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Grapes

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INTRODUCTION, BOTANY, CULTIVATION AND PRODUCT STATISTICS

Introduction

The grape was one of the first fruits to be cultivated by man. Since the dawn of civilisation, the fermented product of grapes, wine, has probably been an important way of consuming grapes (McGovern *et al*. 1995). Wine residues have been identified in 7000-year-old jars in Iran (McGovern *et al*. 1996). However, consumption of the fresh and dried fruit has probably always been popular where the vines grew wild.

This chapter provides some basic information about the cultivation of grapes (this section) before looking at the physiology and biochemistry of the developing and mature grape berry. The final three sections of the chapter deal with post-harvest aspects of grapes pertaining to the three main product types: beverages (wine and juice), fresh fruit (table grapes) and dried fruit (sultanas, raisins and currants). Although today most grapes are converted to wine, the development of the post-harvest technology for grapes has concentrated on the fresh fruit. This is because all the eating-quality parameters (appearance, texture and taste) must be high in the commercial product. The quality of grapes for wine, drying or for other grape products is primarily dependent on harvesting the right varieties at the right time and preventing unwanted chemical changes during processing. The topic of wine production is so huge that this chapter describes only the quality factors required in the harvested grape as the basis of a good wine. Details of processing are omitted but references are given for further reading on this topic. There is relatively little published on the specific post-harvest requirements for grapes used for drying. In the section entitled 'Post-harvest technology for dried grapes', a summary of the basic processing and product preservation is provided.

Botany

The grape plant is a vigorous vine of the family Vitidaceae Juss. (syn. Ampelidaceae; Vitacae) (Watson & Dallwitz 1991, 1992). The European grape *Vitis vinifera* (L.) is hermaphrodite whereas some native North American *Vitis* species are monoecious. Multiple buds are produced laterally on the previous season's cane and flowers are wind pollinated. The fruit clusters mature about 5–7 months after bud burst. The main cultivars in commercial production are described in the product sections below.

Cultivation

Grape is one of the world's most widely grown fruit crops in relatively warm temperate-zone climates (see product statistics below). It is not well adapted to subtropical or tropical areas although special management allows dessert grapes to be harvested 2–4 times per year in tropical countries such as Thailand and Indonesia. Heat accumulation determines the type of grape that can be successfully grown. Between 950°C day and 1500°C day wine production predominates; above 1500°C day table grapes and fortified wines can be produced and above 1950°C day table grapes and dried fruit are dominant (Jackson & Looney 1999).

Crop Post-Harvest: Science and Technology, First Edition. Edited by Debbie Rees, Graham Farrell and John Orchard.

V. vinifera is the dominant species for wine, fresh fruit or drying. This species is thought to originate in the Caucasus Mountains. Of the N. American species, some are used for juicing, some for rootstock and some interspecific hybrids for wine or dessert usage (Jackson & Looney 1999). The selection of sports and hybridisation has traditionally been the only methods of breeding *V. vinifera*, but they are slow because the grape has a long life cycle and high inbreeding depression (Gray *et al*. 1992). Over the past decade, a wide variety of molecular techniques have been developed to improve the identification, breeding and the study of genetic relationships among cultivars (Lefort & Roubelakis-Angelakis 2000; Reisch *et al*. 2000) and the grape genome sequencing (Jaillon *et al*. 2007) is leading to intense works in the grape research community.

The vine has deep roots and is drought tolerant although irrigation is necessary in some production areas. It is also tolerant of many soil types provided they are deep and well drained. Over-fertilisation, especially with nitrogen can lead to too vigorous growth (which may adversely affect wine quality). The vine and developing fruits are susceptible to a number of pests and diseases (Pearson & Goheen, 1988). Some recent concerns have emerged about salt concentrations rising in soils of irrigated vineyards, and their effects regarding the choice of rootstocks and fruit quality have been studied (Walker *et al*. 2007). In Europe and the United States, it is normal to use root stock resistant against the aphid *Phylloxera vastarix* which has caused large losses in these regions. Nematode-resistant root stock is also recommended. Fungal diseases can dramatically affect production. During wet weather, grey mould (*Botrytis cinerea*), downy mildew (*Plasmopora viticola*), anthracnose (*Elsinoë ampelina*) and phomopsis (*Phomopsis viticola*) can be serious. Powdery mildew (*Uncinula necator syn. Erysiphe necator*) can occur in both wet and dry regions. Crown gall (*Agrobacterium* spp.) is the most serious bacterial disease, and there are a number of viruses that damage the vine (Jackson & Looney 1999; Patil *et al*. 1995).

Classic texts on vine cultivation include Winkler *et al*. (1974) and Mullins *et al*. (1992). Other textbooks include Huglin and Schneider (1998), Jackson and Looney (1999), Reynier (1999) and Jackson (2000).

Product statistics

World production of grapes in 2009 was estimated to be about 68 million metric tonnes (MT). The main producer countries were, in order of decreasing production volumes, Italy, China, the United States, France, Spain, Turkey, Chile, Argentina and India (FAO 2009).

Approximately half of the grapes are transformed into wine. The world production of wine was about 27 MT (representing around 36 MT of grapes) with the main producer countries being (in order of decreasing productivity): Italy, France, Spain, the United States, China, Argentina, Australia, South Africa, Chile, Germany and Portugal (FAO 2009). Of this production, about a quarter is exported. Leading exporters were: France, Italy, Spain, Chile, Australia, the United States, Germany, Portugal and South Africa. Only 0.5 million MT of unfermented grape juice was exported world-wide, with the main exporters being Italy, the United States, France, Spain, Argentina, Germany, South Africa and Chile (FAO 2002).

The remaining 32 MT of grapes are either table grapes or raisins (detail unknown). World export volumes of table grapes were in the order of 2.73 million MT with the main exporter countries being Chile, Italy, the United States, South Africa, Mexico, the Netherlands, Greece and Turkey (FAO 2002).

World export volumes of raisins were in the order of 0.6 million MT. The main exporters are Turkey, Iran, the United States, Greece, Chile and South Africa (FAO 2002).

MORPHOLOGY AND PHYSIOLOGY

Introduction

Grape berry physiology and biochemistry was last reviewed in detail by Conde *et al*. (2007) and formerly by Ollat *et al*. (2002). The mature grape berry is a nonclimacteric fruit with a low rate of post-harvest physiological activity. Grapes must therefore be harvested after they have reached optimum levels of colour development and of important solutes such as sugars and acids. Ripening changes occur in a relatively dramatic fashion during the development of the berry on the vine. These changes resemble, in a number of respects, those seen in climacteric fruit after harvest (see section on 'Berry development').

In the last decade, there has been considerable research on the molecular biology of berry development and maturation (Robinson & Davies 2000). The biochemical changes and their control systems that underpin fruit ripening, and thus final fruit quality are gradually being elucidated.

Fruit morphology

Grape fruit develop as clusters (bunches) with each berry attached to the bunch stem (rachis and branches) via a pedicel which contains vascular bundles (also known as the cap stem). There is much variation between cultivars in stem structure (i.e. length of parts, toughness and adherence to berries) (Winkler *et al*. 1974). Impact or shaking of

bunches may cause the loss of berries, leaving an unsightly spray of vascular strands (brush).

Each berry consists of a multi-layered pericarp and may contain up to four seeds, although a number of cultivars for fresh consumption are seedless. The cells of grape berries are tightly packed with an internal gas volume of about 1.2 ml/100 g (Zosangliana & Narasimham 1993). The pericarp can be divided into the exocarp (skin), mesocarp (pulp) and endocarp. The pulp makes up most of the berry weight and cells are highly vacuolated, containing high levels of sugars and other soluble compounds (see below). The seeds contain high levels of tannins (5-8%), oil (10–20%) and phyto-hormones (Winkler *et al*. 1974). The pericarp contains plastids throughout berry development although their morphology changes at around anthesis and lipid-like globules form within (Hardie *et al*. 1996).

Two layers can be distinguished in the exocarp or skin, that is, the epidermis (6.5–10 µm) and the hypodermis (107–246 µm) (Alleweldt *et al*. 1981). The mature epidermis is covered with a cuticle about 3 µm thick which includes an outer 0.5 µm wax layer (Casado & Heredia 2001) and contains stomata which by maturity are not thought to be functional. Just after anthesis, the stomata density was approximately 7±2 stomata per berry for the cultivar Cabernet Sauvignon (Palliotti & Cartechini 2001). The nonliving layers give the grape its 'bloom' which is an important visual quality factor. The thickness and toughness of the skin differ among varieties and can affect the suitability of a cultivar for particular post-harvest uses (Winkler *et al*. 1974). The thickness of the epidermal cell walls is the only parameter showing a positive correlation with resistance to physical stress (Considine 1981). Thus the thickness and toughness of the skin contributes to the resistance of grapes to handling injury. Furthermore, the skin is the main location of the compounds that give the grape its colour, aroma and flavour (see below) (Winkler *et al*. 1974). Despite the stomata present in the epidermis, cuticular transport is thought to be the main route for water loss (Blanke & Leyhe 1987).

