate significant differences.
- 5 Examination parameters: Decay, SO₂ damage and berry split are expressed as percentages of the total mass, with stem condition rated according to a 5-point scale (1 = green stems and 5 = brown and desiccated).
- 6 Inset table: Decay for each of the SO₂ treatments for both bag types.

Table 5b. Decay development on Red Globe table grapes inoculated by placement of *Botrytis cinerea* infected berries in the four corner bunches, after storage for 5 w at -0.5°C, followed by 5 d at 7.5°C.

Examination parameter ⁵	Interaction ² Bag / SO ₂ sheet	Bag type ³ (Factor A)		SO ₂ treatment ⁴ (Factor B)					Prob. > F ¹		
		Non-perf	Perf	Pol 17	Pol 20	Pol 23	Pol 26	Uvasys	A	B	AB
Decay		1.47a	5.93b	3.34	4.41	2.87	3.49	4.40	*	ns	ns

Examination parameter	SO ₂ Sheet	Bag type	
		Non-perf	Perf
Decay ⁶	Pol 17	2.09	4.59
	Pol 20	1.05	7.77
	Pol 23	1.56	4.23
	Pol 26	1.65	5.33
	Uvasys	1.04	7.75

1 - 6 See Table 5a for explanations.

Table 6a. Effect of bag type and SO₂ treatment on the quality of Red Globe table grapes after storage for 8 w at –0.5°C, followed by 5 d at 7.5°C. The grapes were inoculated artificially by placement of decayed *Botrytis cinerea* berries into the four corner bunches, however, decay and the other quality parameters were recorded on the remaining non-inoculated bunches.

Examination parameter ⁵	Interaction ² Bag / SO ₂ sheet	Bag type ³ (Factor A)		SO ₂ treatment ⁴ (Factor B)					Prob. > F ¹		
		Non-perf	Perf	Pol 17	Pol 20	Pol 23	Pol 26	Uvasys	A	B	AB
Decay		0.65	0.87	1.04	0.80	0.74	0.62	0.61	ns	ns	ns
Pedice SO ₂		0.89a	0.14b	0.02	0.72	0.25	1.09	0.32	*	ns	ns
Surface SO ₂	Pol 17	16.96b	0.16c						**	*	*
	Pol 20	16.02b	0.80c								
	Pol 23	16.83b	0.74c								
	Pol 26	30.43a	0.22c								
	Uvasys	3.34c	0.15c								
Calyx SO ₂		2.11a	0.02b	1.24	0.71	0.84	2.04	0.48	*	ns	ns
Stem condition		2.92a	3.53b	3.40a	3.20ab	3.30ab	3.01c	3.14b	**	*	ns

Examination parameter	SO ₂ Sheet	Bag type	
		Non-perf	Perf
Decay ⁶	Pol 17	0.91	1.16
	Pol 20	0.99	0.61
	Pol 23	0.32	1.15
	Pol 26	0.17	1.07
	Uvasys	0.85	0.37

1 - 6 See Table 5a for explanations.

Table 6b. Decay development on Red Globe table grapes inoculated by placement of *Botrytis cinerea* infected berries in the four corner bunches, after storage for 8 w at -0.5°C , followed by 5 d at 7.5°C .

Examination parameter ⁵	Interaction ² Bag / SO ₂ sheet	Bag type ³ (Factor A)		SO ₂ treatment ⁴ (Factor B)					Prob. > F ¹		
		Non-perf	Perf	Pol 17	Pol 20	Pol 23	Pol 26	Uvasys	A	B	AB
Decay		3.68a	8.58b	6.35	7.37	5.72	5.52	5.69	**	ns	ns

Examination parameter	SO ₂ Sheet	Bag type	
		Non-perf	Perf
Decay ⁶	Pol 17	4.85	7.86
	Pol 20	4.19	10.55
	Pol 23	3.60	7.83
	Pol 26	3.21	7.83
	Uvasys	2.54	8.84

1 - 6 See Table 5a for explanations.

Table 7. Effect of bag type and SO₂ treatment on the quality of Dan Ben Hannah grapes after storage for 5 w at –0.5°C, followed by 5 d at 7.5°C. The grapes were not inoculated.

Examination parameter ⁵	Interaction ² Bag / SO ₂ sheet	Bag type ³ (Factor A)		SO ₂ treatment ⁴ (Factor B)					Prob. > F ¹		
		Non-perf	Perf	Pol 17	Pol 20	Pol 23	Pol 26	Uvasys	A	B	AB
Decay		0.40a	1.92b	2.23a	1.31ab	0.95b	0.74b	0.58b	**	*	ns
Pedicel SO ₂	Pol 17	3.27bc	2.09c						***	***	***
	Pol 20	3.68bc	1.76c								
	Pol 23	5.21b	2.58bc								
	Pol 26	14.50a	2.71bc								
	Uvasys	4.42bc	2.53bc								
Surface SO ₂	Pol 17	1.52b	0.26b						***	**	**
	Pol 20	2.42b	0.26b								
	Pol 23	3.43b	0.84b								
	Pol 26	11.02a	0.25b								
	Uvasys	1.34b	0.88b								
Berry split		1.23a	0.75b	0.76	1.19	0.91	1.15	0.93	**	ns	ns
Stem condition		2.72a	2.86b	2.56c	2.75bc	2.71c	2.94ab	2.98a	*	***	ns

1 Two-way ANOVA table for Factor A (Bag type) and Factor B (SO₂ sheet), with ns, *, ** and *** indicating non-significant and significant differences at the 5%, 1% and 0.1% levels, respectively

2 Non-pooled data for significant interaction between factors A and B.

3 Data pooled across SO₂ sheet for non-significant interactions and non-pooled for significant interactions between Factor A (Bag type = perforated and non-perforated bags) & for Factor B (SO₂ sheet = Polymer SO₂ sheets with varying Na₂S₂O₅ concentrations of 17 – 20% and Uvasys SO₂ sheet). Values in the same row, followed by different letters indicate significant differences for pooled data (P<0.05) according to LSD test, whereas for non-pooled data different letters indicate significant differences across rows and columns.

4 Data pooled across bag type for non-significant interactions. Values in the same row, followed by different letters indicate significant differences.

5 Examination parameters: Decay, SO₂ damage and berry split are expressed as percentages of the total mass, with stem condition rated according to a 5-point scale (1 = green stems and 5 = brown and desiccated).

Table 8. Effect of bag type and SO₂ treatment on the quality of Dan Ben Hannah grapes after storage for 8 w at –0.5°C, followed by 5 d at 7.5°C. The grapes were not inoculated.

Examination parameter ⁵	Interaction ² Bag / SO ₂ sheet	Bag type ³ (Factor A)		SO ₂ treatment ⁴ (Factor B)					Prob. > F ¹		
		Non-perf	Perf	Pol 17	Pol 20	Pol 23	Pol 26	Uvasys	A	B	AB
Decay		1.59a	4.66b	5.39a	5.04a	2.24bc	2.15bc	0.80c	***	***	ns
Pedicle SO ₂		4.65a	2.10b	1.44a	2.83b	3.10b	4.77c	4.76c	**	**	ns
Surface SO ₂		4.70a	0.60b	1.61bc	2.12b	3.07ab	4.85a	2.00b	***	**	ns
Berry split		1.23a	0.75b	1.12ab	0.75a	1.68b	1.71b	1.07ab	**	**	ns
Stem condition		2.74	2.76	2.80	2.56	2.79	2.72	2.87	ns	ns	ns

1 - 5 See Table 7 for explanations.

Chapter 4

The effect of infrared-curing as opposed to convection heat curing on the efficacy of the new monolithic sheet in controlling post-harvest *Botrytis cinerea* decay on Dauphine table grapes

Technology developed in this study is confidential and is protected by patent (South African Patent No. 96-2517, International Patents Pending, Provisional No. ZA 2001/1851).

Abstract

Testing of the prototype machine-manufactured SO₂ sheet showed that it compared favourably to the existing commercially available SO₂ sheets. Use of convection ovens for curing the PVC plastisol in the former, however, presented severe limitations regarding production volumes; faster curing times were required. This need for faster curing times led to an investigation into the feasibility of using infrared heaters for curing the PVC matrix. Subsequently, new prototypes were manufactured using infrared curing and tested to determine the efficacy of the infrared-cured polymer SO₂ sheets in comparison to the former convection heat-cured sheets.

The results indicated that curing with an infrared system, instead of convection heat, did not negatively affect the efficacy of the sheet. In fact, use of the infrared curing system could contribute to improving the aesthetics of the final product. Future manufacturing of the polymer sheets could therefore rely on an infrared curing process.

Introduction

Tests conducted with the new machine-manufactured SO₂ sheets showed that they compared favourably with the existing, commercially available SO₂ sheets. Initially, conventional convection ovens were used to heat the plastisol mixture to achieve fusion of the resin-impregnated PVC particles. However, use of convection ovens for this application presented severe practical limitations regarding production volumes. Increased production volumes directly relate to increases in curing oven length. This is in accordance with findings in the paper industry where the need for faster drying rates, and, thereby, faster machine speeds, led to the evolution of infrared drying (Closset, 1986; Stephansen, 1985).

Plastisols convert to a solid and useful state with the application of heat in the 140 to 200°C range (Fig. 1).

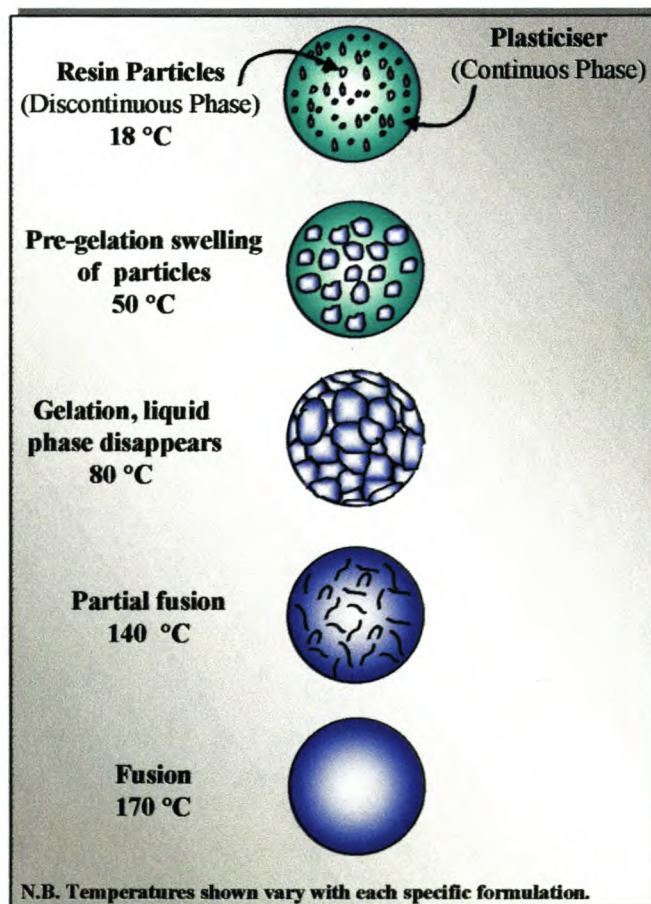


Figure 1 Plastisol gelation and fusion (Werner, 1977).

As the PVC plastisol is increased in temperature from room temperature, plasticiser begins to penetrate each resin particle. When the resin takes up all the plasticiser, the system loses its fluidity. This is referred to as the gelled state. At this stage, the gelled mass has no cohesive strength. As heat continues to penetrate the system the polymer starts dissolving in the plasticiser until all the polymer chains are uniformly dissolved in the plasticiser. This is the fused state, which when cooled provides a plastic material of considerable strength and utility (Werner, 1977).

Besides the increases in production rates, the use of infrared heaters for drying paper coatings had significantly improved the quality of the coated surfaces. Faster curing times can be attributed to the higher penetration of the coating and the substrate by infrared, producing a more uniform temperature distribution in the product compared to convection heat where heat transport from the surface into the product depends on conduction, which is much slower process. Other advantages associated with the use of infrared heating instead of convection heating are related to the small size and ease of installation of the infrared heaters compared to convection ovens (Stephansen, 1985).

Subsequently, new prototypes were manufactured using infrared (IR) curing. This reduced the manufacturing time and costs, and contributed to improving the aesthetics of the product. The effect of IR-curing on the efficacy of the new polymer sheet in controlling decay was, however, yet to be determined.

The objectives of this chapter were to determine the efficacy, of the machine manufactured, infrared-cured polymer SO₂ sheets for controlling: (a) the spread of *Botrytis cinerea* decay from infected grape berries to adjacent sound berries; and (b) the inhibition of new *B. cinerea* infections on the berry surface, with use of perforated and non-perforated bags, in relation to the initial SO₂ concentration incorporated in the sheets, and compare results to those obtained for the former, convection heat-cured sheets.

Materials and methods

Sheet manufacturing

The SO₂ gas-generating sheets were made on a pilot scale production plant purposely built for this project. Manufacturing of the SO₂ gas generating sheets commences with the preparation of the PVC plastisol mixture. Once the desired plastisol mixture is obtained it is applied onto a carrier material by means of a nozzle. The carrier material with the plastisol mixture is sent through a series of curing ovens where the plastisol is cured to a solid state. Once cured, a second layer of non-woven material is laminated onto the carrier layer to enclose the PVC matrix between two sheets of material. A cooling section follows where the product is rapidly cooled down before it is cut to the desired specifications. The finished product is inspected for deviations in quality before it is packaged into poly(ethylene) bags ready to be used.

The infrared-cured SO₂ gas generating sheets were manufactured according to the same process as described above and on the same pilot scale production plant. The only difference was that the convection ovens used for curing were replaced by two infrared units.

Packaging and SO₂ treatment

Fresh Dauphine table grapes were packed at De Doorns and transported to Stellenbosch for inoculation and placement of the different SO₂ sheets in the cartons. The various SO₂ generators that were tested and compared during this trial were:

- i) Machine manufactured polymer sheets of 18 and 23% sodium metabisulphite content;
- ii) Sheets cured either by infrared, or convection heat process; and
- iii) Uvasys 70% (standard) as reference.

The grapes were packaged in cartons as for export in either perforated or non-perforated bags. Individual bunches were packaged in polycote bunch bags. The packaged grapes were kept in cold storage for a period of 5 w at -0.5°C and an additional 5 d at 7.5°C before evaluation.

Inoculation Method

To ensure the presence of a sufficient dosage of *Botrytis cinerea* in the cartons the grapes were inoculated. Inoculation of the grapes were done either by placement of infected berries within three bunches and by spore settlement method.

A settling method with dry conidia was used in order for natural infection and sporulation to take place under the prevailing conditions. Conidia from ten day old *B. cinerea* cultures, grown on Potato Dextrose Agar (PDA) medium, were used to inoculate the grapes. The bottom halves of the inverted petri dish cultures were held in position approximately 5 to 10 cm above the exposed fruit and tapped gently to dislodge individual spores. Water was not used during this inoculation method (Fourie, 1994).

For the placement of infected berries, grapes were inoculated with conidia of *B. cinerea* cultures seven days prior to the day of inoculation. *B. cinerea* cultures were grown under diurnal conditions for ten days on a PDA medium. A spore suspension was made of 1×10^5 spores per mL, by dilution with sterilised water. Sound berries were randomly selected and removed from a number of bunches and the berry surface sterilised with a 70% ethanol and water solution. The berries were allowed to dry naturally for approximately 30 minutes prior to inoculation. A wound, 1mm deep by 1mm wide, was inflicted on the berry surface and a droplet (25 μ l) of the spore suspension placed onto the wound site. Inoculated berries were placed in an incubator/moist chamber at 22 C with a relative humidity of approximately 95% for one week prior to use. The seven day-old inoculated berries were placed amongst the bunches in each of the four corners to obtain a natural spread of decay to adjacent berries (Fourie, 1994).

Experimental layout and statistical detail

Completely randomised two-way ANOVA with bag type and SO₂ sheet Factors A & B, respectively. Data for the different inoculation types were analysed separately. Each treatment consisted of 5 replicate cartons of grapes, with the cartons completely randomised on a pallet. The grapes were examined after storage and factorial analyses of variance performed on the data. Students-T LSD was calculated to compare treatment means at a 5% significance level.

Examination parameters

Examinations took place according to Capespan protocols. Decay and SO₂ damage were expressed by weight as a percentage (%) of the sample mass, while stem condition was rated by visual assessment according to a five point scale, 1 = green and 5 = brown and desiccated.

Results and discussion

The efficacy of controlling post-harvest *Botrytis* storage decay, without affecting other quality defects, was determined for different SO₂ sheets. Trials were conducted with Dauphine table grapes to establish the effect of an infrared curing process in the manufacturing of the polymer SO₂ sheet, opposed to convection heat curing. Two inoculation techniques were used, viz. spore settlement and placement of an infected berry amongst the grapes. Decay, SO₂ damage and the condition of the stems, were assessed after storage for 5 w at -0.5°C, followed by 5 d at 7.5°C.

Statistical analysis of the above quality parameters viz., *Botrytis* decay, SO₂ damage, and stem condition, resulted in a large volume of data. Thus, for clarity purposes and to simplify the discussion of the results, a comprehensive list of tables (Tables 1 and 2) containing the statistical analysis has been included as an "Appendix" at the end of this chapter.

Decay development

Inhibition of the establishment of new infections (inoculation by spore settlement)

All SO₂ sheet treatments confined the establishment of new infections significantly compared to the control (no SO₂ treatment), hence only data for those treatments are shown and discussed (Table 1).

Significantly lower levels of decay, resulting from the establishment of new infections, occurred with use of the Uvasys SO₂ sheet, compared to all other treatments, except the 23% SO₂ infrared (IR)-cured polymer sheet. The latter, in turn resulted in significantly less decay than the 18% SO₂, convection heat-cured sheet. Although no significant reduction in decay was seen when the 23% IR-cured and the 18% IR sheets were compared, a lower level of decay was observed with the aforementioned. This result, and the fact that no differences in decay resulted from packing the grapes in either perforated or non-perforated bags, implied that the SO₂ content of the IR-cured sheets might need to be slightly higher than for the convection heat-cured polymer sheets.

Inhibition of the spread of decay (inoculation by placement of infected berry)

All SO₂ sheet treatments confined the spread of decay significantly compared to the control (no SO₂ treatment), hence only data for those treatments are shown and discussed (Table 2).

A significantly lower level of decay, by spread from an infected berry to adjacent sound berries, occurred with the 23% IR-sheet compared to all other treatments for Dauphine grapes packed in non-perforated bags. Except for the 18% IR-sheet, showing higher decay levels than the 18% convection-cured sheet, no further differences occurred for this bag type. No differences in decay inhibition occurred between any of the treatments for grapes packed in perforated bags.

Inhibition of the decay development on non-inoculated bunches

No differences in decay inhibition on non-inoculated bunches of grapes, representing decay development from natural infections, occurred between any of the SO₂ sheet treatments (Table 2).

SO₂ damage

No significant difference in SO₂ damage occurred as result of the type of SO₂ sheet used, neither at the pedicel end nor on the surface of Dauphine table grapes, for both inoculation types (Table 1 & 2). Besides the fact that no significant differences in SO₂ damage were shown, the actual levels of SO₂ damage incurred at the specific sites of damage assessment were very similar. The level of damage incurred on the surface of Dauphine grapes was generally higher than at the pedicel end of the grapes.

Stem condition

No specific trend with regard to stem condition was observed as result of the use of a specific SO₂ sheet. In the spore settlement inoculation trial (Table 1) the stem condition of the grapes stored with the SO₂ sheets manufactured by IR curing were significantly greener. A similar observation was, however, not established for the second trial.

Conclusions

The results showed that the newly manufactured, infrared-cured polymer sheets could confine the establishment of new infections, as well as the possible spread of decay from previously infected berries, as effectively as the polymer sheets manufactured by a convection heat curing process. It was also shown that the IR-cured sheet controlled decay practically as well as the Uvasys SO₂ sheet

used at present. No differences in decay resulted from packing the grapes in either perforated or non-perforated bags. The results did not show a significant difference in decay control by incorporating different SO₂ concentrations in the infrared-cured sheet, however, slightly improved control was achieved with the sheet of a slightly higher SO₂ content. The exact SO₂ concentration required, however, needs to be established (see chapter 5).

The change in the manufacturing process of the sheet, from heat curing with a convection oven to curing with an infrared system, seems not to have a negative effect on the efficacy of the sheet. Future manufacturing of the polymer sheets could therefore in all probability, rely on an infrared-curing process. Although good results were obtained in the preliminary trial, sufficient results to recommend the exact SO₂ content required are not available. Implementing such a system will be advantageous in that higher production rates will be possible, cutting down on manufacturing time and costs, and assist in improving the aesthetics of the final product.

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Appendix to Chapter 4

To simplify discussion of the results of the statistical analysis, a comprehensive list of tables (Tables 1 to 2) containing the statistical analysis of the quality parameters have been included as an “Appendix” here.

Table 1. Effect of SO₂ sheet and bag type on decay control of Dauphine table grapes, inoculated artificially by settlement of *Botrytis cinerea* conidia onto the berry surface, after storage for 5 w at –0.5°C, followed by 5 d at 7.5°C.

Examination parameter ⁵	Interaction ² Bag type / SO ₂ sheet	Bag type ³ (Factor A)		SO ₂ sheet ⁴ (Factor B)				Prob. > F ¹			
		Non-perf	Perf	18% SO ₂ Convection cured	18% SO ₂ Infrared cured	23% SO ₂ Convection cured	23% SO ₂ Infrared cured	Uvasys	A	B	AB
Decay		6.89	6.44	7.73b	6.81bc	13.67a	3.16cd	1.98d	ns	***	ns
Pedicel SO ₂		0.20	0.22	0.04	0.09	0.26	0.37	0.32	ns	ns	ns
Surface SO ₂		1.68	1.55	1.99	1.66	1.41	1.62	1.40	ns	ns	ns
Stem condition	18% Conv.	3.00a	3.04a						***	**	**
	18% IR	2.14b	3.04a								
	23% Conv.	2.86a	3.08a								
	23% IR	2.44b	2.96a								
	Uvasys	2.82a	3.00a								

- 1 Two-way ANOVA table for Factor A (Bag type) and Factor B (SO₂ sheet), with ns, *, ** and *** indicating non-significant and significant differences at the 5%, 1% and 0.1% levels.
- 2 Non-pooled data for significant interaction between factors A and B.
- 3 Data pooled across SO₂ sheet for non-significant interactions and non-pooled for significant interactions between factor A (Bag type = perforated and non-perforated bags) & factor B (SO₂ sheet = Polymer SO₂ sheets with 18 and 23 % SO₂ content and Uvasys SO₂ sheet, with the polymer sheets cured either by infra-red (IR) or convection heat (Conv.) process). Values in the same row, followed by different letters indicate significant differences for pooled data (P<0.05) according to LSD test, whereas for non-pooled data different letters indicate significant differences across rows and columns.
- 4 Data pooled across bag type for non-significant interactions. Values in the same row, followed by different letters indicate significant differences.
- 5 Examination parameters: Decay and SO₂ damage are expressed as a percentage of the total mass, with stem condition rated according to a 5-point scale (1 = green stems and 5 = brown and desiccated).

Table 2. Effect of SO₂ sheet and bag type on decay control of Dauphine table grapes, inoculated artificially by placement of infected *Botrytis cinerea* berries amongst the four corner bunches, after storage for 5 w at –0.5°C, followed by 5 d at 7.5°C.

Examination parameter ⁵	Interaction ² Bag type / SO ₂ sheet	Bag type ³ (Factor A)		SO ₂ sheet ⁴ (Factor B)					Prob. > F ¹		
		Non-perf	Perf	18% SO ₂ Convection cured	18% SO ₂ Infrared cured	23% SO ₂ Convection cured	23% SO ₂ Infrared cured	Uvasys	A	B	AB
		Decay ⁶ / Inoculated bunches	18% Conv. 18% IR 23% Conv. 23% IR Uvasys	10.95bc 17.74a 15.29ab 5.12d 13.17abc	13.65abc 8.97cd 12.16abc 9.70bcd 12.86abc						ns
Decay ⁶ / Un-inoculated bunches		5.21	3.75	4.69	3.87	6.40	4.93	2.50	ns	ns	ns
Pedicel SO ₂		0.08	0.05	0.10	0.05	0.00	0.11	0.07	ns	ns	ns
Surface SO ₂		1.47	2.03	1.58	1.90	1.18	1.54	2.54	ns	ns	ns
Stem condition	18% Conv. 18% IR 23% Conv. 23% IR Uvasys	2.72cd 3.00ab 3.00ab 3.04ab 2.52d	2.88bc 3.12a 2.96ab 2.94abc 2.96ab						*	**	*

1 - 5 As for Table 1.

6 Decay assessed in the inoculated bunches, as well as the un-inoculated bunches. Decay in un-inoculated bunches represents decay development from natural infections.

Chapter 5

Optimisation of thin-film, machine-manufactured monolithic sheets for in-package SO₂ gas generation to control post-harvest *Botrytis cinerea* decay on South African table grapes

Phase I: Evaluation of the storage quality of semi-commercial export consignments of South African table grapes

Technology developed in this study is confidential and is protected by patent (South African Patent No. 96-2517, International Patents Pending, Provisional No. ZA 2001/1851).

Abstract

Following the successful testing of machine-manufactured monolithic sheets in previous trials, several changes needed to be made to the appearance of the sheet in order to make it aesthetically more acceptable to industry and consumers alike. The three strips of PVC deposit were replaced with a single deposit, covering the entire surface of the sheet. Changes were also made relating to the size of the sheet and the composition of the carrier material used.

The storage quality, with special reference to decay control and SO₂ damage, of semi-commercial export consignments of table grapes using the thin film polymer SO₂ sheet, were evaluated and compared to quality using the Uvasys SO₂ sheet. The results obtained with the polymer SO₂ sheets were good, especially in terms of

Botrytis decay control and resultant SO₂ damage; the levels of both defects were similar to that obtained with the reference Uvasys SO₂ sheet.

Introduction

Following the successful testing of machine-manufactured monolithic sheets in previous trials as described in Chapter 3, consideration was given to making changes to the appearance of the sheet in order to make it aesthetically more acceptable to both industry and consumers. The appearance of the former sheet, containing three strips of the cured polymer matrix, was deemed unacceptable by some of the important role players in the South African table grape industry (personal correspondence with M.A. Taylor, Capespan Technology Development, 2000). In order to make the sheet more acceptable to industry, therefore, three strips of deposit were to be replaced with one single deposit, covering the entire surface of the sheet. This sheet was to be referred to as a thin-film one.

Furthermore, as it was evident from previous observations that localised bleaching of bunches due to uneven distribution of SO₂ gas occurred inside the box (personal correspondence with J.F. Fourie, Capespan Technology Development, 2000), changes to the dimensions of the sheet needed to be made. The dimensions of the sheet were increased from the original size of 300mm by 210mm, to 340mm by 240mm. Although this was a fairly significant increase in size, the sheet still did not cover the entire fruit surface inside the box. Unfortunately, the extent of the changes in dimensions that could be made was limited due to problems associated with the pilot plant design and time constraints. Therefore, only temporary changes were made to sections of the pilot plant until manufacturing of the trial sheets were completed.

The appearance of the carrier material, poly-coated paper, at the end of the storage period was also cause for concern. Moisture penetrated the poly coating through pin holes, created by the absorption of heat during the manufacturing process, rendering the paper, and therefore the SO₂ sheet, aesthetically unacceptable by the end of the storage period. A metalised poly(ester) and poly(ethylene) film with better moisture resistance properties subsequently replaced the poly-coated paper.

The objective of this chapter was to evaluate the storage quality, with special reference to decay control and SO₂ damage, of semi-commercial export consignments of table grapes using the modified thin-film polymer SO₂ sheet, in comparison to the Uvasys SO₂ sheet.

Materials and methods

Sheet manufacture

The SO₂ gas generating sheets were made on a pilot scale production plant built for this project. Manufacturing of the SO₂ gas generating sheets starts with the preparation of the PVC plastisol mixture. Once the desired plastisol mixture is obtained it is applied onto a carrier material by means of a nozzle. The carrier material with the plastisol deposits is sent through a series of infrared curing ovens where the plastisol is cured to a solid state. Once cured, a second layer of non-woven material is laminated onto the carrier layer to enclose the cured PVC matrix between two sheets of material. A cooling section follows where the product is rapidly cooled down before it is cut to the desired specifications. The finished product is inspected for deviations in quality before it is packaged into poly(ethylene) bags ready to be used.

To make the thin-film sheets, a new nozzle was installed that could spread the plastisol mixture over the desired surface area. The rest of the process stayed exactly the same as for the previous sheets.

Packaging and SO₂ treatments

The effect of different SO₂ concentrations incorporated into the polymer sheets were determined on Sunred Seedless, Red Globe and La Rochelle table grapes. The grapes were packed in cartons in either perforated (72 x 4mm configuration) or non-perforated bags and exported by sea to the UK, where they were examined. For individual bunches, polycote bunch bags were used for packing

of Red Globe and La Rochelle, and carrybags for Sunred Seedless. An additional MAM (moisture absorbing material) was placed below all the SO₂ sheet combinations for Red Globe when packed in non-perforated bags. This was not done for Sunred Seedless nor La Rochelle. No MAM was used on grapes packed in perforated bags, irrespective of the cultivar.

