Improving fruit soluble solids content in melon (*Cucumis melo* L.) (reticulatus group) in the Australian production system

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Abstract

Total soluble solids (TSS) is a reliable indicator of melon eating quality, with a minimum standard of 10% recommended. The state of Australian melon production with respect to this quality criterion was considered within seasons, between growing districts and over seasons. It was concluded that improvement in agronomic practice and varietal selection is required to produce sweeter melons. The scientific literature addressing melon physiology and agronomy was summarised, as a background to the work that is required to improve melon production practices in Australia.

The effect of source sink manipulation was assessed for commercially grown and glasshouse grown melon plants. The timing of fruit thinning, pollination scheduling, the application of a growth inhibitor and source biomass removal were assessed in relation to fruit growth and sugar accumulation. Results are interpreted against a model in which fruit rapidly increase in weight until about two weeks before harvest, with sugar accumulation continuing as fruit growth ceases. Thus treatment response is very dependant on timing of application. For example, fruit thinning at 25 days before harvest resulted in further fruit set and increased fruit weight but did not impact on fruit TSS (at 9.8%, control 9.3%), while thinning at 5 days before harvest resulted in a significant (P<0.05) increase in fruit TSS (to 10.8%, control 9.3%) and no increase in fruit weight or number. A cost/benefit analysis is presented, allowing an estimation of the increase in sale price required to sustain the implementation of fruit thinning.
The effect of irrigation scheduling was also considered with respect to increasing melon yield and quality. To date, recommended practice has been to cause an irrigation deficit close to fruit harvest, with the intent of “drying out” or “stressing” the plant, to “bring on” maturity and increase sugar accumulation. Irrigation trials showed that keeping plants stress-free close to harvest and during harvest, facilitated the production of sweeter fruit.

The maintenance of a TSS grade standard using either batch based (destructive) sampling or (non-invasive) grading of individual fruit is discussed. On-line grading of individual fruit is possible using near infrared spectroscopy (NIR), but the applicability of the technique to melons has received little published attention. Tissue sampling strategy was optimised, in relation to the optical geometry used (in commercial operation in Australia), both in terms of the diameter and depth of sampled tissue. NIR calibration model performance was superior when based on the TSS of outer, rather than inner mesocarp tissue. However the linear relationship between outer and middle tissue TSS was strong ($r^2 = 0.8$) in immature fruit, though less related in maturing fruit ($r^2 = 0.5$). The effect of fruit storage (maturation/senescence) on calibration model performance was assessed. There was a negligible effect of fruit cold storage on calibration performance.

Currently, the agronomist lacks a cost-effective tool to rapidly assess fruit TSS in the field. Design parameters for such a tool were established, and several optical front ends compared for rapid, though invasive, analysis. Further, for visualisation of the spatial distribution of tissue TSS within a melon fruit, a two-dimensional, or hyper-
spectral NIR imaging system based on a low cost 8-bit charge coupled device (CCD) camera and filter arrangement, was designed and characterised.
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Sometimes research is a lonely and frustrating pursuit, and I thank all those who offered their friendship and who kept me company during my studies. Much love goes to my family for their continual support and encouragement during my candidature.
Declaration

I declare that this thesis is an original work and no part of this thesis has been previously submitted for the award of another degree.

I also declare that to the best of my knowledge any assistance I received in the experimentation presented in this thesis and all sources of information used in this thesis have been acknowledged.

_____________________
Robert Long

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Robert Long
**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABS</td>
<td>absorbance</td>
</tr>
<tr>
<td>ADC</td>
<td>analogue to digital conversion</td>
</tr>
<tr>
<td>AMA</td>
<td>Australian Melon Association</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>cbar</td>
<td>centibars</td>
</tr>
<tr>
<td>CCD</td>
<td>charge coupled device</td>
</tr>
<tr>
<td>cf.</td>
<td>confer (compare)</td>
</tr>
<tr>
<td>cv.</td>
<td>cultivar variety</td>
</tr>
<tr>
<td>d</td>
<td>days</td>
</tr>
<tr>
<td>d(^2)ABS</td>
<td>second derivative absorbance</td>
</tr>
<tr>
<td>DAA</td>
<td>days after anthesis</td>
</tr>
<tr>
<td>DBH</td>
<td>days before harvest</td>
</tr>
<tr>
<td>DW</td>
<td>dry weight</td>
</tr>
<tr>
<td>e.g.</td>
<td><em>exempli gratia</em> (for example)</td>
</tr>
<tr>
<td>Fig.</td>
<td>Figure</td>
</tr>
<tr>
<td>FW</td>
<td>fresh weight</td>
</tr>
<tr>
<td>HI</td>
<td>harvest index</td>
</tr>
<tr>
<td>i.e.</td>
<td><em>id est</em> (that is)</td>
</tr>
<tr>
<td>LSD</td>
<td>least significant difference</td>
</tr>
<tr>
<td>LWR</td>
<td>leaf weight ratio</td>
</tr>
<tr>
<td>MLR</td>
<td>multiple linear regression</td>
</tr>
<tr>
<td>msec</td>
<td>milliseconds</td>
</tr>
<tr>
<td>n</td>
<td>number</td>
</tr>
<tr>
<td>NIR</td>
<td>near infrared</td>
</tr>
<tr>
<td>NSW</td>
<td>New South Wales</td>
</tr>
<tr>
<td>PAR</td>
<td>photosynthetic active radiation</td>
</tr>
<tr>
<td>PC</td>
<td>principle component</td>
</tr>
<tr>
<td>PDA</td>
<td>photodiode array</td>
</tr>
<tr>
<td>PLS</td>
<td>partial least squares</td>
</tr>
<tr>
<td>QDPI</td>
<td>Queensland Department of Primary Industries</td>
</tr>
<tr>
<td>QLD</td>
<td>Queensland</td>
</tr>
<tr>
<td>r(^2)</td>
<td>coefficient of determination</td>
</tr>
<tr>
<td>R(^2)</td>
<td>coefficient of determination (NIR calibrations only)</td>
</tr>
<tr>
<td>RCBD</td>
<td>randomised complete block design</td>
</tr>
<tr>
<td>RH</td>
<td>relative humidity</td>
</tr>
<tr>
<td>RMSECV</td>
<td>root mean standard error of cross validation</td>
</tr>
<tr>
<td>RMSEP</td>
<td>root mean standard error of prediction</td>
</tr>
<tr>
<td>s/n</td>
<td>signal to noise ratio</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SDR</td>
<td>standard deviation residual</td>
</tr>
<tr>
<td>SE</td>
<td>standard error</td>
</tr>
<tr>
<td>SW-NIR</td>
<td>short wave near infrared</td>
</tr>
<tr>
<td>TSS</td>
<td>total soluble solids</td>
</tr>
<tr>
<td>vs.</td>
<td>versus</td>
</tr>
<tr>
<td>WA</td>
<td>Western Australia</td>
</tr>
</tbody>
</table>
In Australia, consumer dissatisfaction rates of up to 60% have been reported for orange flesh netted melon fruit (*Cucumis melo* L. reticulatus group), which is attributed to product inconsistency and price volatility (Australian Melon Association, 2003). Not only are fruit inconsistent in quality, but they are generally substandard, with mean total soluble solids (TSS) for populations of fruit frequently below 10% TSS (Table 1.1, Fig. 1.1), an accepted quality standard (Mutton, 1981). Even when the population average reaches 10% TSS, it should be noted that only 50% of the population (assuming a normal distribution) will have a TSS level above the minimum quality standard.

For the populations reported in Table 1.1 and Fig. 1.1, TSS was determined using a refractometer, for juice extracted from cores of tissue sampled at equatorial positions on fruit. The amount of tissue sampled (e.g. whole mesocarp including inedible ‘green’ tissue, or the ‘outer’ 1 or 2 cm of tissue) may have varied between populations, which will have introduced an error. The importance of a standardised sampling method is detailed in Chapter 5.

* Two sutured fruit in the foreground are heirloom cultivars of *Cucumis melo* (Janick, 2004).
Table 1.1. TSS Mean and SD for different melon cultivars sampled from locations throughout Australia. (adapted from Guthrie and Walsh, 1999)

<table>
<thead>
<tr>
<th>Year</th>
<th>District</th>
<th>Location</th>
<th>Cultivar</th>
<th>Mean TSS (%)</th>
<th>SD</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998</td>
<td>North Qld</td>
<td>Clare</td>
<td>Dublin</td>
<td>7.7</td>
<td>1.1</td>
<td>356</td>
</tr>
<tr>
<td>1998</td>
<td>South East Qld</td>
<td>Bundaberg</td>
<td>Eastern Star</td>
<td>8.0</td>
<td>1.4</td>
<td>300</td>
</tr>
<tr>
<td>1998</td>
<td>South Qld</td>
<td>Chinchilla</td>
<td>Hammersly</td>
<td>7.5</td>
<td>1.2</td>
<td>326</td>
</tr>
<tr>
<td>1999</td>
<td>North Qld</td>
<td>Gumlu</td>
<td>Dubloon</td>
<td>8.7</td>
<td>0.9</td>
<td>138</td>
</tr>
<tr>
<td>1999</td>
<td>North Qld</td>
<td>Gumlu</td>
<td>Mission</td>
<td>9.9</td>
<td>1.2</td>
<td>106</td>
</tr>
<tr>
<td>1999</td>
<td>North Qld</td>
<td>Gumlu</td>
<td>Eastern Star</td>
<td>9.1</td>
<td>1.1</td>
<td>142</td>
</tr>
<tr>
<td>1999</td>
<td>North Qld</td>
<td>Gumlu</td>
<td>Hammersly</td>
<td>7.6</td>
<td>1.2</td>
<td>160</td>
</tr>
<tr>
<td>1999</td>
<td>North Qld</td>
<td>Gumlu</td>
<td>Eldorado</td>
<td>9.4</td>
<td>1.7</td>
<td>202</td>
</tr>
<tr>
<td>1999</td>
<td>South Qld</td>
<td>Chinchilla</td>
<td>Dubloon</td>
<td>8.0</td>
<td>1.1</td>
<td>276</td>
</tr>
<tr>
<td>1999</td>
<td>South Qld</td>
<td>Chinchilla</td>
<td>Highline</td>
<td>8.7</td>
<td>1.1</td>
<td>180</td>
</tr>
<tr>
<td>1999</td>
<td>South Qld</td>
<td>Chinchilla</td>
<td>Malibu</td>
<td>7.4</td>
<td>1.0</td>
<td>184</td>
</tr>
<tr>
<td>2000</td>
<td>North WA</td>
<td>Kununurra</td>
<td>Hotshot</td>
<td>9.3</td>
<td>0.9</td>
<td>96</td>
</tr>
<tr>
<td>2001</td>
<td>North WA</td>
<td>Kununurra</td>
<td>Hotshot</td>
<td>8.9</td>
<td>0.7</td>
<td>91</td>
</tr>
<tr>
<td>2001</td>
<td>Central QLD</td>
<td>Rockhampton</td>
<td>Eastern Star</td>
<td>9.1</td>
<td>1.5</td>
<td>110</td>
</tr>
<tr>
<td>2001</td>
<td>North NSW</td>
<td>Bourke</td>
<td>na</td>
<td>9.6</td>
<td>2.0</td>
<td>338</td>
</tr>
<tr>
<td>2002</td>
<td>North WA</td>
<td>Kununurra</td>
<td>Hotshot</td>
<td>7.9</td>
<td>1.0</td>
<td>89</td>
</tr>
<tr>
<td>2002</td>
<td>Central QLD</td>
<td>Rockhampton</td>
<td>Eastern Star</td>
<td>10.0</td>
<td>1.6</td>
<td>64</td>
</tr>
<tr>
<td>2002</td>
<td>North NSW</td>
<td>Bourke</td>
<td>Dubloon</td>
<td>10.0</td>
<td>1.3</td>
<td>205</td>
</tr>
<tr>
<td>2002</td>
<td>North VIC</td>
<td>Mildura</td>
<td>Eldorado</td>
<td>8.4</td>
<td>1.7</td>
<td>196</td>
</tr>
<tr>
<td>2002</td>
<td>North VIC</td>
<td>Mildura</td>
<td>Dubloon</td>
<td>10.2</td>
<td>1.3</td>
<td>162</td>
</tr>
<tr>
<td>2003</td>
<td>North NSW</td>
<td>Bourke</td>
<td>Dubloon</td>
<td>9.1</td>
<td>1.7</td>
<td>300</td>
</tr>
<tr>
<td>2003</td>
<td>North VIC</td>
<td>Mildura</td>
<td>Dubloon</td>
<td>10.8</td>
<td>1.5</td>
<td>460</td>
</tr>
</tbody>
</table>

Figure 1.1. Melon fruit TSS (fruit ‘Brix’) as sampled from the fruit markets (Sydney NSW). The plot was updated weekly and presented as part of a quality improvement initiative instigated by the Australian Melon Association (AMA E-news, 2004).