A recently described *in situ* fixation method, that better preserves the membrane integrity, should allow new information to emerge on the internal compartmentation of grape berry cells (Diakou & Carde 2001).

Berry development

Pattern of development

Ollat *et al*. (2002) and Kanellis and Roubellakis-Angelakis (1993) describe the division of the berry development into three stages based on research by a number of researchers, such as Alleweldt (1977) and Coombe (1992). Stage I is a period of very rapid cell division followed by marked cell enlargement. Stage II, the lag phase is a period of slow growth during which the embryos reach their final size, chlorophyll starts to be lost and acidity reaches its highest level. The start of stage III is called 'veraison' and is marked by a rapid acceleration in growth, softening of the berries, an increase in sugars and amino acids and the activity of some enzymes and anthocyanin accumulation in coloured cultivars. Acidity, chlorophyll and ammonia levels and respiration rates all decrease during this stage.

The individual berries within a bunch do not ripen synchronously (Coombe 1992). The variability that this causes has constrained studies of the underlying biochemistry and has commercial implications for fruit quality at harvest (Robinson & Davies 2000). Methods have been developed to extract RNA from grape fruit at different stages of development (Franke *et al*. 1995). Northern blot analysis has shown that some genes are expressed only in berries and only during ripening, whereas others are expressed in a range of grape tissues but are up-regulated during ripening. By homology with known genes, it appears that these grape genes fall into two groups: those that encode proteins involved in cell wall function and structure and those whose products appear in plants under applied stress (Davies & Robinson 2000). Further understanding of the coordination of development at a biochemical level will come as research continues into changing gene expression (Robinson & Davies 2000). The generation of Expressed Sequence Tags (ESTs) has been the basis of microarray analyses (Waters *et al*. 2005; Terrier *et al*. 2005) that many teams are now using, and the development of 'omics networks' (Grimplet *et al*. 2009; Zamboni *et al*. 2010) will speed up research on genes, proteins and metabolisms in the near future.

The recent characterisation of a fruit specific promoter should further help the design of experiments targeted towards study of berry ripening (Burger *et al*. 2006). Another recent study dedicated to a mutation of grapevine leading to fleshless berries will advance our understanding of the genetic and developmental processes involved in the differentiation of an ovary into a fruit (Fernandez *et al*. 2005).

Respiration and photosynthesis

The respiratory rate of the average single grape berry is high early in stage I and then declines rapidly; it then shows a rise at veraison (between stage 2 and 3), with more CO_2 produced than O_2 consumed (Saulnier-Blache & Bruzeau 1967). The rate of gross photosynthesis on a dry weight basis peaks during the early part of stage I and then declines rapidly; on a single berry basis it shows peaks in

the later part of stage I and early part of stage III. Fully ripe berries show practically no photosynthetic activity (Koch & Alleweldt 1978; Niimi & Torikata 1979). Using the cultivar Cabernet Sauvignon, Ollat *et al*. (2000) found that during the whole growth period, the grape berry imported 12 mmoles of carbon. Respiration accounted for 18% of the imported carbon and fruit photosynthesis supplied 10% of the carbon required for fruit development. When fruit of the Pusa seedless variety were harvested at maturity and stored at 1°C, respiration rate declined for 30 days and rose thereafter (Rao *et al*. 1975).

Solute accumulation

Sugars and minerals

The accumulation of sugars is the most important quality change in the ripening fruit. It is these sugars which are converted into alcohol during wine making and which give the sweetness desired in both fresh and dried fruit and fruit juice. It is not therefore surprising that there is considerable interest in understanding the processes that control the production and accumulation of sugars.

From anthesis to veraison, imported carbon (in the form of sucrose) is almost equally partitioned between pericarp, seed growth and respiration. At veraison, carbon imports increase. Then the carbon is mainly allocated to the pericarp and stored as the hexoses, glucose and fructose (Ollat *et al*. 2000). These two sugars are the main carbohydrates of the mature berry pulp. They are present in approximately equal amounts (total sugars = 12–27% fresh weight) although the actual ratio varies between cultivars. Cultivars with more fructose than glucose can be harvested earlier due to the greater sweetness of this sugar compared to glucose. As the fruit become over-mature, the fructose to glucose ratio increases (Winkler *et al*. 1974).

Up to veraison, water is imported mainly through the xylem. At the onset of ripening, the contribution of xylem water is reduced by embolism blockage (Coombe 1992). At this stage, carbon import increases fivefold due to a stimulation of water flow through the phloem.

The back-flow, water movement from the berry to the parent vine, may be an important component of berry weight loss at maturity in some cultivars, such as Shiraz (Tyerman *et al*. 2004). Mineral transport is related to the pathway of water import. Calcium is translocated during early berry growth while potassium is translocated during ripening (Ollat & Gaudillere 1997). Although it has been suggested that berry sink strength increases substantially at the onset of ripening, the factors that control the massive sugar import into the berries and the pathways of assimilate transport are still poorly understood. It is possible that an increase in berry alcohol dehydrogenase activity is linked to fruit ripening (Tesniere & Verries 2000) and that *Adh 2* expression depends upon ethylene signalling (Tesniere *et al*. 2004). There are no clear physiological means to explain these inductions yet. There is also evidence that sucrose transporters may play a role in sugar accumulation (Davies *et al*. 1999). A hexose transporter gene (Vvht1) has been cloned and shows a first peak of expression at anthesis, and a second peak about 5 weeks after veraison. The Vvht1 promoter sequence contains several potential regulating cis elements, including ethylene-, abscisic acid-, and sugar-responsive boxes (Fillion *et al*. 1999).

The effects of hormones on sucrose accumulation and metabolism at different developmental stages (I, II, veraison and III) were investigated by Xia *et al*. (2000). Gibberellic acid (GA), indoleacetic acid (IAA) and abscisic acid (ABA) all significantly facilitated ^{14}C-sucrose import into the berries at all stages studied but caused differing effects on the subsequent transformation of the sucrose. For example, the transformation of ^{14}C-sucrose to reducing sugars was enhanced by IAA whereas GA increased the accumulation of fructose. Recently, ethylene has been proved to regulate the sucrose transport into berries (Chervin *et al*. 2006).

Acids

The acidity level is a very important quality factor in both table grapes and those used for wine production. Consumer acceptance of table grapes and grape juice is strongly influenced by the sweetness to acid balance (Winkler *et al*. 1974). Acidity also determines the suitability of the fruit for wine making. Excessive tartness correlates with low sugar levels which give poor-quality wine (Ruffner 1982). However, in warm climates, grapes with a low pH and high acidity levels are generally desired for table wines. The brilliance and red intensity of coloured grapes is greater at moderate to high acidity and low pH. With low acidity and high pH, they tend to be bluish and dull (Winkler *et al*. 1974).

A review article gives details about the biochemistry of the acidity variations in grape berries (Terrier & Romieu 2001). Tartaric and malic acids constitute over 90% of total acids (% fresh weight); however, the ratio between the two acids varies considerably depending on the grape cultivar. Both acids accumulate before veraison although they show distinct patterns of accumulation (Ruffner 1982). Tartaric acid is thought to be stored both as insoluble calcium tartrates and as the free acid in the vacuole. A recent study demonstrated that ascorbic acid is the precursor of the tartaric acid (DeBolt *et al*.

2006). Malic acid is a very active intermediate in grape metabolism. At veraison acid levels start to go down. The decline in malic acid content is very rapid and is thought to be due to respiration via oxidative phosphorylation. The reduction in acidity is quicker under warm growing conditions (Kanellis & Roubellakis-Angelakis 1993). After veraison, two vacuolar proton pumps have been detected that create a positive membrane potential across the tonoplast resulting in the accumulation of organic acids inside the vacuole. The activity of these pumps increased in parallel during the period of sugar storage, while malic acid content decreased (Terrier & Romieu 2001). IAA, GA and ABA promoted the transformation of ^{14}C-sucrose into organic acid at stage I, significantly inhibiting transformation at stage II (Xia *et al*. 2000).

Phenolic components

Tannins (proanthocyanidins) are the most abundant class of phenolics in grape berries and are the predominant determinants of astringency in red wines (Souquet *et al*. 1996; Cheynier *et al*. 1997). Other major phenolic compounds in grapes include anthocyanins, benzoic acids, cinnamic acids and flavonols (Flanzy 1998). Berry skins contain more hydroxycinnamic tartrates than the flesh, while the latter has more flavan-3-ols and procyanidins. The composition of grape skin proanthocyanidins at different stages of berry development has also been described by Kennedy *et al*. (2001). The seeds have high amounts of phenolics which form a significant proportion of wine tannins and contribute significantly to oxidative browning of grape juice. There has been considerable interest in the chemical properties of grapevine polyphenols, including nonflavonoids (stilbenes, phenolic acid derivatives) and flavonoids (flavanols, flavonols and anthocyanins), and their biological and pharmacological activities (Vitrac *et al*. 2004). A recent work has been published about the expression of different flavonol synthases in grape vines and berries (Fujita *et al*. 2006).