Polymer SO₂ sheets with concentrations of sodium metabisulphite (Na₂S₂O₅) ranging from 10–18%, (in increments of 2%), and of continuous thin-film format, were used. Due to mechanical constraints on the pilot plant the sheets were manufactured with dimensions slightly smaller than the dimensions of the 4.5kg carton; they did not cover the entire surface of the grapes. As a reference, a Uvasys sheet of 70% first stage, the standard sheet recommended and used by the South African table grape industry, was also included.

The grapes were not artificially inoculated at the time of packing. The duration of the storage period was 5 weeks at -0.5°C + 5 days at 10°C.

Experimental layout and statistical detail

Completely randomised two-way ANOVA, with SO₂ sheet and packaging type as Factors A & B, respectively. Each treatment consisted of 5 replicate 4.5kg cartons of grapes, with the cartons completely randomised on a pallet. The grapes were examined after storage and factorial analyses of variance performed on the data. Students-T LSD was calculated to compare treatment means at a 5% significance level.

Examination parameters

Examinations were conducted according to protocols of Capespan Technology Development. Decay, SO₂ damage and berry split were expressed by weight as a percentage (%) of the sample mass, while stem condition was expressed on a scale from 1 – 5, with 1 = green and 5 = brown and desiccated.

Results and discussion

Decay resulting from natural infections, SO₂ damage, berry split and the stem condition of Sunred Seedless (Table 1), Red Globe (Table 2) and La Rochelle table grapes (Table 3) packed with thin-film polymer SO₂ sheets of different concentrations (10–18% SO₂) were determined. The quality of the packaged grapes was assessed after storage for 5 weeks at –0.5°C, followed by 5 days at 10°C.

Statistical analyses of the quality parameters viz., *Botrytis* decay, SO₂ damage, berry split and stem condition resulted in a large volume of data. Thus, for clarity purposes and to simplify the discussion of the results, a comprehensive list of tables (Tables 1 to 3) containing the statistical analyses have been included as an “Appendix” at the end of this chapter.

A. Sunred Seedless

Decay development

There were no significant differences in decay, developing from natural infections, between any of the polymer sheets of varying SO₂ concentrations, nor did the levels differ from results obtained with the Uvasys SO₂ sheet (Table 1). The bag type had no effect on decay development.

SO₂ damage

SO₂ damage occurred at the pedicel attachment area, as well as on the surface of Sunred Seedless berries (Table 1). Significantly higher pedicel-end and surface SO₂ damage was observed with the 18% polymer sheet, compared to the 10–14% polymer sheets. There was no difference in the level of SO₂ damage at either the pedicel-end or on the surface of the berries between the Uvasys sheet treatment and any of the SO₂ concentrations ranging from 10–16%. However, although not shown

to be of statistical significance, relatively high levels of damage also occurred with the 16% polymer sheet.

Significant interactions were indicated for total SO₂ damage (combination of pedicel-end and surface damage) for Sunred packed in perforated or non-perforated bags with regard to the specific SO₂ sheet used. In perforated bags, significantly more total SO₂ damage occurred with the 18% polymer sheet compared to the concentrations of 10, 12 and 14 % SO₂, whereas for the non-perforated bags, significantly higher SO₂ levels occurred with the 18% sheet than with the sheets of 10–16% SO₂, as well as in comparison to the Uvasys SO₂ sheet. A high level of SO₂ damage also occurred on grapes packed in non-perforated bags with the 16% polymer sheet. This result differed significantly from the 10 and 12% concentrations, but not in comparison with the Uvasys sheet. For most of the polymer sheets (10–14% SO₂), no significant difference in total SO₂ damage occurred when packed in either perforated or non-perforated bags, with the exception of the 16 and 18% sheets, where SO₂ damage increased significantly when included in non-perforated bags opposed to when packed in perforated bags.

Berry split

Significantly higher levels of berry split occurred on Sunred Seedless grapes packed in non-perforated bags, compared to perforated bags, using the Uvasys SO₂ sheet (Table 1). This result was not found with any of the polymer SO₂ sheets. A possible explanation for this is that the 1st stage emission of SO₂ from the Uvasys SO₂ sheet (\pm 350ppm), which is much higher than the polymer sheet (\pm 35ppm), causes weakening of the epidermis which results in a higher berry split potential. A significantly higher level of berry split occurred on grapes packed in non-perforated bags with the Uvasys sheet, as opposed to that attained with the different polymer sheets.

Stem condition

The stem condition of Sunred Seedless was not affected by the different SO₂ treatments, but rather by the bag type used (Table 1). Stem desiccation was more severe when packed in perforated than non-perforated bags.

B. Red Globe

Decay development

No difference in decay, developing from natural infections, occurred between any of the polymer sheets of varying SO₂ concentrations, nor did the levels differ from that with the Uvasys SO₂ sheet (Table 2). The extremely low levels of decay recorded after storage for 5 w at -0.5°C + 5 d at 10°C indicated a low decay potential at the time of packing.

SO₂ damage

SO₂ damage was more severe on the surface of Red Globe grapes than on Sunred Seedless or La Rochelle, especially when packed in non-perforated bags, however, damage also occurred at the pedicel attachment area (Table 2). SO₂ damage occurring at the pedicel-end was significantly higher for the 16 and 18% polymer sheets than with the Uvasys SO₂ sheet. Although not statistically different, the levels of pedicel-end SO₂ damage were slightly higher with the 10–14% SO₂ concentrations than with the Uvasys sheet. Significantly more pedicel-end SO₂ damage occurred on Red Globe when packed in non-perforated than perforated bags.

Significantly higher levels of SO₂ damage on the surface of Red Globe, packed in non-perforated bags occurred with the 12–18% polymer sheets, compared to the Uvasys SO₂ sheet. The level of damage with the 10% polymer SO₂ sheet did not differ from that obtained with the Uvasys sheet. On grapes packed in perforated

bags, the levels of surface damage occurring with the 16 and 18% concentrations were significantly higher than with the SO₂ concentrations of 10–14%, and the Uvasys SO₂ sheet. The assessment for total SO₂ damage indicated that an SO₂ concentration of lower than $\pm 10\%$ should probably be used for Red Globe packed in non-perforated bags, as it is extremely sensitive to SO₂ damage. A slightly higher concentration of SO₂ can be tolerated when the fruit is packed in perforated bags.

Berry split

Significantly higher levels of berry split occurred on Red Globe packed in non-perforated bags with use of the Uvasys SO₂ sheet, compared to all the polymer sheet treatments (Table 2). The non-perforated bag generally related to significantly higher levels of berry split than the perforated bags. Similar to Sunred Seedless, berry split was higher in non-perforated bags when the Uvasys SO₂ sheet was used, compared to the polymer sheets. This is possibly because of higher initial levels of SO₂ emitted from the Uvasys sheets, causing damage to the epidermis, which results in a higher split potential.

Stem condition

Stems of Red Globe packed in perforated bags were significantly more desiccated than those in non-perforated bags (Table 2). The SO₂ sheet/ sheet used did not affect the stem condition, irrespective whether the fruit was packed in perforated or non-perforated bags. For some unknown reason, stems of Red Globe packed in perforated bags were more desiccated with use of the 14–18% polymer sheets, while this effect was not evident in non-perforated bags.

C. La Rochelle

Decay development

No difference in decay, developing from natural infections, occurred between any of the polymer sheets of varying SO₂ concentrations, nor did the levels differ from that with the Uvasys SO₂ sheet (Table 3). As for Red Globe, extremely low levels of decay occurred after storage for 5 w at -0.5°C + 5 d at 10°C.

SO₂ damage

SO₂ damage of La Rochelle grapes was generally more severe at the pedicel attachment area than on the surface (Table 3). Significantly higher levels of pedicel-end SO₂ damage occurred with the 18% SO₂ polymer sheet compared to the 10 and 12% polymer sheets, and the Uvasys SO₂ sheet. A significantly higher level of pedicel-end SO₂ damage was also shown with the 16% sheet, as opposed to the Uvasys sheet. Although not statistically different, the levels of pedicel-end SO₂ damage were slightly higher with SO₂ concentrations of 10–14% than with the Uvasys sheet. Pedicel-end SO₂ damage was significantly higher on La Rochelle packed in non-perforated bags than in perforated bags.

Significantly higher levels of SO₂ damage to the surface of La Rochelle packed in non-perforated bags occurred with the polymer sheet of 18% SO₂, compared to the 10 and 12% SO₂ concentrations. The results were similar to those obtained for the Uvasys sheet. The assessment of the overall SO₂ damage for La Rochelle indicated that an SO₂ concentration of ±10 to 12% could probably be used for La Rochelle when packed in non-perforated bags, without causing excessive SO₂ damage, resulting in levels of damage similar to those with the Uvasys SO₂ sheet. The data further suggested that La Rochelle could tolerate a slightly higher concentration of SO₂ when packed in perforated bags, as the level of SO₂ damage was generally significantly lower than when packed in non-perforated bags.

Berry split

There was no difference in berry split between any of the treatments (Table 3). Berry split is inherently not a problem with La Rochelle.

Stem condition

Stem condition of La Rochelle was not affected by the different SO₂ treatments, but rather by the bag type used (Table 3). Stem desiccation was significantly more severe when La Rochelle was packed in perforated rather than in non-perforated bags.

Conclusions

Upon completion of the first phase of semi-commercial export trials with the continuous thin-film polymer sheets it can be concluded that the quality of the grapes was generally acceptable after a storage period of 5 w at -0.5°C, followed by 5 d at 10°C. However, Red Globe was shown to be very sensitive to SO₂ damage when packed in non-perforated bags. The results obtained for the in-package polymer SO₂ sheets of lower concentrations (10–12%) were good, especially in terms of *Botrytis* decay control and resultant SO₂ damage; the levels of both defects were almost similar to those obtained with the reference Uvasys SO₂ sheet. It should be emphasised however, that storage was only for 5 w at -0.5°C followed by 5 d at simulated shelf life conditions, which is not the maximum storage period for the cultivars tested.

From the results it was evident that the recommended SO₂ concentration to be incorporated into the polymer sheet is in the range of 10–12% for grapes packed in non-perforated bags, and slightly higher for grapes packed in perforated bags. The specific grape bag used, whether perforated or non-perforated, does seem to have an affect on the efficacy of decay control of the SO₂ sheets. Generally, higher SO₂ concentrations are required to confine decay development in perforated bags

than in non-perforated bags (comment supported by results found in Chapter 3). Furthermore, lower SO₂ concentrations might be required for more SO₂ sensitive cultivars regardless of which bag type is used.

The exact SO₂ concentration to be used for commercial use will largely be determined by the cultivar, as well as the packaging type, as sensitivity to SO₂ damage differs between cultivars. Further tests are required with a range of lower SO₂ concentrations, and with sheets covering a larger surface area within the 4.5kg grape carton.

Appendix to Chapter 5, Phase I

To simplify discussion of the results of the statistical analysis, a comprehensive list of tables (Tables 1 to 3) containing the statistical analysis of the quality parameters have been included as an “Appendix” here.

Table 1 Effect of SO₂ sheet treatment and bag type on the general quality of Sunred Seedless table grapes exported to the UK and examined after storage for 5 weeks at -0.5°C, followed by 5 days at 10°C.

Examination parameter ⁵	Interaction ² Bag / SO ₂ treatment	Bag type ³ (Factor A)		SO ₂ treatment ⁴ (Factor B)						Prob. > F ¹		
		Perf	Non-perf	Uvasys	Pol 10	Pol 12	Pol 14	Pol 16	Pol 18	A	B	AB
Decay (%)		0.5	0.4	0.5	0.5	0.4	0.6	0.2	0.5	ns	ns	ns
Pedice-end SO ₂ (%)		3.1	7.1	3.7b	1.5b	1.9b	3.4b	6.9ab	13.3a	ns	**	ns
Surface SO ₂ (%)		4.2	8.6	6.7ab	3.8b	3.5b	5.7b	7.1ab	11.8a	ns	**	ns
Berry split (%)	Uvasys	0.2b	4.5a							***	**	***
	Pol 10	0.3b	1.2b									
	Pol 12	0.3b	0.9b									
	Pol 14	0.4b	0.8b									
	Pol 16	0.6b	1.0b									
	Pol 18	0.3b	0.4b									
Total SO ₂ damage (%)	Uvasys	8.1cd	12.6bcd							***	***	*
	Pol 10	4.5d	6.2cd									
	Pol 12	4.3d	6.5cd									
	Pol 14	5.2d	13.0bcd									
	Pol 16	6.9cd	21.0b									
	Pol 18	14.8bc	35.3a									
Stem condition		3.2a	2.5b	2.9	2.8	2.8	2.9	2.8	2.8	***	ns	ns

1 Two-way ANOVA table with Factor A (Bag type) and Factor B (SO₂ sheet). ns, *, ** and *** indicate non-significant and significant differences at the 5%, 1% and 0.1% levels.

2 If itemised, interaction occurs between factor A and factor B. Different letters indicate significant differences between treatment means across rows as well as within columns.

3 & 4 Data pooled across SO₂ treatments for Factor A, and across bag types for Factor B for non-significant interactions. Values in the same row, followed by different letters, indicate significant differences (P<0.05) according to LSD test. Bag type = perforated vs non-perforated and SO₂ sheets = Polymer SO₂ sheets of 10, 12, 14, 16 and 18% SO₂ content vs Uvasys SO₂ sheets.

5 Examination parameters: Decay, SO₂ damage and berry split were expressed as a percentage of the total mass, while stem condition was rated according to a 5-point scale (1 = green stems and 5 = brown and desiccated). Pedicel and surface SO₂ = % SO₂ bleaching to the pedicel-end and surface areas, respectively. Berry split = total % berry split with and without SO₂ damage. Total SO₂ = total % berries showing SO₂ damage.

Table 2 Effect of SO₂ sheet treatment and bag type on the general quality of Red Globe table grapes, exported to the UK, and examined after storage for 5 weeks at -0.5°C, followed by 5 days at 10°C.

Examination parameter ⁵	Interaction ² Bag / SO ₂ treatment	Bag type ³ (Factor A)		SO ₂ treatment ⁴ (Factor B)						Prob. > F ¹		
		Perf	Non-perf	Uvasys	Pol 10	Pol 12	Pol 14	Pol 16	Pol 18	A	B	AB
Decay (%)		0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.1	ns	ns	ns
Pedicle-end damage (%)	SO ₂	4.9b	13.6a	2.0b	7.7ab	7.6ab	9.3ab	13.9a	14.9a	***	**	ns
Surface SO ₂ (%)	Uvasys	4.1e	16.6d							***	***	***
	Pol 10	4.6e	17.1d									
	Pol 12	2.9e	29.1bc									
	Pol 14	4.0e	38.1a									
	Pol 16	20.3d	31.0ab									
	Pol 18	21.5cd	35.0ab									
Berry split (%)	Uvasys	0.3e	7.1a							***	**	**
	Pol 10	0.4e	0.9d									
	Pol 12	0.1e	2.3c									
	Pol 14	0.0e	1.0d									
	Pol 16	0.1e	3.0b									
	Pol 18	0.3e	0.4e									
Total SO ₂ damage (%)	Uvasys	5.9f	18.8de							***	***	**
	Pol 10	6.7f	30.4c									
	Pol 12	5.0f	42.1b									
	Pol 14	10.6ef	50.2ab									
	Pol 16	27.0cd	52.0ab									
	Pol 18	31.4c	54.8a									
Stem condition	Uvasys	3.4a	2.9c							***	**	*
	Pol 10	3.4a	2.8c									
	Pol 12	3.4a	2.8c									
	Pol 14	3.2b	2.8c									
	Pol 16	3.2b	2.8c									
	Pol 18	3.2b	2.8c									

1 - 5

Refer to Table 1 for explanations

Table 3 Effect of SO₂ sheet treatment and bag type on the general quality of La Rochelle table grapes exported to the UK and examined after storage for 5 weeks at -0.5°C, followed by 5 days at 10°C.

Examination parameter ⁵	Bag type ³ (Factor A)		SO ₂ treatment ⁴ (Factor B)						Prob. > F ¹		
	Perf	Non-perf	Uvasys	Pol 10	Pol 12	Pol 14	Pol 16	Pol 18	A	B	AB
Decay (%)	0.1	0.1	0.2	0.1	0.2	0.1	0.1	0.1	ns	ns	ns
Pedicle-end SO ₂ damage (%)	6.1b	14.9a	5.8c	8.1bc	10.2bc	10.9abc	11.6ab	16.4a	***	***	ns
Surface SO ₂ (%)	0.3b	2.7a	1.3ab	0.8b	0.7b	1.9ab	1.8ab	2.8a	***	*	ns
Berry split (%)	0.2	0.5	0.3	0.4	0.5	0.4	0.4	0.3	ns	ns	ns
Total SO ₂ damage (%)	6.5b	17.6a	7.1b	8.9b	10.9b	12.8ab	13.4ab	19.2a	***	***	ns
Stem condition	3.3a	2.7b	3.1	3.0	3.0	3.0	3.0	3.0	***	ns	ns

1, 3-5 Refer to Table 1 for explanations

Chapter 5

Optimisation of thin-film, machine-manufactured monolithic sheets for in-package SO₂ gas generation to control post-harvest *Botrytis cinerea* decay on South African table grapes

Phase II: Establishment of the required SO₂ concentration for Red Globe and Barlinka table grapes

Technology developed in this study is confidential and is protected by patent (South African Patent No. 96-2517, International Patents Pending, Provisional No. ZA 2001/1851).

Introduction

Although good results were obtained upon completion of the first phase, semi-commercial export trials with the continuous thin-film polymer sheets, several questions still needed to be answered regarding the SO₂ concentrations and the aesthetics of the new sheets.

From results of the previous study (Chapter 5, Phase I) it was evident that there was still a degree of uncertainty regarding the correct amount of sodium metabisulphite (Na₂S₂O₅) to be incorporated into the sheets to deliver the optimum concentration of SO₂ gas. Results had indicated that with the new, continuous thin-film sheet lower concentrations of Na₂S₂O₅ than initially expected might be required, to deliver the optimum SO₂ gas concentration needed for maximum decay control and minimum SO₂ damage. This can probably be attributed to improved SO₂ release characteristics of the thin-film sheet, due to the larger surface area of the polymer deposit. Therefore, trials with a series of sheets containing lower

concentrations of $\text{Na}_2\text{S}_2\text{O}_5$ were proposed, to establish the required $\text{Na}_2\text{S}_2\text{O}_5$ to be incorporated into the new polymer sheets.

Aesthetically, the new, continuous thin-film sheet, with the metalised poly film was generally accepted by industry providing that the necessary printing could be done on the sheet in future. At this stage the size of the sheet was increased further, to 345mm by 260mm, in order to cover the entire top surface of the fruit inside the carton, minimising the possibility of localised SO_2 damage to bunches as a result of the uneven distribution of SO_2 gas inside the carton.

The objectives of this section of chapter 5 were to determine the efficacy of a continuous, thin-film polymer sheet, and of lower SO_2 concentrations (7 to 15% SO_2), for controlling: (a) the spread of *Botrytis cinerea* decay from infected to adjacent sound berries, resembling a high decay pressure, (b) decay from natural infections (without artificial inoculation), resembling conditions of low decay potential, and to determine the effect of using a MAM (moisture absorbing material) as an additional barrier below the SO_2 sheet, in an attempt to reduce SO_2 damage.

Materials and methods

Sheet manufacture

The SO_2 gas-generating sheets were manufactured on a pilot scale production plant purposely built for this project. Manufacturing of the SO_2 gas generating sheets starts with the preparation of the PVC plastisol mixture. Once the desired plastisol mixture is obtained it is applied onto a carrier material by means of a nozzle. The carrier material with the plastisol deposits is sent through a series of infrared curing ovens where the plastisol is cured to a solid state. Once cured, a second layer of non-woven material is laminated onto the carrier layer to enclose the cured PVC matrix between two sheets of material. A cooling section follows where the product is rapidly cooled down before it is cut to the desired specifications. The finished product is inspected for deviations in quality before it is packaged into poly(ethylene) bags ready to be used.

Packaging and SO₂ treatments

The effect of different SO₂ concentrations incorporated into the polymer sheets were determined on Red Globe and Barlinka table grapes. The grapes were packed in cartons in either perforated bags (72 x 4mm configuration) with no MAM for all SO₂ sheets or in non-perforated bags, with or without MAM below the different SO₂ sheet combinations. Individual bunches, of both cultivars, were placed in polycote bunch bags. The grapes were kept in cold storage for 7 weeks at -0.5°C followed by 4 days at 7.5°C.

Polymer SO₂ sheets, with concentrations ranging from 7 to 15 Na₂S₂O₅, (in increments of 2%), of the continuous thin-film format were used. As a reference, a Uvasys sheet of 70% first stage, the standard sheet recommended and used by the South African table grape industry, was also included. A treatment containing no SO₂ sheet was also included as a control.

Inoculation Method

To ensure the presence of a sufficient dosage of *Botrytis cinerea* in the cartons some of the grapes were inoculated. Both inoculated (decay development by spread to adjacent berries) and non-inoculated (decay development from natural infections) grapes were included in this trial.

Inoculation of the grapes was done by placement of infected berries within three bunches. For the placement of infected berries, grapes were inoculated with conidia of *B. cinerea* cultures seven days prior to the day of inoculation. *B. cinerea* cultures were grown under diurnal conditions for ten days on a potato dextrose agar (PDA) medium. A spore suspension was made of 1×10^5 spores per mL, by dilution with sterilised water. Sound berries were randomly selected and removed from a number of bunches and the berry surface sterilised with a 70% ethanol and water solution. The berries were allowed to dry naturally for approximately 30 minutes prior to inoculation. A wound, 1mm deep by 1mm wide, was inflicted on the berry surface and a droplet (25 µl) of the spore suspension placed onto the wound site.

Inoculated berries were placed in an incubator/moist chamber at 22°C with a relative humidity of approximately 95% for one week prior to use. The seven day-old inoculated berries were placed amongst the bunches in each of the four corners to obtain a natural spread of decay to adjacent berries (Fourie, 1994).

Experimental layout and statistical detail

Completely randomised two-way ANOVA, with SO₂ sheet and packaging type as Factors A & B, respectively. Each treatment consisted of 5 replicate 4.5kg cartons of grapes, with the cartons completely randomised on a pallet. The grapes were examined after storage and factorial analyses of variance performed on the data. Students-T LSD was calculated to compare treatment means at a 5% significance level.

Examination parameters

Examinations were conducted according to protocols of Capespan Technology Development. Decay, SO₂ damage and berry split were expressed by weight as a percentage (%) of the sample mass, while stem condition was expressed on a scale from 1 – 5, with 1 = green and 5 = brown and desiccated.

Results and discussion

Decay resulting from natural infections, as well as from artificial inoculation, SO₂ damage and berry split on Red Globe (Table 1) and Barlinka table grapes (Table 2) were assessed after storage for 7 weeks at –0.5°C, followed by 4 days at 7.5°C. In general, the results obtained for the continuous thin-film polymer SO₂ sheets of lower SO₂ concentrations were very positive, in terms of identifying the SO₂ content required to achieve control of *Botrytis* decay to levels similar to those attained with the Uvasys SO₂ sheet.

Statistical analyses of the quality parameters viz., *Botrytis* decay, SO₂ damage, berry split and stem condition, resulted in a large volume of data. Thus, for clarity purposes and to simplify the discussion of the results, a comprehensive list of tables (Tables 1 to 3), containing the statistical analyses, have been included as an “Appendix” at the end of this chapter.

A. Red Globe

Decay development

Decay values for artificially inoculated grapes were significantly higher for both the non-perforated (NP) bag treatments not treated with SO₂, and hence, were not included for statistical evaluation.

The general observations was that the levels of decay in both inoculated and un-inoculated grapes decreased with increasing concentration of SO₂ content in the polymer sheets after storage for 7 weeks at -0.5°C + 4 days at 7.5°C (Table 1). Although not consistent, the inclusion of a MAM in the NP bag seemed to decrease decay control efficacy. Overall, the grapes packed in the NP bags developed less decay than those packed in the perforated (P) bags.

Spread of decay (artificial inoculation)

In the P bags, significantly lower levels of decay occurred on grapes packed with the Uvasys SO₂ sheet and a polymer sheet containing 11% SO₂, compared to the 7 or 9% SO₂ sheets (Table 1). Both the 7 and 9% concentrations failed to control decay, compared to the untreated control (not statistically evaluated).

In NP bags, all treatments reduced decay substantially compared to the untreated control, irrespective whether a MAM was included or not (not statistically evaluated). When no MAM was included in the NP bags, control of decay similar to the Uvasys SO₂ sheet was achieved with most of the polymer sheets, except for the 7% and 13% concentrations which showed significantly higher decay levels than the

Uvasys sheet. Decay control comparative to the Uvasys sheet was achieved on grapes packed with the inclusion of a MAM in the NP bag with polymer sheets of 9 to 15% SO₂, while all polymer sheets of 11 to 15% SO₂ reduced decay significantly, in comparison to the 7 and 9% SO₂ concentrations.

When assessing the effect of the different packaging combinations, decay levels generally did not differ significantly for the individual SO₂ treatments. However, decay was significantly lower with the 9% polymer sheet for the NP bag without a MAM than the NP bag with a MAM or P bag packaging combinations. This finding, that 9% SO₂ in the polymer sheet with the NP bag without a MAM combination was effective in reducing the spread of decay from an inoculated berry, indicated that the cut-off point for the SO₂ concentration to be incorporated in the polymer sheet can differ with regard to a specific packaging combination.

In general, it was indicated that SO₂ concentrations in the polymer sheet between 11 to 15% are required to achieve decay control equal to the Uvasys SO₂ sheet. For some as yet unknown reason, slightly higher decay levels occurred with the polymer 13% sheet.

Decay from natural infections

In P bags, significantly lower levels of decay, compared to the untreated control, occurred only with the 15% polymer sheet (Table 1). Neither the Uvasys, nor any of the other polymer sheets, were able to effectively inhibit decay development in P bags. However, decay on Red Globe was significantly reduced by all SO₂ sheet treatments compared to the untreated control when packed in NP bags, irrespective of whether a MAM was used. There was no significant difference in efficacy with regard to the different SO₂ concentrations incorporated in the polymer sheets.

Results indicated that if the inoculum potential is low, a polymer sheet of any concentration could be used for grapes packed in NP bags, and that efficacy would be slightly impaired when using a MAM below the sheet (not substantiated statistically). However, for grapes packed in P bags, a relatively high concentration of SO₂ is required to confine decay development.

SO₂ damage

Typical of the cultivar Red Globe, the surface of the berries was generally more prone to SO₂ damage than the pedicel attachment area. The level of damage incurred was, however, generally lower than normally experienced with this cultivar. Pedicel-end and surface SO₂ damage were significantly lower on grapes packed in P bags, as opposed to the NP bag without a MAM combination. Surface SO₂ damage on Red Globe was slightly lower for the NP bag with a MAM than the NP bag without a MAM combination, but was not of significance. Both pedicel-end and surface SO₂ damage were significantly higher with the 15% polymer sheet, compared to all other treatments. Total SO₂ damage of a similar level to the Uvasys SO₂ sheet occurred with all polymer sheets from 7 to 13% SO₂. However, while not significant, the levels incurred with the 7 and 9% SO₂ concentrations were lower than with the other polymer sheets. SO₂ damage was higher with the polymer 15% SO₂ sheet than any of the other treatments. Significantly more SO₂ damage occurred on Red Globe packed in NP bags, irrespective whether an MAM was included, than when packed in P bags.

Berry split

Significantly higher levels of berry split occurred on Red Globe grapes packed in NP bags, irrespective of the use of a MAM, than when packed in P bags. Significantly higher levels of berry split also occurred with the 13 and 15% SO₂ concentrations than with the 9 and 11%. Although not clearly indicated, it seems that berry split could be accentuated by use of higher SO₂ concentrations. No difference in berry split occurred between the polymer sheets of the higher concentrations and the Uvasys SO₂ sheet.

B. Barlinka

Decay development

High levels of decay occurred on Barlinka grapes not treated with SO₂, irrespective of the packaging combination (Table 2). Decay values for artificially inoculated grapes were significantly higher for both the NP bag treatments not treated with SO₂, and hence, were not included for statistical evaluation.

Spread of decay (artificial inoculation)

Barlinka grapes packed in P bags with an Uvasys SO₂ sheet showed significantly lower levels of decay than when packed in the polymer sheets containing 7, 9 or 13% SO₂ (Table 2). All treatments, except the 7% polymer sheet, reduced decay substantially, compared to the untreated control (not statistically evaluated). The 11 and 15% polymer sheets, but not the 13%, were as effective in confining the spread of decay as the Uvasys SO₂ sheet was. Slightly higher levels of decay occurred with both these polymer sheets, but the differences were not significant. This finding corresponds to that for Red Globe, where the 13% polymer sheet, for no obvious reason, showed higher levels of decay.

In NP bags, with or without using a MAM, all treatments reduced decay substantially compared to the untreated control (not statistically evaluated). Effective control of decay in comparison to the Uvasys SO₂ sheet was achieved with all the polymer sheets (11 to 15%), except the 7% and 9% treatments.