Fruit quality will vary between days within a given planting and between plantings within a single farming season. This variation is illustrated in the quality control data.
from a One-Harvest ‘sweet melon’ marketing program for fruit from a commercial farm in Kununurra WA during the growing seasons of 2000, 2001 and 2002 (with destructive measurement of 20 to 35 fruit per day for a number of consecutive plantings, Fig. 1.2). During the 2000 season, plantings 8 and 9 yielded fruit high in TSS, with 11 out of 12 days for planting 8 achieving an average TSS above a quality standard of 10%, whilst 5 out of 12 days for planting 9 were above the 10% cut off (Fig. 1.2). Plantings 10 and 11 of 2000 yielded fruit low in TSS, with all days from these populations being well under the 10% cut off. Plantings 12, 13, 14 and 15 produced fruit high in TSS during the initial harvest days, but mean TSS dramatically decreased during the later part of the harvest period. Fruit sweetness varied between years, with 2000 and 2001 producing fruit above the 10% quality cut off, whilst fruit produced during 2002 was very poor, with all plantings averaging below 10% TSS.

Melon TSS will vary for a range of reasons. For example, the picking of immature (prior to fruit abscission) fruit will guarantee lower fruit TSS (Long et al., 2004), whilst warmer weather may shorten fruit development time at the expense of sugar accumulation. The effects of temperature on melon plant growth and fruit quality have been detailed previously (Jenni et al., 1996; Amuyunzu et al., 1997; Ventura and Mendlinger, 1999; Baker and Reddy, 2001), and are elaborated on in Chapter 2. Certainly for those plantings sampled in Kununurra (Fig. 1.2), average fruit sweetness trended higher during the cooler period of July and August compared to plantings later in the season. Different cultivars will respond differently to temperature, and the most appropriate cultivars should be selected for a specific location, or for specific times within the growing season at a single location. Another cultivar (other than
Figure 1.2. Cultivar Hotshot (cultivar Dubloon for 2002 plantings 1 to 3) mean TSS and SE ($n = 20-35$) sampled from plantings (pl) when NIR sorting was in progress, from a farm in Kununurra WA for the 2000, 2001 and 2002 seasons. Dotted line is the quality TSS cut off. Daily maximum and daily minimum temperatures are reported (Australian bureau of meteorology - Kununurra airport).
Hotshot) may be more appropriate to cultivate during the warmer parts of the Kununurra season.

The indeterminate production of female flowers and subsequent cyclic nature of fruit set ensures that a single broad acre melon crop consists of plants bearing fruit varying in stages of maturity. As a result, a single crop will take a number of consecutive days to harvest. As fruit are harvested, the source to sink relationship of a plant will change and the agronomic requirements such as irrigation (Chapter 4) and nutrition will also differentially change according to individual fruit maturity status. The presence of commercial pickers will also impact on plant integrity, and changing environmental conditions during the period of harvest are likely to affect the quality of fruit. For example, damage to vines by picking crews may account for the decrease in fruit TSS observed towards the end of some of the plantings in Kununurra (Fig. 1.2). Chapter 3 details the effects of manipulating the source to sink ratio on melon fruit quality and yield, with emphasis placed on the timing of application. A supporting parallel study assesses source sink manipulation on stone fruit (Appendix 1).

To improve the TSS quality of melon fruit at market, either a rigorous batch testing protocol or the individual sorting of fruit can be implemented. The labour intensive protocol involved in juice refractometry has been a stumbling block to the adoption of TSS standards. Near infrared (NIR) spectroscopic analysis allows the assessment of individual pieces of fruit non-invasively and rapidly (Maruo et al., 2002). Such analysis not only sorts fruit into quality grades, but it also enables the quality of produce to be recorded. Additionally, this information assists agronomic research,
augmenting the understanding of crop physiology in relation to fruit sweetness. The ability of NIR equipment to assess melon fruit TSS depends on the thickness of outer rind tissue and the homogeneity of melon tissue, and how these issues affect the spectral sampling of a representative portion of fruit pertaining to the TSS of the edible mesocarp (Chapter 5). This is also affected by the postharvest attributes of a given melon population, such as the physiological stage of maturity at the time of harvest, and the time that fruit spend in cold storage prior to NIR assessment (Chapter 5). Potentially the rind problem could be circumvented by employing an invasive NIR probe, allowing rapid, if destructive, analysis (Chapter 7). Finally, the use of NIR hyperspectral 2D imaging of the cut surface of melon fruit (Chapter 6) could prove to be a useful tool to physiologists seeking to understand fruit ripening and sucrose accumulation.
2. Cucumis melo L. a review

Melon classification and form

The general taxonomy, morphology and anatomy of melon is detailed herein primarily as that summarised by Whitaker and Davis (1962) and Robinson and Decker-Walters (1999). Melon occur as cultivar varieties of Cucumis melo L, classified within the Cucurbitaceae family, subfamily Cucurbiloideae, tribe Melothrieae, subtribe Cucumerinae (Robinson and Decker-Walters, 1999).

Plants are typically trailing vines, having simple, palmately veined, five-lobed leaves occurring one per stem node. Along the stem there are five leaves for two twists of the stem before one leaf is directly above another (phyllotaxy of 2/5). Stems are round, centrally hollow and contain bicollateral vascular bundles, meaning that phloem tissue occurs uniquely on the outside and inside of the xylem. Plants consist of a main or primary stem, from which lateral stems or branches are produced. In turn these branches may bear more lateral stems. Coiled tendrils occur at leaf axils, and act to help the plant cling to trellises and other supports. The plant has a relatively strong tap root which is thought to penetrate to approximately 1 m depending on soil type and irrigation arrangement. The secondary roots however make up most of the root mass and occur in the first 60 cm of soil.
C. melo is either monoecious, bearing separate staminate and pistillate flowers on the same plant, or andromonoecious, bearing separate staminate and hermaphroditic flowers on the same plant. Staminate flowers occur in axillary clusters on the main stem and laterals, whilst ovary bearing flowers occur at the first node of each lateral branch. Pollination of ovary bearing flowers is required, and facilitated by naturally occurring pollinating agents (commonly commercial honey bees).

Fruits vary in shape and size depending on cultivar variety. Typically, monoecious plants produce elongated fruit, whilst andromonoecious plants produce round or ovate fruit. Some cultivars possess longitudinal sutures on fruit, whilst in others sutures are suppressed. The fruit surface may be smooth, or possess lenticels, which form a netted suberised cork-like texture. Seeds vary in number between cultivars, but are usually in the multiple hundreds per fruit. Each seed has a testa of several layers, a thin collapsed perisperm and endosperm, and an embryo which consists of two large flat cotyledons and a small radicle. Seeds have a high percentage of lipids (32.3%) and proteins (19.3%) (de Mello et al., 2001).

Whitaker and Davis (1962) gave an extensive account of classical genetics and breeding for melons. C. melo possesses 12 pairs of chromosomes. Although considered to be a highly polymorphic species they are classified as cytologically stable, with extensive sampling of sub-species and cultivars revealing no deviation from a regular meiosis with twelve pairs of chromosomes. The earliest known genetic studies on C. melo were reported to be those by Sagaret in 1826 (40 years prior to Mendel’s pea experiments), which focused on characterising fruit traits of economic
importance; identifying yellow skin colour as dominant and white as recessive, netted epidermis dominant and smooth recessive, sutures dominant over no sutures, and acid flavour dominant and sweet flavour recessive. Early commercial breeding work on *C. melo* focused on disease resistance, and included the development of melon rust resistant plants in 1908, and the famous and successful development of the powdery mildew resistant (PMR) no. 45 cultivar in 1937 by Jagger and Scott (U.S. Department of Agriculture). Breeding programs still continue, addressing disease resistance, and more recently, quality and yield parameters (Norton, 1975; Bowers *et al.*, 1981; Iglesias *et al.*, 2000). Currently the majority of commercial *C. melo* production is obtained from F1 hybrids. Such hybrids are proprietary inbreeds and yield unreliable seed, thus allowing seed companies to control their genetic intellectual property.

The geographical origin of *C. melo* is unclear, but it is traditionally considered to be a ‘desert’ plant, and is thought to have originated in Africa and first domesticated as a food source in Egypt and Iran 3000 years BC. Historical records show that melon spread throughout India, China and Afghanistan, eventually being introduced to the papal estate of Cantaluppe near Rome in the fifteenth century. In the 1700s, melon cultivars were under cultivation in British glasshouses, and by the 1800s had been introduced to ‘new world’ countries (Robinson and Decker-Walters, 1999; Goldman, 2002).

Today, *C. melo* utilised as food are classified under the scheme suggested by Naudin in 1859 (Whitaker and Davis, 1962), which are groups based on fruit characteristics and use. The common groups are:
1. Cantalupensis (true Cantaloupe common in Europe, rough or scaly surface but not netted)
2. Reticulatus (Rockmelon, Muskmelon or known in the U.S. as ‘Cantaloupe’, fruit are netted and slip from the vine)
3. Inodorus (Winter Melon or Honeydew Melon in Australia)
4. Flexuosus (Snake Melon or Serpent Melon)
5. Conomon (Oriental Pickling Melon)
6. Chito (Mango Melon or Garden Lemon)
7. Dudaim (Pomegranate melon or Queen Anne’s melon)

Rockmelon, as known in Australia, or Muskmelon or Cantaloupe in the U.S., come from plants belonging to the reticulatus group. Mature fruit have a reticulated lenticel netting over the epidermis, although they may be sutured and abscise or “slip” from the vine at maturity. Flesh is typically orange and is sweet to taste with a musky flavour (Goldman, 2002). The experimental chapters in this thesis concern proprietary andromonoecious F1 hybrid cultivar varieties (cv.) of *C. melo* (reticulatus group), which herein is simply referred to as melon.