Anthocyanins give rise to the red and purple colouration of certain grape cultivars and are thus important quality factors in table grapes and wine. The malvidin derivatives are generally the most abundant anthocyanins in grapes. Interesting reviews have been published recently about anthocyanin content in wine grapes (Mazza 1995) and table grapes (Carreño *et al*. 1997). During the last 20 years, there have been several studies on the gene expression of anthocyanins summarised by Holton and Cornish (1995). In grapes, the cloning of various genes of the anthocyanin pathway was performed by Sparvoli *et al*. (1994). They found that most of them were induced by light in grape seedlings. The induction of the main genes involved in the anthocyanin pathway is probably resulting from complex interactions between various signals such as light, sugar, abscisic acid and ethylene among others (Mol *et al*.1996). Boss *et al*. (1996a) observed the expression of these genes in white and red grapes, the non-expression of some of these genes being correlated to the absence of anthocyanins. The transcription of most of these genes was clearly induced at veraison (Boss *et al*. 1996b). The UDP-flavonoid glycosyl transferase plays an important role in the redness of the berry tissues (Boss *et al*. 1996a; Kobayashi *et al*. 2004 and refs herein), catalyzing a step that is known to stabilise the anthocyanins (Piffaut *et al*. 1994). A recent study points out the role of a transcription factor involved in phenylpropanoid pathways (Deluc *et al*. 2006). Plant hormones like auxin and abscisic acid may play a role on the expression of these genes (Davies *et al*. 1997). From a physiological point of view, some competition between the anthocyanin and stilbene synthesis has been highlighted (Jeandet *et al*. 1995).

Some recent advances in anthocyanin analysis by electrospray ionisation mass spectrometry have been reported (Sarni-Manchado *et al*. 1997). In the last decade, some studies have focussed on the antioxidant properties of the grape anthocyanins and associated variations between red and white, young and old wines (Tubaro *et al*. 1999).

Aromatic compounds

The aroma of grapes is attributed to over a hundred different compounds, mostly located in the skin. Some cultivars from the species *V. labrusca* and *V. rotundifolia* have very distinct aromas, as do the Muscat types of *V. vinifera* although other cultivars of this species are not highly aromatic. Some aroma compounds are isoprenoid secondary metabolites such as monoterpenes and damascenone (Jackson 2000). It has been suggested that their synthesis is linked to the formation of lipid-like globules in plastids found in the pericarp (Hardie *et al*. 1996). Some other aroma precursors are present in a glycosylated form (Williams *et al*. 1995).

Hormonal changes

The hormonal changes from anthesis to maturation of the berry are well summarised in the review by Kanellis and Roubellakis-Angelakis (1993). Very little is known about the post-harvest role of phyto-hormones in grapes.

Gibberellic acids

The size of mature berries correlates well with the number of seeds and parthenocarpic or stenospermocarpic cultivars

generally have small fruit unless treated artificially with growth hormones. The seeds have high levels of hormones such as abscisic acid and gibberellic acid-like compounds. Girdling of certain cultivars such as the parthenocarpic black Corinth is known to increase gibberellic acids (GAs) in the fruit and increase the size of the berries. GAs are sprayed on the developing grape bunch to control bunch shape and berry size (Lynn & Jensen 1966). Transcripts potentially involved in seedlessness have been described recently (Costenaro-da-Silva *et al*. 2010).

Ethylene

Grapes have been classified as nonclimacteric fruit as their ripening phase was apparently not triggered by ethylene and not associated with a respiratory burst. In fact ethylene levels are very low in grapes, within the range of pmoles. g^{-1}_{FW}, however the use of a specific inhibitor of ethylene receptors has shown that ethylene signalling is involved in some ripening metabolisms such as the increase of the berry volume during the second growth phase and the anthocyanin accumulation (Chervin *et al*. 2004). Indeed the ethylene signalling modulates the berry expansion, via the transcription regulation of many genes, among which xyloglucan endotransglucosylases and aquaporins seem critical (Chervin *et al*. 2008) and changes occur in transcripts related to ethylene signals (Chervin & Deluc 2010). Wounding and water deficit have been shown to induce ethylene emission by grapevines, but the influence on fruit ripening was not assessed (Boschetti *et al*. 1997). Goldschmidt (1998) has published a review about the possible involvement of ethylene in the ripening of nonclimacteric fruit.

It has been suggested that the levels of ethylene and abscisic acid (ABA) act synergistically to promote pre-harvest ripening (Coombe & Hale 1973). The grape industry has already adopted the use of an ethylene precursor (2-chloroethylphosphonic acid), also known as ethephon, whose spray applications around veraison enhance colour development, in pigmented cultivars, with a concomitant fall in acid levels and sometimes a rise in sugar levels (Weaver & Montgomery 1974; Shulman *et al*. 1985). The impact on anthocyanin accumulation may be due to ethylene effects on several enzymes involved in anthocyanin synthesis, among which the UDP glucose-flavonoid 3-O-gucoslyltransferase (El-Kereamy *et al*. 2003). Ethephon also stimulates abscission and is used to chemically thin just after full bloom and to improve berry removal during mechanical harvesting for wine production (Szyjewicz 1984).

Abscisic acid and auxins

Abscisic acid (ABA) levels increase in the maturing fruit and are thought to induce *de novo* synthesis of gluconeogenic enzymes (Palejwala *et al*. 1985). ABA-specific binding sites have been located in the endomembranes of grape berry mesocarp with maximum binding values coinciding with development phase II and dropping off at veraison (Zhang *et al*. 1999).

The abscisic acid is involved in the grapevine response to partial root zone drying (Stoll *et al*. 2000), a method that consists in boosting grape ripening by altering the irrigation schedule, thus creating a stress beneficial to grape quality when well managed.

On the contrary, auxins act primarily in the young berry formation, thus the conjugation of auxin to amino acid leading to low auxin levels in the berry may be part of the ripening induction (Bottcher *et al*. 2010).

Jasmonates

There is an increasing interest in the study of jasmonate roles in grape berry physiology. Their levels seem related to the presence of seeds (i.e. more jasmonates in seeded berries), and they seem to follow an accumulation kinetic that resembles the ethylene one (Kondo & Fukuda 2001; Chervin *et al*. 2004). They are also known to stimulate stilbene accumulation, with a more pronounced effect on leaves (Larronde *et al*., 2003).

Brassinosteroids

A novel series of compounds has been determined as potential grape hormones, brassinosteroids are indeed produced at relatively high level at the onset of berry ripening and stimulated this berry developmental process (Symons *et al*. 2006).

Defence mechanisms

Immature fruit of all plant species contain preformed and/or inducible defence systems (production of phytoalexins). Usually however, these systems become less effective as the fruit ripens. This appears to be true in grape berries for the best studied grape phytoalexin, a stilbene called resveratrol (Jeandet *et al*. 1991). Maximum levels of resveratrol were shown to be induced by UV light from 1–5 weeks post-flowering, dramatically declining in maturing berries sampled from 10–16 weeks post-flowering. It was suggested that this might be a major factor in the increasing susceptibility of ripening grape berries to *Botrytis cinerea* infection (Bais *et al*. 2000). On the other hand, a study has shown that levels of defence-related protein, basic chitinase and a thaumatin-like protein (grape osmotin) rise proportionally with fruit reducing sugar content (Derckel *et al*.

1998; Salzman *et al*. 1998). The timing of the accumulation of grape osmotin correlates with the inability of the fungal pathogen powdery mildew (*Uncinula necator*) to initiate new infections of the berry (Tattersall *et al*. 1997). Loulalakis (1997) showed that an osmotin-like gene was expressed in grape cell cultures exposed to ethylene.

Soluble proteins

Proteins in the fruit pulp contribute to clouding of juice and wine. Recently some authors have described the proteome of berry skin (Deytieux *et al*. 2007). The major soluble proteins appear to be the pathogenesis-related chitinases as described previously (Pocock *et al*. 2000). Polyphenol oxidase (PPO) activity in grapes has been well characterised (Okuda *et al*. 1999; Yokotsuka *et al*. 1988). The role of PPO in berry and juice browning is discussed further in sections on wine and juice grapes and on table grapes respectively. There is evidence of only one PPO gene in grape with high levels of expression in young developing berries, leaves and roots, but little expression in mature tissues (Dry & Robinson 1994). The compartmentation of a number of key enzymes in grape berries during development is described by Famiani *et al*. (2000).