When assessing the effect of the different packaging combinations for each of the SO₂ treatments, significantly lower levels of decay occurred on grapes packed in the NP bag without a MAM combination for all the SO₂ treatments, compared to the P bag. The 9% polymer sheet used in the NP bag combination reduced decay, compared to when the MAM was included. In the NP bags, inclusion of a MAM consistently reduced decay control efficacy. This was, however not always significant for the sheets of lower SO₂ content.

In contrast to Red Globe, the cut-off point for the SO₂ concentration required to effectively confine the spread of decay on Barlinka grapes packed in NP bags, under conditions of high inoculum pressure, was shown to be 11% and not 9% SO₂. This findings confirmed previous recommendations (see Chapter 3) that slightly higher SO₂ concentrations are required in the polymer sheet to effectively control decay of grapes packed in P bags. Although only speculative, the question remains whether perhaps the SO₂ levels reached with the polymer sheets in the P bag during the initial stages (shortly after closure of the bag), is not too low. Hence, the slightly lower levels of decay with the Uvasys SO₂ sheet, which is known to have a high emission of SO₂ during the early stages (see Chapter 8 for release rates).

Decay from natural infections

Decay from natural infections was significantly reduced by all SO₂ sheet treatments compared to the untreated control, with no differences in efficacy with regard to the SO₂ concentrations incorporated in the polymer sheets. Effective decay control, comparative to the Uvasys sheet, was achieved with all of the polymer sheets. No differences in decay could be related to a specific packaging combination for any of the individual SO₂ treatments.

SO₂ damage

The surface of Barlinka berries was generally more prone to SO₂ damage than the pedicel attachment area. Pedicel-end and surface SO₂ damage were both significantly higher for grapes packed in non P bags and treated with a polymer sheet containing 15% SO₂, compared to all other treatments. Total SO₂ damage of a level similar to, or lower than, the Uvasys SO₂ sheet occurred with all polymer sheets, except the 15% concentration, on grapes packed in P or NP bags, irrespective whether a MAM was included or not.

Berry split

Berry split was not perceived as being a problem on Barlinka.

C. Pooled data for Red Globe and Barlinka

To acquire some indicators of the general efficacy of the polymer sheets for a range of cultivars, statistical evaluation was performed on pooled data of the cultivars Red Globe and Barlinka. Only general findings are discussed.

As previously mentioned for the individual cultivars, decay values for artificially inoculated grapes were significantly higher for both the NP bag treatments not treated with SO₂, and hence, were not included for statistical evaluation.

Significantly lower levels of decay, by spread from an infected berry, occurred on grapes packed either in P or NP bags (with or without MAM), with inclusion of an Uvasys SO₂ sheet or polymer sheets ranging between 11 to 15%, compared to the 7 and 9% concentrations (Table 3). Where the MAM was excluded, the 9% polymer sheet was as effective in controlling decay as was the Uvasys SO₂ sheet. On grapes packed in P bags, all polymer sheets of 11 to 15% controlled decay to an extent similar to that of the Uvasys sheet, with only the 15% polymer sheet reducing decay significantly in comparison to the 7% SO₂ sheet.

No differences in decay originating from natural infections occurred between any of the SO₂ treatments. This result implies that for conditions of low decay pressure, even the polymer sheet of 7 to 9%, which has a relatively low SO₂ concentration, could possibly be sufficient for decay control.

The use of a MAM as an additional barrier below the SO₂ sheet had no negative effect on decay control for polymer sheets of 11 to 15% SO₂. Generally, however, the use of a MAM lowered decay control efficacy. Total SO₂ damage in NP bags was significantly reduced by inclusion of a MAM with the 15% polymer sheet.

Generally, total SO₂ damage increased when a SO₂ concentration of more than 13% was incorporated into the polymer sheets, when using a NP bag for packaging.

Conclusions

The second phase trials, with the new, continuous thin-film, polymer sheets of larger deposit, carried out to determine the most suitable SO₂ concentrations to be incorporated for commercial use have been completed and the following conclusions can be made from the results obtained.

The machine-manufactured, thin-film deposit of an SO₂ polymer matrix is potentially a viable option for controlling post-harvest *Botrytis* decay; it compares favourably to the currently used Uvasys SO₂ sheet. The most suitable SO₂ concentration to be incorporated into the polymer sheet is in the range of 11 to 13% for NP bag packaging. SO₂ damage generally increased when levels higher than 13% SO₂ were incorporated in the polymer sheet, especially when used in NP bag packaging.

The type of grape bag, whether of P or NP nature, can affect the efficacy of decay control. A higher SO₂ concentration is required to confine decay in P bags. For conditions of low inoculum pressure, a polymer SO₂ sheet of relatively low SO₂ concentration could possibly be sufficient for decay control, although not advisable. The use of an additional MAM as barrier below the polymer sheets had no negative effect on decay, nor did it have any real benefit in reducing SO₂ damage.

References

Fourie, J.F. (1994). Control of *Botrytis cinerea* with new and existing SO₂ sheet formulations. *Annual report*, Pp. 330-343, Unifruco Research Services.

Appendix to Chapter 5, Phase II

To simplify discussion of the results of the statistical analysis, a comprehensive list of tables (Tables 1 to 3) containing the statistical analysis of the quality parameters have been included as an “Appendix” here.

Table 1 Efficacy of new, continuous thin-film polymer SO₂ sheets of different SO₂ concentrations, compared to the Uvasys SO₂ sheet, in confining post-harvest *Botrytis* decay, and the effect of using an additional barrier between the grapes and the sheet, on the general quality of Red Globe table grapes after storage for 7 w at -0.5°C, followed by 4 d at 7.5°C.

Examination parameter ⁵	Interaction ² Barrier/ SO ₂ treatment	Bag type / MAM combination/ ³ (Factor B)			SO ₂ treatment ⁴ (Factor A)							Prob. > F ¹		
		Perf (-MAM)	NP (-MAM)	NP (+MAM)	Uvasys	P 7	P 9	P 11	P 13	P 15	No SO ₂	A	B	AB
Decay Berry inoc.	Uvasys	5.3def	2.8f	6.5def								***	ns	**
	Pol 7	11.6abc	12.9ab	15.9a										
	Pol 9	12.3ab	4.4ef	9.6bcd										
	Pol 11	6.5def	3.2f	3.7ef										
	Pol 13	8.4bcde	9.5bcd	2.7f										
	Pol 15	6.8cdef	3.8ef	4.2ef										
	No SO ₂	12.1	25.7	25.3										
Decay Un- inoc.	Uvasys	5.1bc	2.1cd	2.3cd								***	ns	**
	Pol 7	4.4bcd	2.7cd	4.6bcd										
	Pol 9	4.7bcd	3.8bcd	2.5cd										
	Pol 11	4.2bcd	2.4cd	4.4bcd										
	Pol 13	3.5bcd	1.4cd	0.8d										
	Pol 15	2.1cd	1.4cd	3.1bcd										
	No SO ₂	7.1b	16.3a	17.6a										
Pedicle SO ₂		0.2b	0.6a	0.5ab	0.5b	0.2b	0.4b	0.4b	0.3b	1.1a	0.0b	*	*	ns
Surface SO ₂		0.7b	3.2a	2.3a	1.8b	1.3bc	1.5bc	1.7bc	2.9b	5.1a	0.0c	***	***	ns
Berry split		0.5b	2.6a	2.2a	1.7ab	2.3a	1.3bc	1.2bc	2.7a	2.9a	0.3c	**	***	ns
						b								
Total SO ₂ damage		0.8b	3.8a	2.8a	2.3b	1.5bc	1.9bc	2.1bc	3.3b	6.1a	0.0c	***	***	ns

1 Two-way ANOVA table with Factor A (SO₂ sheet) and Factor B (Bag type/ MAM combination). ns, *, ** and *** indicate non-significant and significant differences at the 5%, 1% and 0.1% levels.

2 If itemised, interaction occurred between factor A and factor B. Different letters indicate significant differences between treatment means across rows as well as within columns.

3 & 4 Data pooled across barrier types for Factor A, and across SO₂ treatments for Factor B, for non-significant interactions. Values in the same row, followed by different letters indicate significant differences (P<0.05) according to LSD test. Barrier type/ bag type = P(-MAM) for P bag with no MAM, NP(-MAM) and NP(+MAM) for NP bag without or with an additional MAM, respectively). SO₂ sheets = Polymer SO₂ sheets of 7, 9, 11, 13 and 15% SO₂ content vs Uvasys SO₂ sheet.

5 Examination parameters: Decay, SO₂ damage and berry split were expressed as a percentage of the total mass, while stem condition was rated according to a 5-point scale (1 = green stems and 5 = brown and desiccated). Pedicle and surface SO₂ = % SO₂ bleaching to the pedicle end and surface areas respectively. Berry split = total % berry split with and without SO₂ damage. Total SO₂ = total % berries showing SO₂ damage. Bunches of grapes were either inoculated artificially by placement of an infected berry amongst three bunches within a carton, or left un-inoculated.

Table 2 Efficacy of the new, continuous thin-film polymer SO₂ sheets of different SO₂ concentrations, compared to the Uvasys SO₂ sheet, in confining post-harvest *Botrytis* decay, and the effect of using an additional barrier between the grapes and the sheet, on the general quality of Barlinka table grapes after storage for 7 w at -0.5°C, followed by 4 d at 7.5°C.

Examination parameter ⁵	Interaction ² Barrier/ SO ₂ treatment	Bag type / MAM combination/ ³ (Factor B)			SO ₂ treatment ⁴ (Factor A)							Prob. > F ¹		
		Perf (-MAM)	NP (-MAM)	NP (+MAM)	Uvasys	P 7	P 9	P 11	P 13	P 15	No SO ₂	A	B	AB
Decay Berry inoc.	Uvasys	16.2efg	3.1i	12.8fghi								***	***	*
	Pol 7	33.8abc	20.0def	29.4bcd										
	Pol 9	27.9cd	14.3fgh	26.7cd										
	Pol 11	25.9cde	5.9hi	9.7ghi										
	Pol 13	27.9cd	7.8ghi	11.2fghi										
	Pol 15	21.1def	6.9ghi	9.2ghi										
	No SO ₂	38.6	43.6	43.6										
Decay Un-inoc.		2.2	2.4	2.7	0.1b	0.9b	0.7b	0.2b	0.3b	0.2b	14.6a	***	ns	ns
Pedicel SO ₂	Uvasys	0.0c	0.4c	0.4c								***	**	***
	Pol 7	0.3c	0.1c	0.2c										
	Pol 9	0.1c	1.2c	0.0c										
	Pol 11	0.1c	0.9c	1.3c										
	Pol 13	1.5bc	2.0bc	2.3bc										
	Pol 15	1.3bc	12.9a	4.1b										
	No SO ₂	0.0c	0.0c	0.0c										
Surface SO ₂	Uvasys	2.3efgh	5.7bc	3.2defg								***	***	***
	Pol 7	0.3h	1.1gh	4.1bcdef										
	Pol 9	0.8gh	3.9bcdef	3.9bcdef										
	Pol 11	1.9fgh	3.8cdef	3.1defg										
	Pol 13	3.1defg	4.5bcde	5.1bcd										
	Pol 15	2.4efgh	12.0a	6.3b										
	No SO ₂	0.0h	0.0h	0.0h										
Berry split		0.2b	0.3b	0.5a	0.6	0.2	0.2	0.4	0.6	0.2	0.1	ns	*	ns
Total SO ₂ damage	Uvasys	2.3dfefg	6.0cd	3.6cdefg								***	***	***
	Pol 7	0.6g	1.1efg	4.3cdefg										
	Pol 9	0.9fg	5.1cde	3.9cdefg										
	Pol 11	2.0efg	4.7cdef	4.3cdefg										
	Pol 13	4.6cdefg	6.4bc	7.3bc										
	Pol 15	3.7cdefg	24.9a	10.4b										
	No SO ₂	0.0	0.0	0.0										

1 - 5 Refer to Table 1 for explanation

Table 3

Pooled data for the cultivars Barlinka and Red Globe to illustrate the efficacy of polymer SO₂ sheets of different SO₂ concentrations in confining post-harvest *Botrytis* decay, and the effect of the barrier type used between the grapes and the sheet, on the general quality of the grapes after storage for 7 w at -0.5°C, followed by 4 d at 7.5°C.

Examination parameter ⁵	Interaction ² Barrier/ SO ₂ treatment	Bag type / MAM combination/ ³ (Factor B)			SO ₂ treatment ⁴ (Factor A)							Prob. > F ¹		
		Perf (-MAM)	NP (-MAM)	NP (+MAM)	Uvasys	P 7	P 9	P 11	P 13	P 15	No SO ₂	A	B	AB
Decay Berry inoc.	Uvasys	10.7cdefg	2.9h	9.7defgh								***	**	*
	Pol 7	22.7a	16.4abcd	22.7a										
	Pol 9	20.1ab	9.3defgh	18.2abc										
	Pol 11	16.2abcde	4.6gh	6.7fgh										
	Pol 13	18.2abc	8.7efgh	6.9fgh										
	Pol 15	14.0bcdef	5.4gh	6.7fgh										
	No SO ₂	25.3	34.6	34.5										
Decay Un-inoc.		7.5	7.7	9.0	1.6a	2.2a	2.2a	19.a	1.1a	1.1a	14.1b	*	ns	ns
Berry split		0.3b	1.4a	1.4a	1.1a	1.3a	0.8ab	0.8ab	1.6a	1.5a	0.2b	*	***	ns
Total SO ₂ damage	Uvasys	1.2efg	5.0cd	3.2cdefg								***	***	***
	Pol 7	1.1efg	1.5efg	2.6defg										
	Pol 9	0.5fg	3.7cdef	3.5cdef										
	Pol 11	1.2efg	4.4cde	3.0cdefg										
	Pol 13	2.5defg	6.0bc	5.6bcd										
	Pol 15	3.4cdef	16.7a	8.7b										
No SO ₂	0.0g	0.0g	0.0g											

1 - 5 Refer to Table 1 for explanation

Chapter 5

Optimisation of thin-film, machine-manufactured monolithic sheets for in-package SO₂ gas generation to control post-harvest *Botrytis cinerea* decay on South African table grapes

Phase III: Evaluation of the storage quality of Barlinka table grapes packed with a 13% SO₂ polymer sheet

Technology developed in this study is confidential and is protected by patent (South African Patent No. 96-2517, International Patents Pending, Provisional No. ZA 2001/1851).

Introduction

Results obtained from the second phase trials revealed that a sodium metabisulphite (Na₂S₂O₅) concentration of approximately 13%, incorporated into the new, continuous thin-film sheets, delivered levels of decay control and SO₂ damage similar to the results achieved by using the Uvasys sheet. There remained, however, a degree of uncertainty as to whether or not it would be advantageous to use a MAM (moisture absorbing material) in combination with the new polymer sheets, especially when high *Botrytis* inoculum levels were present.

Aesthetically, the new, continuous thin-film sheet, with the metalised poly film, was generally accepted by industry providing that the necessary printing could be printed onto the sheet in future. The problems associated with localised SO₂ bleaching on bunches detected with the smaller sheets were eliminated by the

increased size of the sheets; no localised bleaching was detected on the fruit evaluated during Phase II.

Phase III trials were therefore aimed at testing the new SO₂ sheets containing 13% Na₂S₂O₅ on less sensitive cultivars like Barlinka, and comparing the results to those obtained with the Uvasys sheet. Positive results would mean a fast track to conducting larger semi-commercial trials for sensitive cultivars and possibly even for commercial consignments for less sensitive cultivars early in the coming 2001/2002 season.

The objective of this section of chapter 5 was to evaluate the storage quality of Barlinka table grapes of six producers in the Hex River production area packed with a polymer SO₂ sheet of 13% SO₂, in comparison to the Uvasys SO₂ sheet.

Materials and methods

Sheet manufacture

The SO₂ gas generating sheets were manufactured on a pilot scale production plant built for this project. Manufacturing of the SO₂ gas generating sheets starts with the preparation of the PVC plastisol mixture. Once the desired plastisol mixture is obtained it is applied onto a carrier material by means of a nozzle. The carrier material with the plastisol deposits is sent through a series of infrared curing ovens where the plastisol is cured to a solid state. Once cured, a second layer of non-woven material is laminated onto the carrier layer to enclose the cured PVC matrix between two sheets of material. A cooling section follows where the product is rapidly cooled down before it is cut to the desired specifications. The finished product is inspected for deviations in quality before it is packaged into poly (ethylene) bags ready to be used.

Packaging and SO₂ treatments

The effect of a 13% SO₂ concentration incorporated into the polymer sheet on Barlinka table grapes was determined. The grapes were packed as for export in packhouses at six producers in the Hex River production area. The grapes were packed in 4.5kg cartons, in non-perforated bags, with or without a MAM below the SO₂ sheet. Individual bunches were placed in polycote bunch bags.

A polymer SO₂ sheet with a concentration of 13% Na₂S₂O₅, of the continuous thin-film format was used. As a reference, a Uvasys sheet of 70% first stage, the standard sheet recommended and used by the South African table grape industry, was also included. A treatment containing no SO₂ sheet was also included as control.

The grapes were not inoculated after packing. Assessment of the storage quality, by determining the incidence of decay and SO₂ damage, was conducted after 8 weeks at -0.5°C, followed by an additional 4 days at 7.5°C.

Experimental layout and statistical detail

Completely randomised one-way ANOVA. Each treatment consisted of 5 replicate 4.5kg cartons of grapes, with the cartons completely randomised on a pallet. The grapes were examined after storage and factorial analyses of variance performed on the data. Students-T LSD was calculated to compare treatment means at a 5% significance level.

Examination parameters

Examinations were conducted according to protocols of Capespan Technology Development. Decay, SO₂ damage and berry split were expressed by weight as a percentage (%) of the sample mass, while stem condition was expressed on a scale from 1 – 5, with 1 = green and 5 = brown and desiccated.

Results and discussion

For clarity purposes and to simplify the discussion of the results, comprehensive results for the individual producers (Table 1), and the table of statistical analysis for data pooled across the six producers (Table 2) have been included as an “Appendix” at the end of this chapter.

Decay development

The decay potential of grapes packed without an SO₂ sheet was exceptionally high for most producers, as indicated by the high decay incidence levels (from initial observations, as well as further examinations) (Table 1). Decay control with all the SO₂ sheets tested exceeded expectations; natural *Botrytis* decay was reduced by ± 80%. From a marketing point of view, however, this total decay level (obtained by combining the defect value for the top and bottom sectors of the bunches), was regarded as being a little too high. Very little difference in total decay incidence, expressed as an average for the six producers, occurred with use of the 13% polymer sheet applied without MAM (11% decay), in comparison to decay with the Uvasys SO₂ sheet (8.4%).

Use of the 13% polymer sheet along with a MAM resulted in 16.4% total decay, which indicated that the MAM does reduce decay control efficacy. The severity level of decay for the Uvasys and 13% polymer sheet treatments were 0.9 and 1.2, respectively, which relates to a decay manifestation rating of “low-low” (rating of 1 indicates low severity) for both treatments, opposed to “high” (rating of 6 indicates high severity) for the untreated grapes (personal communication with J. Fourie, Capespan Technology Development, 2001).

Decay incidence was generally higher for all SO₂ treatments (Table 1) at the bottom of the bunches (data expressed as “further scrutiny”) compared to the top of the bunches (indicated as “initial observation”). The observations, from the initial or succeeding assessment, that no extraordinary differences seemed to occur in decay control between the Uvasys sheet and the 13% polymer sheet, by measurement of decay incidence or severity, was substantiated by statistical analysis of the data

pooled across the six producers (Table 2). All the SO₂ treatments reduced decay significantly in comparison to the untreated control, with no further significant differences occurring between any of the treatments.

SO₂ damage

The incidence of SO₂ damage during storage was relatively high for both the Uvasys and 13% polymer sheet (40 and 44%, respectively). It was slightly lower when the polymer sheet was used in combination with the Capespan MAM (27% SO₂ damage). Of importance is that the incidence of SO₂ damage was very similar for both the Uvasys and 13% polymer sheet treatments.

Apart from the incidence of SO₂ damage, the severity of SO₂ damage was similar for both treatments (2.9), which relates to a "low-medium" occurrence rating of SO₂ damage (a rating of 4 indicates medium severity), but perhaps slightly too high for acceptance without any reservations by most supermarkets (personal communication with J. Fourie, Capespan Technology Development, 2001).

Similar to decay control, the observations that no extraordinary difference in SO₂ damage appeared in total SO₂ damage between the Uvasys sheet and the 13% polymer sheet, by measurement of SO₂ damage incidence or severity, was substantiated by statistical analysis of the data pooled across the six producers (Table 2). SO₂ damage was increased significantly by all the SO₂ treatments in comparison to the untreated control, with no further significant differences occurring between any of the treatments.

Conclusions

Decay control, and subsequent level of SO₂ damage, with the polymer SO₂ sheet of 13% was comparable to that achieved with the Uvasys SO₂ sheet. This is an indication that the polymer sheet is as effective as the Uvasys SO₂ sheet in conditions of a high decay potential.

The tested polymer sheet of 13% SO₂ concentration is considered suitable for use in non-perforated bag packaging. While the use of a MAM slightly reduces decay control, its inclusion in non-perforated bag packaging drastically reduces SO₂ damage. It is, therefore, suggested that the use of the MAM along with the polymer sheet, especially with SO₂ sensitive cultivars, be considered.

General conclusions to Chapter 5

Numerous trials were conducted during the 2000/2001 grape season in an attempt to optimise the new polymer SO₂ gas-generating sheets. In phase I dramatic aesthetic changes to the new polymer sheets were implemented. In phase II the exact concentration of Na₂S₂O₅ to be incorporated into the sheets was determined. Final testing of the new polymer sheet was done in phase III. During all three phases the efficacy of the new sheets regarding SO₂ damage and the ability to control *Botrytis cinerea* decay were compared to the Uvasys sheet.

The aesthetics of the new polymer sheet was improved during the 2001 season; it progressed from a three-deposit strip to a single-deposit of the polymer matrix, covering the entire surface of the carrier material. The first tests were conducted with a sheet covering ± 65% of the surface area on top of the grapes, while in later trials sheets of a larger dimension, covering ± 90% of the surface area, were used. The change from a three-deposit sheet to a single-deposit sheet, and from a smaller to a larger dimension, was required to reduce the possible risk of SO₂ damage occurring directly below the SO₂ deposit strips, and to reduce decay development in bunches not covered by the SO₂ deposits.

Apart from the size of the SO₂ deposits, the carrier material for the polymer matrix was shown to be of great importance. Water-soaked areas, which are totally unacceptable in appearance, occurred with the paper-type carrier, and hence had to be changed. The first alternative carrier material, a combination of poly(ester), aluminium foil and poly(ethylene), did not show similar problems. However, slight discolouration occurred at the sealed edges, and was ascribed to oxidation of the exposed aluminium film. The carrier material was subsequently changed to a metalised poly(ester) and poly(ethylene) combination, with no further problems

occurring. The plastic carrier type material used did not affect the efficacy of the SO₂ sheet.

From the results obtained in this study it was evident that the exact SO₂ concentration to be used, for trial purposes or future commercial use, will largely be determined by the cultivar, as well as the packaging type, as sensitivity to SO₂ damage differs between cultivars. The SO₂ concentration to be incorporated into the polymer sheet is in the range of 10 to 13%, for grapes packed in non-perforated bags, and slightly higher for grapes packed in perforated bags.

The specific grape bag used, whether perforated or non-perforated of nature, seems to have an effect on the efficacy of decay control of the SO₂ sheets. Generally, higher SO₂ concentrations are required to confine decay development in perforated than in non-perforated bags (comment supported by previous results in Chapter 3). Furthermore, lower SO₂ concentrations might be required for more SO₂ sensitive cultivars, regardless of which bag type is used.

There is still a degree of uncertainty whether or not it is advantageous to use an additional MAM in combination with the new polymer sheets, especially where high *Botrytis* inoculum levels are present. The results found during these trials were inconclusive in this regard. It was suggested that the use of an additional MAM as barrier below the polymer sheets had no negative effect on decay development, nor any real benefit in reducing levels of SO₂ damage. There were, however, some indications that the use of an additional MAM might be advantageous, especially for the more SO₂ sensitive cultivars packed in non-perforated bags.

Depending on the outcome of the trials conducted at the end of the 2001 season, which will possibly include consignments by various exporters, commercial applications are envisaged. These should be limited, however, and planned for cultivars less sensitive to SO₂ damage. Small scale, semi-commercial export trials (1000 to 2000 cartons), using a range of SO₂ concentrations on selected cultivars should first be considered before embarking on large, commercial applications. Stringent control will be required throughout such an operation to ensure effective handling and control of the consignments, proper feedback and examination, to determine the condition of the grapes upon arrival on the overseas market and at the point of sale.

Appendix to Chapter 5

Phase III

For clarity purposes and to simplify the discussion of the results, comprehensive results for the individual producers (Table 1), and the table of statistical analysis for data pooled across the six producers (Table 2) have been included as an “Appendix” here.

Table 1 Incidence of decay and SO₂ damage on Barlinka table grapes sampled from six producers in the Hex River area after storage for 8 weeks at -0.5°C followed by 4 days at 7.5°C, with use of a polymer sheet of 13% SO₂ vs the commercially used Uvasys SO₂ sheet.

Prod. No.	Treatment	Decay incidence (%)				SO ₂ incidence (%)			
		Initial ¹ observation	Further ² scrutiny	Total decay ³	Decay ⁴ severity	Initial ¹ observation	Further ² scrutiny	Total SO ₂ damage ³	SO ₂ severity ⁴
		(top of bunches)	(bottom of bunches)	(top & bottom combined)	(rating 0-7)	(top of bunches)	(bottom of bunches)	(top & bottom combined)	(rating 0-7)
1	No SO ₂	100.0	100.0	100.0	6.7	0.0	0.0	0.0	0.0
H0410d	No SO ₂	100.0	100.0	100.0	5.7	0.0	0.0	0.0	0.0
H417	No SO ₂	100.0	100.0	100.0	6.3	0.0	0.0	0.0	0.0
H408b	No SO ₂	94.4	100.0	96.3	6.0	0.0	0.0	0.0	0.0
H416	No SO ₂	100.0	100.0	100.0	6.3	0.0	0.0	0.0	0.0
6	No SO ₂	51.6	44.4	49.3	2.3	0.0	0.0	0.0	0.0
	Avg.	91.0	90.7	90.9	5.6	0.0	0.0	0.0	0.0
1	Uvasys	22.0	44.4	28.8	3.0	13.7	11.1	12.7	2.3
H0410d	Uvasys	4.8	33.3	13.7	2.0	15.1	33.3	20.7	1.0
H417	Uvasys	0.0	0.0	0.0	0.0	58.9	100.0	73.1	5.0
H408b	Uvasys	0.0	22.2	7.9	0.7	35.6	44.4	38.9	3.3
H416	Uvasys	0.0	0.0	0.0	0.0	44.4	88.9	59.3	4.3
6	Uvasys	0.0	0.0	0.0	0.0	30.2	55.6	37.8	1.7
	Avg.	4.5	16.7	8.4	0.9	33.0	55.6	40.4	2.9

Table 1 (continued)

1	Pol13+MAM	10.3	44.4	21.5	1.7	4.8	0.0	3.3	0.7
H0410d	Pol13+MAM	9.7	77.8	32.2	3.7	5.6	11.1	7.9	0.7
H417	Pol13+MAM	16.7	33.3	22.7	2.0	12.2	22.2	15.3	1.0
H408b	Pol13+MAM	5.6	11.1	7.4	1.0	22.2	44.4	29.3	2.3
H416	Pol13+MAM	0.0	11.1	3.3	0.3	42.9	100.0	61.1	5.0
6	Pol13+MAM	5.6	22.2	11.1	0.7	38.1	66.7	47.4	2.7
Avg.		8.0	33.3	16.4	1.6	21.0	40.7	27.4	2.1
1	Pol13%	14.3	22.2	16.7	2.7	9.5	0.0	6.7	0.7
H0410d	Pol13%	5.6	44.4	17.0	1.7	15.1	44.4	23.7	1.0
H417	Pol13%	5.6	11.1	7.4	1.3	28.9	44.4	34.3	2.7
H408b	Pol13%	15.0	11.1	14.4	0.7	41.7	33.3	39.4	2.7
H416	Pol13%	5.6	11.1	7.0	0.7	47.6	100.0	63.4	5.7
6	Pol13%	0.0	11.1	3.3	0.3	94.4	100.0	96.3	4.7
Avg.		7.7	18.5	11.0	1.2	39.5	53.7	44.0	2.9

1 Initial observation (% incidence of the defect) = first impression of the occurrence of decay or SO₂ damage on the visible sector of the bunches.

2 Further scrutiny (% incidence of the defect) = occurrence of decay or SO₂ damage on three bunches not showing the defect from the top. The bunches were turned around to dispose the bottom half of the bunch.

3 Total incidence = total incidence of the defect as determined from combining the occurrence of the defects for the visible sector (top half) and the disposed bottom half.

4 Severity = rating of the severity of the defect according to a scale of 0-7, where 7 = total area of visible sector showing the defect.