*Melon eating quality*

Sweetness or sugar content is the most important factor determining the eating quality of melon fruits (Mutton *et al.*, 1981), although melons also exhibit unique ‘musky’ flavour components based on aroma volatile esters, acetates, saturated and unsaturated aldehydes, alcohols and sulphur compounds (Jordan *et al.*, 2001; Shalit *et al.*, 2001; Flores *et al.*, 2002). Work by Burger *et al.* (2000) demonstrated that variation in fruit sucrose content accounted for practically all the variation in total sugar content in the
mature fruit, and hence concluded that sucrose metabolism is important in
determining sugar content and eating quality of melon fruit. TSS of melon flesh, as a
refractometer reading, is an acceptable measure of sugar content and thus a measure
of quality (Chapter 1, Appendix 2). To improve melon quality an understanding of
melon photoassimilate source and sink physiology is required. Specifically, an
appreciation of assimilate usage during fruit development is required, in addition to
how physical extrinsic mechanisms, such as agronomic practices and disease,
influence assimilate delivery to the fruit sink.

*Photoassimilate production and transport*

As plants grow and develop, assimilated carbon (carbohydrate) is redistributed
between plant tissues for usage and storage. Plant structures can be divided into two
broad groups on the basis of these processes (Sonnewald and Willmitzer, 1992).

1. Areas of assimilate production or ‘sources’, being the photosynthetically active
portions such as mature leaves, with are net exporters of photoassimilates.

2. Photoassimilate ‘sink’ regions that draw assimilate to them, being net importers of
fixed carbon for vegetative and reproductive structural growth and maintenance.
Sink tissue can be further classified as utilisation or storage sinks. Utilisation sinks
are highly metabolically active, rapidly growing tissues such as meristems and
immature leaves, whilst storage sinks deposit incoming carbohydrates as storage
compounds like starch, sucrose, fatty acids or proteins.
Through the course of plant growth, organ structures will vary in assimilate metabolising behaviour. For example, a newly expanding leaf is a net importer of fixed carbon before maturing into a net exporter (Hay and Walker, 1992).

Assimilated carbohydrates are transported throughout the plant via phloem tissue. Phloem consists of several structurally distinct cell types: sieve elements, companion cells, parenchyma cells and fibres (Esau, 1965; Crafts and Crisp, 1971; Salisbury and Ross, 1992). Sieve elements are living cells linked end to end to form the conduit for the long distance movement of assimilate. During their maturation the nucleus and other organelles degenerate, facilitating a flow through structure. The end walls of a sieve element are modified to form a porous sieve plate (Pessarakli, 1995). Companion cells are fully functional highly metabolically active cells which act to maintain the structural integrity of sieve elements. Companion cell protoplasts are connected to sieve elements via plasmodesmata, providing a cytoplasmic connection between the two cell types (Crafts and Crisp, 1971; Taiz and Zeiger, 1991).

In source leaves, photosynthetic mesophyll cells are never more than a few cells away from phloem tissue, minimising the distance that assimilate must travel to effect efficient phloem loading. There are three types of companion cells found in source leaves for different plant groups (Pessarakli, 1995). Type 1 companion cells or intermediary cells display large numbers of plasmodesmata linking these cells through to photosynthetic cells, facilitating symplastic assimilate transfer. Type 2a and 2b companion cells do not use plasmodesmatal transfer, but rely on a membrane transport system known as apoplastic phloem loading, to move the majority of assimilate molecules. Melons exhibit type 1 companion cells and the associated
symplastic assimilate transfer (Pessarakli, 1995). The type 1 (symplastic loading) condition is hypothesised to be the (less efficient) ancestral condition, with apoplastic loading arising later in temperate and arid climates (Gamalei, 1991).

To achieve symplastic loading, the disaccharides sucrose and galactinol are passed from photosynthetic cells to companion cells via plasmodesmata, where the galactose residues of galactinol are transferred to sucrose to form raffinose and stachyose with the release of myo-inositol. Rafinose and stachyose then pass into the sieve element but are prevented from passing back into the photosynthetic cell because of the smaller diameter of the plasmodesmata connecting this cell to the companion cell (Fig. 2.1). Smaller amounts of other sugars such as sucrose and galactose also move into sieve elements. Chrost and Schmitz (1997) measured the sugar composition of the phloem exudate from the peduncle, which is the stem directly attached to the fruit, and reported that stachyose and raffinose were the main sugars (at 20 days before harvest DBH, stachyose 5.0, raffinose 3.2, sucrose 2.2, glucose 2.5 mg·g·mL⁻¹). They also reported that total sugar content was 15-18 mg·mL⁻¹, a magnitude less than that observed in most other plants. Indeed Schmitz et al. (1987) fed radiolabelled ¹⁴CO₂ to melon plants and reported that the dominant sugars in stem translocate were stachyose and raffinose (% ¹⁴C of sugar fraction: raffinose, stachyose and verbascose 88.7, sucrose 7.5, hexoses 3.9).

Assimilate loading into the sieve element creates an osmotic potential in the sieve cells. Water thus follows osmotically, causing pressure to develop. In the sink, photoassimilates are unloaded symplastically, with water following. Overall, these
processes generate a pressure drop from source to sink, facilitating the bulk flow mechanism of phloem transport (Crafts and Crisp, 1971).

**Figure 2.1.** In melon, sucrose and galactinol are symplastically loaded from photosynthetic cells through companion cells, to sieve elements, with subsequent conversion to raffinose and stachyose (adapted from Pessarakli, 1995).

Source sink interactions

The translocation of assimilate from source to sink is controlled by processes operating at both the source and sink, such that the number of, the yield and quality of, economically important sinks will be primarily limited by the supply of assimilate (source-limited) or by the capacity of sinks to import and utilise assimilate (sink-limited). During the development of a sink the relationship between source and sink changes, such that final economic yield can be attributed to a series of sequentially determined yield components, each being the product of source sink interactions occurring during successive phases of plant development (Hay and Walker, 1992).
Perturbing sink or source function in melon facilitates an understanding of source sink interactions and assists in effecting greater economic production (i.e. fruit quality and yield) (e.g. as presented in Chapter 3).

To examine the sink strength of growing melon fruit relative to the whole plant, Valintine et al. (1998) examined the effect of fruit number on leaf area and photosynthetic activity in two melon cultivars. Flowers were removed to provide a treatment of one fruit per plant, whilst another treatment constituted unrestricted fruit growth. Whole plant net photosynthesis, measured weekly from flowering until fruit harvest, was reduced by 30% under high fruit load, although light use efficiency remained unaffected. Twenty four days after pollination, the leaf area of plants with unrestricted fruit number decreased (less new leaves developed), while leaf area of single fruit plants kept increasing. Thus, more fruit on plants diverted assimilate away from and reduced the production of source leaves, although the photosynthetic rate of remaining leaves was not affected. Similar work by El-Keblowy and Lovett-Doust (1996) presented corroborating evidence for developing fruit sinks inhibiting new leaf production. To quantify the origin of assimilate that translocate to fruit sinks, Hughes et al. (1983) fed $^{14}$CO$_2$ to melon leaves and reported that leaves which were 3 nodes acropetal to fruit exported 65% of the label in 6 hours, while those further from the fruit retained the label longer. They generalised that the influence of melon fruit on the movement of $^{14}$C label was limited to a few internode lengths along the branch.
**Source sink – temperature**

Temperature will affect source and sink physiology, such as leaf expansion rate, photosynthetic rate, photorespiration rate, and source developmental physiology (Hay and Walker, 1992). Amuyunzu *et al.* (1997) showed that the relative growth rate of melon (based on main stem length) was affected by temperature, whilst Baker *et al.* (2001) developed a melon phenology model using a base temperature of 10 °C (zero leaf appearance rate), an optimum temperature of 34 °C and an upper threshold temperature of 45 °C. Similarly, Jenni *et al.* (1996) reported a multiple linear regression model that used air and soil temperature data to predict the growth of ‘Earligold’ melon from transplanting to anthesis. Bode Stoltzfus *et al.* (1998) propagated melon seedlings at different root temperatures and reported a root zone temperature threshold for optimum melon seedling growth of 35-36°C. Ventura and Mendlinger (1999) reported that melon plants grown in unheated greenhouses (compared to normal in-winter heated houses) produced a greater number of smaller fruit per plant, which were higher in TSS. They attributed this to a longer fruit development time in turn enabling more assimilate to accumulate.

**Source sink – light intensity and photoperiod**

According to Robinson and Decker-Walters (1999) ‘high light intensity is needed for optimum yield of cucurbits’, which reflects the desert dwelling origin of melon. Inadequate light intensity affects the assimilate producing ability of leaves; under prolonged shade conditions vigorous vegetative growth may re-distribute assimilate stored in fruit (Robinson and Decker-Walters, 1999). Melon plants under 50% shade treatments implemented 10 to 15 days prior to fruit maturation produced fruit low in sucrose content and with a “water-soaked” appearance of flesh (Nishizawa *et al.*, ...
Whitaker and Davis (1962) reported that the reduction of light intensity or shortening the period of illumination lead to a smaller leaf area compared to control plants. Deliberately cultivating crops at different latitudes is one strategy that allows photoperiod/illumination period control, however potential variations in temperature, rainfall and disease that accompany latitudinal change need to be considered (Hay and Walker, 1992).

**Fruit development**

The early stages of melon fruit growth include ovary development, fertilisation and fruit set (Gao *et al.*, 1999). Chrost and Schmitz (1997) showed that the fresh weight of fruit increased over time until 7–10 days before abscission or at about 46 days after anthesis (DAA), with fresh weight (FW) declining thereafter (Fig. 2.2), reflecting the typical sigmoidal growth curve described previously (Sinnott, 1945; McGlasson and Pratt, 1963). Between 39 and 46 DAA, an initial dramatic increase in sucrose concentration was recorded (for endocarp: 5 mg·g FW⁻¹ at 39 DAA compared to 40 mg·g FW⁻¹ at 46 DAA) (Fig. 2.2). This sucrose increase was 3.5% of the fresh weight change (585 g) over the same time frame, suggesting that fruit fresh weight is insignificantly affected during maximum sucrose accumulation and flesh sweetness. The subsequent decrease in fresh weight was probably due to a break down in peduncle vascular tissue, while water continued to be transpired, without being replenished. Lester (1998a) measured melon fruit longitudinal and equatorial circumference in the morning and evening of each day during the course of fruit development, and noted that melon fruit growth was significantly greater during the night than during the day. However the study did not differentiate between carbohydrate or water accumulation in the sampled fruit.
Figure 2.2. Melon fruit fresh weight and total sugar content of the exocarp (▼), endocarp (○) and placenta (●) tissue. Fruit were sampled from anthesis to fruit abscission (adapted from Chrost and Schmitz, 1997).

Fruit development – sugar enzyme biochemistry

Enzymes involved in the metabolism of assimilate within melon fruit can be classified into two basic groups (Fig. 2.3). The first group includes those enzymes involved with the initial hydrolytic removal of galactose from raffinose and stachyose with the subsequent metabolism of galactose. These enzymes are the galactosidases, galactokinase, UDPgal pyrophosphorylase, epimerase, and UDPglu pyrophosphorylase. The second group affect hexose and sucrose metabolism. This group includes the invertases, sucrose synthase and sucrose phosphate synthase (SPS) (Schaffer et al., 2000).