Cell wall changes

Grape cell walls are composed of about 90% polysaccharide and less than 10% protein. The two main types of polysaccharides, cellulose and polygalacturonans show considerable varietal differences in their relative abundance (Nunan *et al*. 1997). The firmness of table grapes is an important quality attribute. Grape berries begin to soften at veraison and the degree of softening at maturity is determined largely by cultivar. Although most post-harvest berry softening has been attributed to loss of water (Nelson 1979), the softening associated with ripening is considered to result from changes in the composition of the cell walls (Robinson & Davies 2000). During softening, depolymerization of pectin and xyloglucan molecules and a decrease in the amount of hemicellulose and cellulose have been detected (Yakushiji *et al*. 2001). Large changes in protein composition also occur (Nunan *et al*. 1998). There is a steady decrease in total pectin substances during grape ripening and a decrease in methyl-esterification of insoluble pectins (Barnavon *et al*. 2001). Furthermore levels of calcium, a mineral which stabilises plant cell walls, decrease during berry ripening (Cabanne & Doneche 2001). Soluble pectic polymers from mesocarp activity in juices (also named musts) may play a detrimental role in white wine making by restricting juice extraction (Robertson *et al*. 1980) and delaying must clarification (Saulnier & Brillouet 1988). The expression patterns of cell wall modifying enzymes during berry development have been described by Nunan *et al*. (2001).

POST-HARVEST TECHNOLOGY FOR WINE AND JUICE GRAPES

Introduction

Wine grapes have not been the subject of much post-harvest research so far. This may change in the future with trends towards longer transport times of grapes to wineries, more mechanically harvested grapes that are more exposed to post-harvest deterioration and the increasing need to preserve very delicate fruit flavours. The production of wine is a complex process which transforms the grape berries into an alcoholic beverage. Recent books published on the science of wine making include Jackson (2000) for North America, Rankine (1997) for Australia and New Zealand and Ribéreau-Gayon *et al*. (1998) and Flanzy (1998) for France. A quite comprehensive and practical book about most laboratory analyses has been published by Australian academics (Iland *et al*. 2000). A book listing the OIV official methods for must and wine analyses is also available (Anonymous 1999).

Fewer than 30 cultivars provide the world's classic quality wines, all from *V. vinifera*. Hundreds more are used to a limited extent. Some important cultivars are: red: pinot noir, merlot, cabernet sauvignon, shiraz, grenache and tempranillo; and white: chardonnay, riesling, sauvignon blanc, chenin blanc, muller thurgau, chasselas, Semillon and palomino (Galet 2002). There might be interesting aromas to be gained from the use of some other Vitis species.

Harvest maturity requirements

The timing of wine grape harvest is very critical. Too early and the grapes are too acid. Too late and they may lack acidity or suffer reduced yields from bird damage or rots. The berries must contain the correct balance of flavour and aromatic compounds. The typical maturation levels of sugars should lie between 16% and 24% and acid between 0.6% and 1%. The yield of juice depends primarily on the cultivar's degree of pulpiness. Other factors influence yield such as the stage of ripeness, size of berries, seediness, thoroughness of fermentation and efficiency of crushing, pressing and other operations (Winkler *et al*. 1974).

Sampling

The most important source of variation is in the individual vine (Rankine 1997). A detailed set of precautions is given by Rankine to enable the most representative sample to be

achieved. Various effects of freezing and homogenising the sample with various durations and tools has been reported recently (Cynkar *et al*. 2004).

Sugars

Sugar level at harvest depends on the region of production. For classical dry wines, a global figure of 200 g of reducing sugars/kg of fresh grapes can be given (Jackson 2000), this will roughly give a 12% alcohol wine. But cool climate areas will produce grapes which are usually less sweet than warmer and sunnier areas. Moreover the type of desired wine will also influence the date of harvest (thus the sugar level), for example late harvested grapes (with sugar levels above 300 g / kg) will be used to produce sweet wines. An optimal harvest date is critical to achieving the right sugar level.

There are pre- or post-harvest practices known to increase the sugar levels like leaving the grapes on the vines for one or two months beyond the optimal harvest date, thus leading to natural sugar enrichment by berry desiccation. The vineyard has to be in a suitable climatic area (dry afternoons avoiding strong rot development).

Acids

The acid level varies according to the region and type of wine, but as described in *Berry Development* (acidity) it is inversely proportional to the sugar level i.e. as the acid content drops, the sugar content increases. To give an idea of the range of acidity found in musts, one can cite average acidity of Cabernet Sauvignon in Bordeaux over 20 years: 100 ± 20 g of meq. / L (Ribéreau-Gayon *et al*. 1998). Jackson (2000) states that must pH should be below 3.3 for whites and 3.5 for reds. The acid content is important in various ways: it affects sulphur dioxide efficiency, the freshness of the taste and the ability of the wine store well, among other factors. An optimal harvest date is critical to achieving the right acid level.

Polyphenols

Polyphenols are of great importance to wine quality and can be assessed using various methods (see *Berry Development*: *phenolic components*). However, rapid and accurate measurement of polyphenol berry content is still difficult (Jackson 2000), and a matter for further research. An optimal harvest date and good post-harvest management are critical to achieving and preserving the polyphenol content. There are very few studies on the polyphenol changes over the post-harvest period; one report least shows a global decrease of most of them during this period (Borsa & Di Stephano 2000), but an ethylene postharvest treatment was shown recently to increase polyphenols in wine grapes (Botondi *et al*. 2011); this effect was associated to a partial dehydration. Furthermore, polyphenolics are substrates of enzymes that may alter wine quality (Flanzy 1998). Another aspect, linked to the food science, is the optimisation of polyphenol extraction in the case of red wines using various processes that will not be detailed here.

Aroma potential

Aroma potential depends on several compounds which vary according to the grape cultivar. Jackson (2000) cited the terpenes and the glycosyl-glucose contents, as being among the markers for aroma potential which have received attention. Their assessment by simple means is still a matter for research and development (Flanzy 1998). A suitable harvest date and good postharvest management are critical to optimise this potential (see 'Harvesting and post-harvest management; below). Some other aromatic componds, like methoxypyrazines that are typical of green pepper, are already present in grape berry tissues in the aromatic form (Allen *et al*. 1990), but rarely desired in the resulting wines. However, in white wines, there are also some aromas produced during the fermentation, that are derived from precursors, whose levels in grape depend on cultivation conditions as some volatile thiols in Sauvignon blanc (Gachons *et al*. 2005).

Phytopathogens

One of the main post-harvest problems due to grape moulds and *Botrytis cinerea* in particular is the oxidative action of fungal laccase (Nair & Hill 1992; Jackson 2000). This extracellular enzyme induces browning of white musts by oxidising polyphenols and causes off-flavours in red wines (Rankine 1997). Laccase activity in musts can be assessed by various automated systems which are already in use in many wineries (Ribéreau-Gayon *et al*. 1998). Good pre-harvest and post-harvest control of fungal infections is critical to managing this problem. Laccase has been used as a marker for successful disease control in grapes (Dubos *et al*. 1996). Recent efforts have been made for the assessment of gluconic acid as an indicator of rotten grapes (Crachereau 2004).

Waxes

Waxes from the berry epidermis are the primary source of waxes in wine and may contribute to colloidal turbidity of wines (Rosenquist & Morrison, 1988). The waxes offer anchoring points for large numbers of spores of various microorganisms (Zahavi *et al*. 2000) that will influence the post-harvest life of the berries and the wine quality.

Harvesting and post-harvest management

Temperature control

The temperature of freshly picked grapes should be as low as possible to limit biochemical alteration processes (Rankine 1997). Grapes are often picked at night with mechanical harvesters and transported in refrigerated trucks when long distance trips are necessary. Solid carbon dioxide (CO_2) or dry ice is sometimes used to cool harvested grapes (see below).

Oxidation control

Some antioxidants (ascorbic acid, sulphur dioxide) are sometimes introduced into the transport bin just after harvest, but SO_2 addition is not recommended before de-stemming as it favours the extraction of compounds with a grassy taste (Ribéreau-Gayon *et al*. 1998). Limiting oxygen access to the grapes after mechanical harvest can also help to minimise unwanted oxidative changes (Flanzy 1998) and inert gases (CO_2, N_2) have been tested during transport.

Before pressing grapes used for white wines, CO_2 is sometimes added to protect the grapes from oxidation and cool them down (for example using solid CO_2). On-line addition of CO_2 gas in the must as an antioxidant is sometimes set-up between the hopper and the storage tank, or the pre-fermenting vat.

Sorting

Sorting is a crucial step to get the best of hand-harvested grapes, and may limit the quantity of rotten bunches to be sent to a given tank, thus increasing the must quality and limiting the amount of sulphur dioxide required. Ideally sorting is carried out in the vineyard or it can be done on arrival in the winery. For mechanically harvested grapes, systems separating stems from juice are recommended to avoid a grassy taste (Ribéreau-Gayon 1998).

Drying

Natural drying on shelves or on the ground, for example as carried out in Jerez, Spain, is another post-harvest step aiming to concentrate the sugars and obtain a higher alcohol degree. The yield in juice is usually very low, as little as 300 L / ton of grapes (Ribéreau-Gayon *et al*. 1998). The vineyard has to be in a suitable climatic area (at least dry autumn afternoons to avoid strong rot developments). Alternatively, artificial drying using forced warm air may be used. The association of partial dehydration and changes in protein patterns has been studied recently (Di Carli *et al*. 2010).