Table 2 Efficacy of the polymer SO₂ sheet of 13% SO₂ in relation to the Uvasys sheet for control of decay, and subsequent SO₂ damage related to the treatment, on Barlinka table grapes after 8 weeks at –0.5°C followed by 4 days at 7.5°C. The table represents pooled data of six producers.

Examination parameter ³		SO ₂ Treatment ²				Prob. > F ¹
		No SO ₂ sheet	Uvasys	Pol 13% +MAM	Pol 13%	
Decay	Initial observation (% incidence)	91.0a	4.5b	8.0b	7.6b	***
	Further scrutiny (% incidence)	90.7a	16.6b	33.3b	18.5b	***
	Total decay (%)	90.9a	8.4b	16.4b	10.9b	***
	Decay severity (rating 0-7)	5.6a	1.0b	1.6b	1.2b	***
SO ₂ damage	Initial observation (% incidence)	0.0a	33.0b	21.0b	39.5b	**
	Further scrutiny (% incidence)	0.00a	55.6b	40.7b	53.7b	**
	Total SO ₂ damage (%)	0.00a	40.4b	27.4b	44.0b	**
	SO ₂ damage severity (rating 0-7)	0.00a	2.9b	2.1b	2.9b	*

1 One-way ANOVA table, where *, ** and *** indicate significant differences at the 5%, 1% and 0.1% levels.

2 Different SO₂ sheets were placed in the packed cartons of un-inoculated grapes before onset of forced-air cooling. The grapes were packed in non-perforated bags.

3 Examination parameters: Initial observation (% incidence of the defect) = first impression of the occurrence of decay or SO₂ damage on the visible sector of the bunches; Further scrutiny (% incidence of the defect) = occurrence of decay or SO₂ damage on three bunches not showing the defect from the top. The bunches were turned around to dispose the bottom half of the bunch; Total incidence = total incidence of the defect as determined from combining the occurrence of the defects for the visible sector (top half) and the disposed bottom half; Severity = rating of the severity of the defect according to a scale of 0-7, where 7 = total area of visible sector showing the defect.

Chapter 6

The stage of storage and level at which SO₂ damage manifests on Barlinka table grapes during cold storage, determined with the use of in-package SO₂ gas generating sheets of varying concentrations

A condensed version of this chapter was submitted to the American Journal of Enology and Viticulture for publication.

Abstract

SO₂ is used in South Africa, as in most other grape-exporting countries of the world, for the control of *Botrytis cinerea* during post-harvest storage of table grapes. The presence of unacceptably high SO₂ residues in grapes has become more important since the Food and Drug Administration (FDA) revoked the generally regarded as safe (GRAS) status of SO₂. To determine at which stage SO₂ damage manifests on table grapes, the level of SO₂ damage associated with different SO₂ concentrations, and whether SO₂ damage manifests more readily at a particular position on the bunch, grapes were packed with SO₂ gas-generating sheets of varying SO₂ concentrations. The possible effect of an increase in storage temperature, from an initial storage at -0.5°C to 10°C, on the levels of SO₂ bleaching was also investigated.

Levels of SO₂ damage were significantly affected by the storage period, SO₂ concentration in the SO₂ generating sheet and temperature fluctuations. For Barlinka grapes kept at -0.5°C, or when followed by a shelf life period at 10°C after the initial cold storage period, no significant differences in the levels of decay development and SO₂ damage were observed in relation to the orientation of the bunches in the carton.

Introduction

In South Africa, sulphur dioxide (SO₂) gas is used for post-harvest preservation of export table grapes. SO₂ is regarded by many researchers to be the most practical solution for the prevention of decay caused by *Botrytis cinerea* during the post-harvest storage of table grapes (Nelson, 1979; Marois *et al.*, 1986). However, SO₂ has lately been the focus of increasing concern, especially since it was discovered that food containing sulphite residues poses a health risk to sulphite-sensitive individuals (Anon., 1986a). This concern was illustrated when the United States Food and Drug Administration (USFDA) revoked the generally regarded as safe (GRAS) status of SO₂ for use on fresh grapes (Anon., 1986a). Furthermore, a residue tolerance level of 10 µg SO₂ per gram off fresh grapes was established by the Environmental Protection Agency (EPA) (Anon., 1986a; Anon., 1989).

The implementation of these strict measures created the need for a better understanding of the mechanism of decay control with SO₂ and the effects associated with its use. Peiser and Yang (1985) concluded that sulphite residues are present in either free or bound sulphite. Smilanick *et al.* (1990a) indicated trends in the detection of sulphite residues, and that sulphite residues were located in the grape tissue after fumigation with SO₂. The effect of SO₂ on surface bleaching of the berries has been reported by various researchers (Marois, *et al.*, 1986; Nelson, 1979). Guelfat-Reich *et al.* (1975) also noted that the effect of SO₂ treatment on the quality of the grapes varies greatly with both the cultivar used and the method of SO₂ application. However, very little is known about at which stage during the post-harvest storage period SO₂ bleaching occurs, and how temperature differences during storage influence SO₂ bleaching.

The objectives of this chapter were to determine the following: (a) the stage at which SO₂ damage manifests on table grapes with use of a variety of SO₂ sheets; (b) the level of SO₂ damage associated with different SO₂ concentrations; (c) whether SO₂ damage manifests more readily at a particular position on the bunch, either at the top or bottom half, in relation to the orientation of the bunch in the

carton; and (d) the effect that fluctuations in storage temperatures have on decay control and SO₂ damage.

Materials and methods

Packaging and SO₂ treatment

Fresh, unblemished Barlinka table grapes were packaged in corrugated cardboard cartons as for export, either in perforated (P) or non-perforated (NP) bags. Individual bunches were packaged in carry bags. The packed grapes were kept in cold storage for 7, 21, 35 and 49 days at -0.5°C, and for 7, 21, 35 and 49 days at -0.5°C, followed by 5 days at 10°C

The various SO₂ generators that were tested and compared during this trial were:

- i) Sheet 1*, new polymer sheet of 14% sodium metabisulphite content (abbreviated, henceforth, as 14% polymer sheet);
- ii) Sheet 2*, new polymer sheet of 20% sodium metabisulphite content (abbreviated, henceforth, as 20% polymer sheet);
- iii) Sheet 3*, new polymer sheet of 26% sodium metabisulphite content (abbreviated, henceforth, as 26% polymer sheet);
- iv) Uvasys SO₂ sheet (Grapetek, South Africa); and
- v) Fresca 6g dual release SO₂ sheet (Quimetal, Chile).

**These are experimental sheets manufactured by the University of Stellenbosch in conjunction with the South African Deciduous Fruit Producers Trust.*

The polymer SO₂ gas generating sheets were manufactured on a pilot scale production plant. Manufacturing of the SO₂ gas generating sheets commences with the preparation of a PVC plastisol mixture. Once the desired plastisol mixture is obtained, it is applied onto a carrier material by means of a nozzle. The carrier

material with the plastisol deposit is sent through a series of curing ovens where the plastisol is cured to a solid state. Once cured, a second layer of non-woven material is laminated onto the carrier layer to enclose the PVC matrix between two sheets of material. A cooling section follows, where the product is rapidly cooled down to room temperature before it is cut to the desired specifications. The finished product is inspected for deviations in quality before it is packaged into poly(ethylene) bags, ready to be used.

Experimental layout and statistical detail

Completely randomised two-way ANOVA with storage condition and SO₂ sheet as Factors A & B, for trial 1, respectively, and bunch orientation and SO₂ sheet as Factors A & B, for trial 2, respectively. Each treatment consisted 5 replicate cartons of grapes. Factorial analyses of variance were performed on the data and Students-T LSD calculated to compare treatment means at a 5% significance level.

Examination parameters

Examinations were conducted according to Capespan protocols, with special emphasis on decay development and SO₂ damage. SO₂ damage and decay was expressed by weight as a percentage of the original sample mass. The position at which the damage occurred, either at the top or bottom of the bunch, was also recorded.

Results and discussion

The potential control of post-harvest *Botrytis* storage decay, and other quality defects, with the use of different SO₂ sheet treatments, was determined. Trials were conducted with Barlinka table grapes harvested at optimum harvest maturity and packed by a commercial pack house. Decay development, SO₂ damage manifestation, berry split and the particular position at which SO₂ damage occurred on the bunch, were assessed after various storage periods.

Statistical analysis of the above quality parameters resulted in a large volume of data. Thus, for clarity purposes and to simplify the discussion of the results, a comprehensive list of tables (Tables 1 to 4) containing the statistical analysis has been included as an "Appendix" at the end of this chapter.

Decay development

No difference in the level of decay (inhibition of the spread of decay) occurred on grapes as result of usage of a range of in-package SO₂ emitting sheets (Table 1). A significantly higher level of decay occurred on grapes stored for 35 days at -0.5°C compared to those stored for 7 days and 21 days, as well as the 49 days storage periods (Factor B). The reduction in decay associated with the 49 days storage period compared to 35 days cannot be explained.

Essentially similar results occurred for grapes kept at -0.5°C for different periods, followed by 5 days at 10°C (Table 2). However, in this case the grapes stored for 49 days exhibited higher levels of decay than those subjected to a 7-day storage period. The decay levels were generally very low; typical of a year of a low decay potential. Differences as result of varying treatments are seldom found in this scenario.

SO₂ damage

No difference in the level of SO₂ damage occurred between any of the SO₂ sheet combinations for storage periods of 7 or 21 days at -0.5°C (Table 1). However, when kept for 35 or 49 days at -0.5°C, a significantly higher level of SO₂ damage occurred with the polymer SO₂ sheet of 26%, compared to all other treatments, including the 14 and 20% polymer sheets. A relatively high level of SO₂ damage also occurred with the Uvasys SO₂ sheet after 35 and 49 days at -0.5°C. Both the former as well as the 20% sheet resulted in significantly more SO₂ damage than with the Fresca 6g sheet. No differences in the levels of SO₂ damage occurred between the Uvasys and the 14 and 20% polymer sheets after 35 or 49 days of

storage. The 20% polymer SO₂ sheet also showed significantly more SO₂ damage than the 14% sheet, when kept for 49 days at -0.5°C, but not for shorter storage duration.

Differences in SO₂ damage levels before (Table 1) and after simulated shelf life conditions (Table 2) were small, whereas no differences occurred for both 7 or 21 days storage at -0.5°C only. Significantly more SO₂ damage occurred with the Uvasys SO₂ sheet than the 14% polymer sheet when the grapes were subjected to a shelf life period after the initial cold storage period of 21 days at -0.5°C. Significantly higher levels of SO₂ damage also occurred with the 20 and 26% polymer sheets after storage for 49 days + 5 days shelf life, compared to all other SO₂ treatments, while after 35 days, higher levels of SO₂ damage were observed for the 26% polymer sheet and all treatments, except the Uvasys treatment.

Between storage periods for a particular SO₂ sheet no significant differences in SO₂ damage occurred with extended storage from 7 to 49 days for either the Fresca 6g or 14% polymer sheet (Table 1). Significantly higher levels of SO₂ damage occurred with the 26% polymer sheet, as well as the Uvasys SO₂ sheet, for both the 35 and 49 days storage periods, compared to 7 and 21 days at -0.5°C. The 20% polymer sheet also showed higher levels of damage after 49 days compared to a cold storage period of 35 days.

The results for storage at -0.5°C followed by 5 days at 10°C was essentially similar to those kept for different periods at -0.5°C only. No differences were observed over time for the 14% polymer sheet. Significantly higher levels of SO₂ damage occurred for the 21, 35 and 49 days followed by 5 day shelf life storage, compared to 7 days storage with use of the 26% polymer sheet, Uvasys and Fresca 6g SO₂ sheets.

The above results indicated an increase in SO₂ damage with use of the polymer SO₂ sheet of 26% SO₂ concentration with prolonged storage, from 35 days onwards, compared to the level of SO₂ damage of polymer sheets of lower SO₂ concentration, as well as other SO₂ sheets used in practice. A potential risk of increased SO₂ damage could also arise with the 20% polymer sheet, as well as the Uvasys SO₂ sheet, by keeping the grapes for longer than 35 days at -0.5°C. Whereas differences in the level of SO₂ damage only occurred after 35 days storage

at -0.5°C , differences were already observed after 21 days storage when followed by a period of shelf life.

The results further showed that SO_2 damage to grapes could increase throughout the storage period, even if kept at -0.5°C for the entire storage period. Similar findings were reported by Harvey *et al.* (1988), who also found that higher levels of SO_2 damage occurred with longer storage periods. Similarly, Nelson (1979) reported that it became increasingly difficult to keep the damage to fruit, due to SO_2 bleaching, within acceptable levels as the storage period was extended.

Warming of grapes, whether accidental or due to exposure to shelf life conditions, could enhance and exacerbate SO_2 damage. This will be even more appropriate for SO_2 sensitive cultivars. Nelson (1979) and Smilanick *et al.* (1990b) made similar observations.

The levels of sulphite residues present in the grapes were determined for non-sensitive cultivars by the Department of Health according to the modified Monier-Williams method (Anonymous, 1995). The levels of sulphite residues detected were below the 10 ppm tolerance level imposed by the FDA and EPA for all the polymer sheets tested (report attached in appendix). However, this work may in future need to be repeated for specific SO_2 sensitive cultivars.

Position of decay and SO_2 damage

The effect that bunch orientation has on decay development and SO_2 damage, related to damage incurred at the top or bottom of the bunch, was determined. No differences in the level of decay occurred in relation to the orientation of the bunches in the carton, for any of the four storage periods, for Barlinka grapes kept at -0.5°C (Table 3), or when followed by a shelf life period at 10°C after the initial cold storage period (Table 4). No differences in decay occurred between any of the SO_2 sheets used for any of the storage periods (Tables 3 and 4).

No differences in the level of SO_2 damage occurred in relation to the orientation of the bunches in the carton, for any of the four storage periods, for Barlinka grapes kept at -0.5°C (Table 3), or when followed by a shelf life period at

10°C after the initial cold storage period (Table 4). However, slightly higher levels of SO₂ damage generally occurred on the top half of the bunches. This corresponds to conclusions made by Nelson and Ahmedullah (1972), that there is a sharp decrease in the concentration of SO₂ gas away from the generator, suggesting that the fruit nearer the generator is exposed to higher concentrations of SO₂ gas, therefore, resulting in more SO₂ damage. The level of SO₂ damage was determined by the SO₂ sheet, increasing in most cases with use of the polymer sheet of a higher SO₂ concentration (see detailed results for specific SO₂ sheets (Tables 1 to 4) as described above). This corresponds to the findings of Marois *et al.* (1986) who indicated that higher levels of SO₂ damage occurred with higher levels of SO₂ gas.

Berry split

Berry split increased significantly with longer storage periods. Significantly higher levels of berry split occurred for both the 35 and 49 days storage periods compared to storage for 7 or 21 days at -0.5°C (Table 1). No difference in berry split occurred for any of the storage periods on Barlinka cooled initially to -0.5°C followed by 5 days at 10°C (Table 2). A possible explanation for the increase in the levels of berry split is that berry split can possibly be related to SO₂ damage, as the increased levels of berry split correspond to increased levels of SO₂ damage (personal communication with J. Fourie, Capespan Technology Development, 2001).

Conclusions

From the results it can be concluded that SO₂ damage generally increases over time, irrespective of the SO₂ sheet used. The increase in SO₂ damage was more pertinent for the polymer sheet of a higher SO₂ content (26%). This is particularly relevant for long term storage. The level of SO₂ damage caused by the Uvasys SO₂ sheet did not differ significantly from the polymer sheets of <20% SO₂ content. Higher SO₂ concentration resulted in more damage than caused by the

Uvasys sheet. It is therefore most important that, with the use of SO₂ sheets, the duration of the storage period must be taken into account and form part of a decay and SO₂ management strategy when selecting a specific SO₂ sheet.

The level of SO₂ damage generally increased when the grapes were subjected to increases in temperature, as depicted by the simulated shelf life conditions (5 days at 10°C). This is an indication that the efficacy of decay control and SO₂ damage related to the use of the polymer SO₂ sheets could be affected by the storage period and temperature fluctuations. Decay control comparable to that with the Uvasys SO₂ sheet was achieved with most of the polymer sheet combinations tested.

The orientation of the bunches in the carton did not affect the level of decay, nor the level of SO₂ damage of Barlinka grapes kept at -0.5°C, or when subjected to a shelf life period at 10°C, irrespective of the storage period. Although not statistically significant, slightly higher levels of SO₂ damage were observed on areas of the bunches directly below the SO₂ sheets/sheets.

Sulphite residues detected in the grapes after the storage period were below the 10 ppm tolerance level imposed by the FDA and EPA.

The use of in-package SO₂ generators are imperative for controlling post-harvest *Botrytis* decay on table grapes. However, exposure of the grapes to too high levels of SO₂ will lead to the presence of unacceptably high levels of SO₂ residues. These results confirm that a very fine balance exists between decay control and SO₂ damage and table grape producers and marketers need to take cognisance of it.

Temperature fluctuations during storage need to be avoided, as increased and excessive SO₂ damage may result from such practices, while prolonged storage of 6 to 8 weeks of table grapes may also result in increased SO₂ damage, especially at high SO₂ concentrations.

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Appendix to Chapter 6

To simplify discussion of the results of the statistical analysis, a comprehensive list of tables (Tables 1 to 4) containing the statistical analysis of the quality parameters have been included as an “Appendix” here.

Table 1 The level of SO₂ damage incurred on Barlinka grapes packed with polymer SO₂ sheets of varying concentrations, compared to the Uvasys SO₂ sheet and an imported Fresca 6g sheet, after storage for different periods at –0.5°C. (Decay and SO₂ damage recorded at the top and bottom of the bunch were pooled.)

Examination parameter ⁵	Interaction ² SO ₂ sheet / Storage	Storage period (days) ³ (Factor A)				SO ₂ treatment ⁴ (Factor B)					Prob. > F ¹		
		7	21	35	49	Pol 14	Pol 20	Pol 26	Uvasys	Fresca6g	A	B	AB
Decay		0.01b	0.04b	0.38a	0.13b	0.29	0.09	0.10	0.08	0.13	***	ns	ns
Berry split		0.12b	0.04b	0.34a	0.27a	0.13c	0.17bc	0.34a	0.28ab	0.05c	***	**	ns
Total SO ₂	Pol14	0.45g	0.40g	1.01defg	1.11defg						***	***	***
	Pol20	0.84efg	1.10defg	1.33def	2.30bc								
	Pol26	0.79efg	0.55fg	2.95ab	3.27a								
	Uvasys	0.94efg	0.73fg	1.69cd	1.66cd								
	Fresca6g	0.87efg	0.45g	0.39g	0.98defg								

1 Two-way ANOVA table for Factor A (Storage period) and Factor B (SO₂ sheet), with ns, *, ** and *** indicating non-significant and significant differences at the 5%, 1% and 0.1% levels.

2 Non-pooled data for significant interaction between factor A and B.

3 & 4 Data pooled across storage period or SO₂ sheet for non-significant interactions for Factor A (storage period 7d, 3w, 5w and 7w) and for Factor B (SO₂ sheet = Polymer SO₂ sheets with varying concentrations of 14, 20 and 26% SO₂ content, the Uvasys SO₂ and Fresca 6g SO₂ sheets). Values in the same row, followed by different letters, indicate significant differences for pooled data (P<0.05) according to LSD test, whereas for non-pooled data different letters indicate significant differences across rows and within columns.

5 Examination parameters: Decay, SO₂ damage and berry split are expressed as percentages of the total mass. Total SO₂ indicates a combined value for pedicel and surface SO₂ damage.

Table 2 The level of SO₂ damage incurred on Barlinka grapes packed with polymer SO₂ sheets of varying concentrations, compared to the Uvasys SO₂ sheet and an imported Fresca 6g sheet, after storage for different periods at –0.5°C + 5 d at 10°C. (Decay and SO₂ damage recorded at the top and bottom of the bunch were pooled.)

Examination parameter ⁵	Interaction ² SO ₂ sheet / Storage	Storage period (days) ³ (Factor A)				SO ₂ treatment ⁴ (Factor B)					Prob. > F ¹		
		7	21	35	49	Pol 14	Pol 20	Pol 26	Uvasys	Fresca6g	A	B	AB
Decay		0.04b	0.14ab	0.09ab	0.21a	0.12	0.23	0.10	0.03	0.13	*	ns	ns
Berry split		0.03	0.01	0.13	0.11	0.06	0.06	0.08	0.12	0.04	ns	ns	ns
Total SO ₂	Pol14	0.29g	0.73defg	0.50efg	1.60bc						***	***	***
	Pol20	0.69defg	1.28bcde	0.84cdefg	3.55a								
	Pol26	0.34fg	1.51bcd	1.69b	3.51a								
	Uvasys	0.67defg	1.86b	1.24bcde	1.57bc								
	Fresca6g	0.06g	1.17bcdef	0.71defg	1.48bcd								

1 - 5 See Table 1.

Table 3 Effect of orientation of the bunches in the carton on decay and SO₂ damage, related to damage incurred at the top or bottom of the carton, on Barlinka grapes packed with polymer SO₂ sheets of varying concentrations, compared to the Uvasys SO₂ sheet and an imported Fresca 6g sheet, after storage for 7 d at –0.5°C.

Storage period (days) ⁵	Examination parameter ⁴	Orientation of the bunch in the carton at which defect occurs ² (Factor A)		SO ₂ treatment ³ (Factor B)					Prob. > F ¹		
		top	bottom	Pol 14	Pol 20	Pol 26	Uvasys	Fresca 6g	A	B	AB
7	Decay	0.02	0.00	0.00	0.00	0.00	0.04	0.00	ns	ns	ns
	Tot. SO ₂	0.86	0.70	0.45	0.84	0.79	0.94	0.87	ns	ns	ns
21	Decay	0.07	0.01	0.09	0.04	0.00	0.03	0.02	ns	ns	ns
	Tot. SO ₂	0.18	0.05	0.40b	1.09a	0.55b	0.73ab	0.45b	ns	*	ns
35	Decay	0.26	0.49	1.00	0.25	0.10	0.24	0.29	ns	ns	ns
	Tot. SO ₂	0.45	0.43	1.01bc	1.33b	2.95a	1.69b	0.39c	ns	***	ns
49	Decay	0.21	0.04	0.05	0.07	0.28	0.00	0.22	ns	ns	ns
	Tot. SO ₂	1.92	1.77	1.11c	2.30ab	3.27a	1.56bc	0.98c	ns	***	ns

1 Two-way ANOVA table for Factor A (Bunch orientation) and Factor B (SO₂ sheet), with ns, *, ** and *** indicating non-significant and significant differences at the 5%, 1% and 0.1% levels.

2 & 3 Data pooled across bunch orientation or SO₂ sheet for non-significant interactions for Factor A (bunch orientation), top or bottom half of the carton, and for Factor B (SO₂ sheet = Polymer SO₂ sheets with varying concentrations of 14, 20 and 26% SO₂ content, the Uvasys SO₂ and Fresca 6g SO₂ sheets). Values in the same row, followed by different letters, indicate significant differences for pooled data (P<0.05) according to LSD test, whereas for non-pooled data different letters indicate significant differences across rows and within columns.

4 Examination parameters: Decay and SO₂ damage expressed as percentages of the total mass. Total SO₂ indicates a combined value for pedicel and surface SO₂ damage.

5 Storage periods were analysed independently. For simplification, the data for different storage periods were included into the same table.

Table 4 Effect of orientation of the bunches in the carton on decay and SO₂ damage, related to damage incurred at the top or bottom of the carton, on Barlinka grapes packed with polymer SO₂ sheets of varying concentrations, compared to the Uvasys SO₂ sheet and an imported Fresca 6g sheet, after storage for 7 d at –0.5°C + 5 d at 10°C.

Storage period (days) ⁵	Examination parameter ⁴	Orientation of the bunch in the carton at which defect occurs ² (Factor A)		SO ₂ treatment ³ (Factor B)					Prob. > F ¹		
		top	bottom	Pol 14	Pol 20	Pol 26	Uvasys	Fresca 6g	A	B	AB
7	Decay	0.02	0.06	0.00	0.03	0.08	0.05	0.04	ns	ns	ns
	Tot SO ₂	0.47	0.35	0.29ab	0.69a	0.34ab	0.67a	0.06b	ns	*	ns
21	Decay	0.09	0.20	0.33	0.25	0.09	0.03	0.01	ns	ns	ns
	Tot SO ₂	1.30	0.32	0.73	1.28	1.51	1.86	1.17	ns	ns	ns
35	Decay	0.08	0.10	0.07	0.18	0.04	0.00	0.18	ns	ns	ns
	Tot SO ₂	1.16	0.84	0.50	0.84	1.69	1.24	0.71	ns	ns	ns
49	Decay	0.26	0.16	0.07	0.45	0.19	0.04	0.30	ns	ns	ns
	Tot SO ₂	2.62	2.07	1.60b	3.55a	3.51a	1.57b	1.48b	ns	***	ns

1 - 5 See Table 3.

Chapter 7

Physical changes to cell-wall structures of table grapes caused by post-harvest fumigation with sulphur dioxide as observed by low-temperature scanning and transmission electron microscopy

A condensed version of this chapter was submitted to the International Journal of Food Science and Technology for publication.

Abstract

The exposure of table grapes to high levels of sulphur dioxide gas (SO₂) during post-harvest cold storage as a method of decay control could pose various quality problems. The extent of damage incurred to grape tissue by the absorption of SO₂ gas was determined with low temperature scanning (LTSEM) and transmission electron microscopy (TEM) techniques. LTSEM and TEM micrographs of areas damaged by SO₂ gas, compared to undamaged areas, revealed that exposure to SO₂ gas may lead to plasmolysis and the loss of cellular fluids. These observations imply that absorption of SO₂ gas by grape tissue may lead to the excessive degradation of cell walls, cell wall structures, cell membranes and cause ultrastructural damage to the chloroplasts, other than natural senescence occurring during storage. Although damage to the cell walls, cell wall structures and cell membranes, caused by SO₂ gas, was more prominent in the tissue layers nearer to the fruit surface, damage also occurred to a lesser extent in deeper tissue layers.

Introduction

The use of SO₂ gas during post-harvest cold storage of table grapes is considered essential for their protection against *Botrytis cinerea* decay (Ballinger & Nesbitt, 1984; Harvey, 1956; Morris *et al.*, 1992; Mustonen, 1992; Nelson, 1979; Winkler & Jacob, 1925). The application of SO₂ at the correct concentration to control *Botrytis* infections is critical, as the levels required for effective control of *Botrytis* decay are very close to the levels which may damage the fruit (Harvey, 1956; Nelson, 1979). Excessive levels of SO₂ damage the berries by bleaching, and furthermore causes premature browning of the stems and increased water loss. These factors have a negative influence on the marketability of table grapes, especially the SO₂ sensitive cultivars (Marois *et al.*, 1986; Mustonen, 1992; Nelson, 1979).

Several researchers have expressed their views on the inhibitory activity of SO₂ during post-harvest storage of table grapes (Peiser & Yang, 1985; Smilanick *et al.*, 1990), and on the uptake and retention of SO₂ by the grapes (Austin *et al.*, 1997; Lagunas-Solar *et al.*, 1992). The distribution of sulfite residues, both within and on the surface of grape berries, and the kinetics of SO₂ uptake and retention has also been documented (Lagunas-Solar *et al.*, 1992). However, little work has been conducted to establish the extent of the damage, if any, caused by the absorption of SO₂ gas to the grape tissue.

The extent of damage caused by SO₂ fumigation to the tissue of various other fruit types, other than table grapes, has been documented (Fourie, 1996; McBean *et al.*, 1964; Theart & Smit, 1983). From the results it was concluded that the absorption of SO₂ by fruit tissue leads to softening of the fruit tissue as a result of the degradation of cell walls, cell wall structures, cell membranes and intercellular layers (Fourie, 1996; McBean, 1976; McBean *et al.*, 1971; Theart & Smit, 1983).

Fruit texture as a function of quality is determined by cell wall composition, cell turgor and cellular anatomy (Hand *et al.*, 1977; Bartley & Knee, 1982). Therefore, in order to study the process of fruit damage the structural changes taking place in the cell walls and membranes must be determined. The cell wall structure is normally composed of a rigid skeleton of cellulose microfibrils embedded in a gel-like matrix composed of several non-cellulosic polysaccharides and glycoproteins (Fry, 1986). Cell to cell cohesion is made possible by the middle lamella, a region rich in pectinaceous materials (Dey & Brinson, 1984). Membranes are envisaged as continuous bi-layers, comprised primarily of lipids and proteins (Singer & Nicolson, 1972). Besides the obvious trauma of harvest, stress may be imposed by mechanical, chemical (SO₂ damage) or pathogenic injury.

The objectives of this chapter were to observe the effect of SO₂ damage on the ultrastructure of the cell walls and membranes and to establish the extent of damage caused to the grape tissue by the absorption of SO₂ during post-harvest treatment with SO₂ gas, by using low temperature scanning electron microscopy (LTSEM) and conventional transmission electron microscopy (TEM) techniques.