Schaffer et al. (2000) suggested that the enzyme α-galactosidase Type 1, which catalyses the metabolism of stachyose to galactose, might control a key step in
determining fruit development and quality. This enzyme is capable of hydrolysing both raffinose and stachyose at neutral-alkaline conditions, and displays a distinct developmental pattern different to \( \alpha \)-galactosidase Type 2. As Type 2 decreases in activity throughout fruit development, \( \alpha \)-galactosidase Type 1 increases in activity, and is the predominant \( \alpha \)-galactosidase enzyme during the period of sucrose accumulation. This enzyme is also more prevalent in sweeter melon cultivars, as compared to less sweet cultivars (Schaffer et al., 2000).

**Figure 2.3.** Melon fruit enzymatic metabolism of sucrose from stachyose and raffinose (adapted from Schaffer et al., 2000).

The transition from growth to sucrose accumulation in the fruit is characterised by the developmental loss of the vacuolar form of acid invertase (AI) (Schaffer et al., 2000). This enzyme hydrolyses sucrose, and its absence allows vacuolar sucrose storage.
The quantity of sucrose accumulated in fruit flesh is due to both the length of the sucrose accumulation period and the rate of sucrose accumulation during this period, the former is determined by the timing of the developmental decrease in AI activity (Burger et al., 2000). Importantly there is genetic variability in this enzymatic behaviour, indicating that the early decrease in AI activity might be a desirable genetic trait.

Lester et al. (2001) concluded that both SPS and AI were key enzymes responsible for sugar accumulation in melon. They showed that during early fruit development for both a known high sugar accumulating cultivar and a known low sugar cultivar, no sucrose was detected which coincided with less SPS activity than AI activity. At maturity fruit from the high sugar cultivar had higher sucrose content and SPS activity, and lower AI activity, compared to the low sugar cultivar.

To date no known recombinant DNA technology has been implemented to modify the sucrose accumulating patterns in melon. There may be potential to create a genetically modified melon plant that has the transformed regulation of specific sugar accumulating enzymes (e.g. up-regulation of SPS).

*Fruit development – softening*

Softening is typically associated with the ripening of many fruits including melon (Miccolis and Saltveit, 1995) which is represented by a decrease in tissue firmness, a measure of fruit quality (Appendix 2). Softening is a product of decreased cell adhesion caused by the degradation of cell wall and middle lamella polysaccharides such as polyurinides and xyloglucans (Simandjuntak et al., 1996). Xyloglucan
breakdown is responsible for the initial process, while polyurinide degradation is associated with further excessive softening (Wakabayashi, 2000). Following abscission, melon fruit will continue to soften and senesce. Senescence is primarily associated with the loss of hypodermal mesocarp tissue membrane integrity which is attributed to lipoxygenase activity (Lester, 1990; Lester and Stein, 1993).

*Fruit development - ethylene*

Melon fruit are considered to be climacteric exhibiting a distinct increase in the production of ethylene late in fruit maturation (Lyons *et al.*, 1962; McGlasson and Pratt, 1964; Pratt, 1971), which is associated with changes in flesh colour, fruit sugar content, aroma and flavour, and peduncular abscission (Seymour and McGlasson, 1993). The extent of the effects of ethylene on specific maturation/ripening events has been addressed via the assessment of ethylene-suppressed antisense transgenic melon cultivars (Flores *et al.*, 2001a; Flores *et al.*, 2001b; Flores *et al.*, 2002; Perin *et al.*, 2002). Specifically, Flores *et al.* (2001a) reported that rind degreening and cell separation in the peduncle were ethylene-dependent processes, whilst tissue softening and membrane deterioration were ethylene-independent. Flores *et al.* (2001b) reported differences in the maturation/ripening of the ‘rind’ and ‘pulp’ (whole mesocarp) for transgenic melon in contrast to control fruit. During the climacteric period (commencing 30 days after pollination – DAP) chlorophyll levels continued to increase in the rind of antisense fruit whilst levels decreased in control fruit. In contrast chlorophyll in the pulp for both fruit types steadily decreased from pollination to maturation. Sucrose level dramatically increased while citric acid levels decreased in pulp tissue for both antisense and control fruit, although sucrose and acid levels were higher in antisense fruit at maturation. However for rind tissue, acid and
sugar in control fruit plateaued at the climacteric, but continued to linearly increase in antisense fruit.

**Cultural practice**

Cultural practices will affect plant growth, assimilate partitioning and subsequent melon quality and yield. Fruit thinning practice is likely to influence assimilate distribution to remaining fruit. Accurate irrigation scheduling ensures the maintenance of appropriate soil moisture levels at different stages in the melon life cycle. Nutrition management is equally important in the maintenance of a growing melon plant that will produce an appropriate amount of assimilate-producing leaves relative to an appropriate number of fruit capable of maturing into a quality product. Plant disease parasitises assimilate carbon and thus detrimentally affect fruit development by either directly infecting the fruiting body or by competing for assimilate by infecting vegetative organs.

**Cultural practice – fruit thinning**

Fruit thinning, either implemented manually or chemically, is a common technique employed in the commercial production of a number of fruit commodities (Link, 2000). For example, fruit thinning increases apple fruit sugar and acid levels (Link, 2000; Basak, 2002). To date, thinning is not commercially practiced in the Australian melon growing industry. Robinson and Decker-Walters (1999) reported that melon fruit thinning may allow remaining fruit on the vine to grow larger, but did not report specific details on implementing the practice. Fruit thinning at appropriate times in melon fruit development, might allow more source leaves to be present relative to fruit sinks, hence allowing more photoassimilate to be available to sink organs. The
timing of fruit thinning will probably affect how the fruit sink will behave (e.g. affecting size and/or sweetness). Lester and Dunlop (1985) showed that within the first 40 days from anthesis, assimilate is used for fruit growth, and that the subsequent time until maturity is when sugar accumulates. Hence thinning during early fruit development may affect fruit weight, whilst thinning later in fruit development may affect fruit sweetness. Such issues are considered further in Chapter 3.

Cultural practice – soil and irrigation

According to Gobat et al. (2004) three states of water are distinguished in the soil according to the strength with which the soil retains it: gravitational water, plant available water and plant unavailable water. Gravitational water is that influenced by gravity, and when the force of gravity is balanced by the retentive force of the soil, the soil water is at field capacity. Plant available water is more strongly retained and fills pores 0.2 to 50 µm in diameter. Plant roots can extract this water to the permanent wilting point, occurring when the retentive force of the soil for water equals the maximum suction force exerted by the plant. Plant unavailable water is that occurring as hydrated minerals.

Melon plants like other cucurbits prefer well drained soils with a high organic content at a pH of between 5.6-6.8 (Robinson and Decker-Walters, 1999). Irrigation practice will affect melon root form. Typically furrow or flood irrigation, which involves saturation of the whole soil profile, promotes a more wide spread root system, both laterally and in depth. Trickle irrigation usually consists of a porous tube buried 10 to 30 cm under the ground and feeds the root system water more directly. This method is thought to use much less water due to the more efficient direct feed to plant roots,
which tend to concentrate around the trickle line. However, as less soil volume is explored by roots, plant access to soil nutrients is altered, and typically the delivery of fertiliser is also delivered via trickle (fertigation). Appropriate soil moisture management is required to maximise yield and quality of melon (Chapter 4).

*Cultural practice – mineral nutrition*

Mineral elements essential for plant function are classified as macro or major elements (N, P, S, K, Ca, Mg) and micro or trace elements (Fe, Mn, Cu, Zn, Mo, B, Al) (Gobat *et al.*, 2004). Such elements play key roles in cellular function and structure. For example, N constitutes amino acids and thus proteins, P is important in the cellular transport of energy (ATP and ADP) and K plays an important role in regulating osmotic pressure. For melon production, as for other cucurbits, a base fertiliser comprising the major elements (e.g. N:P:K) is ploughed into beds before planting. Additional fertiliser is delivered as a side dressing (for furrow irrigation prior to significant vegetative growth) or via trickle fertigation throughout different stages of crop growth. Further, foliar applications of certain nutrients may also be applied during the crop cycle (Robinson and Decker-Walters, 1999). Nutrition management will affect fruit yield and quality.

*Cultural practice – disease*

Melon plants are susceptible to a range of diseases, including various fungal, bacterial, viral and insect infestations (Robinson and Decker-Walters, 1999). Common fungal diseases include Fusarium wilt, powdery and downy mildew and gummy stem blight. Fusarium wilt is caused by *Fusarium oxysporum*, and symptoms include yellowing and dying-off of leaves, dark lesions on fruit and a brown exudate
from stems (Punja et al., 2001). Powdery mildew is primarily caused by *Sphaerotheca fuliginea*, and is characterised by white talcum powder-like growth on leaves, petioles and stems; the fungus parasitises nutrients from epidermal cells and eventually causes plant death (Perez-Garcia et al., 2001). Downy mildew is caused by *Pseudoperonospora cubensis* and effects dark brown lesions on leaves (Thind and Mohan, 2001). *Didymella bryoniae* is the causal agent of gummy stem blight, and is also responsible for leaf and stem destruction, exhibiting a characteristic ‘gum’ which oozes from stems and branches (Lovatt et al., 1997).

Sudden wilt syndrome is a name that collectively categorises disease that contributes to rapid wilting and vine decline late in fruit development, due to root destruction and thus an incapacity of root xylem to deliver water to leaves during the period of heavy fruit load and the associated high level of CO₂ assimilation (Pivonia et al., 2002). The casual agent for sudden wilt has been reported to be a mix of different fungal and bacterial infections (Iglesias et al., 2000), although some fungi species have been more closely associated, including *Fusarium oxysporum, Verticillium dahliae, Acremonium cucurbitacarum* and *Monosporascus cannonballas* (Garcia-Jimenez et al., 1999; Aegerter et al., 2000; Cohen et al., 2000a, b). Poor irrigation management early in the crop cycle, in particular too much water and the development of a small root system, has been associated with sudden wilt.

Two bacterial infections common to the Australian production system are bacterial spot, which is caused by *Xanthomonas campestris* pv. *cucurbitae*, and angular leaf spot, which is caused by *Pseudomonas syringe* pv. *lachrymans*. Both diseases effect necrotic black patches on leaves and fruit (Lovatt et al., 1997). Four common virus
infections are papaya ringspot (PRSV-W), watermelon mosaic (WMV-2), zucchini yellow mosaic (ZYMV), and squash mosaic virus. The latter is seed borne and spread by the 28-spot ladybird, the other viral infections are transmitted by aphids (Lovatt et al., 1997; Klingler et al., 2001). Aphids (Aphis gossypii) also destroy plant tissue during feeding, as do other insect pests such as pumpkin beetle (Aulacophora foveicollis) (Rajak, 2000), white fly (Bemisia tabaci) (Blackmer and Byrne, 1999), cucumber moth larvae (Phakellura indica) and spider mite (Leptoglossus australis) (Robinson and Decker-Walters, 1999).