Juice and jelly production

Juice is an important product in some countries (see section *Product statistics*). For white grapes, the juice is extracted from the crushed fruits using a basket press. The juice is filtered through cloth and bottled. It may be preserved either by adding SO_2, sodium benzoate or by pasteurisation. Although the flavour of the juice is better from cold-pressed fruits, hot pressing can increase yields by as much as 20%. Coloured grapes need to be heated for 10–15 min at 60–63°C in order to extract the coloured anthocyanins treatment (Patil *et al*. 1995).

Browning is one of the most important quality changes in grape juice, especially from white grapes (Yokotsuka *et al*. 1988). Intensity of browning depends on the activity of polyphenol oxidase and the type and concentration of certain phenolic compounds in the juice (Sapis *et al*. 1983a). Soluble proteins can cause haze and sediments in the final product and levels are exacerbated by damage to the fruit caused by mechanical harvesting and long distant transport which delays pressing (Pocock *et al*. 1998). Sediments in the juices can be removed by clarification with kaoline or bentonite treatment, freezing or enzyme treatment (Patil *et al*. 1995).

Some grape juice is clarified for use in blended juices. The prevention of 'wine stone' or the crystallisation of tartrate is a problem for long-term storage of the juice. Exchanging sodium for potassium in the juice by ion exchange will change the relatively insoluble potassium hydrogen tartrate for the more soluble sodium salt (Arthey & Ashurst 2001).

In the United States, about 30% of juice produced is used to make jelly. The most important cultivar in the United States for juice and jelly production is Concord (Patil *et al*. 1995). Grape juice is occasionally concentrated following de-acidification and removal of water usually by evaporation under a partial vacuum. Concentrated grape juice can have a sugar precipitate if it is concentrated above about 55° Brix but this will quickly re-dissolve on dilution (Arthey & Ashurst).

POST-HARVEST TECHNOLOGY FOR DRIED GRAPES

Introduction

The main dried products of grapes are raisins, sultanas and currants. Raisins are the second most important product of the grape vine after wine (Shanmugavelue 1989).

Cultivars

The main grape types used for commercial drying are all *V. vinifera* cultivars (Jackson and Looney 1999). Currants

are universally produced from the small dark seedless Zante type grape (known as Black Corinth in California) although other grapes (e.g. the Australian Carina are used; Arthey & Ashurst 2001). In Australia both Thompson's seedless and Sultana grapes are used to make sultanas. In California, Thompson's seedless are used to make raisins. In both California and Europe, Sultanas are used to make sultanas. Muscat raisins are known as such in Europe and America, while in Australia, they are known simply as raisins. These raisins are sweeter than other types. The Muscat grapes have large berries with seeds which may or may not be removed after drying (Arthey & Ashurst 2001).

Harvest maturity indices

A high-quality dried product depends on the harvest quality. This is determined by the berry size, the uniformity and brilliance of the berry colour, the texture of the skin and pulp, the moisture content, chemical composition and presence of decay and foreign matter. There is evidence that the timing of harvest can be critical to final quality, not just in terms of berry sugar content but that it affects other parameters such as product colour (Uhlig & Clingeleffer 1998).

Harvesting

Grapes for drying are usually hand-picked. Generally, machine harvesting causes too much berry damage but the canes can be pruned mechanically with the bunches still attached and hung to dry on the vine (Jackson & Looney 1999).

Drying technology

A review of raisin production is given by Arthey and Ashurst (2001), Patil *et al*. (1995) and Waskar (1993). For efficient drying, grapes should have a high sugar content of 20–24° Brix. The grapes may be dried naturally (common in California, Iran and the USSR) or they may be pre-treated to speed up the drying process. A solar drier that is substantially more efficient than natural drying for grapes has been described by Fuller *et al*. (1990). The moisture reduces from about 70% to about 15%. Differences in the thickness and toughness of the skin between varieties influence the rate of water loss in raisin making (Winkler *et al*. 1974). The raisins are then winnowed mechanically to remove the capstem, leaves and stem pieces. After washing and grading, the raisins are filled into packs ranging in size from a few grams to bulk packs of about 12.5 kg for other food manufacturers to use.

For some products it is normal to dip the grapes before drying in a solution of potassium carbonate (2.5–4.5%) containing a 'dipping' oil. Other dips include sodium hydroxide (NaOH), citric acid or a mixture such as an alkaline, oil-in water emulsion. There may be benefits to combining alkali dipping with a microwave pre-treatment to reduce total drying time (Kostaropoulos & Saravacos 1995).

It appears that the dip changes the structure of the waxy bloom making it more permeable to water (Rojchev & Botiyanski 1998). It also seems that it makes the grape more transparent to infrared rays, allowing a better radiant heat uptake. It can speed up the drying process by several weeks (from 4–5 weeks to 8–14 days). Pre-treatments such as NaOH and citric acid have been shown to cause a substantial reduction in cell wall pectins (Femenia *et al*. 1998).

Muscat grapes are usually alkaline treated due to their larger size. Those that are not treated are carefully handled so as not to damage the bloom. The resulting dried fruits are used for high-class outlets such as health food stores (Arthey & Ashurst 2001). To optimise quality, various combinations of pre-treatments may need to be evaluated for particular cultivars and under local conditions (Gowda 2000).

Improving product quality

A light colour for dried grape products is considered highly desirable. The extent of browning in the dried product is determined amongst other things by the activity of polyphenol oxidase (PPO) particularly in the skin of the berry. Cultivars with a naturally low level of PPO dry to a lighter colour than others (Rathien & Robinson 1992). Low (<21° Brix) or very high sugar levels (>23° Brix) can increase browning in the dried fruit (Uhlig & Clingeleffer 1998). Berries exposed to the sun before harvest tend to produce darker dried product than shaded fruits (Uhlig 1998).

In some countries, sultanas and occasionally raisin grapes are treated with sulphur dioxide to bleach the fruit and give a more golden colour. The fruits are placed in purpose-built fumigation chambers (houses). Sulphur is then burned in a draught channel under the chamber, the gas enters the chamber and treats the fruit. Residues up to 2000 mg/kg are permitted. The fruits are then dried by one of the methods described above.

Some products may be given a light coating of mineral oil to improve handling and prevent stickiness and clumping when packaged.

Problems of dried grapes

An important quality problem of dried grapes is the migration and crystallisation of sugars on the outside known as sugaring. Skin characteristics influence the degree of sugaring of natural raisins during storage. The delicate skin of Monukka raisins renders them susceptible to sugaring,

whereas the tough skins of Black Corinth and Thompson Seedless usually prevent sugaring (Winkler *et al*. 1974).

Ecchymosis is when the raisins contain more than 16% water and are stored in bags or in deep bins, the lower part is pressed and the skin may be interrupted. Under these conditions the syrup migrates out of the raisin. Very soon it takes a dark colour and a lot of yeasts etc. start to ferment the molasses.

Pests, moulds and mycotoxins

A wide range of pests and diseases may be found on dried grape products if the product is not treated chemically with insecticidal compounds or is not protected by suitable packaging. Typical fungi include *Penicillium*, *Aspergillus*, *Cladosporium*, *Erotium and Alternaria* spp. Pests (insects and mites) may attack the grape berry before, during and after drying. Insecticide treatments before harvest may control some of these pests post-harvest (Buchanan *et al*. 1984); however, attentions should be paid to the fate of these chemical as drying can increase pesticide residue levels fourfold (Cabras *et al*. 1998). A biocontrol method with a granulosis virus has been shown to be highly effective against *Plodia* spp (Vail *et al*. 1991) and attempts have been made to combine effective packaging with parasitic wasps to control the almond moth *Cadra cautella* in commercially packaged raisins (Cline & Press 1990).

Ochratoxin A and aflatoxins may be found in dried grape products and methods for measuring concentrations of Ochratoxin A in these products and aflatoxin have been described (Bacigalupo *et al*. 1994; MacDonald *et al*. 1999).

POST-HARVEST TECHNOLOGY FOR TABLE GRAPES

Introduction

Table grapes are a high-value fresh-fruit commodity. Consumers will pay a premium for a quality product that has a display value as well as being a convenient and tasty fruit for consumption. 'White' (green/yellow) and red/purple/'black' cultivars are internationally popular (Figure 9.1). China grows by far the most fresh table grapes in the world. In 2006 China produced over treble the amount of table grapes (6.5 million MT) grown by the next largest producer, Turkey (USDA 2007). In both cases, the majority of this fruit is consumed internally.

World export volumes of table grapes are in the order of 2.73 million MT with the main exporter countries being, in descending order, Chile, Italy, the United States, South Africa, Mexico, the Netherlands, Greece and Turkey (FAO 2002). In many major importing markets, there is a consumer preference for seedless cultivars (Perl *et al*. 2000) and in some countries (e.g. the United Kingdom) the market for seeded table grapes has contracted substantially.

Figure 9.1 Packaging three colours of grapes (rainbow pack) is becoming very attractive to consumers.