Materials and methods

Barlinka table grapes from the De Doorns area in South Africa were packed under export conditions. After packing, commercial dual-release SO₂ sheets (Fresca 6 gram sheet, Quimetal, Chile) were included before storage at – 0.5°C for 5 weeks. This procedure is not the standard commercial practice used in South Africa for exporting table grapes; SO₂ sheets with slightly lower SO₂ concentrations are generally used. The higher SO₂ concentration sheets (Fresca 6 gram) were used to assure and induce high levels of SO₂ damage (personal communication with J. Fourie, Capespan Technology Development, 1999). At the end of the storage period, the berries containing areas damaged by SO₂ gas

were selected for damage assessment. Sections of 2 x 2 x 4 mm in size were cut inwards from the cuticle, with a scalpel. As a reference (control), a similar sample was cut from an area from a berry where no SO₂ damage occurred.

Sample preparation for LTSEM

Sections of tissue were selected from damaged areas and from undamaged areas (control). The sections were then placed on the same cryo-stub, with their cuticles facing each other. The latter action facilitated the location of complementary areas of fractured sections for observation, comparison and photography. The sections were mounted to the cryo-stub with a mixture of cryo-adhesive (Tissue-Tek™; Miles Scientific, Naperville, Ill., USA) and colloidal graphite. These sections were simultaneously submerged and rapidly frozen in liquid nitrogen (N₂) slush.

To examine internal tissues, frozen sections had to be fractured using a pre-cooled scalpel while kept submerged in the liquid N₂ slush. A vacuum was drawn in the chamber and both the fractured sections were transferred under vacuum to the cryo-unit (Fisons LT 7400). The fractured surfaces of the samples were then viewed using a LEO S440 Analytical Scanning Electron Microscope.

The tissue sections were initially not sputter coated and the stage was kept at -175°C. If ice crystals formed, the sections were sublimated for 15 min at -90°C. Images were recorded at 2.5 kV and a pro-current of 35 pA.

Sample preparation for TEM

Selected tissue sections as described above were fixed overnight in a solution of 2.5% glutaraldehyde in phosphate buffered saline solution (PBS) (pH 7.4). The sections were then removed from the glutaraldehyde and washed twice with the PBS (pH 7.4) for a period of 5 min.

Sections were postfixed in a solution of 1% osmium in PBS for a period of 1 hour. The sections were then removed from the osmium and washed twice with PBS (pH 7.4) for a period of 5 min each time. The sections were then removed from the PBS and washed twice with distilled water for 5 min.

Sections were then taken through an ethanol dehydration procedure; they were placed in solutions containing 30, 50, 70, 80, 90 and 95% ethanol (v/v) for a period of 5 min in each concentration. On completion of the dehydration, the samples were washed in 100% ethanol for a period of 10 min, followed by 100% acetone for a further 10 min.

Sections were then transferred to a mixture of 50% resin and 50% acetone, overnight. The acetone was gradually replaced with resin over a period of 2 or more days and the sections embedded in resin blocks and solidified by placing the resin blocks containing the embedded sections in an oven at 60°C for 16 hours.

Thin sectioning of the embedded sections were made using a Reichert Ultracut S (Leica) ultra microtome. The 100 nm thin sections of the embedded sections were then stained with 2% uranyl acetate and Reynolds Lead Citrate. Sections were viewed and photographed in a JEOL 200CX Transmission Electron Microscope.

Results and discussion

For clarity purposes and to simplify the discussion of the results, the figures containing the SEM and TEM micrographs (Figure 1 and 2) have been included as an "Appendix" at the end of this chapter.

In this study it was observed that areas with mechanical or pathogenic injuries were more susceptible to damage caused by exposure to SO₂ gas. The position of the bunch in the box had a significant effect on the occurrence of damage caused by SO₂ gas. Higher levels of SO₂ damage were observed where

the berries were either directly in contact with, or closer to the SO₂ gas generating pads. Similar observations were made for berries in the bottom of the carton, which were often flattened or bruised due to pressure from above (personal communication with J. Fourie, Capespan Technology Development, 1999).

It appears that factors determining access of SO₂ gas, such as berry density and bunch shape, as well as physical damage to the berries, have a significant effect on the occurrence of SO₂ damage. A higher occurrence of *Botrytis cinerea* decay with little SO₂ damage was observed within densely packed bunches, suggesting that the berries on the inside of these bunches are exposed to lower levels of SO₂ gas than the berries towards the outside of the bunch. Vail & Marois (1991) made similar observations with regard to decay development. However, where the berry attachment (pedicel) area is affected by specific growing conditions, increased SO₂ damage could occur. Observations of SO₂ damage occurring mostly at the pedicel end of Thompson Seedless table grapes suggests the importance of these observations (personal communication with J. Fourie, Capespan Technology Development, 1999).

It has been reported that exposure of table grapes to high levels of SO₂ gas during post-harvest cold storage has a negative impact on fruit quality (Marois *et al.*, 1986; Mustonen, 1992; Nelson, 1979). During post-harvest SO₂ gas treatment, the SO₂ gas enters the fruit through lenticels on the berry surface and through lenticels and stomata in the pedicel tissue (Nelson, 1979). Damaged areas, caused by mechanical or pathogenic injury, in the cuticle and pedicel are important pathways for the penetration of SO₂ gas into the grape tissue (Ballinger & Nesbitt, 1984; Mustonen, 1992; Nelson, 1979).

According to observations made by several researchers, absorption of SO₂ gas leads to localised bleaching of the skin tissue (Ballinger & Nesbitt, 1984; Jooste, 1987; Mustonen, 1992; Nelson, 1979). Furthermore, penetration of SO₂ gas into the epidermal cells causes localised bleaching of colour pigments (yellow carotene in the case of white grapes and red anthocyanin, carotene or

chlorophyll in the case of red grapes) that may be present (Nelson, 1979). Data from this study confirmed the findings of other researchers, namely that the severity of SO₂ treatment determines the extent of damage to fruit tissue (Marois *et al.*, 1986; Mustonen, 1992; Nelson, 1979).

In this study LTSEM was used to provide a structural basis to explain the extent of the damage caused to grape tissue by the absorption of SO₂ gas during post-harvest storage of table grapes (Fig. 1). For a better understanding of the changes to the cell wall and intercellular layers that took place, additional observations were conducted using TEM (Fig. 2). While LTSEM and TEM provided different types of information, the observations in each instance tended to correlate with and complement the other.

In the LTSEM images of both the SO₂ damaged and control tissue, essentially all cells on the fractured surface were broken, exposing cross fractures of the walls and internal contents (Fig. 1A and B). In the LTSEM images, taken from tissue samples of control grapes, mesocarp cells had thin, well-defined cell walls, but other cellular components and organelles were difficult to discern (Fig. 1A). The opposite was found for LTSEM images taken of tissue samples of damaged grapes (Fig. 1B). Cell walls of mesocarp cells were observed as thick and incoherent structures, presumably due to the loss of structural unity of the cell walls or by an altered state of hydration caused by the absorption of SO₂ gas (Fig. 1B).

In the TEM images taken from tissue samples of the control grapes, mesocarp cells had a densely stained cell wall and dark stained fibrillar middle lamella (Figs. 2A and C), suggesting greater cell cohesion. The cell membranes of the control fruits were undamaged and fixed to the cell walls (Fig. 2A). In contrast, the SO₂ damaged cells showed breakdown of the middle lamella (Figs. 2B and D) while cell membranes lost structure, showed distension and loosening from the cell walls (Fig. 2B).

Chloroplasts of the control fruits were clearly intact structures (Fig. 2E) while in the SO₂ damaged fruit, chloroplast degeneration was nearly complete,

showing loss of membrane, granal structure and remnants of the thylakoid system (Fig. 2F). No starch granules were observed in the SO₂ damaged fruit, but these were clearly visible in the control fruit, indicating that the chloroplast function was likely unhindered (Fig. 2E).

Observations made from the LTSEM and TEM micrographs in this study revealed that the extent of the damage caused by the absorption of SO₂ gas by the fruit tissue is not limited to localized bleaching of the fruit tissue and colour pigments. There are positive indications that plasmolysis and the loss of cellular fluids had occurred, leading to degradation of cell walls, cell membranes and cellular structures. Variations in cell wall thickness between the undamaged control and the SO₂ damaged samples were observed in both the LTSEM and TEM micrographs. The cell walls of the damaged samples were much thicker, with a total loss in internal structure, compared to the thinner, well defined cell walls of the undamaged control samples. This suggests that the absorption of SO₂ gas by the grape tissue interfered with the structural composition of the intercellular membranes. The changes could also be as a result of the toxicity of SO₂ to the physiological processes in the cell, leading to cell death. Structural effects would therefore be a secondary effect. However, the aim of this study was only to determine the extent of the damage to fruit tissue caused by SO₂ gas and not to determine whether the damage was a primary or secondary effect.

Variations were observed on the LTSEM micrographs between the ice crystal structure found in the samples selected from the berries which were damaged by SO₂ gas (Fig. 1B), as apposed to the samples selected from berries that were not damaged by SO₂ gas (Fig. 1A). Ice crystals from the undamaged samples seemed to have a smoother, more uniform structure (Fig. 1A) compared to the smaller, more diverse structure found with the damaged samples (Fig. 1B). These variations can possibly be attributed to differences in hydration or differences in the composition of the cellular content of the damaged cells as a result of plasmolysis, caused by the absorption of SO₂ by the fruit tissue.

Data obtained in this study using LTSEM and TEM techniques and illustrated in the micrographs show that absorption of SO₂ gas by fruit tissue causes damage to cell walls, cell wall structures and intercellular layers. These results correlate to observations made by McBean *et al.* (1971), namely that during sulphuring fruit tissue softens and plasmolysis occurs. These results also confirm observations found in similar studies by McBean (1976) and Fourie (1996) on other fruit types.

The findings of the present study indicate that damage caused by SO₂ to cell walls and cell wall structures are not limited only to the surface tissue layers. Observations in this study suggest that damage to cell structures is more prominent in the surface layers and that with continual exposure to SO₂ gas the damage will spread to the deeper tissue layers, but to a lesser extent. These observations suggest that penetration of SO₂ gas into grape tissue is mainly due to a mechanism of gaseous diffusion, in correlation with the views expressed in previous reports by various authors (Lagunas-Solar *et al.*, 1992; McBean *et al.*, 1964; Rosselló *et al.*, 1993). Disorganization of tissue by plasmolysis results in the disruption of intercellular pathways and this probably has a blocking effect on the further diffusion and uptake of SO₂ gas by the grape tissue (McBean *et al.*, 1964). This interpretation is supported by the studies of Austin *et al.* (1997), who reported that after an initial uptake, levels of SO₂ residues might remain constant or even slowly start to decline during the latter stages of storage.

Conclusions

The extent of the damage caused by the absorption of SO₂ gas to grape tissue is not simply limited to bleaching of color pigments present in the skin tissue or underlying surface layers. LTSEM and TEM micrographs of areas damaged by SO₂ gas, compared to undamaged areas, revealed that exposure to SO₂ gas led to plasmolysis and the loss of cellular fluids. These observations imply that absorption of SO₂ gas by grape tissue leads to the degradation of cell

walls, cell membranes and cell wall structures. Our view is that damage to these cell components is due to excessive absorption of SO₂ gas and not by natural senescence during storage. Depending on the level of SO₂ exposure or injury, the destructive effects of SO₂ gas are more prominent in the surface tissue layers, although damage caused by SO₂ gas is also visible in the deeper tissue cells, but to a lesser extent.

The presence of certain quality defects found during post-harvest storage of table grapes, such as soft tissue breakdown and berry split viz., destruction of cell walls and cell wall structures, may also be attributed to the absorption of SO₂ gas by the grape tissue. The results recorded were emphasized by using a SO₂ generator that would accentuate SO₂ damage (personal communication with J. Fourie, Capespan Technology Development, 1999).

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Appendix to Chapter 7

For clarity purposes and to simplify the discussion of the results, the figures (Fig. 1 and 2) containing the SEM and TEM micrographs have been included as an "Appendix" here.

Legend to figure 1

Figure 1 LTSEM observations of fractured grape tissue surfaces with and without SO₂ damage. **A:** Fractured mesocarp cells of control with thin, well-defined cell walls (cw). **B:** Fractured mesocarp cells of SO₂ damaged tissue with thick, incoherent cell walls (cw), presumably due to plasmolysis (pl) and the loss of structural integrity. Bars = 10µm.

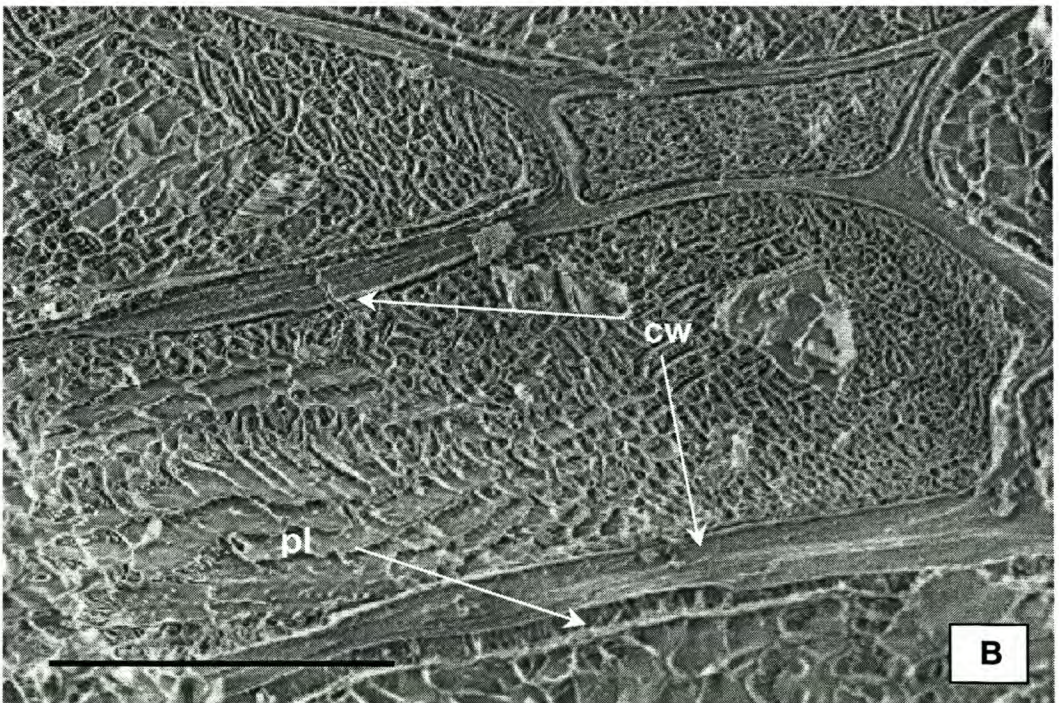
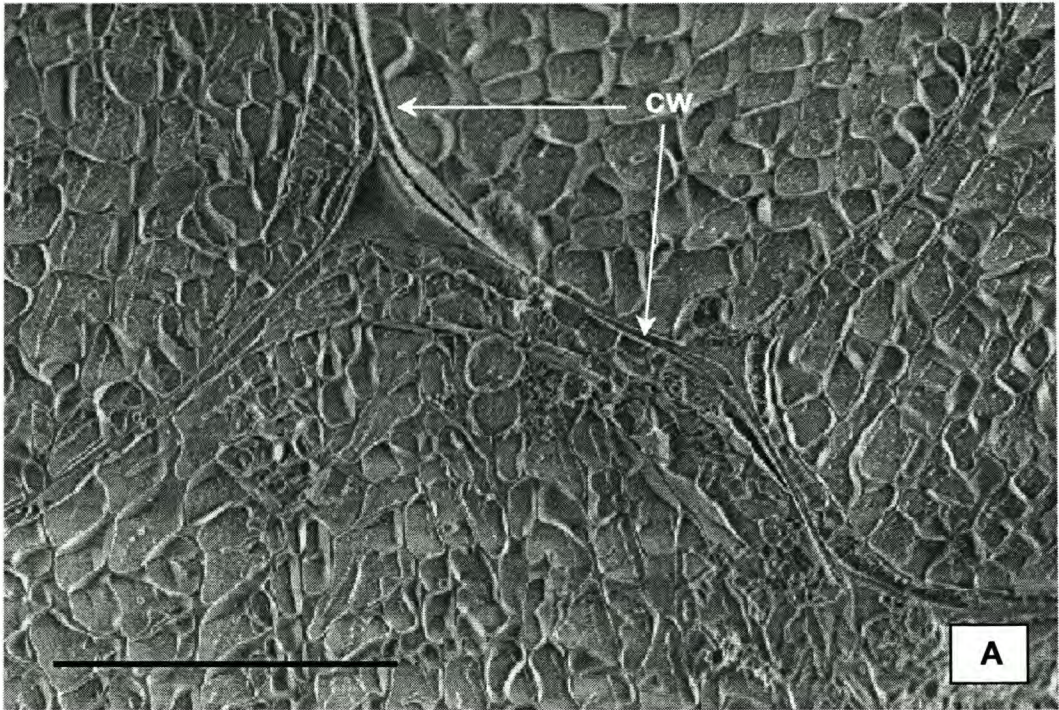


Figure 1

Legend to figure 2

Figure 2 Ultrastructure of cell wall and chloroplasts of grape tissue with and without SO₂ damage as observed by TEM. **A:** Cell walls (cw) of adjacent cells from control tissue showing tightly packed and darkly stained cell membranes (cm), and middle lamella (ml). **B:** Cell walls (cw) from SO₂ damaged grape tissue showing distension and loosening of cell membranes (cm), formation of empty regions (er), and the middle lamella (ml) disintegrates. **C:** Ultrastructure of cell walls (cw) from adjacent cells from control tissue showing darkly stained middle lamella (ml). **D:** Cell wall (cw) of damaged tissue indicating complete degradation of cell wall ultrastructure and disappearance of middle lamella (ml). **E:** Ultrastructural appearance of chloroplast (ch) for control tissue showing perfect thylakoid system (th) and presence of starch granules (s). **F:** Ultrastructural appearance of chloroplasts (ch) from SO₂ damaged tissue showing loss of thylakoid system (th) and disappearance of starch granules. Bars = 1µm.

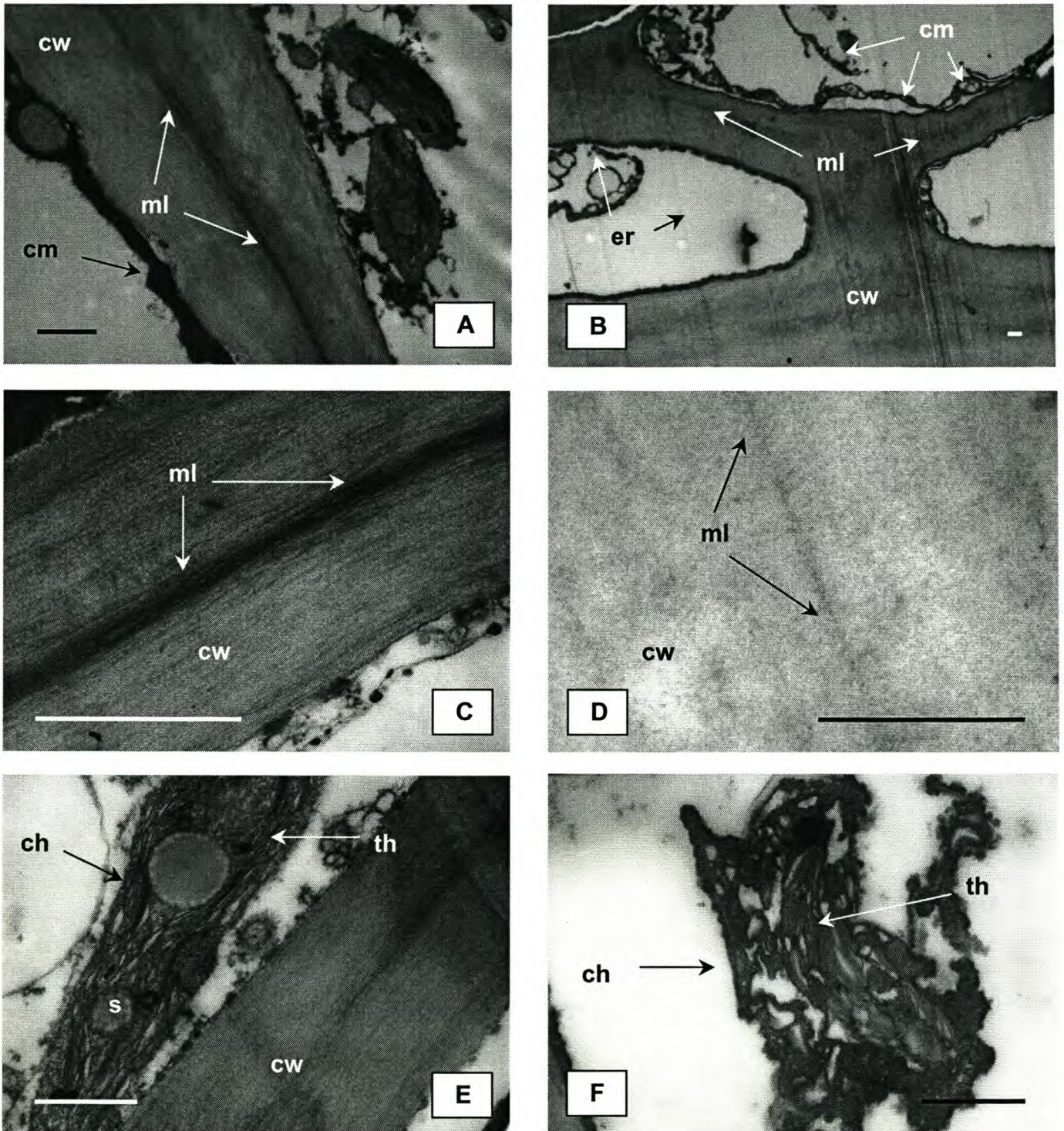


Figure 2

Chapter 8

In-package SO₂ release rate determinations of thin-film, machine manufactured SO₂ sheets

Technology developed in this study is confidential and is protected by patent (South African Patent No. 96-2517, International Patents Pending, Provisional No. ZA 2001/1851).

Abstract

Fumigation with SO₂ gas in combination with cold storage is considered essential to protect table grapes from *Botrytis cinerea* decay during post-harvest storage. Maintaining the correct in-package concentration of SO₂ gas is critical to fruit quality. To determine the in-package SO₂ concentration for various SO₂ sheets, grapes were packed with SO₂ gas generating sheets of varying concentrations SO₂, and the concentrations of SO₂ gas emitted by the sheets were monitored by placing SO₂ gas sensors inside the packaging. The levels of SO₂ gas emitted by the various sheets were recorded. Levels of SO₂ gas released by the polymer SO₂ sheets were dependent on the levels of Na₂S₂O₅ incorporated into the sheets. Higher levels of SO₂ gas were released from polymer sheets which contained higher concentrations Na₂S₂O₅. The release curve for the commercial Uvasys SO₂ sheet was significantly different to that of the polymer sheets.

Introduction

Fumigation with SO₂ gas in combination with cold storage is considered essential to protect fruit from *Botrytis cinerea* decay during post harvest storage (Ballinger & Nesbitt, 1984; Harvey, 1956; Morris *et al.*, 1992; Mustonen, 1992; Nelson, 1979; Winkler & Jacob, 1925). Too low levels of SO₂ gas present during post-harvest storage do not give sufficient protection against *Botrytis cinerea* decay while, on the other hand, too high levels of SO₂ gas will adversely affect the quality of the fruit (Marois *et al.*, 1986; Mustonen, 1992; Nelson, 1979). Obtaining the correct concentration SO₂ gas within the grape package is therefore essential to fruit quality. There is a very fine balance between obtaining the correct concentration of SO₂ gas for maximum decay control, yet low enough to ensure minimum SO₂ damage (Harvey, 1956; Nelson, 1979).

Although the use of SO₂ generating sheets inside the grape package, to deliver constant levels of SO₂ gas during post-harvest storage, has been well documented (Ballinger & Nesbitt, 1982; Morris *et al.*, 1992; Nelson & Ahmedullah, 1976; Perkins-Veazie *et al.*, 1992), there is some uncertainty as to what the optimum release curve for SO₂ should look like. Is the height of the initial peak the main contributing factor, or is it the area under the peak (personal communication with J.F. Fourie, Capespan Technology Development, 2000). Research conducted by Taylor *et al.* (1990) suggested that the optimum release curve for SO₂ would be one where low levels of SO₂ gas are periodically released during storage.

The objective of this chapter was to determine the in-package SO₂ release rates of the thin-film polymer sheets and compare it to the release rate of the commercial Uvasys SO₂ sheet.

Materials and methods

The SO₂ release rates of thin-film polymer SO₂ sheets was studied by packing fresh, export-quality table grapes in cartons and including polymer SO₂ sheets containing various Na₂S₂O₅ concentrations. The commercially available Uvasys SO₂ sheet was used as reference.

Packaging and SO₂ treatment

Fresh, unblemished Barlinka table grapes were packaged in corrugated cardboard cartons, as for export, in non-perforated bags. Individual bunches were packaged in carry bags. The packed grapes were kept in cold storage for the duration of the release rate measurements.

The various SO₂ generators that were tested and compared during this trial were:

- i) Sheet 1*, new polymer sheet of 13% sodium metabisulphite content (abbreviated, henceforth, as 13% polymer sheet);
- ii) Sheet 2*, new polymer sheet of 15% sodium metabisulphite content (abbreviated, henceforth, as 15% polymer sheet); and
- iii) Uvasys SO₂ sheet (Grapetek, South Africa).

**These are experimental sheets manufactured by the University of Stellenbosch in conjunction with the South African Deciduous Fruit Producers Trust.*

The polymer SO₂ gas generating sheets were manufactured on the pilot scale production plant. Manufacturing of the SO₂ gas generating sheets commences with the preparation of a PVC plastisol mixture. Once the desired plastisol mixture is obtained, it is applied onto a carrier material by means of a nozzle. The carrier material with the plastisol deposits is sent through a series of curing ovens in which the plastisol is cured to a solid state. Once cured, a

second layer of non-woven material is laminated onto the carrier layer to enclose the PVC matrix between two sheets of material. The product is rapidly cooled down before it is cut to the desired specifications. The finished product is inspected for deviations in quality before it is packaged into poly(ethylene) bags, ready for use.

Monitoring of in-package SO₂ concentration

The in-package SO₂ concentration was monitored constantly, throughout the storage period, using Sensor Stik[®] (Exidene Instrumentation Technologies) electrochemical gas sensors. Continuous measurements were taken by placing the sensors amongst the fruit, towards the middle of the box prior to its closure. A small hole was cut into the side of the plastic liner through which the wire protruded. Once the sensor and wire were in position, the hole in the plastic liner was sealed with adhesive tape. The SO₂ sheets were placed into position before closing the cartons. The sensors were connected to a data logger and the cartons were placed in the cold storage rooms.

The signals from the sensors are transferred, by wire, to the data acquisition equipment (Data Taker[®] DT 500, Measurement and Control Solutions) where the signals are amplified, measured, processed and stored. Stored data is then transferred, either at intervals or continuously, to a computer where data can be displayed or manipulated with software programmes.

Results and discussion

SO₂ gas release rates for the various sheets (13% and 15% polymer sheets, and the Uvasys SO₂ sheet) were recorded by SO₂ gas sensors for 40 days at -0.5°C. Results are shown in Figures 1 to 5. The sensors used have

a sensitivity factor of 95%, therefore, allowing a 5% error in the reading. For clarity purposes and to simplify the discussion of the results, the figures containing the SO₂ release rates (Figs. 1 to 5) have been included as an “Appendix” at the end of this chapter.

The levels of SO₂ gas released by the 13% polymer sheet are depicted in Figure 1. The 13% polymer sheet responded quickly after closure. A peak of SO₂ gas, approximately 50 ppm, was released within hours after closure. The level of SO₂ gas decreased within 24 h to a level of between 5 to 15 ppm, where it remained for approximately 240 h (10 d). No SO₂ was detected by the sensors after approximately 480 h (20 d).

The levels of SO₂ gas released by the 15% polymer sheet are depicted in Figure 2. The 15% polymer sheet released a SO₂ gas peak of approximately 80 ppm. The maximum level was reached after approximately 48 h. The level of SO₂ gas decreased slowly to a level of approximately 20 ppm after 5 d. For the remainder of the storage period varying levels of between 0 to 10 ppm SO₂ were detected by the sensors.

When the SO₂ release rate of the 15% polymer sheet was compared to that of the 13% polymer sheet (Fig. 3), it was noted that higher levels of SO₂ gas were recorded for the 15% polymer sheet throughout the storage period. The levels of the initial peak of SO₂ gas were higher and, more significantly, remained at a higher level for a longer period of time (wider peak). The higher levels of SO₂ damage recorded by the 15% polymer sheet (see chapter 5) can be attributed to this.

The levels of SO₂ gas released by the Uvasys sheet are depicted in Figure 4. A high peak of SO₂ gas of approximately 175 ppm was released within the first hour after closure, but it decreased to zero within 24 h. No further levels of SO₂ gas release were detected by the sensors.

Compared to the Uvasys sheet (Fig. 5), both of the polymer sheets tested released SO₂ gas for a longer period of time than the Uvasys sheet did. The initial peak of SO₂ gas released by the Uvasys sheet was, however, much higher (and for a shorter period of time) than the peak released by any of the other polymer sheets. The higher levels of SO₂ damage recorded in previous

chapters for the polymer sheets containing higher SO₂ concentrations (15% and higher) compared to the Uvasys sheet, can most likely be attributed to the higher levels of SO₂ gas released for longer periods of time.