Assessing TSS

As discussed, measuring the TSS of melon fruit tissue is a reliable indication of tissue sugar content and thus eating quality (Chapter 1). NIR spectroscopy is a technology that has been adopted for the on-line sorting of intact (thin skinned) fruit for TSS (Walsh et al., 2004). However the thick lenticels and other rind components of melon fruit limit the application of this technique for the assessment of the TSS of inner mesocarp in melon fruit. Melon fruit flesh TSS is inhomogenously distributed (Peiris et al., 1999) and this characteristic also impedes the successful performance of NIR based sorting technology. Chapters 5, 6 and 7 specifically address the measurement of melon fruit TSS using NIR spectroscopy, including the use of 2D NIR imaging that will aid in visualising the spatial distribution of flesh TSS in melon fruit.
Conclusion

Melons are typical cucurbits, displaying hollow stems with bicollateral vascular bundles, and transport photosynthate via symplastic assimilate loading. Stachyose and raffinose are translocated into fruit as sucrose, which is primarily responsible for melon flesh taste. The fruit enzymes $\alpha$-galactosidase Type 1, acid invertase and sucrose phosphate synthase are thought to play important roles in sucrose metabolism and are potential candidates for transgenic regulation in attempts to engineer a sweeter melon.

Sucrose content and yield can be influenced by employing cultural practices that increase assimilate transport to fruit. Fruit thinning practice will alter the source to sink relationship of plants and will make assimilate more available to developing fruit. Implementing the most appropriate irrigation method for particular growing regions, and scheduling watering events at optimal rates, will influence production. Similarly, optimising nutrition and disease management will increase economic returns.

NIR-based sorting technology has been recently introduced into the Australian fresh fruit industry (first used in 2000). The ability for a market to provide ‘guaranteed sweet’ fruit for sale places pressure on producers to consistently provide quality fruit. The work in this thesis was part of a national Horticulture Australia Limited (HAL) program entitled: Development of a Crop Management Program to Improve the Sugar Content and Quality of Rockmelons ref no. VX00019. The program consisted of field trials at melon growing locations in Australia (Kabra, QLD; Kununurra, WA and Bourke, NSW). The aim of the project was to streamline those cultural practices
already employed, which included irrigation scheduling, mineral nutrition, cultivar
selection and source sink manipulative experiments. This thesis focused on two
components, source sink manipulation, which was augmented by glasshouse and
additional field experiments, and irrigation scheduling, which was supported by soil
moisture monitoring technology.

Further, detailed characterisation of the distribution of TSS within melon fruit, as
related to the NIR-based measurement of TSS, was undertaken. Invasive NIR
techniques were also explored, including the design of a ‘front end’ acquisition
apparatus for a portable instrument, as well as the design and characterisation of a low
cost NIR imaging system.
Source-sink manipulation to increase melon fruit biomass and soluble sugar content

Abstract
Various source-sink perturbations were employed to alter partitioning to orange flesh melon (*Cucumis melo* L. reticulatus group) and thus to influence fruit biomass and soluble sugar content (indexed as total soluble solids of fruit juice, % TSS), with attention given to the timing of treatment application. A strong relationship existed between harvest index and fruit mass ($r^2 = 0.88$) in control plants, while the relationship with fruit TSS was poor ($r^2 = 0.11$). Augmentation of assimilate supply to fruit early in fruit development (before approx. 21 days before harvest, DBH) resulted in more fruit set and increased fruit biomass, while augmentation after 21 DBH resulted in increased fruit TSS. Thus, fruit biomass was increased (1644 cf. 1442 g FW per fruit for control, $P = 0.02$), but not TSS, on plants in which fruit set was delayed (source biomass increased, harvest index decreased from 59% for control to 38%). Treatment of plants with a cytokinin based vegetative growth inhibitor at 14 DBH produced fruit with higher TSS (11.3 cf. 10.7% for control). Thinning fruit to leave one fruit per plant one week before harvest increased the proportion of fruit in a population that exceeded a quality control standard of 10% TSS from 20 to 80%. Variations in plant response with timing of treatment application are interpreted in terms of fruit development (cell division, cell expansion and sugar accumulation phases). Although a detriment to yield (15 cf. 31 t/ha for control), the fruit thinning treatment was recommended for commercial use and a simple model was developed to calculate the required farm gate price of fruit to make thinning economically viable.

Introduction

Biomass and carbohydrate content of fruit are manipulated by agronomic practices that increase assimilate partitioning to fruit in a number of crops. For example, hand and chemical thinning of fruits is common in the apple (Basak, 2002), citrus (Stover et al., 2001), and stone fruit (Byers et al., 2003) industries to increase fruit size and TSS. Plant growth regulator applications are also used to improve partitioning to fruit (Looney, 1997) and pollination scheduling can be used to alter the number of fruit set, and thus the source to sink ratio (eg. in apple, Benedek and Nyeki, 1996). Thus a number of manipulation options are available, although in all examples the timing of treatments in relation to the development of the fruit is critical. Similar treatments should be applicable for the manipulation of rockmelon fruit yield and quality, however, the literature base on source-sink manipulation of rockmelon is sparse, with more focus on the effect on fruit biomass than carbohydrate content.

Melon fruit display a typical sigmoidal growth curve (McGlasson and Pratt, 1963), with four separate phases to fruit development (Higashi and Ezura, 1999): (1) ovary development; (2) cell division (this phase being a primary determinant of fruit size in terms of sensitivity to temperature); (3) cell expansion; and (4) sugar accumulation. Fruit fresh weight reaches a plateau, and may decline, during the last 2 weeks of development (Chrost and Schmitz, 1997), while total sugar accumulation continues until fruit abscission (Lester et al., 2001). Total sugar accumulation throughout the life of a melon fruit may be either linear (Lester and Dunlap, 1985), or have a distinct increase in the final stages of development (Miccolis and Saltveit, 1991; Chrost and Schmitz, 1997).
Broadly, sugar accumulation in a fruit can be influenced by source availability and or competing sink activity. However, a change in source availability from early plant development may result in a change in the number of fruit set and in biomass per fruit, with similar sugar content (e.g. Hubbard et al., 1990). Thus, when Eischen et al. (1994) used floating row covers to exclude bees from field grown cantaloupe plants, delaying pollination by 0, 6, or 12 days, the source to sink balance was manipulated. Delayed pollination was reported to generally result in a greater total fruit biomass and greater number of fruit per plant, with no effect on fruit TSS. A change in source availability late in fruit development may, therefore, be more likely to impact fruit TSS.

Melon fruit are described as ‘dominant’ sinks, relative to vegetative growth (El-Keblawy and Lovett-Doust, 1996). When fruit are removed from a melon plant, the plant will re-invest the available photosynthate into the remaining fruit, or into vegetative growth (e.g. Valantin et al., 1998), although photosynthetic rate may decrease through a negative feedback loop (product inhibition). Removal of competing sinks early in fruit development is likely to lead to the setting of subsequently more fruit, while removal in later fruit development is likely to result in increased fruit TSS and weight, but decreased overall yield.

Plant growth regulators can also be used to influence source-sink balance in melons. Application of ethrel (Sidhu et al., 1982) or the synthetic cytokinin 1-(2-chloro-4-pyridyl)-3-phenlurea (CPPU) (Hayata et al., 2001) to newly pollinated ovaries has been reported to increase fruit set and yield per plant, but to have no effect on fruit TSS. However, application of paclobutrazol to plants at a later stage in fruit
development inhibited vegetative growth and resulted in increased fruit TSS, but not fruit weight (Nerson et al., 1989).

Thus a range of techniques for manipulating the source-sink ratio and assimilate partitioning to the melon fruit exist. In this study we re-examine such manipulations with a focus on the effect on fruit ‘sweetness’.

**Materials and methods**

*Plant material and culture*

Experiments were conducted on 3 commercial farms, located in Kununurra, Western Australia (15° 46’ S 128° 44’ E), Bourke, New South Wales (30° 2’ S 145° 57’ E) and in Kabra, Queensland (23° 28’ S 150° 23’ E), and in a glasshouse at Central Queensland University, Rockhampton, Queensland (23° 22’ S 150° 32’ E). For field plantings, fertiliser was delivered per normal commercial practice, based on soil nutrient analysis. In Kununurra, seeds were directly sown singly into uncovered beds 1.8 m apart, at 3-4 cm depth and 40 or 50 cm spacing. Soil was a heavy cracking black clay. Fertiliser was delivered as a pre-plant base comprising 44 kg N/ha, 60 kg P/ha, 49 kg K/ha, 20 kg S/ha, 56 kg Ca/ha and 18 kg Zn/ha. Furrow irrigation was delivered for 6 h at germination, 6 h at first male flower production, and for 6 h late in fruit development. In Kabra, seedlings were transplanted approximately 14 days after sowing, 60 cm apart into plastic covered beds 2.0 m apart. Soil was a sandy loam. Pre-plant base and fertigated nutrition totalled 88 kg N/ha, 167 kg P/ha, 56 kg K/ha, 63 kg S/ha and 20 kg Ca/ha. A surface trickle line delivered irrigation at seedling transplant, and again after 14 days, for 1.5 h per day until early to mid fruit development, and for 2 h per day for the remainder of the crop. At Bourke, single
seedlings were transplanted at 40 cm spacing into rows at 2 m centres. Soil was a red sandy clay. Beds were covered with plastic mulch containing a surface trickle line. The delivery of pre-plant base and fertigated nutrition totalled 50 kg N/ha, 17 kg P/ha, 113 kg K/ha and 15 kg Ca/ha. Irrigation was subjectively delivered to meet the crop requirements.

For glasshouse propagation, 8.3 L plastic draining pots were lined with shade cloth and filled with steam-sterilised sand. Two seeds (cultivar Hot Shot) were sown at 15 mm depth, per pot. Full strength hydroponic solution (elemental concentration - ppm: N 215, P 37, K 218, Ca 152, S 54, Mg 42, Fe 4.08, Mn 0.96, Zn 0.48, Cu 0.36, B 0.036, and Mo 0.012) (N: P: K ratio of 5.8: 1: 5.9) was delivered to pots via automated flooding benches (100 mm depth, flooding daily for 1 h, 10 to 15 pots per bench), with re-circulated nutrient solution replaced every three to four weeks. Solution pH was adjusted weekly to 6.5 using 1M KOH or 1M H$_2$SO$_4$.

Fruit measurements

Unless otherwise stated, fruit were harvested when they abscised (‘slipped’) from the vine, fruit number and fresh weight (and dry weight for some experiments) were recorded, and the TSS of fruit mesocarp tissue determined. For the cultivars used, fruit were between 150 and 250 mm in diameter at harvest. For TSS assessment, a 22 mm diameter core of mesocarp tissue was extracted from a randomly located equatorial position for glasshouse fruit (not possessing a ground spot), or located at 180° relative to the ground spot for field grown fruit. For each core approximately 5 mm of outer rind and green inedible tissue, and inner seeds, were removed and juice was extracted from the remaining edible orange mesocarp tissue using a hand
operated garlic press. TSS of the juice was determined using a Bellingham and Stanley RFM 320 temperature compensated digital refractometer.

Plant partitioning

For some experiments, plants were harvested and partitioned before drying at 70°C. Plants were partitioned into

1) Main leaves: leaves of the main branch.

2) Main stems: stems, petioles and tendrils of the main branch.

3) Lateral leaves: leaves from all lateral branches.

4) Lateral stems: stems, petioles and tendrils from all lateral branches.

5) Fruits

6) Roots

7) Male flowers

8) Female flowers

Roots were only recovered from glasshouse grown plants.