Cultivars

The major cultivar of table grapes is probably Italia (Muscat) with around 700 000 tons produced per year in Italy in the early 1990s; the main table grape cultivars grown in France are Chasselas, Muscat de Hambourg and Alphonse Lavallée (Vidaud *et al*. 1993). Another important cultivar is Regina Bianca also known as Razaki in Turkey and Rosaki in Greece. In California, which produces 90% of US table grapes, the major cultivars are 'Thompson Seedless' ('Sultanina') and 'Flame Seedless', marketed mostly during the summer months. The early season market in the Coacheilla Valley is dominated by 'Perlette', 'Sugraone' ('Superior Seedless'), Midnight Beauty and Flame Seedless. 'Princess', 'Ruby Seedless', 'Crimson Seedless' and 'Autumn Royal' make up the bulk of the remaining production. There is also increasing production of the seeded 'Red Globe' cultivar which is important for export in the mid-to-late season. The Chilean and Spanish industries are being developed based on California cultivars while the South Africa grape industry has its own cultivars such as Sunred Seedless, Regal Seedless, La Rochelle, Dauphine, Bonheur and Bien Donné (ARC 2001).

Maturity and quality indexes

The table grape is a nonclimacteric fruit with a relatively low rate of physiological activity. Optimal flavour attributes are usually obtained at commercial maturity. The main maturity index is the sugar content, determined as the %

total soluble solids (TSS), otherwise known as Soluble Solid Concentration (SSC) or °Brix. For certain specific cultivars and situations, the titratable acidity (TA) and SSC-TA ratio are used as maturity indices (Guelfat-Reich & Safran 1971; Crisosto *et al*. 1994). Cultivars other than 'white' ones also have minimum colour maturity requirements, based on the percentage of berries in the cluster that show a certain minimum colour intensity and coverage. Other quality criteria for table grapes are good appearance, free of decay, thin skin, large size, good texture and flavour. The rachis should be fresh and green (i.e. not desiccated and brown). The bloom is also an important quality factor. It is destroyed by over-handling and rubbing which causes the berries to become shiny rather than lustrous.

Minimum maturity requirements vary with cultivar, growing area and market; however, standards are gradually being harmonised within the major market places. The Economic Commission for Europe, for example, has its own standards for table grapes (UNECE 2003) but work is underway to align these standards with draft FAO/WHO Codex Standards on table grapes by the end of 2007. The EU standards define table grapes as fruits grown from cultivars of *Vitis vinifera*. L.. Minimum SSC levels are given as 12° Brix for the Alphonse Lavalleé, Cardinal and Victoria varieties, 13° Brix for all other seeded varieties, and 14° Brix for all seedless varieties. By contrast, in California, United States the minimum SSC is generally 16.5° Brix and in early production areas, an SSC/TA ratio of 20 or more is used to determine maturity for cultivars with a minimum required SSC less than 16.5° Brix.

EU standards classify cultivars into greenhouse grown varieties and field grown varieties. This latter classification is being further divided into large-berried and small-berried types. The berries of 'Extra Class' grapes must be evenly spaced along the rachis and have the bloom virtually intact. Lower classes (I-III) are determined by the bunch shape and the presence or absence of colouring defects, bruising and sun-scorch. In all classes the berries must be firm and firmly attached to the stalk. The larger the berries, the higher the class, provided other quality factors are met.

Ethnic background can influence the factors that determine consumer acceptance as shown in a study of the acceptability of 'Red Globe'. For example, TA played an important role in American and Chinese consumer acceptance (Crisosto & Crisosto 2002).

Harvesting and packaging

Detailed information on the post-harvest handling of table grapes is well described by Nelson (1985). Traditionally most California table grapes have been packed in the field while a high proportion of grapes in South Africa and Chile were shed packed (Crisosto & Mitchell 2000). Recently, a combination of shed and field ('avenue') packing is developing in California and Chile.

Before harvesting, irrigation is usually withheld and the avenues between vines are treated to reduce dust contamination. The picker is trained to select appropriate bunches on the basis of the maturity indices described above. Grapes that fail to meet minimum standards are usually taken to local wineries or for use by other local industries such as cattle feed. Higher levels of efficiency can be obtained by training the vines at an appropriate height for convenient harvesting.

Field packing

The most common field-packing system is the 'avenue pack' (Figure 9.2a). The picker usually trims the fruit to remove defective berries and obtain a better bunch shape and size. The bunches are then placed carefully into field crates ('lugs') or baskets which are made of wood or plastic. The picking lugs are then transferred a short distance to the packer, who works at a small, shaded portable stand in the avenue between vineyard blocks (Figures 9.2b). It is common for the packer and several pickers to work as a crew. Packing materials are located at the packing stand, which also shades the packer (Figure 9.2c). The bunches may be packed directly into shipping cartons which reduces damage from repeated handling. With many packing stands around the vineyard supervision to maintain quality standards is more difficult than in a packing shed.

Shed packing

Shed-packed fruit is harvested by pickers and placed in field lugs. These are then moved into the shade of the vines to await transport to the shed. At the packing shed the field lugs are distributed to packers who select, trim and pack the fruit. In some operations, trimming, colour sorting, and a first quality sorting may have occurred in the field.

Packaging

Whether field or shed packed, grapes are nearly always packed on a scale to facilitate packing to a precise net weight. Generally two grades are packed simultaneously by each packer. High quality bunches, often destined for export, will be individually packaged, increasingly either in 'zip and slide' polyethylene bags, or plastic 'clam shells'. Both forms of packaging provide consumer-sized units and reduce the drop of loose berries onto produce department floors.

The use of the plastic cluster bags greatly reduces fruit damage during marketing (Luvisi *et al*. 1995). Bags and

(a)

(b)

(c)

Figure 9.2 (a) The most common field-packing system in California is the 'avenue pack'. (b) The grapes are picked and placed into shallow plastic picking lugs. The picking lug is then transferred a short distance to the packer, who works at a small, shaded portable stand in the avenue between vineyard blocks. (c) It is common for the packer and several pickers to work as a crew. Packing materials are located at the packing stand, which also shades the packer.

Figure 9.3 Loaded pallets coming from the field often pass through a "pallet squeeze," a device that straightens and tightens the stacks of containers. These pallet loads are unitized, usually by strapping or netting.

clam shells are perforated to maintain ventilation and thus reduce microbial decay. A restricted cluster bag with 1.4% perforation (compared to about 60% perforation for standard bags) was patented in 2000. Research has shown that these bags can restrict water loss and slow shrivelling of the fruit and stem browning without affecting decay and phytotoxicity levels (Davis *et al*. 2000).

Individual bunch packs are then placed into cartons which come in a variety of forms including wooden ended technical kraft veneer (TKV) crates, returnable plastic crates ('RPCs'), corrugated cardboard 'shoe' boxes and polystyrene (Styrofoam) boxes. TKV and Styrofoam boxes are mainly used for grapes destined for longer storage periods because they maintain their structural integrity in high-humidity conditions better than corrugated boxes.

Carton dimensions will depend on the pallet size in use in particular markets (Vidaud *et al*. 1993). Detailed studies of the relationship between pack volume and packing height in the box versus grape quality have been carried out for the different box materials and sizes (Luvisi *et al*. 1995). Additional cushioning separators in cartons have been found to reduce physical damage to the grapes but the use of absorbent materials can accelerate weight loss (Mencarelli *et al*. 1994).

Palletisation

After packing with grapes, cartons are palletised on disposable or re-cycled pallets. Often loaded pallets coming from the field pass through a 'pallet squeeze', a device that straightens and tightens the stacks of containers (Figure 9.3). These pallet loads are unitised, usually by strapping or netting. In shed-packing operations, some

palletising glue is used to bond the corrugated containers vertically on the pallet so that only horizontal strapping is required.

Cooling, storage and transportation

Cooling and storage

Because rachises and berries are susceptible to deterioration due to water loss (see 'Physiological disorders'), grapes are normally forced-air cooled as soon as possible after harvest. Grapes do not tolerate the wetting associated with hydro-cooling (bunches are not sufficiently robust and the presence of free water encourages grey mould and other diseases). The use of fruit coatings to control water loss in grapes has given inconclusive results and some bloom damage has been observed so it is not recommended (D. Lydakis, personal communication).

After palletisation is completed, the pallets are moved either to a fumigation chamber for immediate sulphur dioxide (SO_2) treatment, to a forced-air cooler and fumigation, or to a forced air cooler where fumigation is done at the end of the day's packing (Figure 9.4a). In any case, cooling must start as soon as possible and SO_2 applied within 6–12 hours of harvest (see *Diseases and their control*). After forced air cooling is completed, the pallets are moved to a storage room to await transport (Figure 9.4b).

Ideally the storage room operates at −1°C to 0°C (30° to 32°F) and 90 to 95 percent RH, with a moderate air flow 20–40 cubic feet per minute (CFM) per ton stored grapes. The constant low temperature, high RH and moderate air flow are important to limit the rate of water loss from fruit stems. Stores should be regularly monitored for physiological deterioration, fruit rot, SO_2 injury, and stem drying.