When the release rates for the thin-film sheets (Figs. 1 to 3) were compared to results of similar studies done by the author on the hand-made sheets (Opperman, 1995), higher levels of SO₂ gas were released by the thin-film sheets during the early stages of storage. Although the thin-film sheets had a higher initial peak, the hand-made sheets released higher levels of SO₂ gas during the latter stages of storage. This confirms the findings mentioned in previous chapters that lower concentrations of SO₂ need to be incorporated into the thin-film sheets, compared to the hand-made sheets, due to better release characteristics of the thin-film polymer sheets.

Conclusions

Increasing the concentration of Na₂S₂O₅ incorporated into the thin-film sheets has a significant effect on the level and the duration of SO₂ gas released.

Higher levels of SO₂ gas are released for longer periods of time when a polymer sheet of higher SO₂ concentration is used.

The Uvasys sheet releases a high SO₂ peak for a short period of time, while the polymer sheets generally release lower, but wider, peaks, followed by a steady low level release of SO₂.

The higher levels of SO₂ damage recorded with polymer sheets containing higher SO₂ concentrations (15% and higher) in previous chapters can probably be attributed to the longer exposure of the grapes to relatively high levels of SO₂ gas.

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Appendix to Chapter 8

For clarity purposes and to simplify the discussion of the results, the figures (Figs. 1 to 5) containing the SO₂ release rates have been included as an "Appendix" here.

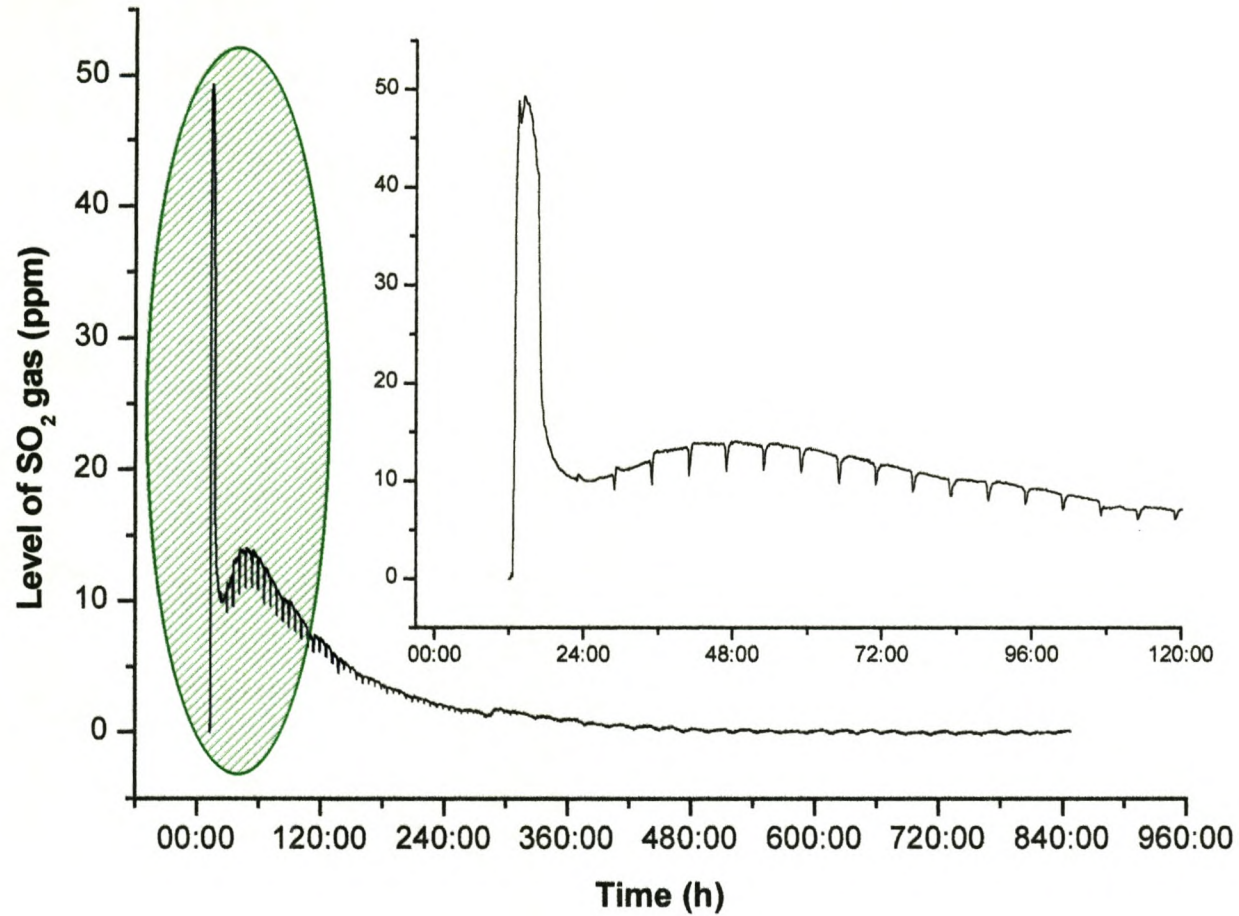


Figure 1 SO₂ gas release rate of the 13% polymer sheet as recorded by SO₂ gas sensors for 40 days (960 h) at -0.5 °C. Inset graph represents expanded view of shaded area, for the first five days (120 h) at -0.5 °C.

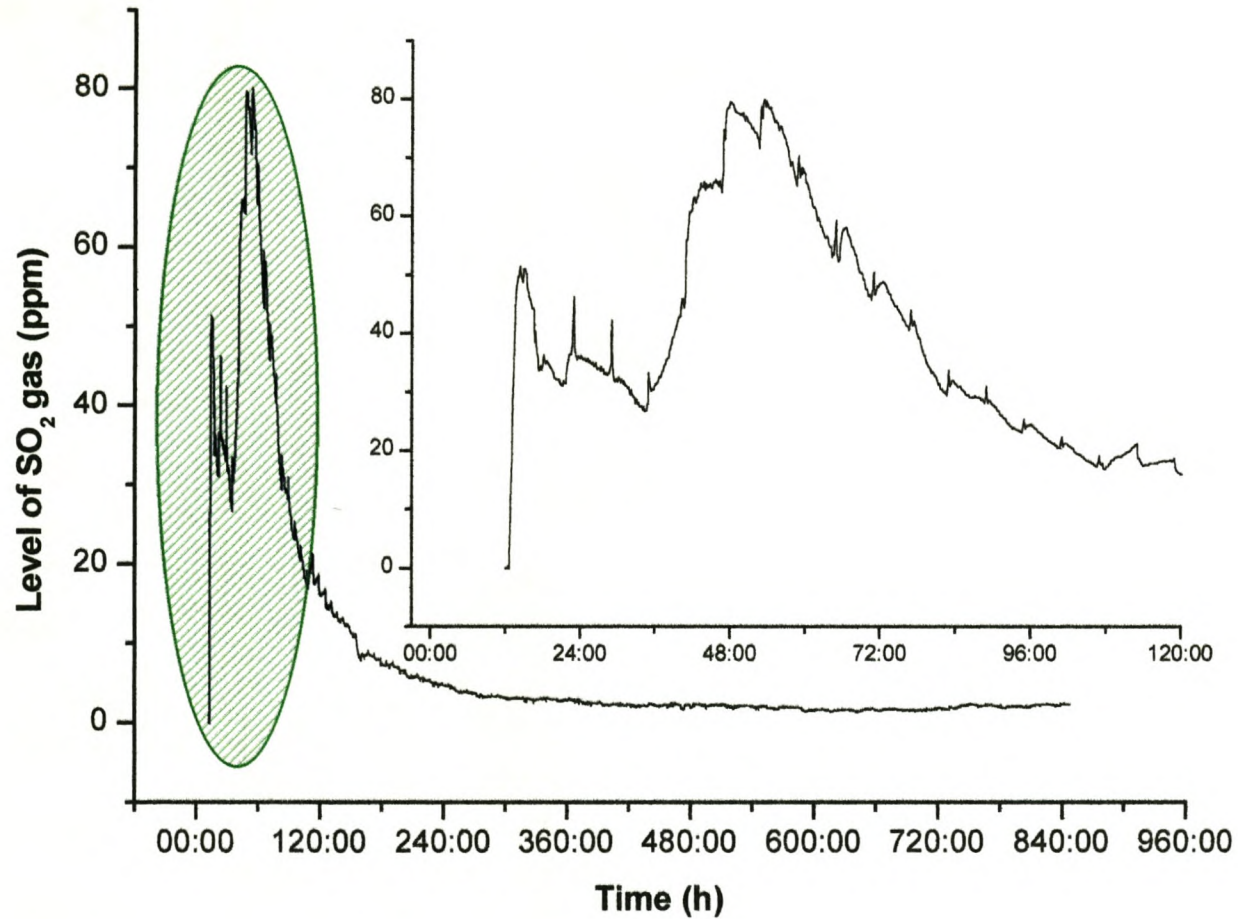


Figure 2 SO₂ gas release rate of the 15% polymer sheet as recorded by SO₂ gas sensors for 40 days (960 h) at -0.5 °C. Inset graph represents expanded view of shaded area, for the first five days (120 h) at -0.5 °C.

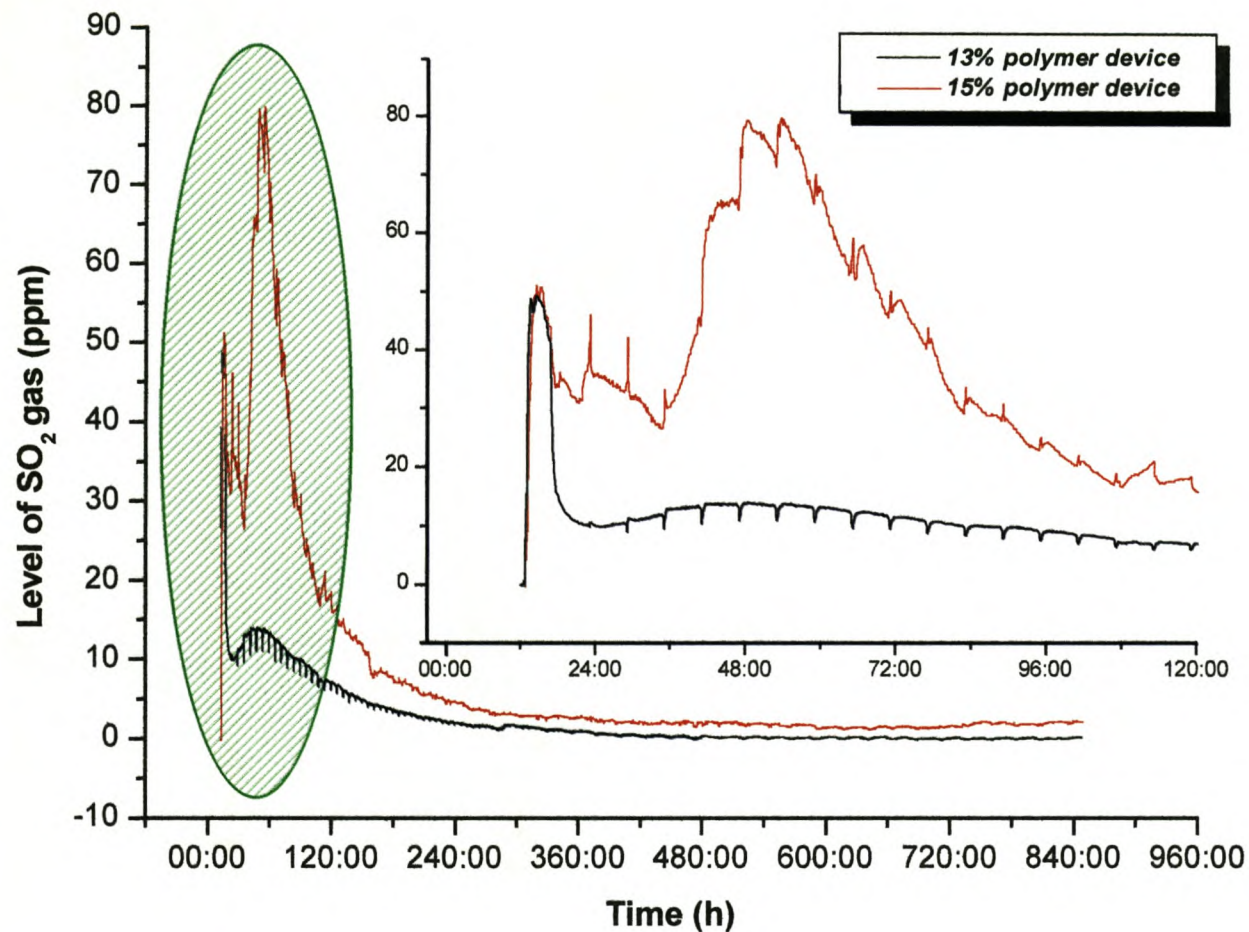


Figure 3 SO₂ gas release rates of the 13% and 15% polymer sheets as recorded by SO₂ gas sensors for 40 days (960 h) at -0.5 °C. Inset graph represents expanded view of shaded area, for the first five days (120 h) at -0.5 °C.

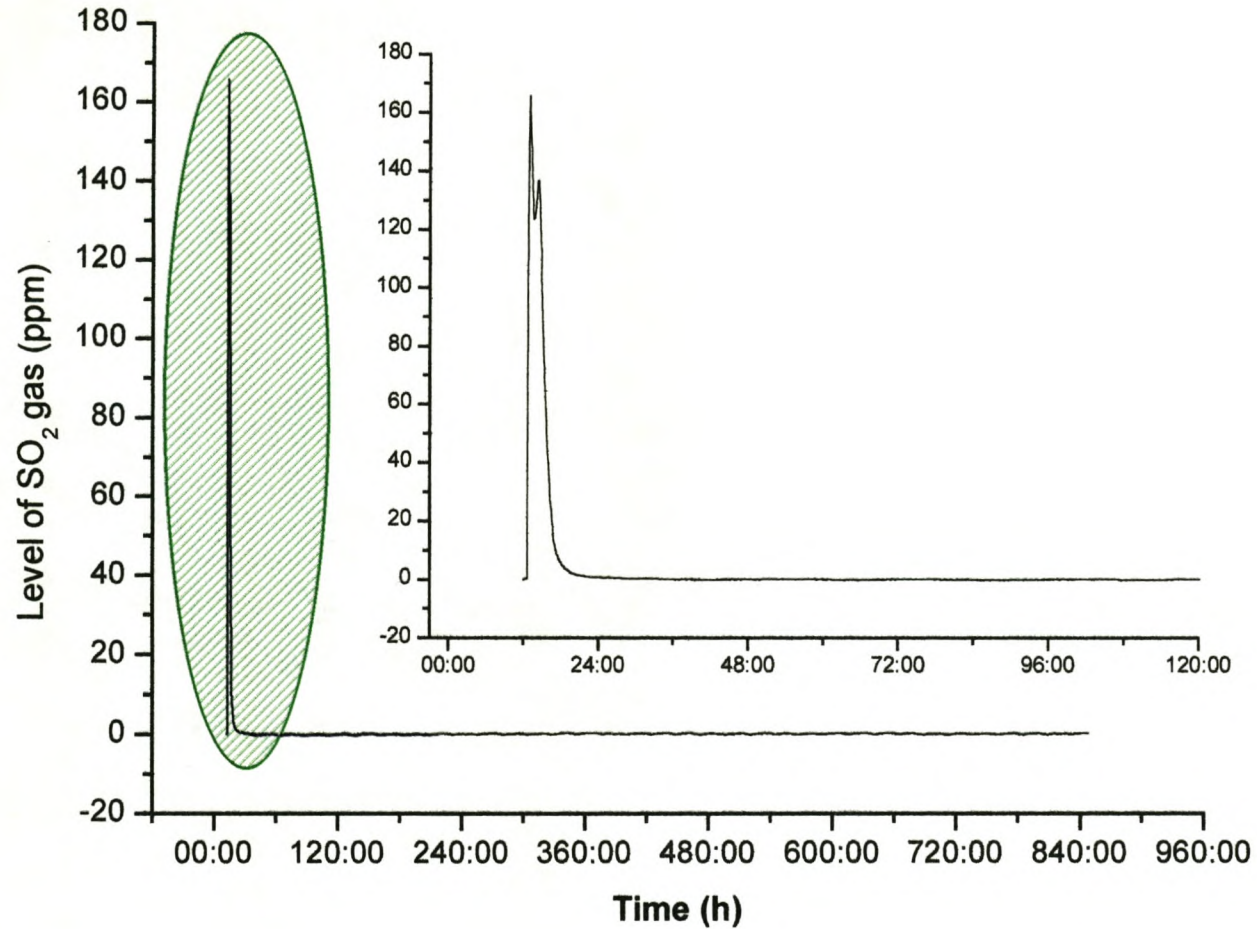


Figure 4 SO₂ gas release rate of Uvasys sheet as recorded by SO₂ gas sensors for 40 days (960 h) at -0.5 °C. Inset graph represents expanded view of shaded area, for the first five days (120 h) at -0.5 °C.

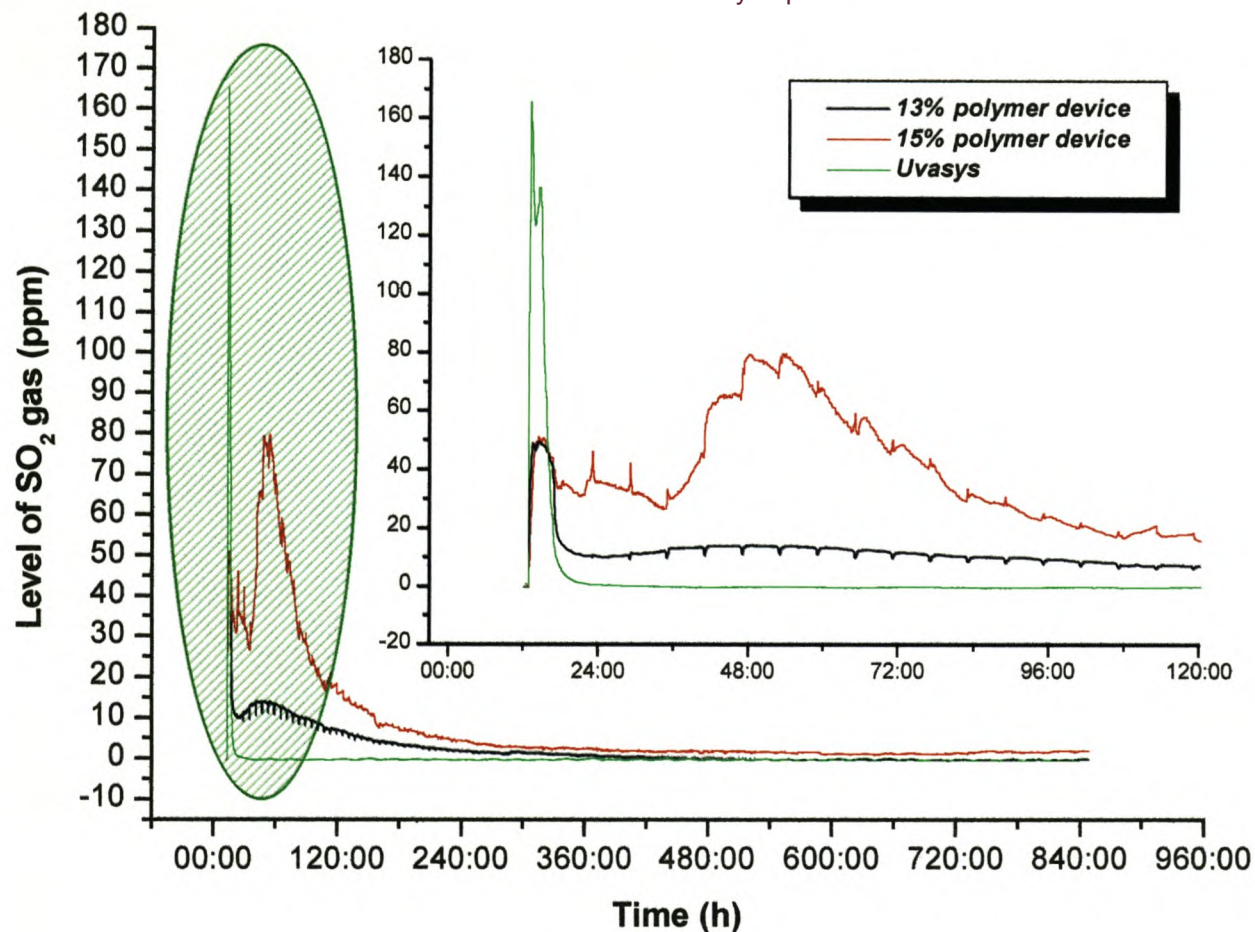


Figure 5 SO₂ gas release rates of the 13% and 15% polymer sheets compared to that of the Uvasys sheet as recorded by SO₂ gas sensors for 40 days (960 h) at -0.5 °C. Inset graph represents expanded view of shaded area, for the first five days (120 h) at -0.5 °C.

Chapter 9

Construction of a pilot-scale production plant for the manufacture of slow-release SO₂ gas sheets

This process by which the polymer SO₂ gas sheets were prepared, was designed as a pilot scale production plant. In practical terms it is impossible to reflect on all development work conducted during the course of the project, hence, only the end result is discussed here. Development work is still being conducted to optimise key areas of the production plant. Changes are being made to the design of the pilot plant as new ideas and technologies become available.

Technology developed in this study is confidential and is protected by patent (South African Patent No. 96-2517, International Patents Pending, Provisional No. ZA 2001/1851).

Introduction

A new sheet for the in-package generation of SO₂ gas was developed for the table grape industry. The sheet consisted of active SO₂ particles (in the form of Na₂S₂O₅) dispersed evenly throughout a polymer matrix, releasing SO₂ gas based on a monolithic type release mechanism. The rate at which SO₂ gas was released from the polymer matrix was subject to the reaction of the active SO₂ particles with moisture and the ease with which the formed SO₂ gas can escape from the polymer matrix. The SO₂ gas release rate could therefore be manipulated by changing the composition of the polymer matrix, by making it more, or less, accessible to moisture.

Initially the sheets were manufactured individually, by hand, in the laboratory, according to a batch process documented by the author as part of his M.Sc. thesis (1995). Following the successful earlier testing of the hand-manufactured SO₂ gas sheets (Opperman *et al*, 1999) attention turned to investigating the possibilities of upscaling the research project into a commercial venture. Testing a wider range of table grape varieties and in larger volumes than was the case with the hand-manufactured sheets, had therefore to be conducted. Results obtained from these tests should indicate whether the new gas sheets would perform as well as the commercially available gas sheets. To manufacture the larger volume of sheets needed for conducting more extensive trials, a pilot-scale manufacturing plant had to be designed and built.

The greatest challenge in building the pilot plant was to take the manual manufacturing process from the laboratory as a starting point and then to design a process and production plant from which the sheets could be manufactured continuously. Proper quality assurance mechanisms had to be installed to ensure that every sheet manufactured by the mechanical coating process was of the highest quality, had the same release characteristics as the hand-manufactured sheets and was aesthetically acceptable to the table grape industry clients.

The coating technology on which the hand-manufactured sheets was based is widely used in the plastics industry. Examples of similar coating processes can be found in lamination and coating plants (Vynide Pty(Ltd.)). The implementation of existing coating technology for the purpose of the slow release of SO₂ gas had however never been attempted before. No existing process or plant could therefore be referred to. The majority of components for the pilot-scale production plant had therefore to be modified, designed or built ab initio. The unique properties of some of the raw materials and strict quality requirements of the final product necessitated that existing standard industrial manufacturing practises had to be modified to suit specific needs.

The process that was subsequently developed for manufacturing the SO₂ gas sheets on the pilot scale production plant is schematically presented in Figure 1.

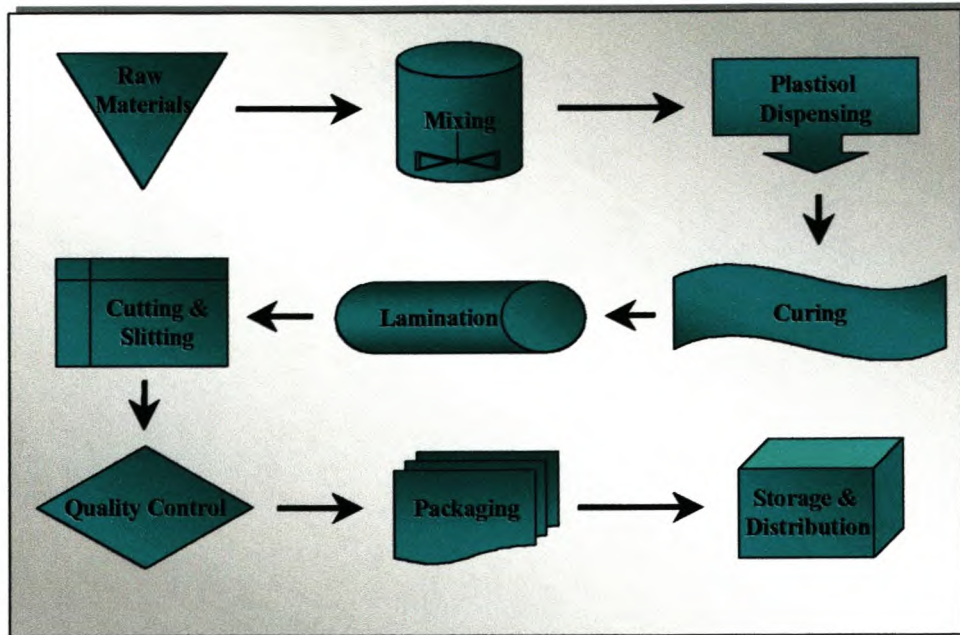


Figure 1 Schematic flow diagram of all the key aspects of the pilot scale production process for the manufacture of SO₂ gas sheets.

Although the production process consists of nine steps, as illustrated in Figure 1, in order to discuss the manufacturing process of the SO₂ gas sheets, the process will be arranged into three stages:

- i) Selection and acquiring of suitable raw materials, followed by the preparation and handling of the plastisol mixture;
- ii) Application of the prepared plastisol mixture onto a carrier material, curing of the plastisol mixture to a solid state, lamination of a non-woven material onto the carrier layer to enclose the PVC matrix, followed by a cooling section where the product is rapidly cooled down before it is cut to the desired specifications; and
- iii) Quality control, packaging, storage and distribution.

Stage 1: Plastisol preparation and handling

a). Handling and storage of raw materials

Purchasing of raw materials was conducted in such a manner as to ensure that all raw and packaging materials were of an agreed quality and were procured at quantities required to ensure uninterrupted production for trial purposes. All raw materials were accompanied with the relevant documentation on delivery, to ensure quality conformance.

Raw materials were stored and handled in such a way as to prevent any damage to the packaging and/or the product. Raw materials were used on a first-in-first-out basis and materials of which the packaging was damaged were not used.

b). Mixing process

The objective of mixing is to increase the probability of finding a particle of any one of the ingredients within any small sample of the total mass. The degree of mixing is essential to the quality of the final product. Inadequate mixing will result in variations in quality that may include variations in colour, porosity and changes in physical properties such as tensile strength and hardness (Roesler & Metz, 1977).

The task of selecting a mixer to suit specific needs can be very demanding, as there is a wide selection of mixer types to prepare plastisols available to industry. Some conventional paste mixers are dough mixers, ribbon blenders, disc impellers, and high-intensity mixers (Park, 1977).

The mixer selected to do the mixing for this pilot plant was a Dynaflo paste mixer with a mixing capacity of 500 litres (Fig. 2). This is a combination top-entry mixer fitted with both a variable speed paddle and a variable speed high shear blade.