Two plant indexes were calculated based on dry weight (DW) partitions, harvest index (HI) and leaf weight ratio (LWR).

\[ HI = \left( \frac{\text{fruit DW}}{\text{total plant DW}} \right) \times 100 \]

\[ LWR = \left( \frac{\text{leaf DW}}{\text{total plant DW}} \right) \times 100 \]
**Phenology trial**

From a field of plants (cultivar Malibu) seeded in March 02 at the Kabra farm, eight plants were selected at random from a single row at 7 day intervals, to monitor biomass partitioning and fruit TSS. Plants were partitioned into organs and weighed (following drying at 70°C for up to 5 days). In addition, 30 fruit selected at random from adjacent rows were harvested each week for TSS assessment. The TSS determination method was unique to the phenology experiment. Tissue cores (22 mm diameter) were sampled as previously described and divided into approximately 10 mm thick slices, starting from the exocarp/ rind. TSS was determined for the juice extracted from each slice. At each harvest, a single whole edible mesocarp core tissue sample was taken from each of three fruit and diced into approximately 5 mm sided cubes and freeze dried for 48 h in a Virtis Sentry freeze dryer for sugar analysis. Each sample was ground into a known amount of 80% ethanol (approximately 10 mL), agitated for 2 min, allowed to extract for 30 min in a 65°C water bath, and then centrifuged for 10 min. A sub-sample of the supernatant was stored for HPLC determination of sucrose, glucose and fructose, using a Waters carbohydrate column and a refractive index detector.

**Sink manipulation – fruit thinning**

Two field trials were conducted at each of the Kabra and Kununurra farms to examine the effect of fruit thinning, implemented at different times before fruit maturation, on the sugar content and yield of the remaining fruit. Treatments for each trial were arranged in a randomised complete block design (RCBD).

During the 2000 season in Kununurra, fruit were removed either 5 or 10 days before harvest (DBH) from cultivar Hotshot vines to leave one fruit per plant. Each
treatment was imposed over a 10 m portion of a row, and replicated three times. An identical treatment was also imposed on two successive plantings (2001 and 2002) of cultivar Malibu at the Kabra field site, except that in the 2001 experiment, plants were thinned at 4, 11, 18, 25 and 32 DBH, while in the 2002 planting, thinning was implemented at 12, 19 and 26 DBH. Each treatment was imposed on 10 plots, with each plot consisting of six and four plant positions (two plants per planting hole) in 2001 and 2002, respectively.

A fourth field experiment was established in 2002 on the Kununurra farm using cultivar Hotshot. Two levels of planting density (25 and 50 cm seed spacing, or 11,111 and 22,222 plants/ha) were combined factorially with three fruit removal treatments (control, thinning of fruit to one per plant, and thinning to leave two fruit per plant) implemented 21 DBH. Treatments were replicated three times, with each replicate consisting of a 2 m portion of a planting row.

Fruit thinning treatments were also imposed on cultivar Hotshot grown under glasshouse conditions (May to August 2001). Plants were hand pollinated to set one fruit on each of 17 plants and two fruits on each of 15 plants. Four plants were left unpollinated. At approximately 31 days after pollination (21 DBH), one fruit was removed from seven of the plants bearing two fruit, and eight plants that had been set with one fruit were thinned to no fruit. Treatments were completely randomised. Photosynthetic rate of a single leaf, eight to ten node positions from the apex of the main branch, was assessed for each plant, seven days after fruit thinning (14 DBH). Measurements were made from 12:00 to 14:30 hours on a cloudless day using an
ADC Limited LCA-4 infrared gas analyser. At harvest, root tissue was recovered as well as shoot and fruit organs.

*Source manipulation – leaf removal*

The effect of removal of photosynthetic source organs on yield and sugar content of fruit of cultivar Hotshot was determined in a trial conducted in Kununara during 2000. The following five treatments were implemented at early to mid fruit development and at late fruit development (21 and 8 DBH, respectively). Source organs were removed basipetal relative to the branch apex: (i) 25% of leaves were removed from each branch; (ii) 50% of leaves were removed from each branch; (iii) 25% of each branch was removed; (iv) 50% of each branch was removed; and (v) no treatment (control). Each treatment comprised a 5 m plot within a row and the ten treatments were randomly allocated within a 50 m portion of a row. The treatments were replicated four times throughout the planting as a RCBD.

*Chemical inhibition of vegetative growth*

‘NBX’, manufactured by Stoller Australia Pty. Ltd, is marketed as a chemical inhibitor of vegetative growth. According to the manufacturer, NBX inhibits the movement of auxin from the growing tips, which in turn limits assimilate movement toward the growing tips, allowing partitioning to other sink organs. Active ingredients are reported as B (10% w/v), Mo (0.007% w/v), polyamine complexing agents and seaweed derived cytokinin. The chemical was applied via a backpack sprayer at an approximate rate of 3 L/ha (recommended rate 2–4 L/ha) in one glasshouse and three field trials.
For the three field trials, treatment plots (arranged in a RCBD) were surrounded by 2 m buffer zones. At Bourke (April 2001), NBX was applied to a 5 m portion of a row of cultivar Dubloon, with four replicates, at 15 DBH. At Kabra (September 2001), NBX was applied to 5 m plots of cultivar Eastern Star, with eight replicates, at 3 and 7 DBH. At Kununurra (September 2002), NBX was applied to a 2 m portion of a row of cultivar Hotshot, with six replicates, at 10 DBH. In the glasshouse trial (August to November 2001), NBX was applied 7 DBH to eight cultivar Hotshot plants which had been set with one fruit per plant. Treatment and control plants were completely randomised.

*Pollination scheduling*

The effect of delaying fruit set on fruit TSS and weight was examined in two glasshouse experiments (December to March, and June to October 2002, cultivar Hotshot) and one field experiment (cultivar Sahara, at Kabra from August to November 2002). In the first glasshouse experiment, one fruit was set close to the centre (‘crown’) of each of 13 plants. Twenty one days after this event, eight plants that had been denied fruit set (‘delayed plants’) were pollinated to set one fruit per plant. In the second experiment, two plantings (of 29 and 20 pots) were staggered by 20 days. When the later planting began to produce female flowers, one fruit per plant was set for both groups of plants.

In the field trial, white Marix thermal net cover (VP Trade Goods, Brisbane; 20% shade) was used to cover plants within three treatments: (i) control plants (with covers in place but with open sides); (ii) first or ‘crown’ fruit set (sides of covers were closed after first fruit set); (iii) pollination delayed by 14 days (sides of net covers closed
until 14 days after treatment ii, then opened for 5 days). Each treatment comprised ten plants per plot, with four replicate plots in a RCBD.

Statistics

The SAS System and SPSS 11.5 for Windows were used for ANOVA statistical testing. For significant (P<0.05) and for marginally significant (P<0.10) ANOVA, means were separated by either the least significant difference (LSD) or Dunnett’s test at 5% significance level. Microsoft Excel was used for regression analysis.

Results and discussion

Phenology

In a field trial involving cultivar Malibu, the crop cycle was completed in 11 weeks (79 d) (Fig. 3.1). The first noticeable flower count was at 23 days after transplant, and flower number peaked at 37 d. Fruit became the dominant organ (by mass) at 51 days (28 DBH). The biomass of main branch stems was fairly static from this point, while main leaf and lateral stem biomass increased, peaking 7 to 14 DBH. Lateral leaves contributed the most to total vegetative plant biomass, and had a biomass comparable to that of fruit. At harvest, fruit accounted for 51% of plant above ground biomass (dry weight basis).

Fruit development (pollination to fruit abscission) occurred over a period of approximately 42 days. Total fruit weight (DW and FW) rapidly increased until three weeks before harvest (8 weeks after transplanting), and then plateaued, but fruit TSS continued to increase (Fig. 3.1), consistent with the reports of Hubbard et al. (1990), Miccolis and Saltviet (1991), and Chrost and Schmitz (1997). The TSS of inner,
middle and outer mesocarp tissue was similar during early fruit development, increasing in all tissues from 4 to 6% TSS within the first 4 weeks of development. In later development, middle and inner tissue accumulated sugar at a greater rate, with middle tissue TSS increasing by 2 TSS units, and inner tissue increasing by 4 TSS units during the last three weeks. The total amount of soluble sugar per fruit increased linearly during fruit development, with the mature fruit containing approximately 100 g sugar per 1000 g FW fruit.

Glucose and fructose concentrations were always similar, and increased from 160 to 200 mg/g DW during the initial three weeks of fruit development, but then decreased to about 150 mg/g DW at fruit abscission. Sucrose concentration was very low during early fruit development (between 0 and 10 mg/g DW), but increased dramatically within the last three weeks to equal hexose concentration (Fig. 3.1). This result is consistent with that of Lester and Dunlap (1985), who also noted that sucrose content continued to increase at the expense of the monosaccharides following fruit harvest.

The effect of a manipulation of the source or sink is expected to be specific to the developmental stage of the fruit and the plant. Thus, for example, an increase in carbohydrate availability to fruit seven weeks before harvest might allow more cell division, resulting in larger fruit, but with no increase in fruit TSS. In contrast, increasing carbohydrate availability close to harvest, after fruit had passed their cell division and expansion phases, should result in increased sugar storage.
Figure 3.1. Phenology of cultivar Malibu melon plants grown on a commercial farm at Kabra Queensland from 30 March until 15 June 2002. For dry weight and flower number, each data point represents the mean and se for eight replicate plants. For TSS and fruit fresh weight, each data point represents the mean and se for 30 replicate fruit. Sugar per fruit data are the average fresh weight multiplied by the average whole mesocarp TSS. Specific sugar data are mean and se values for three tissue samples.
Patterns of partitioning

In control (pooled glasshouse grown) plants, a positive linear relationship ($r^2 = 0.94$) was noted between leaf weight ratio and fruit fresh weight per plant, and a similar but negative relationship ($r^2 = 0.88$) existed between harvest index and fruit fresh weight (Fig. 3.2). The relationship between either leaf weight ratio or harvest index and TSS was poor, although as fruit development time advanced, leaf weight ratio decreased and harvest index increased. Thus, in non-manipulated plants, source availability was linked to fruit biomass, rather than fruit sweetness.

Figure 3.2. The linear relationship ($r^2$) between fruit fresh weight, TSS or fruit development time, and either leaf weight ratio or harvest index. For control melon plants and treatment plants not subject to post pollination source or sink manipulation. Data are from combined glasshouse experiments (common with Tables 3.1, 3.3 and 3.4).
Total plant biomass was similar for plants with one or two fruits and plants that never set fruit (average 65 g DW, glasshouse trial, Fig. 3.3), however biomass was partitioned differently. For plants with fruit, about 80% of total biomass was apportioned to fruit, and only 5% was apportioned to lateral branch stems and leaves. In contrast, plants that were denied fruit set invested resources into lateral branch production, such that at harvest 50% of total plant biomass was in the form of branches and flowers (Fig. 3.3). For plants in which all fruit were removed 21 DBH, resources were again invested into branch production, such that at maturity 38% of total biomass was allocated to branches and flowers (Fig. 3.3).

**Figure 3.3.** Fruit, shoot and root dry weight partitioning (mean plus se), and the percentage of dry matter partitioned within plants, for glasshouse grown cultivar Hotshot melons sampled at the time of fruit maturation. Data set is common with Table 3.1.