Transportation

Domestic transportation is mainly by refrigerated truck (Figure 9.4c) but sometimes grapes are transported using refrigerated rail cars. Exported grapes may be transported by truck but most are transported by sea freight using cold stores or containers. When the price is justified, air freight is used. Throughout transportation, fruit pulp temperatures should be maintained at −0.5 to 0°C (31–32°F).

Physiological Disorders

Stem, berry browning and water loss

In general, cumulative water loss during post-harvest handling results in weight loss, fruit stem (rachis or peduncle and pedicels) browning, berry shatter and even shrivelling of berries.

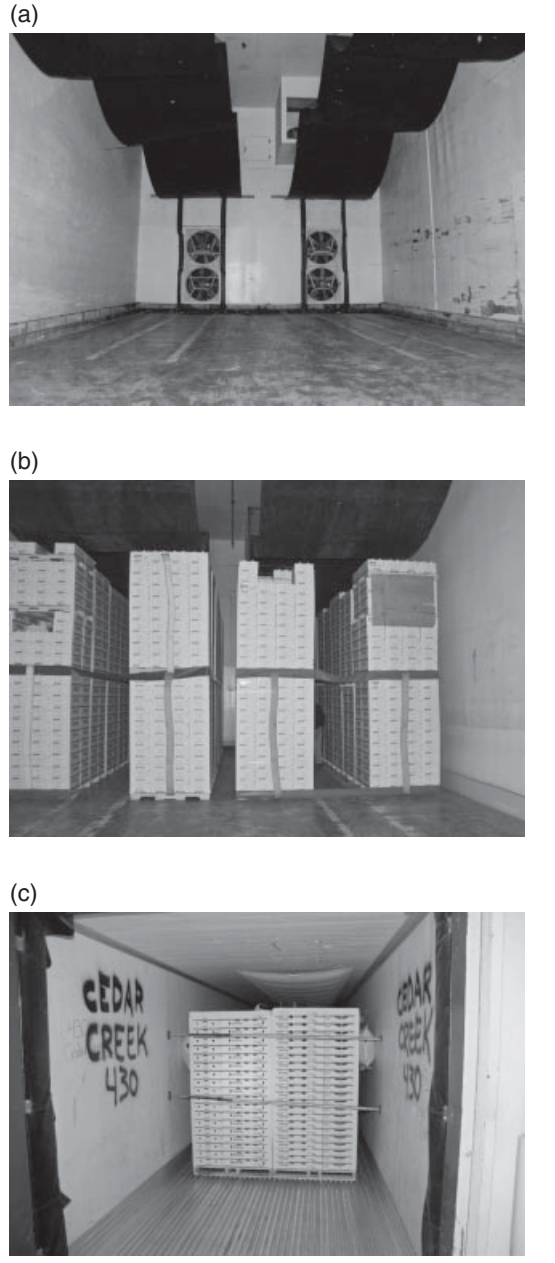

Figure 9.4 (a) After palletisation is complete, the pallets are moved to a fumigation chamber for immediate SO_2 treatment, to a forced-air cooler or fumigation or to a forced-air cooler where fumigation is done at the end of the day's packing. (b) After cooling is completed, the pallets are moved to a storage room to await transport. (c) During transportation, a central loading technique is utilized to maintain cold temperature during the transportation period.

Stems are particularly susceptible to water loss due to their high surface to volume ratio. The high rate of respiration of stems may also be a contributor to stem browning, as the respiration rate of the stems may be 15 times or more that of berries. Although stem browning does not affect the eating quality of the berries, it is a serious quality defect, as it reduces the overall attractiveness of the bunch. Varieties differ greatly in the rate at which post-harvest stem browning occurs (Winkler *et al*. 1974).

There is a strong correlation between cluster water loss and stem browning. A survey indicated that water loss ranged from 0.5 to 2.1% based on the initial weight (measured at harvest) within the 8-hour period before cooling. The magnitude of the losses was directly related to the length of delay, temperature during the delay before cooling, and type of box material. Even a few hours delay at high temperatures can cause severe drying and browning of cluster stems, especially on the hottest days. When cluster water loss reaches 2.0% or more for 'Perlette', 'Superior', 'Flame Seedless', 'Thompson Seedless', 'Ruby Seedless', and 'Fantasy Seedless', stems will show symptoms of browning approximately seven days later in cold storage (Crisosto *et al*. 2001). In cultivars growing in France, water loss of as little as 3% can cause a reduction in firmness and shrivelling of berries (Chapon *et al*. 1991). In all the cases, excessive water loss leads to berry shatter. A recent study shows that chlorophyll fluorescence is well correlated to water loss at the cluster level (Wright *et al*. 2009).

Browning in both damaged and intact berries and stems is almost certainly due to oxidation of phenolics via quinones to brown pigments by the action of polyphenol oxidase (Sapis *et al*. 1983a, 1993b). The severity may be determined by the level of membrane permeability and injury of cells (Burzo *et al*. 1998). Severe desiccation causes the breakdown of cell membranes and the oxidation of phenolics in the cell sap. Berries may suffer from skin and pulp browning if they become bruised during handling. Rachis and berry browning is inhibited by SO_2 treatment (Morris *et al*. 1992). Berries that have not been treated with SO_2 are also more susceptible to gradual browning of the pulp over time (Luvisi *et al*. 1992). This may be exacerbated by the use of high levels of carbon dioxide during storage for fungicidal or quarantine purposes (Ahumada *et al*. 1996; Yahia *et al*. 1983; Crisosto *et al*. 2002c).

Berry Shatter

Berry loss or shatter can be a significant problem with certain cultivars of table grapes such as Thompson Seedless (Wagener 1985; Berry & Aked 1996). The high losses of berries from Thompson Seedless have been linked to the late (post fruit-set) application of gibberellin (GA3) before harvest (Ben Tal 1990), however, GA3 treatments can have opposite effects depending on the cultivar (Jeong *et al*. 1998). There appear to be three types of berry shatter: physiological, pathological and mechanical. The first is associated with the thickening and hardening of the pedicel and production of an abscission layer (Ben Tal 1990; Nakamura & Hori 1981; Xu *et al*. 1999). The presence of fungi such as *B. cinerea*, *Rhizopus stolonifer* and *Alternaria* spp. can cause wet abscission without an abscission layer (Xu *et al*. 1999). Control of fungi with fungicides, acetic acid or SO_2 fumigation reduces shatter in stored table grapes (Xu *et al*. 1999; Sholberg *et al*. 1996; Morris *et al*. 1992). Some researchers reported that ethylene stimulates berry shatter (Nakamura & Hori 1981; Lydakis & Aked 2003b). Cold storage, GA, NAA or aminooxyacetic acid treatments were found to inhibit shatter (Wu *et al*. 1992). In California, berry shatter is mainly triggered by mechanical damage occurring during harvesting, packaging and transportation (Luvisi *et al*. 1995).

Diseases and their control

Causal organisms

The primary cause of post-harvest loss in table grapes is grey mould disease or Botrytis rot caused by *Botrytis cinerea* (Pearson & Goheen 1988; Snowdon 1990) (Plate 9.1). This disease occurs wherever the crop is grown. The fungus can grow at temperatures as low as –0.5°C (31°F) and so may spread from one berry to another during storage and transportation even if adequate pre-cooling is carried out and suitable temperatures are maintained. Botrytis rot can be identified by the characteristic 'slipskin' condition that develops, and later, by 'nests' of decayed berries encased in white mycelium.

Post-harvest berry infection is primarily caused by conidial infection at or after veraison (Kock & Holz 1991a) although some authors suggest it may happen at the flower stage (Nair & Allen 1993). The fungus remains quiescent in the developing fruit, with symptoms only appearing on the mature fruit. It is thought that loss of berry resistance is due to the decreasing ability of the maturing berry flesh to synthesise antimicrobial stilbenes and also due to the fall in proanthocyanidin concentration during development (Hill *et al*. 1981; Creasy & Coffee 1988). Berry cracking in certain cultivars also encourages infection.

Other less important fungal post-harvest diseases of table grapes include: Aspergillus rot (*Aspergillus niger*) which doesn't grow below 5°C, blue mould rot, (*Penicillium* spp), Rhizopus rot (*Rhizopus oryzae; R. stolonifer*),

Alternaria rot (*Alternaria alternata*), anthracnose (*Elsione ampelina*, *Glomerella cingulata*), bitter rot (*Greenaria uvicola*), black rot (*Guignardia bidwelii*), Botryodiplodia rot (*Botryodiplodia theobromae*), Cladosporium rot (*Cladosporium herbarum*), Coniella rot (*Coniella diplodiella*), Phompsis rot (*Phomopsis viticola*) and ripe rot (*Botryosphaeria ribis* and others) (Snowdon 1990).