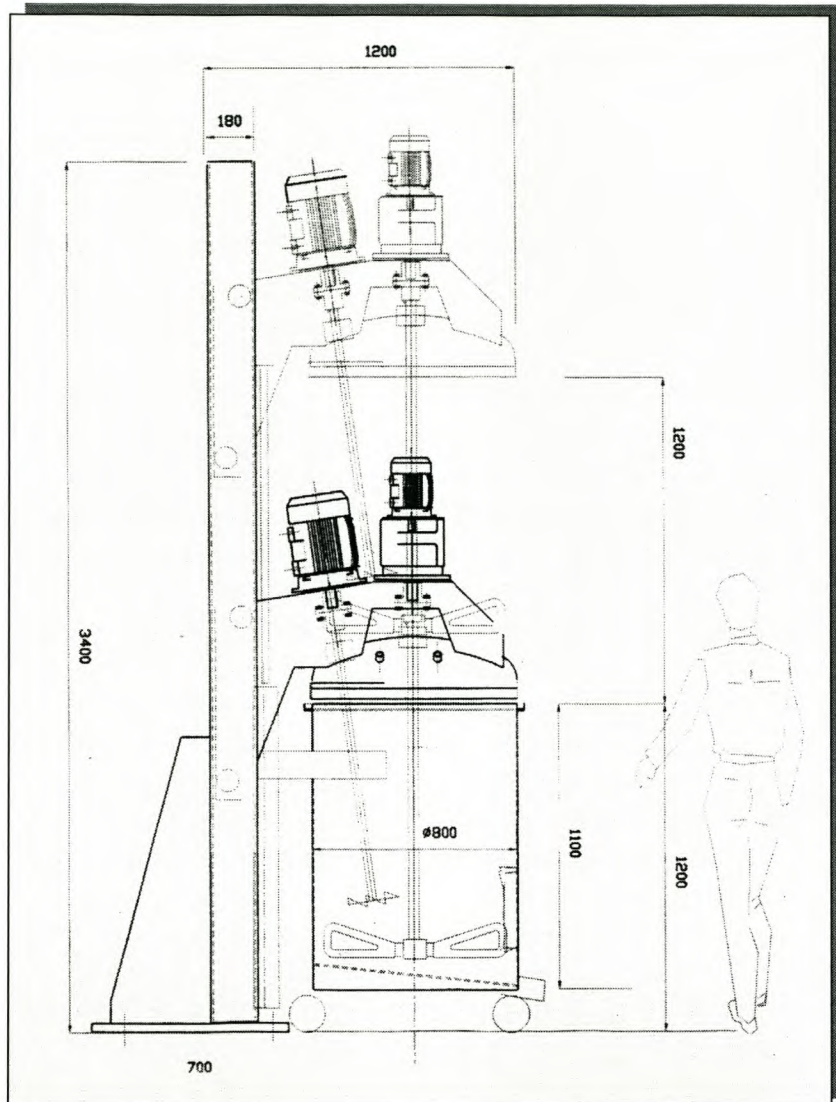


Figure 2 Drawing of the Dynaflo paste mixer with 500 litre mixing pot (Drawing courtesy of Cape Industrial Trading).

Both the variable speed drives and gearboxes are mounted onto the lid of the mixing pot, using double cartridge seals to enable a vacuum to be drawn during the mixing process. The entire assembly containing the lid, seals and fittings, drives and gearboxes, and shafts with mixing blades, are fitted to a frame and can be lifted up and down by way of a rack and pinion system.

The mixing process for this plant is based on a batch system. All the plastisol components are individually weighed and loaded into the mixing pot. Proper quality control measures ensure that the consistency of the plastisol mixture remains the same for every batch. After verifying that all the plastisol components are present in the mixing pot, it is taken to the mixer and secured in the correct position before the lid and mixing blades are lowered into position. A vacuum is drawn on the inside of the mixing pot to ensure that no air bubbles are trapped in the plastisol mixture during the mixing process. The timer is set to the desired mixing time and the mixer is switched on. On completion of the mixing process, the vacuum is broken and the lid and mixing blades are hoisted to the top position. The mixing pot is removed from its attachments to the mixer, taken to the machine where the mixing pot is directly coupled to the dispensing system by means of a valve located at the bottom of the mixing pot. To alleviate handling and mobility of the 500 litre mixing pot, it was fitted with heavy-duty castors.

c). Plastisol storage and handling

Plastisols are generally easy to work with but may vary greatly in viscosity and viscosity stability. The most important factors affecting viscosity and viscosity stability are, particle size, particle size distribution, and shear rate (Davis, 1977). The addition of sodium metabisulphite crystals, which is the active reagent for the release of SO₂ gas, to the plastisol significantly increases the viscosity. The hygroscopic sodium metabisulphite crystals may react with moisture in the air and release SO₂ prematurely. Thus, from a quality perspective, it is essential to protect the plastisol mixture from coming into contact with moisture.

To alleviate the factors that can be detrimental to the quality of the final product, the plastisol storage reservoir, dispensing head and circulation pump were designed to form a closed system (Fig. 3). This effectively isolates the plastisol mixture from moisture and keeps it in motion, to keep the

viscosity constant and to keep the sodium metabisulphite crystals dispersed evenly throughout the mixture.

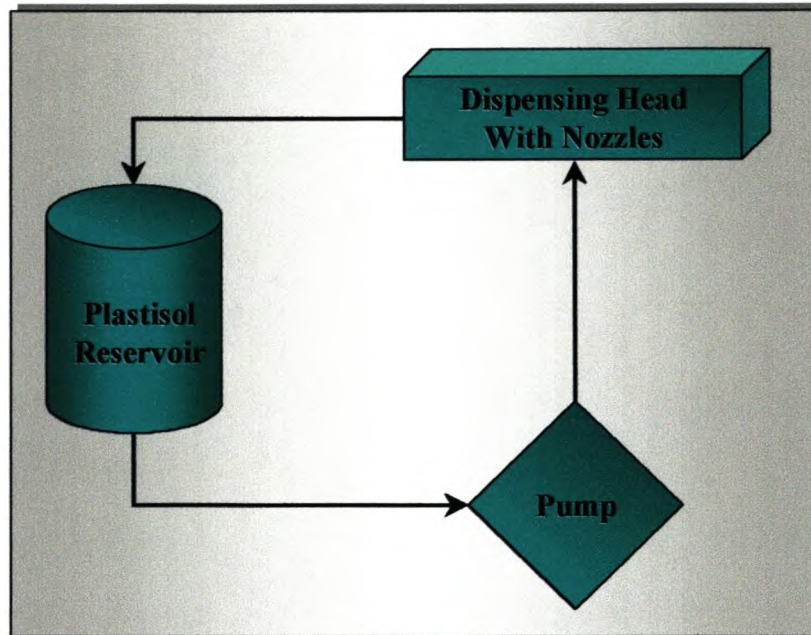


Figure 3 Schematic diagram of the plastisol storage and handling system.

The plastisol storage and dispensing system consists of a plastisol storage reservoir, a circulation pump (Flexopal FP 100), and the dispensing head with nozzles. All the components are coupled to each other by 32 mm plastic hosing. Threaded stainless steel fittings were used to connect the hosing to the individual components. The dispensing head is also fitted with a pressure transducer, coupled to the control program, to monitor the pressure inside the closed system.

Stage 2: Pilot-scale SO₂ gas sheet production plant

The pilot scale production plant, depicted in Figure 4, consists of a paper wind-off and tension control system, a curing tunnel consisting of four infrared curing ovens, a cloth wind-off and lamination system, a cooling section and a cutting and slitting system. A reel cylinder, driven by a master motor, is used to provide a wide range of constant running speeds of the carrier sheet. At the other end, a slave motor with a brake, coupled to the carrier tension sensor, controls the take off speed to maintain a constant carrier tension throughout the manufacturing process. The exposure distance between the infrared emitter surface and the paper surface can be adjusted to optimise the heat transfer from the infrared emitters to the plastisol mixture.

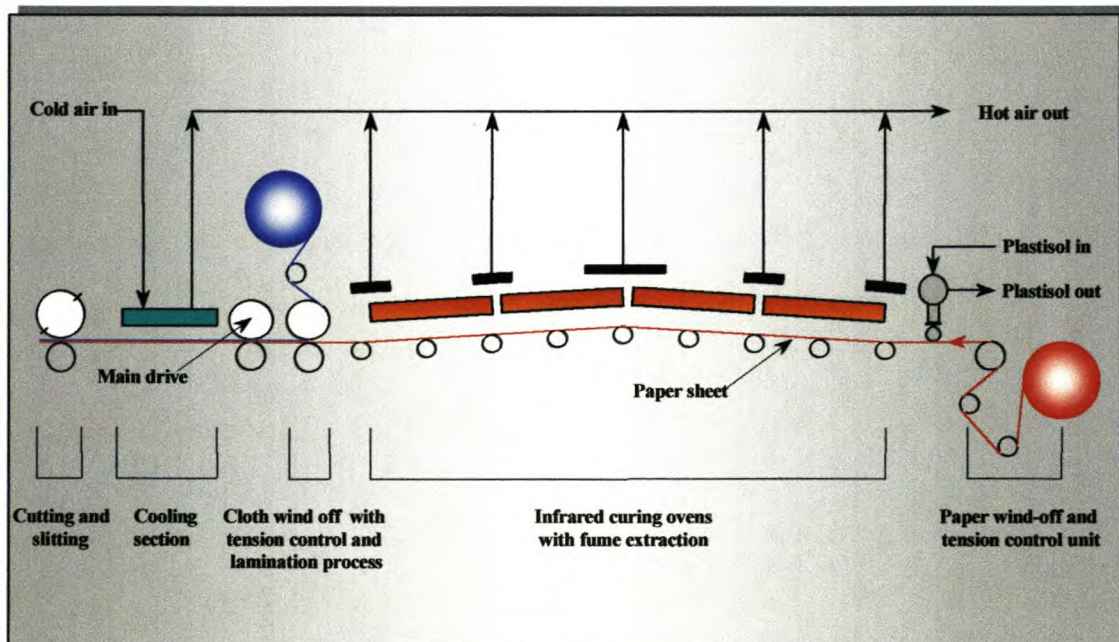


Figure 4 Schematic diagram depicting the layout of the pilot scale SO₂ gas sheet production plant.

a). Carrier-material wind-off and tension control unit

The unit consists of the wind-off frame with the carrier-material roll fitted onto an air shaft (Fig. 5). The wind-off frame is constructed from 12 mm steel plating, to ensure rigidity. The air shaft fits into two safety chucks that prevent the air shaft from moving sideways, aiding in the alignment of the carrier through the machine. One of the safety chucks is connected to a pneumatic brake, which is controlled by a current to pressure converter (I to p converter). The I to p converter receives a signal from the load cell, detecting the tension between the coating roller and the pneumatic brake. The desired tension can be set on a control panel and a programmable logic controller (plc) will ensure that the set value is maintained. Upper and lower limits can also be programmed into the control system.

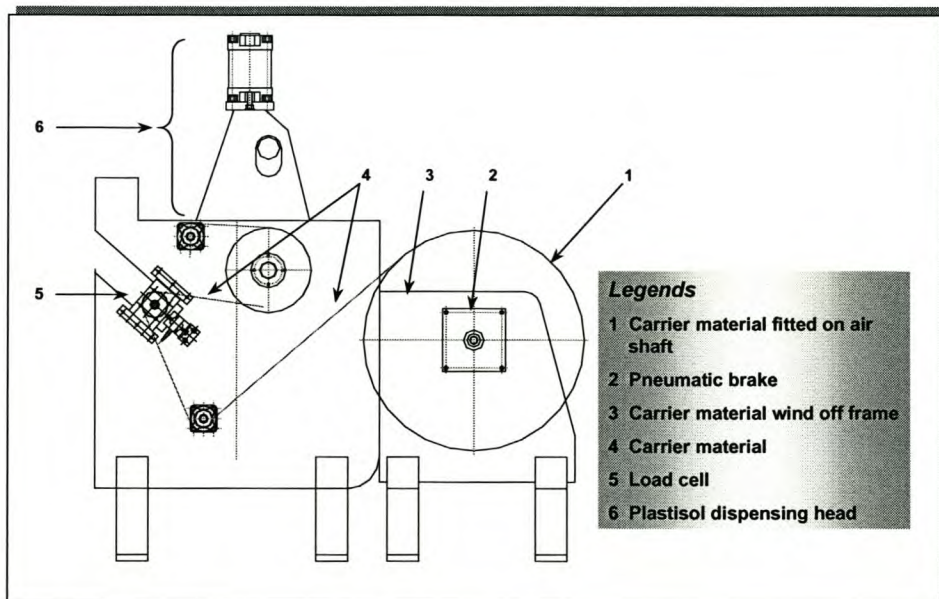


Figure 5 Side-view drawing of the carrier material wind-off and tension control unit (drawing courtesy of C. Butcher, B+ Projects).

The purpose of the carrier-material wind-off and tension control unit is twofold. Firstly, it feeds the carrier material into the machine and ensures that it stays aligned during the course of the manufacturing process. Secondly, it places tension onto the carrier material between the coating roller and the wind-off roller. This reduces the amount of creasing and shrinkage of the carrier material caused by the increase in temperature as the carrier material with the plastisol deposits move through the curing ovens.

b). Plastisol dispensing system

From an aesthetic and quality perspective, the application of the plastisol mixture onto the paper can be considered as the most important action. This will not only determine the physical appearance of the sheet, but will also ultimately determine the SO₂ gas release characteristics. The plastisol must be applied in such a manner that each deposit on the paper is symmetric in shape, of uniform thickness, and of the same volume.

None of the common plastisol application systems used by industry, e.g. dipcoating, spraying, spreading knife or calendering, were suitable for producing the particular design of the final product. This was due to specific design requirements of the final product, the high viscosity and high crystalline content, due to the addition of sodium metabisulphite crystals of the plastisol mixture.

Moving parts, thin diameter piping, sharp edges and bends, all had to be kept to a minimum as a hard, crystal-like deposit formed around these specific areas after continuous pumping of the plastisol mixture. The deposit that formed was probably a mixture of sodium metabisulphite crystals and PVC powder. Deposit forming was accelerated when the pressure was increased and in severe cases resulted in the blockation of non-return valves, fittings and pipes. Since no commercially available system could be used for this specific application, a dispensing system in which all these factors were eliminated, aiding deposit forming, had to be designed and built.

To facilitate maintenance and to reduce down time on the plant the dispensing head was designed in such a manner that the nozzle was easily accessible and could be removed separately without having to stop the production process.

The dispensing head consists of a manifold containing nine outlets leading to the nozzle which is mounted on a moveable frame (Fig. 6). The manifold consists of a 50 mm inside diameter stainless steel pipe with a length of 900 mm, fitted with threaded stainless steel fittings at both ends. The latter facilitate easy connection to the pipes from the circulation pump and to the storage reservoir. To monitor the dispensing pressure the manifold is fitted with a pressure gauge and a pressure transducer coupled to the control computer. The manifold contained nine outlets leading to the nozzle. Nozzle inlet pipes simply slide into the manifold fittings. Leakage of the plastisol mixture from the inlet pipes is prevented by the use of o-rings.

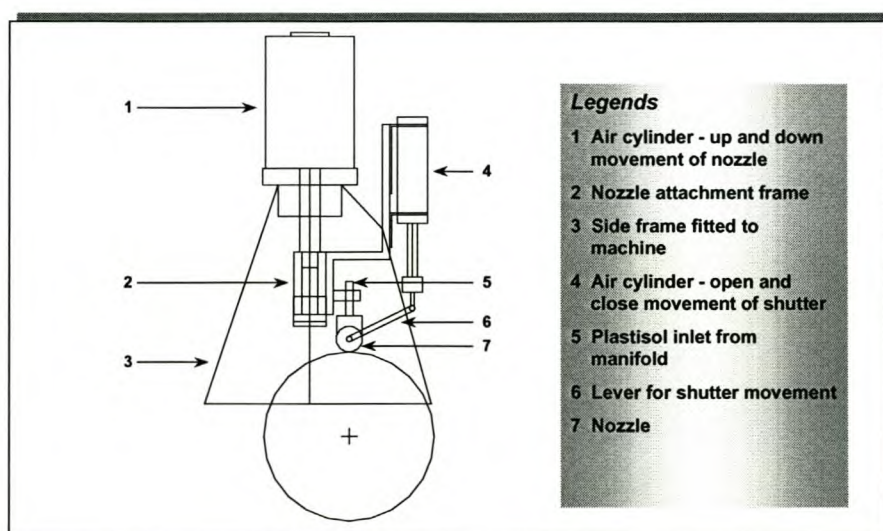


Figure 6 Side-view drawing depicting the assembly of the dispensing head for dispensing the plastisol and sodium metabisulphite mixture onto the paper (drawing courtesy of J.J. vd. Westhuizen, Stellenbosch University).

The nozzle consists of a stainless steel inlet pipe, leading from the manifold to a stainless steel housing containing a shutter with a lever connected to the shutter at one end and to the frame at the other end for opening and closing of the shutter. The shutter fits tightly into the housing and operates on the same principle as a gate valve.

At the bottom end, opposite the inlet pipe, the surface is milled flat. The flat surface contains a single row of holes, 2 mm in diameter, through which the plastisol mixture is deposited onto the paper sheet.

The nozzle is mounted onto a frame by way of a clamping system that allows for height adjustments to the nozzle. This height adjustment will determine the distance of each nozzle from the surface of the carrier during the deposition process, which in turn determines the thickness of the layer of plastisol mixture that is deposited. The downward movement of the frame, with the nozzles connected to a lever system, triggers the depositing process. The downward movement causes the lever system to turn the shutter to the open position, allowing the plastisol mixture to flow through the openings in the housing. Moving the frame upwards will turn the shutter to the closed position, stopping the flow of the plastisol mixture. The movement of the frame is created by a pneumatic cylinder and the control computer controls the sequence of the process by sending a signal through a plc controller to a solenoid valve.

c). Infrared curing process

Plastisols convert to a solid and useful state with the application of heat in the 140 to 200°C range. As the temperature of the PVC plastisol is increased from room temperature, plasticiser begins to penetrate each resin particle. When the resin takes up all the plasticiser, the system loses its fluidity. This is referred to as the gelled state. At this stage the gelled mass has no cohesive strength. As heat continues to penetrate the system the polymer starts dissolving in the plasticiser until all the polymer chains are dissolved uniformly in the plasticiser. This is the fused state, which when

cooled provides a plastic material of considerable strength and utility (Werner, 1977).

Initially, conventional convection ovens were used to heat the plastisol mixture to achieve fusion. However, use of convection ovens for this application presented severe limitations regarding production volumes. This is in conjunction with findings in the paper industry where the need for faster drying rates, and, thereby, faster machine speeds, led to the use of infrared drying (Closset, 1986; Stephansen, 1985).

Besides the increases in production rates, the use of infrared heaters for drying paper coatings significantly improved the quality of the coated surfaces. Faster curing times can be attributed to the higher penetration of the coating and the substrate by infrared, producing a more uniform temperature distribution in the product compared to convection heat where heat transport from the surface into the product relies upon conduction, which is much slower process. Other advantages associated with the use of infrared heating instead of convection heating are related to the small size and ease of installation of the infrared heaters compared to convection ovens (Stephansen, 1985).

Extensive testing was conducted, in conjunction with Industrelek, on a smaller, temporary plant, to establish the efficacy of infrared heating as a method for curing this specific plastisol sodium metabisulphite mixture. Important parameters that needed to be determined were the exact energy requirements and exposure time to achieve curing. The results obtained from these tests proved conclusively that the desired level of curing could be achieved by using infrared heating, at significantly shorter exposure times, than those of conventional convection heating. Conclusions made regarding energy requirements and curing times contributed to making accurate estimations of the specific requirements needed for the design and manufacture of the infrared curing tunnel.

The electric infrared heater units consist of 27 ceramic Elstein FSR infrared emitters, mounted onto anodised aluminium reflectors (Fig. 7). The infrared emitters are mounted into a steel housing with cooling fans.

Externally, the outer housing of each unit measures 1050 mm wide by 1050 mm long by 135 mm in height.

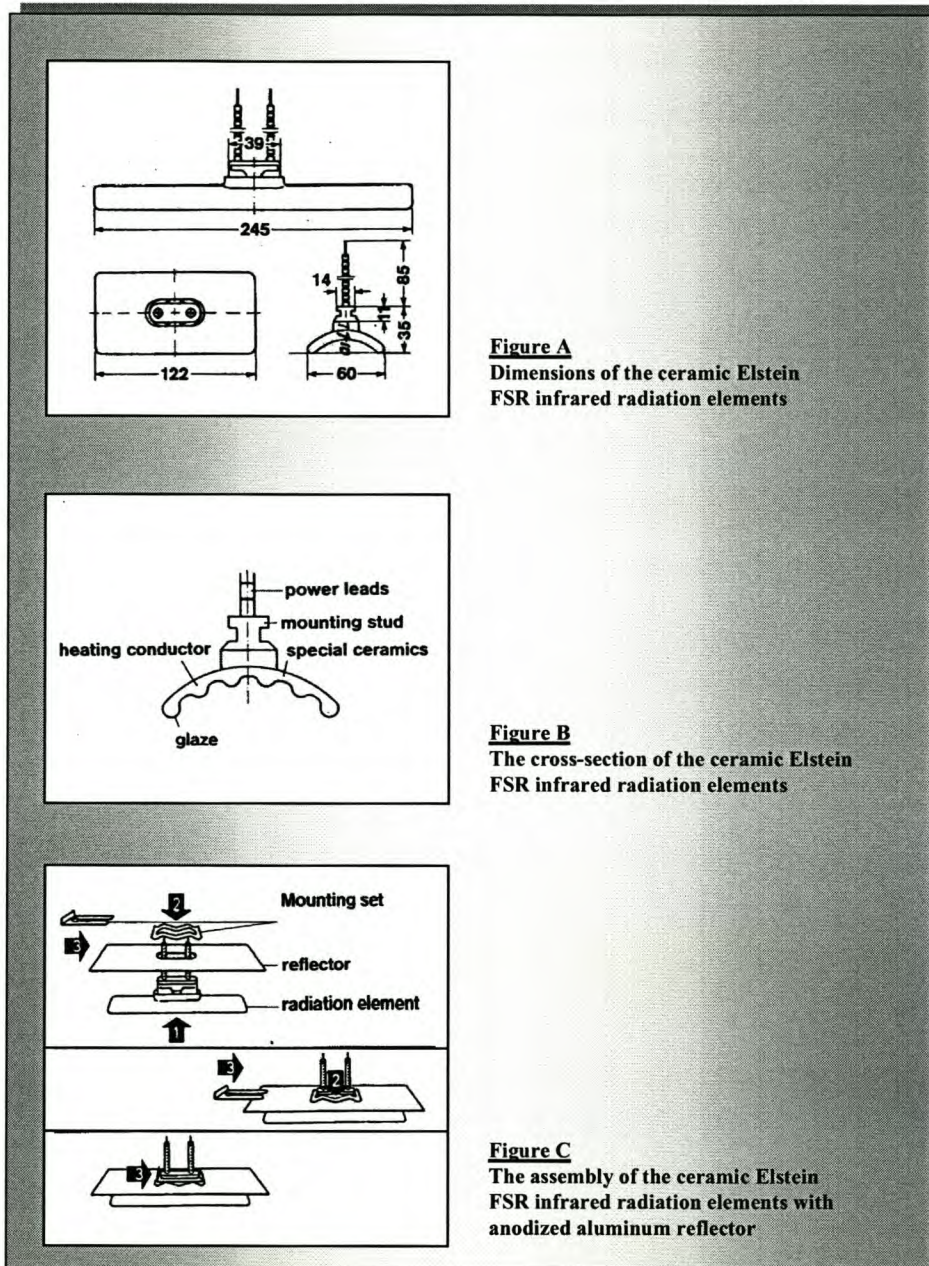


Figure 7 Construction and assembly of the long-wave infrared elements (Elstein technical data sheet).

Internally the unit is wired for 3-phase power. Each of the emitters has a power output of 650 W and draws a current of 2.82 amp. Therefore,

each infrared heater unit with 27 emitters draws a current of 76.3 amp per unit, wired over three phases, so each phase has a current load of 25.43 amps. The arrangement of the 27 emitters underneath each unit is depicted in Figure 8.

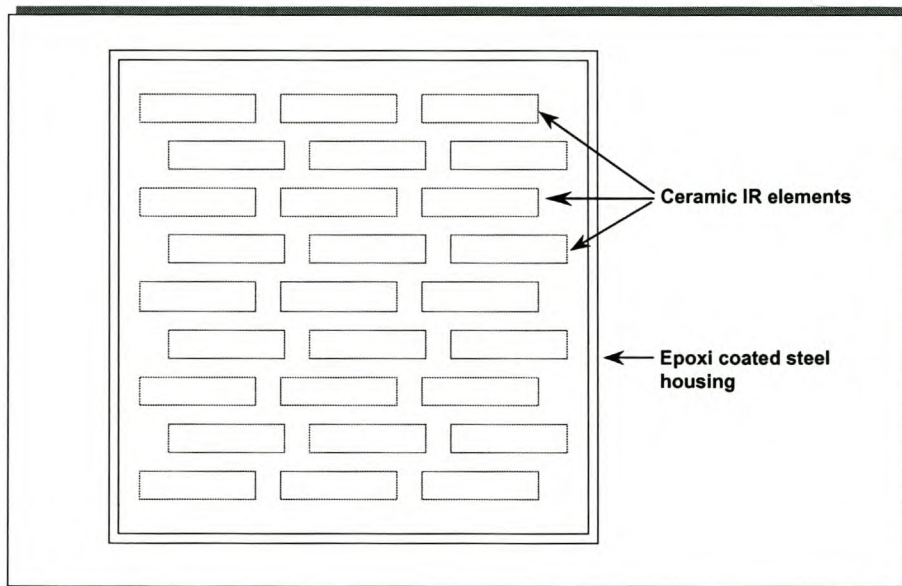


Figure 8 Schematic representation of the arrangement of the 27 IR emitters underneath each infrared curing unit (drawing courtesy of J.J. vd. Westhuizen, Stellenbosch University).

Each ceramic infrared emitter is manufactured by a special process and has a firmly burnt in heating coil consisting of highly heatproof resistance wire with almost constant resistance over the whole temperature range. The ceramic surface is glazed with a special glaze to improve the radiation properties of each emitter (Elstein technical data sheet).

The four infrared heater units are mounted onto a steel frame to form a continuous heated zone with a total length of 4m (Fig. 9). Slotted side brackets on each side of every unit allow for adjustments in height to regulate the exposure distance between the infrared emitters and the heated surface.

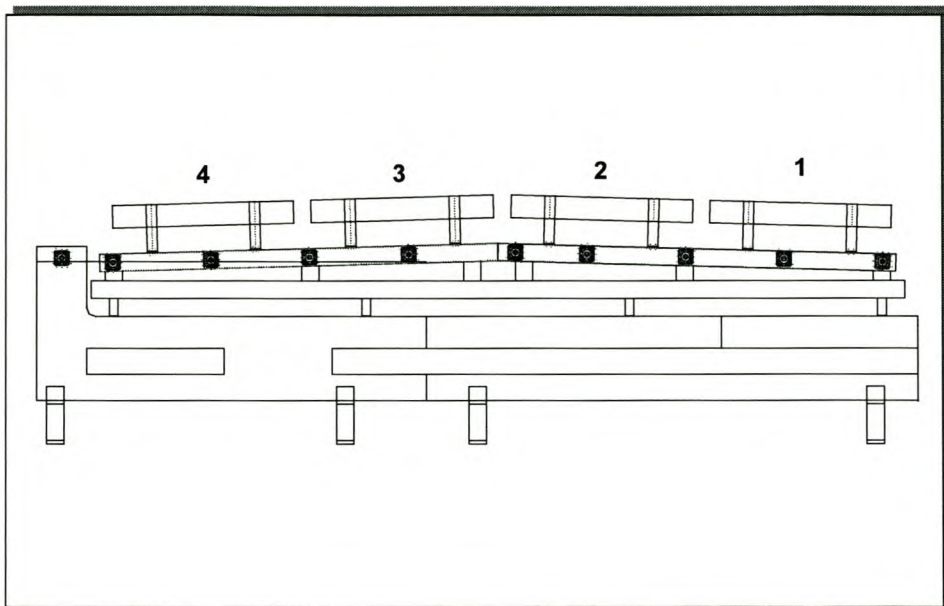


Figure 9 Side-view drawing depicting the assembly of the long wave infrared curing tunnel consisting of units 1 to 4 mounted on a steel frame (drawing courtesy of C. Butcher, B+ Projects).

The temperature control system in each infrared unit detects the temperature of the compound when it passes the pyrometer. The signal is passed to a Gefran controller which in turn, via solid state relays, adjusts the power output. Since each infrared unit has a pyrometric temperature sensor, overheating of the PVC plastisol mixture is not possible and sections of the process can individually be controlled.

d). Cloth wind-off with tension control and lamination process

The cloth wind-off unit resembles the same construction as was used for the paper wind-off unit and is situated above the welder roller (Fig.10). The non-woven material is fed into position, on top of the paper, where it is laminated onto the carrier material by means of the rotary heat-sealing unit.

This unit consists of the wind off frame with the non-woven roll fitted on an air shaft. The cloth wind-off frame is constructed from 12 mm steel plating to ensure rigidity. The air shaft fits into two safety chucks that prevent the shaft from moving sideways, aiding in the alignment of the non-woven to the drum welder. One of the safety chucks is connected to a pneumatic brake, which is controlled by a I to p converter. The I to p converter receives a signal from the load cell, detecting the tension between the welding roller and the pneumatic brake. The desired tension can be set on a control panel and a plc controller will ensure that the set value is maintained. Upper and lower limits can also be programmed into the control system.