<table>
<thead>
<tr>
<th>Fruit load treatment</th>
<th>Dry weight (g)</th>
<th>Partitioned dry weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 fruit</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>79.9</td>
<td>8.9</td>
</tr>
<tr>
<td></td>
<td>3.8</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>1.8</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>0.0</td>
<td>2.4</td>
</tr>
<tr>
<td>2 fruit</td>
<td>80.4</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>3.9</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>0.0</td>
<td>1.9</td>
</tr>
<tr>
<td>no fruit</td>
<td>0.0</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>0.0</td>
<td>0.3</td>
</tr>
<tr>
<td>thin to 1</td>
<td>72.6</td>
<td>13.8</td>
</tr>
<tr>
<td></td>
<td>19.5</td>
<td>28.5</td>
</tr>
<tr>
<td></td>
<td>0.0</td>
<td>0.3</td>
</tr>
<tr>
<td>thin to none</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Thus, at fruit maturity, leaf weight ratio was two to three fold higher in plants with no fruit than in plants bearing fruit, and the ratio was also higher for plants denied fruit set compared to plants which were thinned of all fruit (45 vs. 39%, respectively)
Source availability is determined by leaf area and photosynthetic rate per unit area. Photosynthetic rate is expected to vary in response to the source – sink (mass) ratio through a product inhibition response. In glasshouse plants in which fruit were removed 21 DBH, photosynthetic rate (taken at 14 DBH) was not significantly different between plants bearing one or two fruits (average 9.9 μmol CO$_2$/m$^2$.s), but was markedly lower in plants thinned of all fruit (thinned to no fruit, 3.6 μmol CO$_2$/m$^2$.s) (Table 3.1). In plants that were not allowed to set fruit, the photosynthetic rate was lower again, at 1.5 μmol CO$_2$/m$^2$.s. These results are consistent with those of Valantin et al. (1998) who recorded a maximum photosynthetic rate of 15 μmol CO$_2$/m$^2$.s in melon leaves, with no variation in plants thinned of fruit load, and proposed that the family Cucurbitaceae is characterised by a loose connection between sink demand and specific photosynthetic rate. Marcelis (1991) also noted that leaf net photosynthesis was only reduced in cucumber plants when all fruit were removed. Leaf photosynthetic rate is therefore not expected to decline with time from fruit thinning, although leaf area will increase, driving an increase in plant photosynthetic capacity.

On balance, however, thinning of fruit increased the availability of assimilate to remaining fruit, and fruit of plants set with one fruit and plants thinned to one fruit possessed higher TSS than that of plants bearing two fruit (one fruit 9.0, thin to one fruit 8.8, two fruit 7.8% TSS) (Table 3.1).

3. Source sink manipulation
Table 3.1. The effect of fruit load on fruit TSS, fresh weight, development time, photosynthetic rate (14 DBH), harvest index and leaf weight ratio, on glasshouse grown cultivar Hotshot melons. 'No fruit' treatment involved plants denied fruit set. Thinning was implemented 21 days before harvest. Probability values are reported for ANOVA analyses. Means with the same letter are not significantly different at LSD p<0.05.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Mean individual fruit FW (g)</th>
<th>Total fruit FW per plant (g)</th>
<th>Fruit development time (days)</th>
<th>Harvest index (%)</th>
<th>Photosynthetic rate (µmol CO₂/m².s)</th>
<th>Leaf weight ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>One fruit</td>
<td>9</td>
<td>9.0 a</td>
<td>486 a</td>
<td>52</td>
<td>79 a</td>
<td>9.11 a</td>
<td>12.2 a</td>
</tr>
<tr>
<td>Two fruit</td>
<td>8</td>
<td>7.8 b</td>
<td>312 b</td>
<td>52</td>
<td>80 a</td>
<td>11.23 a</td>
<td>11.8 a</td>
</tr>
<tr>
<td>Thin to one fruit</td>
<td>7</td>
<td>8.8 a</td>
<td>395 c</td>
<td>52</td>
<td>72 b</td>
<td>9.24 a</td>
<td>15.9 b</td>
</tr>
<tr>
<td>Thin to no fruit</td>
<td>8</td>
<td></td>
<td>395 c</td>
<td>52</td>
<td></td>
<td>3.56 b</td>
<td>45.0 c</td>
</tr>
<tr>
<td>No fruit</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.51 b</td>
<td>38.8 d</td>
</tr>
<tr>
<td>P (ANOVA)</td>
<td></td>
<td>0.07</td>
<td>0.00</td>
<td>0.86</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

3. Source sink manipulation
Mean fruit FW was also greater for plants which were set with a single fruit compared to plants thinned to one fruit or plants with two fruit (486, 395 and 312 g, respectively), although total FW yield per plant was significantly greater for two fruit plants (625 vs. 486 g for plants bearing two and one fruit, respectively) (Table 3.1).

*Sink manipulation – fruit thinning*

Thinning close to harvest (4 to 12 DBH) was more effective at increasing the TSS of fruit (typical increase of 2% TSS, e.g. from 9.8 to 12% in the Kununurra trial), than earlier thinning events (Fig. 3.4A), but also had the most detrimental impact on fruit yield per plant. TSS increased by 22, 15 and 21% in the Kununurra (5 DBH), Kabra 01 (thinned 4 DBH), and Kabra 02 (12 DBH) trials, respectively (Fig. 3.4A, B, C), in which fruit number per planting hole was reduced by 42%, 56% and 47%, respectively. These fruit had passed their cell expansion phase, so fruit did not increase in size, and assimilate was partitioned to sugar storage.

Thinning during the cell expansion phase (14 to 21 DBH) resulted in larger fruit, but had less effect on fruit TSS (Fig. 3.4B,C). For the 2001 Kabra cultivar Malibu population, mean fruit fresh weight was greater for treatments imposed at or before 18 DBH (1050 cf. 800 g for control), while in the 2002 trial fruit weight was greater for treatments imposed at or before 26 DBH (1600, cf. 1100 g for control). Similarly, in a glasshouse trial (Table 3.1), thinning was implemented 21 DBH. Plants that were initially set with one fruit, or thinned to one fruit, bore fruit with TSS higher than fruit from plants bearing two fruit.
Figure 3.4. The effect of the timing of fruit removal (leaving one fruit per plant) on the fresh weight and TSS of remaining fruit. Data are from field trials at (A) Kununurra 2000 with cultivar Hotshot, (B) Kabra 2001 with cultivar Malibu, and (C) Kabra 2002 with cultivar Malibu. Data points are displayed as mean values with se. Numbers above bars indicate the number of fruit harvested at maturity per planting replicate. ANOVA for mature fruit TSS and FW for the three locations were significant (P<0.001). LSD$_{0.05}$ values for (A) 0.3%; (B) TSS 0.8%, FW 0.1 kg; (C) TSS 0.9%, FW 0.2 kg.
Single fruit from the thinned treatment were also larger (395 g FW) than the two fruit treatment (312 g FW), although not as large as one fruit treatment (486 g FW) (Table 3.1). Thus, more assimilate was made available to the remaining fruit during the cell expansion and sugar accumulation phases.

Thinning at even earlier stages allowed for more vegetative growth, enabling plants to produce female flowers and set additional fruit (e.g. where fruit were thinned 32 DBH, leaving 2 fruit per plant hole, 3 fruit were harvested; Fig. 3.4B). Competition for assimilate between these fruit would result in lower TSS, relative to the treatment of thinning close to fruit maturity.

Thus fruit thinning in the last weeks of fruit development increased the proportion of melons in a population that exceeded a quality control standard of 10% TSS (from approximately 20% in the control population to 80% and 70% in the Kabra 01 (4 DBH) and Kabra 02 (12 DBH) trials, respectively; Fig. 3.5). However, thinning treatment reduced harvestable yield. Yield was reduced from 31 t/ha to between 15 and 20 t/ha, increasing to between 23 to 25 t/ha with increasing time before harvest for thinning implementation (Fig. 3.5). The converse of this observation is seen in the record of the weight of fruit removed in thinning operations (e.g. Fig. 3.4B, lower panel).
Figure 3.5. Yield (t/ha) and the percentage of melons with flesh TSS ≥10%, from thinning treatments leaving one fruit per plant, for two plantings (2001 and 2002) of cultivar Malibu melons grown on a commercial farm at Kabra Queensland. Data are common to Fig 3.4 B, C. Yield values are presented as means and se. ANOVA analysis was significant for yield (p<0.01); LSD0.05 between dates were 6.0 (2001) and 7.5 t/ha (2002).

Source manipulation – removing leaves

Manipulating the source, by reducing or impeding branch growth, has been examined as a method of controlling flower production, but the effects on fruit yield or TSS were either unsuccessful or not reported (Lloyd, 1920; Wolf and Hartman, 1942). In the one published report on the effect of source removal on fruit TSS, Hubbard et al. (1990) removed 50% of plant leaves 28 DBH, and noted a significant reduction in fruit TSS, although neither fruit weight nor yield data were reported.
In the current study, source removal was implemented after fruit growth had plateaued, while sugar was still accumulating (8 DBH), and at the end of the cell expansion period (21 DBH). Thus source removal only had a marginally significant depression of fruit FW (P = 0.08, Table 3.2), but markedly reduced fruit TSS. The effect of 50% defoliation treatments on TSS was severe (producing fruit lower in TSS by 1%; Table 3.2), while 25% defoliation had a negligible effect. There was no significant interaction between the amount of leaves or branches removed (25 or 50 %), the timing of source removal (21 or 8 DBH), and the structure of the removed source (leaf or branch) (P > 0.30 for interactions).

Table 3.2. Fruit TSS, fresh weight and yield mean values for source organ removal treatments applied at either 21 or 8 days before harvest, implemented on field grown cultivar Hotshot melon in Kununurra WA 2000. ANOVA P and LSD$_{0.05}$ values are documented.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TSS (%)</th>
<th>FW (g)</th>
<th>Yield (t/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>10.1</td>
<td>1456</td>
<td>21.6</td>
</tr>
<tr>
<td>25% branch removal 21 dbh</td>
<td>9.9</td>
<td>1468</td>
<td>21.8</td>
</tr>
<tr>
<td>25% branch removal 8 dbh</td>
<td>9.8</td>
<td>1461</td>
<td>24.3</td>
</tr>
<tr>
<td>25% leaf removal 21 dbh</td>
<td>9.7</td>
<td>1347</td>
<td>21.9</td>
</tr>
<tr>
<td>25% leaf removal 8 dbh</td>
<td>9.6</td>
<td>1410</td>
<td>23.5</td>
</tr>
<tr>
<td>50% leaf removal 21 dbh</td>
<td>9.4</td>
<td>1347</td>
<td>20.0</td>
</tr>
<tr>
<td>50% branch removal 21 dbh</td>
<td>9.3</td>
<td>1337</td>
<td>19.8</td>
</tr>
<tr>
<td>50% leaf removal 8 dbh</td>
<td>9.0</td>
<td>1318</td>
<td>22.0</td>
</tr>
<tr>
<td>50% branch removal 8 dbh</td>
<td>8.9</td>
<td>1338</td>
<td>20.8</td>
</tr>
<tr>
<td>P (ANOVA)</td>
<td>0.00</td>
<td>0.08</td>
<td>0.27</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>0.7</td>
<td>175</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Source manipulation – chemically inhibiting vegetative growth

Plant bioregulators can improve fruit size, appearance and internal fruit quality, by directly affecting fruit growth and development, or by indirectly affecting fruit load, plant vigour and canopy architecture (Looney, 1993). The timing of application is important, with reference to fruit growth and sugar accumulation phases.
Table 3.3. The effect of NBX (organic cytokinin) applied as a foliar spray on TSS (%), fruit fresh weight (g) and yield (t/ha) for melon plants grown on three commercial farms in Bourke NSW, Kununurra WA, and Kabra QLD. For the glasshouse trial, vegetative DW (g), harvest index (%) and leaf weight ratio (%) were recorded. NBX was delivered at the recommended rate of 2-4 L/ha. The time of application varied for each location from three days before harvest up to 14 DBH.