General disease control

Strict hygiene in the vineyard is necessary to minimise the amount of crop debris on which fungi can survive and form spores. Thinning of bunches helps to prevent overcrowding of berries and the resultant cracking which allows ready infection. Pre-harvest fungicide sprays can give some control of post-harvest fungal infections (Snowdon 1990). It is recommended not to harvest until at least 3 days after rain. After this period, berries infected with grey mould and other fungi should be visible and can be removed during bunch trimming. Harvesting tools should be disinfected between rows of vines to reduce the transmission of viral and bacterial diseases. After harvest it is vital to cool the grapes as rapidly as possible and to handle carefully to minimise injuries to the berries. Obviously a good control of fungus development over the growing period is critical for a good postharvest storage; however, a recent study suggested that postharvest treatments are required even with a good pre-harvest management (Smilanick *et al*. 2010).

Sulphur dioxide (SO_2) fumigation

Table grapes are treated with SO_2 primarily to control grey mould which is not inhibited sufficiently by rapid cooling alone. Standard practice is to fumigate with sulphur dioxide immediately after harvesting and/or packing followed by lower dose SO_2 treatments weekly during storage. Usually this initial fumigation uses a high level of SO_2 (up to 5000 ppm) and may be carried out in specially constructed rooms. Cold storage fumigation uses lower concentration (2500 ppm or lower) and is carried out every seven to ten days. In this traditional system the excess SO_2 is removed from the treatment chamber by venting or scrubbing through water or sodium hydroxide aqueous solution after a treatment period of about 20 min. Formulas for calculating SO_2 fumigation dosages are available in the publications by Nelson (1985) and Luvisi *et al*. (1992).

Recently it has been demonstrated that the amount of SO_2 needed to kill *Botrytis* spores, or to inactivate exposed mycelium is dependent on both the SO_2 concentration and fumigation time. A cumulative concentration, calculated as the product of the concentration and contact time, called 'CT product', describes the SO_2 exposure needed to kill *Botrytis cinerea*. A CT of at least 100 ppm-hour is the minimum required to kill spores and mycelium of *Botrytis* at 0°C (32°F) or approximately 30 ppm-hour at 20°C (68°F). The CT-100 dose can be obtained with an average concentration of either 100 ppm for 1 hour, 200 ppm for ½ hour, 50 ppm for 2 hours or an equivalent combination of concentration and time. This finding was the basis for the development of the total utilization system.

The total utilization system differs from the traditional system in that there is no excess SO_2 fumigant at the end of the fumigation treatment, reducing both air pollution and sulphite residues in the fruit. It can be used with forced air cooling for initial fumigation and in cold storage for subsequent periodic treatments. Total utilization typically uses about half as much sulphur dioxide as the traditional method, and improves uniformity and effectiveness of the SO_2 fumigant. Details on this work are available in the Luvisi *et al*. (1992). Inexpensive SO_2 dosimeter tubes are available to enable fumigant penetration and distribution to be monitored in store. These dosimeters were originally designed for human safety monitoring.

When grapes are loaded for transport/shipment they may receive an additional SO_2 fumigation before loading to assure a longer market life because fumigation is seldom available in receiving markets. During ocean shipment period longer than 10 days or long retail handling in which SO_2 fumigation cannot be applied, the use of SO_2 generating pads in combination with a box plastic liner is advised (Crisosto *et al*. 1994). Sodium or potassium metabisulphite is incorporated into the pads, allowing the release of SO_2 when exposed to moisture during transit and marketing. The amount of SO_2 released is also affected by the temperature and the effective use of these pads depends on a good cool-chain being maintained. Dual-release pads give a rapid initial release of SO_2 from part of the pad while another part of the pad releases SO_2 slowly over a period of 8–10 weeks (Mustonen 1992). In France has been reported that SO_2 levels within the carton usually reach approximately 10 ppm within the first week of cold storage and then stabilise at around 2 ppm (Vidaud *et al*. 1993). A special low dosage has been developed for fumigation in trucks and overseas containers (Crisosto *et al*. 2002b). Unless SO_2 fumigation is available, the receiver must order grapes for immediate needs, and must complete distribution and marketing within a reasonable time after arrival.

Problems with sulphur dioxide treatments

One of the problems associated with SO_2 fumigation of grapes is the constant potential for injury to the berries and rachis. Injured tissue first shows bleaching of colour,

followed by sunken areas where accelerated water loss has occurred. These injuries first appear on the berry where some other injury has occurred, such as a harvest wound, transit injury or breakage at the cap stem attachment. Symptoms may also be seen around the cap stem and slowly spread over the berry. Careful attention to SO_2 treatment procedures is necessary to minimize this damage. Additionally, treated berries sometimes develop a sulphurous taint (Austin *et al*. 1997).

Another problem with SO_2 fumigation of grapes is the level of sulphite residue remaining at time of final sale. Sulphur dioxide was once included on the 'generally recognized as safe' (GRAS) list of chemicals, for which no registration is required (Anon 1986). Heavy usage of sulphites in some other foods has caused a change in regulation, because some people are highly allergic to sulphites. Sulphite residues in grapes are currently limited to <10 ppm, (10 µg SO_2/g) and there are limits on the number of repeat SO_2 fumigations allowed, depending upon cultivar (Anon 1989).

Alternatives to the use of sulphur dioxide

Considerable research has been conducted to find alternatives to SO_2 treatments for grey mould control. This is especially desirable for organic table grape growers who are prohibited from using SO_2 (USDA 2001). Despite many treatments showing promise in the laboratory, none is being used routinely in a commercial setting. Limitations include phytotoxicity and difficulties in getting good penetration of the treatment through the grape bunches. Methods tried include fumigation with hydrogen peroxide (Rij & Forney 1995), ozone (Sarig *et al*. 1996), chlorine (Zoffoli *et al*. 1999), chlorine dioxide (Crisosto *et al*. 1994), volatiles of natural origin such as hexenal (Archbold *et al*. 1999), acetaldehyde (Avissar & Pesis 1991), ethanol (Lichter *et al*. 2002) or acetic acid (Sholberg *et al*. 1996). Tripathi and Dubey (2004) have reviewed the potential of a large selection of natural products to control post-harvest rots.

High carbon dioxide levels have long been known to inhibit the growth of fungi. In the last few decades, controlled or modified atmospheres have been shown to have promise for the control of *Botrytis* in table grapes (Yahia *et al*. 1983, Crisosto *et al*. 1995; Retamales *et al*. 2003; Artés-Hernández *et al*. 2004). Levels of CO_2 at 15% or above can completely suppress the growth of *Botrytis* Levels above 10% CO_2 for too long a period, however, were found to generate off-flavours and accelerate stem browning. Sensitivity to CO_2 was, however, dependent on cultivar and maturity (Crisosto *et al*. 2002c, 2002d). In some cases the return to air atmosphere at the end of storage can be followed by strong rot development (P. Westercamp, personal communication). More work in this area is strongly recommended.

Vapour heat treatments, for example 52°C for 20 minutes have been found to be highly effective at eradicating *Botrytis* from grape berries without causing damage to the fruit (Lydakis & Aked 2003a, 2003b). Kock and Holz (1991b) found that gamma irradiation could control grey mould and Thomas *et al*. (1995) tested with some success, combinations of hot water dip and irradiation against various moulds. UV-C light has also been applied successfully to control *Botrytis* on table grapes (Nigro *et al*. 1998). A number of researchers have found a number of bacteria, yeasts and fungi to be effective as biocontrol agents against grey mould and other pathogens of table grapes (Ferreira 1990; Latorre *et al*. 1997; Lima *et al*. 1999; Zahavi *et al*. 2000). Ethanol dips showed some promise for control of *Botrytis* (Gabler *et al*. 2005), and the use of ethanol vapours (Chervin *et al*. 2005) may be developed with the pad systems that are already in use for SO_2 delivery. The use of pre-harvest ethanol sprays may also have positive impacts of the postharvest shelf life (Chervin *et al*. 2009).

Interesting alternatives using edible herb extracts (Gatto *et al*. 2011) or electrolyzed oxidizing water (Guentzel *et al*. 2010) have been reported recently.

Insect quarantine treatment

Several insects that attack table grapes are of quarantine concern and they must be eradicated prior to/or during shipment to other countries. Novelties in sanitation or quarantine treatments have been noticeable over the past ten years. This has been primarily in response to the likelihood that the use of the fumigant methyl bromide, which depletes stratospheric ozone, will be phased out over the next few years.

A number of combination treatments are being developed in order to benefit from additive and synergistic effects. For example, an official approved protocol based on CO_2 and SO_2 fumigation is being used in California to export grapes to Australia, England and New Zealand. This quarantine treatment kills black widow spiders in packaged table grapes (Mitcham 2005). A potential area of development for disinfestations studies is the use of semiochemicals, chemicals that mediate interactions between organisms (Cox 2004) and that can be used to repel or attract and kill insects. Another area of development are 'systems approaches': taking into account the initial pest count in a fruit load, which can be estimated by monitoring pests in the vineyard, to adapt the post-harvest treatment (US EPA 2000).

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