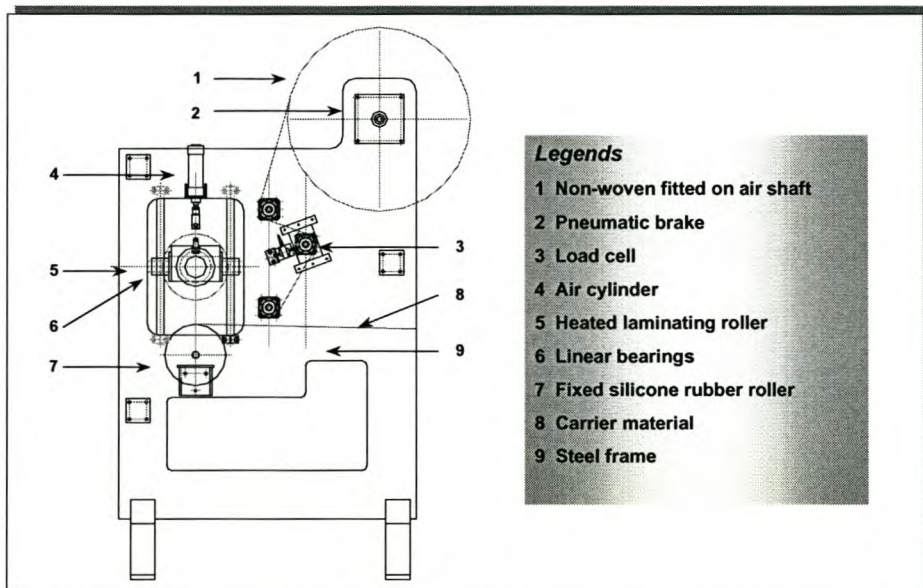


Figure 10 Side-view drawing depicting the assembly of the cloth wind off with tension control unit and the two rollers of the lamination unit (drawing courtesy of C. Butcher, B+ Projects).

The lamination unit assembly consists of two rollers: an adjustable heated roller and a fixed silicone rubber coated roller (Fig. 10). The heated roller is mounted directly above the coated roller on linear bearings and is fitted with a pneumatic cylinder to allow up and down adjustment of the heated

roller. The heated roller is constructed from three sections of pipe: the outer pipe, the mounting pipe with infrared heater elements and reflectors, and the inner pipe. The inner pipe fits inside the mounting pipe and these two sections form a stationary unit inside the outer pipe.

1). Heated roller assembly

The outer pipe is constructed from 270 mm outside diameter steel tubing with a wall thickness of 7.5 mm and a length of 1 016 mm. The surface of the pipe contains nine rectangular pockets, 345 mm by 360 mm in dimension, that are machined into the surface to accommodate the squares of cured plastisol mixture. The ends of the tube are closed with two flanges. Each end flange contains a centered stub fitted with bearing housings. One of the stubs is hollow to allow the wires for the infrared heater elements through while the stub on the opposite side is solid and fitted with a flexible coupling that is connected to a drive and a gearbox. The entire drum assembly rests on two linear bearings secured to the 12 mm side plate that forms part of the machine frame. The linear bearings are used to allow up and down movement of the lamination drum for alignment purposes and also to regulate the pressure under which lamination of the two layers will take place. The drum is moved upwards by way of a pneumatic cylinder. Proximity sensors detect and relay the position of the drum to the control system. For the downward movement, air pressure is released and the drum is allowed to move under its own weight. If necessary, air pressure can also be used to control the downward movement.

The mounting pipe is a 1 000 mm electroplated steel pipe with an outside diameter of 60 mm and a wall thickness of 3 mm. This pipe fits inside the outer pipe and is attached to the end flanges of the outer pipe by a bearing on each side. Therefore, the inner pipe and the mounting pipe form a stationary unit fitted inside the outer pipe. The mounting pipe acts as a support for the 16, 1000 watt Elstein ceramic infrared heater elements with their anodized aluminum reflectors. The dimensions of the Elstein ceramic

infrared emitters are identical to those used for the infrared curing units except that these ones have a power output of 1000 W each (see Fig. 7). The infrared emitters and reflectors are mounted on short studs to the surface of the pipe and configured in 8 rows of 2 emitters staggered along the length of the pipe. The configuration of the infrared emitters and reflectors along the length of the mounting pipe is depicted in Figure 11 (For explanatory reasons the pipe was rolled open).

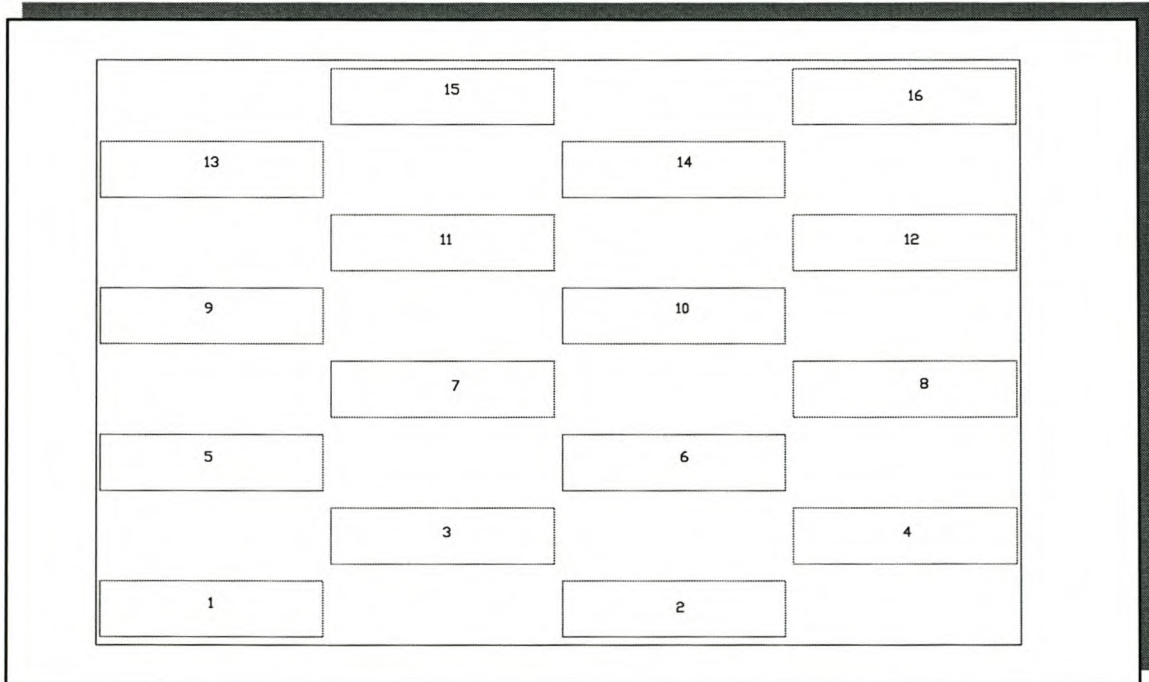


Figure 13 Schematic representation of the mounting pipe rolled open to depict the configuration of the 16 ceramic infrared emitters and aluminum reflectors along the length of the pipe (drawing courtesy of J.J. vd. Westhuizen, Stellenbosch University).

The inner pipe is constructed from 142 mm outside diameter electroplated steel pipe with a wall thickness of 3 mm and a length of 1200 mm and fits inside the mounting pipe. It is secured, at both ends, to the mounting pipe by two sets of bearings and contains the 34 wires leading from

the infrared heater elements and 2 thermocouples (type k) mounted on the surface of the mounting pipe.

2). Coated roller assembly

The coated roller is constructed from a 250 mm outside diameter steel pipe and coated with a 5 mm thick coating of silicone rubber. To prevent hot materials sticking to the roller surface during the lamination process, the rubber surface is covered with a 0.3mm thick layer of self-adhesive Teflon tape.

e). Cooling section

The cooling section consists of a steel frame fitted with a plenum box below the paper, and a cooling unit. Cold air is blown onto the paper by the fan of the cooling unit. Hot air is dissipated by the cold air.

f). Sheeting and slitting unit

The unit consists of a frame constructed from 12 mm steel plating, to ensure rigidity; two shafts fitted with the 4 rotary slitter blades and anvils; the main drive unit; and the sheeter (Fig. 12).

Main drive unit

The main drive unit assembly consists of two rollers; an adjustable stainless steel roller, coupled to a variable speed drive and a gearbox, and a fixed silicone rubber coated roller (Fig. 12). The speed and position of the carrier material during the production process are determined by the speed and

position of the variable speed drive and controlled via the main control system.

The stainless steel roller is mounted directly above the coated roller on linear bearings and is fitted with a pneumatic cylinder to allow up and down adjustment of the stainless steel roller. When in the down position, the laminated layers of non-woven and carrier material are gripped between the stainless steel and rubber rollers and pull the materials through the plant. When the stainless steel roller is in the up position, the materials are stationary and can be placed into position manually. The stainless steel roller has four raised segments on the surface that matches the polymer segments that were deposited onto the carrier material. This is to prevent the polymer matrix from being damaged by the pulling action of the main drive.

Slitting unit

Slitting the laminated layers of non-woven and carrier material is achieved by a series of four rotating blades that are fixed and held in position on a shaft by means of a locking mechanism and four rotating anvils that are fixed on a second shaft (Fig. 12). The slitting blades consist of two parts, a blade holder or top knife and an anvil or bottom knife (Fig. 13). The shafts and the blades are driven via the main drive of the machine to synchronise the speed of the laminated layers of material with that of the blades. To allow the laminated layers of material or the carrier material to pass through the machine without being slit, the slitting blades can be opened and closed by means of a mechanical cam action.

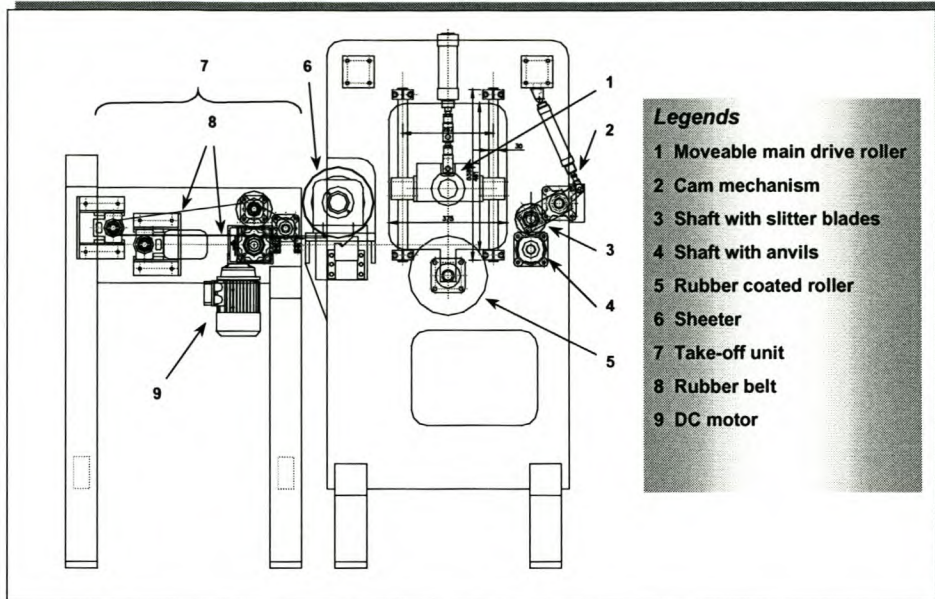


Figure 12 Side-view drawing depicting the assembly of the sheeting and slitting section (drawing courtesy of C. Butcher, B+ Projects).

Sheeter

The sheeter works on the principle of a stationary anvil and a rotary blade (Fig.12). The stationary anvil stands straight while the rotating cutting blade is angled in two planes, x and y planes, allowing the blade to shear the material as it moves over the anvil. A separate drive and gearbox drives the blade.

Two sensors control the cutting action. A photo sensor detects the index mark where the material is to be cut while a proximity sensor detects the position of the blade. The motor will receive a signal from the control system when to start and at what speed to turn.

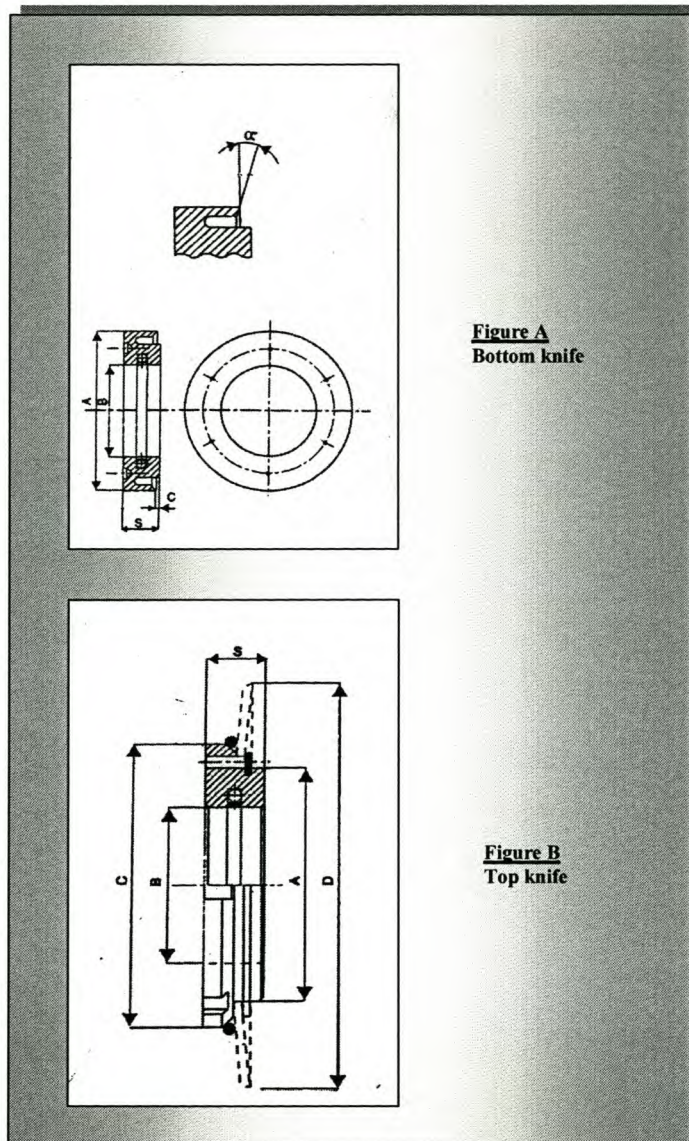


Figure 13 Assembly of the bottom (Fig. A) and top (Fig. B) knives for the slitting section (Barkev Coatings technical data sheet).

g). Take-off unit

The take-off unit consists of two rubber belts running on the top and bottom of the paper (Fig. 12). An ac motor drives the belts via a gearbox. The speed of the belts is 10% higher than the speed of the machine, creating the necessary tension needed at the sheeter. The sheets are presently collected at the end of the take-off unit in three containers. In future,

depending on production volumes, an automated stacking and packing system may be implemented.

Stage 3: Handling and storage of the manufactured SO₂ gas sheets

a). Product inspection (Quality control)

The final product was visually inspected to ensure that there were no deviations from predetermined quality parameters. Special attention was given to the strength of the lamination between the poly film and the non-woven, the appearance of the PVC deposit, mass of the PVC deposit and the size of the sheets. Sheets were taken randomly from the production line and tested. Further measures to be implemented in future to ensure product quality are discussed extensively in the next section, Quality management.

b). Product packaging and storage

The quantity of SO₂ gas sheets required by industry is dependent on the seasonal availability of fresh table grapes. As the table grape season reaches its peak, the demand for SO₂ gas sheets far exceeds the industrial production capabilities, creating a demand for imported sheets. The only way in which it may be possible to supply SO₂ gas sheets throughout the entire grape season is by building up a stockpile of SO₂ gas sheets during the off season and distributing them on demand during the table grape season.

Due to the physical properties of the SO₂ gas sheets, special precautions must be undertaken during packaging and storage of the sheets to ensure that optimum quality prevails during long-term storage. From a quality perspective, it is essential that the SO₂ gas sheets be packaged in such a manner that exposure to any kind of moisture be eliminated. Contact with moisture during storage will trigger the release of SO₂ gas prematurely.

Ultimately this will have a negative influence on the ability of the gas sheets to produce SO₂ gas during post harvest storage of table grapes. The presence of SO₂ gas during post harvest storage of table grapes is considered to be essential for the prevention of *Botrytis cinerea* rot (Nelson, 1979). Absence of SO₂ gas during the post harvest storage period of table grapes will have serious financial and quality implications. Lower fruit quality ultimately leads to lower prices (Nelson, 1979).

The SO₂ gas sheets that were manufactured on the pilot plant were stacked in three trays by the take-off unit. The sheets were collected from the trays in bundles of 50 sheets and placed in heavy poly(ethylene) bags, 100µm in thickness, and taken to the Multivac packaging machine. To eliminate the presence of any moisture inside the poly(ethylene) bags, air was removed from the bag and the contents flushed with nitrogen before the bag was closed by means of heat-sealing.

As a quality control criterion, each bag was weighed to ensure that it contained the correct amount of sheets. A label was attached to each bag stating the batch number and date of manufacture. The bags were placed in corrugated carton boxes and palletised for distribution. Proper stock rotation practises must be implemented to ensure that the storage period of the gas sheets is kept to a minimum.

c). Distribution

The SO₂ gas sheets produced on the pilot plant will, for the time being, be exclusively used for trial purposes. Distribution by the Deciduous Fruit Producers Trust (DFPT) to selected producers or marketing companies is foreseen for the near future. In future, the relative parties involved in the proposed commercial venture will distribute the SO₂ gas sheets accordingly.

Quality Management System

The raw materials used in PVC processing are considered to be process variables to the same extent as machine cycles and temperatures. Knowledge of the variables of the materials used, and how these variables may affect the quality of the final product, is essential in controlling the process. Raw materials variation can be limited by identifying the important characteristics of the various plastisol components and then selecting the components with the desired properties from the normal sources of supply (de Groot, 1977).

The quality management system is still in the developmental stage and will be completed before production commences. A documented quality management system will be established and maintained as means of ensuring that the product and processes conform to the ISO 9001:2000 requirements.

ISO 9001:2000 is based on the so-called Business Excellence Module. Quality is no longer seen as the responsibility of the Quality Control Department, which used to be associated with the operational areas of the business. It now includes the management of all factors that influence quality and runs through the organisation like a golden thread. It is a philosophy of all work being a process, a sequence of inputs and outputs linked by the work activities. All organisations therefore have an internal chain of customers and suppliers reflecting the fact that every work activity involves being both a customer and supplier. Those activities linked together form business processes which, if measured, analysed and understood, present a continuous improvement of business tools available. ISO 9001:2000 is process driven and it is therefore essential that the core business processes are defined and that the Total Quality Management System is then built around these (personal correspondence with S. Viljoen, Vynide Pty(ltd), 2001).

The following process is to be followed when setting up the ISO 9001:2000 documentation (unpublished, S. Viljoen, Vynide Pty(ltd), 2001).

Developing a quality policy manual

Management needs to define their business principle and fundamental rules by which it operates. These, together with the Quality Objectives, form the input to the Quality Policy Statement.

Developing a quality procedure manual

The procedure manual shall detail what is done and who is responsible where required, and refer to the relevant operating/working instructions. It is based on the identified core business processes/support processes and will cover all the elements of the Quality Management Systems.

Developing works instructions

Whenever special instructions are required to perform processes defined in the procedures, operating/works instructions will be generated which will describe in detail how, when and where the various activities are to be performed.

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Chapter 10

General discussion and overall conclusions

General discussion

In the table grape industry the importance of sulphur dioxide as a preservative was identified during the early 1920s. Today, the use of SO₂ gas in combination with cold storage is still considered to be the most practical and economical method by which to control the development of *Botrytis cinerea* decay during post-harvest storage of table grapes.

However, during the past decade concern increased regarding the effect of sulphurous compounds present in foods on certain individuals. The health effect of concern is sulfite sensitivity. The Food and Drug Administration (FDA) and Environmental Protection Agency (EPA) have established that the use of sulfites as preservatives on fresh fruits and vegetables poses a risk to that small segment of the population which is sulfite sensitive and placed a 10 parts per million (ppm) tolerance level on sulfite residues found in grapes to be sold to the public.

Exposure of table grapes to high levels of SO₂ gas during post-harvest cold storage also poses various quality problems. Excessive levels of SO₂ gas damages the berries by bleaching, and furthermore causes premature browning of the stems and increased water loss. These factors have a negative influence on the marketability of table grapes, especially for SO₂ sensitive cultivars. The extent of the damage caused by the absorption of SO₂ gas by grape tissue is not merely limited to bleaching and general loss of quality, but leads to the degradation of cell walls, cell membranes and cell wall structures. Depending on the level of SO₂ exposure or injury, the destructive effects of SO₂ gas are more prominent in the surface tissue layers,

however, damage caused by SO₂ gas is also visible in the deeper tissue cells, but to a lesser extent. The presence of certain quality defects found during post-harvest storage of table grapes, such as soft tissue breakdown and berry split viz., destruction of cell walls and cell wall structures, may also be attributed to the absorption of SO₂ gas by the grape tissue.

The stricter food legislation and export requirements necessitated that thorough research be performed to ensure that South Africa remain competitive in the world market with regards to exporting top-quality fruit. In addition to the above, several problems were experienced with the presently-available, locally manufactured SO₂ sheets i.e. Uvasys, regarding lack of availability, tremendous increase in cost and frequent unacceptably high SO₂ gas-emission levels. This gave rise to the need to find new technology for the in-package production of SO₂ gas that must be cheap and offer a wider versatility in its gas generating mechanism, in order to manufacture sheets that can change along with the ever-changing demands of the table grape industry. The development and manufacturing of a locally manufactured, alternative SO₂ sheet, which is cheaper but as effective as the SO₂ sheets used at present, is of major importance for the South African Table Grape industry. Therefore, a research program was initiated by the South African Table Grape industry to develop, manufacture and test an alternative SO₂ generating sheet.

Prototype sheets were developed, based on slow-release technology that is widely used in the pharmaceutical industry, and initially, hand-made in the laboratory. The sheets consisted of three deposit-strips of a PVC plastisol/Na₂S₂O₅ mixture enclosed in a poly-coated paper and non-woven envelope. SO₂ gas is released from the sheet through a monolithic release mechanism once the Na₂S₂O₅, incorporated into the PVC matrix, comes into contact with moisture. The release rate can be manipulated by changing the internal structure of the PVC matrix, thereby changing the accessibility of the matrix to moisture, or by altering the movement of the generated SO₂ gas through the matrix. These hand-made prototype sheets were successfully tested in a series of small-scale trials on various cultivars.

To thoroughly investigate the true potential of these new sheets, a

production process was developed and a pilot scale production unit assembled in order to prototype manufacture sheets with more consistent properties so as to conduct semi-commercial and possibly commercial trials.

The aesthetics of the new polymer sheet was improved during the 2001 season; it progressed from a three-deposit strip to a single-deposit of the polymer matrix, covering the entire surface of the carrier material. The first phase tests were conducted with a sheet covering $\pm 65\%$ of the surface area on top of the grapes, while in later trials sheets of a larger dimension, covering $\pm 90\%$ of the surface area, were used. The change from a three-deposit sheet to a single-deposit sheet, and from a smaller to a larger dimension, was required to reduce the possible risk of SO₂ damage occurring directly below the SO₂ deposits strips, and to reduce decay development in bunches not covered by the SO₂ deposits.

Apart from the size of the SO₂ deposits, the carrier material for the polymer matrix was shown to be of great importance. Water-soaked areas, which are totally unacceptable in appearance, occurred with the paper-type carrier, and hence had to be changed. The first alternative carrier material, a combination of poly(ester), aluminium foil and poly(ethylene), did not show similar problems. However, slight discolouration occurred at the sealed edges, and was ascribed to oxidation of the exposed aluminium film. The carrier material was subsequently changed to a metalised poly(ester) and poly(ethylene) combination, with no further problems occurring. The plastic carrier type material used did not affect the efficacy of the SO₂ sheet.

The thin-film, single-deposit, SO₂ polymer sheet generally reduced post-harvest *Botrytis* decay developing by spread from artificial inoculation, which resembles conditions of high inoculum pressure, as well as from natural infections. Generally, with most of the sheet types tested, good results were achieved with regard to reduction of decay and SO₂ damage. However, the concentration of SO₂ incorporated into the sheet and the nature of the deposit strip affected both these quality parameters. The concentration of SO₂ had to be decreased from $\pm 20\%$ for the three-deposit strip to $\pm 13\%$ for the single-deposit strip to ensure that any levels of SO₂ damage were within acceptable limits. The use of too low concentrations of SO₂ gas, in turn, resulted in

increased decay levels. Control of decay was comparable to that obtained with the Uvasys SO₂ sheet for most of the polymer sheets tested.

The efficacy of decay control and the subsequent level of SO₂ damage will depend on the type of grape bag used, whether perforated or non-perforated, which is directly associated with the grape cultivar and its sensitivity to decay development and SO₂ damage.

It was shown that, generally, the SO₂ concentration to be incorporated into the polymer sheet was likely to be in the range of 11-13% for non-perforated bag packaging, and 14-15% for perforated bags. Ideally one would prefer to have only one sheet for all packaging conditions, therefore, any future work should include investigating the using a sheet of one SO₂ concentration for all cultivars and packaging conditions.

Efficacy of the polymer sheets for storage of grapes up to 5 w, and for conditions of low decay potential, was similar to that of the Uvasys sheet, irrespective of the SO₂ content in the sheet. However, for long storage periods (> 8 w) and high decay potential, too low SO₂ concentrations ($\pm 10\%$ SO₂) were not advisable, as decay in perforated bags is inclined to increase with extended storage periods (> 6 w), and more so for the polymer sheet than the Uvasys sheet. Therefore, sufficient SO₂ concentration needs to be incorporated into the sheet for it to be effective.

Small differences in the incidence of total decay for Barlinka grapes from six producers in the De Doorns area occurred with use of the 13% polymer sheet. Decay levels were, however, generally high. This was ascribed to the fact that the grapes were packed immediately after rain, which tested the ability of the sheet under conditions of natural, high inoculum pressure. Decay control was comparable to the Uvasys sheet.

SO₂ damage, with use of polymer sheets, inevitably increases with storage beyond 5 w, especially on grapes packed in non-perforated bags. Therefore, a polymer sheet of a too high SO₂ concentration (> $\pm 14\%$) cannot be used.

SO₂ damage generally increased over time, irrespective of the SO₂ sheet used. The increase in SO₂ damage was more pertinent for the polymer

sheet of a higher SO₂ content (26%). This is particularly relevant for long-term storage of table grapes. The level of SO₂ damage caused by the Uvasys SO₂ sheet did not differ significantly from the polymer sheet of <20% SO₂ content, while a higher concentration led to more damage than the Uvasys sheet. It is therefore important that with the use of SO₂ sheets, the duration of the storage period must be taken into account and form part of a decay and SO₂ management strategy when selecting a specific SO₂ sheet.

Packing of grapes at a relatively high pulp temperature ($\pm 30^{\circ}\text{C}$) would not readily cause increased levels of decay or SO₂ damage, if a polymer sheet is used where the SO₂ concentration incorporated into the sheet does not exceed 14%. During post-harvest storage, the level of SO₂ damage generally increased when the grapes were subjected to increases in temperature as depicted by the simulated shelf-life conditions (5 days at 10°C). This is an indication that the efficacy of decay control and SO₂ damage related to the use of the polymer SO₂ sheets could be affected by the storage period and temperature fluctuations. Packaging materials had a significant effect on the levels of SO₂ damage. Generally, increased SO₂ damage occurred on grapes packed in non-perforated bags when compared to grapes packed in perforated bags.

There is still a degree of uncertainty whether or not it would be advantageous to use an additional MAM in combination with the new polymer sheets, especially when high *Botrytis* inoculum levels are present. The results recorded during these trials were inconclusive in this regard; they suggested that the use of an additional MAM as barrier below the polymer sheets had no negative effect on decay development, nor any real benefit in reducing levels of SO₂ damage. However, there were some indications that the use of an additional MAM might be advantageous, especially for the more SO₂ sensitive cultivars packed in non-perforated bags.

The orientation of the bunches in the carton did not affect the level of decay, nor the level of SO₂ damage of Barlinka grapes kept at -0.5°C or when subjected to a shelf life period at 10°C, irrespective of the storage period. Although not statistically significant, slightly higher levels of SO₂ damage were observed on areas of the bunches directly below the SO₂ generators.

According to independent analysis conducted by the Department of Health on non-sensitive grape cultivars, levels of sulphite residues detected were below the 10 ppm tolerance level imposed by the FDA and EPA for all the polymer sheets tested. However, this work may in future need to be repeated for specific SO₂ sensitive cultivars.

Overall conclusions

Each chapter in this dissertation is an individual entity with its own conclusions. However, the overall conclusions of the present study were:

The manufacturing process and pilot-scale production plant that was developed and constructed can be used to manufacture polymer SO₂ generating sheets that are both technically efficient and aesthetically acceptable to industry.

The efficacy of the original hand-made polymer sheet, with regards to preventing post harvest *Botrytis cinerea* decay development, does not seem to be affected by the manufacturing process; similar levels of efficacy are achieved with the new machine-manufactured polymer sheet.

Control of post harvest *Botrytis cinerea* decay development with the new polymer SO₂ generating sheet was comparable to that obtained with the commercially available Uvasys SO₂ sheet.

Increased levels of SO₂ damage and *Botrytis cinerea* decay development are more likely to occur when grapes are kept for longer storage periods and are exposed to conditions causing temperature fluctuations.

The SO₂ concentration to be incorporated into the polymer sheet is likely to be in the range of 11-13% when using the non-perforated bag packaging and 14-15% when using perforated bags.

The exact SO₂ concentration to be used for semi-commercial and possible commercial applications will largely be determined by the sensitivity of the cultivar to SO₂, as well as the packaging type used.