<table>
<thead>
<tr>
<th>Application time:</th>
<th>14 DBH</th>
<th>10 DBH</th>
<th>7 DBH</th>
<th>3 DBH</th>
<th>7 DBH</th>
<th>Location and date: Bourke 01</th>
<th>Kununurra 02</th>
<th>Kabra 01</th>
<th>Kabra 01</th>
<th>CQU glasshouse 01</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSS &amp; FW Yield</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Vegetative DW</td>
<td>HI</td>
<td>LWR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10.7</td>
<td>1325</td>
<td>27</td>
<td>8.4</td>
<td>1509</td>
<td>34</td>
<td></td>
<td>9.1</td>
<td>1596</td>
<td>22</td>
</tr>
<tr>
<td>NBX</td>
<td>11.3</td>
<td>1241</td>
<td>25</td>
<td>8.8</td>
<td>1512</td>
<td>37</td>
<td></td>
<td>9.2</td>
<td>1603</td>
<td>20</td>
</tr>
<tr>
<td>P (ANOVA)</td>
<td>0.02</td>
<td>0.06</td>
<td>0.65</td>
<td>0.24</td>
<td>0.97</td>
<td>0.28</td>
<td></td>
<td>0.46</td>
<td>0.70</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>0.59</td>
<td>0.88</td>
<td>0.16</td>
<td>0.12</td>
<td>0.82</td>
<td>0.88</td>
<td></td>
<td>0.12</td>
<td>0.82</td>
<td>0.92</td>
</tr>
</tbody>
</table>

3. Source sink manipulation
When NBX was applied at 14 DBH (toward the end of fruit growth, 2001 Bourke trial), fruit TSS was improved (by 0.6% TSS), and fruit FW was also marginally significantly higher (P = 0.06, increase of 100 g) (Table 3.3). Applications closer to the time of harvest (10, 7 and 3 DBH) had no effect on TSS (with probability values for TSS further from significance as NBX was applied closer to the time of harvest; Bourke at 14 DBH P=0.02, Kununurra at 10 DBH P=0.24, Kabra at 7 DBH P=0.46, and Kabra at 3 DBH P=0.88) (Table 3.3).

This result is consistent with a diversion of assimilate from vegetative growth to fruit storage, with the time frame of the response expected to be longer than that achieved through thinning fruit from vines. It is recommended that further work be conducted with respect to the timing of application of the growth retardant, and with more widely known retardants, such as paclobutrazol (gibberellin biosynthesis inhibitor).

**Source manipulation – delaying pollination**

Delaying pollination in melon plants was expected to result in the setting of fruit on plants with a greater than normal amount of source biomass (e.g. 30 and 165 g DW total plant biomass in control and delay set plants, corresponding to Table 3.4B). In the first glasshouse trial involving pollination scheduling, single fruit were set on ‘delayed’ and ‘normal’ plants. However, fruit were set on normal plants first, such that fruit development in the two treatments was confounded with time. Fruit TSS and FW were higher for the delayed treatment (9.5% TSS, 1543 g for delayed cf. 6.3% TSS, 1221 g for control). Harvest index was greater for the normal treatment,
but there was no significant difference between treatments for leaf weight ratio (Table 3.4A).

In a second glasshouse experiment, the simultaneous setting of one fruit per plant for both normal plants and delayed plants allowed fruit to develop under the same environmental conditions, with the only difference being that delayed plants had more source biomass. Harvest index was greater for normal plants (59 cf. 38% for delayed), whilst leaf weight ratio was lower for normal plants (23 cf. 26%). Fruit development was approximately two days longer for delayed treatments (Table 3.4B). However, fruit TSS did not significantly differ between the two treatments, although delayed plants produced heavier fruit (1644 g FW) than normal plants (1442 g) (Table 3.4B).

**Table 3.4.** The effect of delaying pollination in glasshouse grown melon plants by 20 days, and the subsequent setting of one fruit per plant for plants differing in vegetative biomass, on fruit TSS, fruit fresh weight, fruit development time, total plant dry weight, harvest index and leaf weight ratio. Experiment A treatment plants were seeded on the same day, whilst experiment B plants were seeded 20 days apart.

<table>
<thead>
<tr>
<th></th>
<th>TSS (%)</th>
<th>FW (g)</th>
<th>Fruit development time (days)</th>
<th>Total plant DW (g)</th>
<th>Harvest index (%)</th>
<th>Leaf weight ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Expt A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delayed</td>
<td>9.5</td>
<td>1543</td>
<td>35</td>
<td>289.5</td>
<td>42.4</td>
<td>24.8</td>
</tr>
<tr>
<td>Normal</td>
<td>6.3</td>
<td>1221</td>
<td>30</td>
<td>129.7</td>
<td>49.8</td>
<td>23.9</td>
</tr>
<tr>
<td>P (ANOVA)</td>
<td>0.00</td>
<td>0.03</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.18</td>
</tr>
<tr>
<td><strong>Expt B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delayed</td>
<td>12.3</td>
<td>1644</td>
<td>47</td>
<td>434.4</td>
<td>38.2</td>
<td>26.4</td>
</tr>
<tr>
<td>Normal</td>
<td>12.5</td>
<td>1442</td>
<td>45</td>
<td>230.5</td>
<td>59.2</td>
<td>22.7</td>
</tr>
<tr>
<td>P (ANOVA)</td>
<td>0.48</td>
<td>0.02</td>
<td>0.04</td>
<td>0.00</td>
<td>0.00</td>
<td>0.01</td>
</tr>
</tbody>
</table>

This result is consistent with the interpretation that the extra photoassimilate made available during the fruit set and cell division and expansion phases will result in enhanced fruit weight rather than enhanced fruit TSS. The result is in agreement with
that of Eischen et al. (1994), who also noted that there was no effect on fruit TSS in plants in which fruiting was delayed through use of net covers to exclude pollinators.

Delaying pollination in-field (Table 3.5) meant that vines were able to direct assimilate resources into the continual promotion of lateral branch and female flower production, such that when netting covers were opened, more female flowers than normal were available for pollination. The additional assimilate supply facilitated the setting of more fruit per plant (7.1 fruit per delay plant cf. 4.3 control), but these fruit were smaller (by approximately 400 g FW) and lower in TSS than control fruit (by about 1.0% TSS) (Table 3.5).

**Table 3.5.** The effect of netting covers to exclude pollinating agents from field grown cultivar Sahara melon plants, Kabra Queensland 2002. Covers were implemented before fruit set to delay normal pollination, and implemented after the first or ‘crown’ fruit set to inhibit secondary fruit set. ANOVA P and LSD0.05 values are reported for TSS, fresh weight, the number of fruit per plant, fruit yield, harvest index and leaf weight ratio, for plants harvested at fruit maturity.

<table>
<thead>
<tr>
<th></th>
<th>TSS (%)</th>
<th>FW (g)</th>
<th>Fruit per plant</th>
<th>Yield (g/plant)</th>
<th>Harvest index (%)</th>
<th>Leaf weight ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.1</td>
<td>1588</td>
<td>4.3</td>
<td>6784</td>
<td>51.5</td>
<td>23.6</td>
</tr>
<tr>
<td>Crown set</td>
<td>7.9</td>
<td>1789</td>
<td>2.7</td>
<td>4831</td>
<td>42.5</td>
<td>32.5</td>
</tr>
<tr>
<td>Delayed set</td>
<td>7.1</td>
<td>1112</td>
<td>7.4</td>
<td>8257</td>
<td>71.9</td>
<td>11</td>
</tr>
<tr>
<td>P ANOVA</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.21</td>
<td>0.06</td>
</tr>
<tr>
<td>LSD0.05</td>
<td>0.6</td>
<td>215</td>
<td>1.4</td>
<td>1812</td>
<td>n.s.</td>
<td>17.8</td>
</tr>
</tbody>
</table>

Where net covers were used to restrict pollination, allowing the setting of the first set (or ‘crown’ set) fruit only, fruit TSS and average FW were not significantly different from control treatment fruit (Table 3.5). The number of fruit per plant was significantly less for plants set with the first crown fruit only (2.7 fruit per plant) than for control plants (4.3 fruit per plant), and consequently fruit yield per plant was 30% lower in crown set plants than control plants (Table 3.5). This result was not
expected. We interpret this result as being a confounding of treatment effect with
time, similar to the first glasshouse experiment (Table 3.4A). Further work should be
undertaken using staggered plantings to avoid this problem.

The viability of fruit thinning as an agronomic tool

Of all the techniques considered, fruit thinning in the final one to three weeks before
harvest gave the greatest increase in fruit TSS. However, it also resulted in the
greatest decrease in overall yield.

To remedy this drawback, plant density could be increased to maintain ‘normal’
yields (t/ha) of fruit, although it was anticipated that increased plant density could
cause a light (source) limitation in the crop. Kultur et al. (2001) propagated melons at
72,600 plants/ha and 36,300 plants/ha plant spacing, and reported no difference in
fruit TSS between treatments, although fruit number, yield per plant and average fruit
weight, were higher for less dense plantings, but yield (t/ha) was lower. Nerson
(2002) reported that fruit TSS and average fruit weight decreased with increasing
plant density (13.5% TSS at 50,000 plants/ha, 12.1% at 80,000 plant/ha, 10.4% at
160,000 plants/ha). In the Kununurra 2002 trial, thinning increased fruit TSS only at
the greater plant spacing of 50 cm (9.9% for plants with 1 fruit cf. 8.6% for control)
and harvestable fruit yield was not significantly different at the two planting densities
employed (31.4 cf. 36.9 t/ha for control plants, 28.5 cf. 35.4 t/ha for thinned plants,
Table 3.6).
Table 3.6. The effect of thinning fruit at 21 DBH from plants to leave one and two fruit per plant (cv. Hotshot). Plants were grown at 25 cm and 50 cm seed spacing, on a commercial farm in Kununurra WA in 2002. Mean values for fruit TSS, fruit FW and yield are reported. LSD is reported for the significant ANOVA result for TSS (P<0.05).

<table>
<thead>
<tr>
<th></th>
<th>50 cm spacing</th>
<th>25 cm spacing</th>
<th>LSD&lt;sub&gt;0.05&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSS (%)</td>
<td>Control 8.6</td>
<td>Control 9.2</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>2 fruit 9.3</td>
<td>2 fruit 9.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 fruit 9.9</td>
<td>1 fruit 9.6</td>
<td></td>
</tr>
<tr>
<td>FW (g)</td>
<td>1568</td>
<td>1608</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>1516</td>
<td>1579</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1692</td>
<td>1676</td>
<td></td>
</tr>
<tr>
<td>Yield (t ha&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>31.4</td>
<td>36.9</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>30.3</td>
<td>31.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>28.5</td>
<td>35.4</td>
<td></td>
</tr>
</tbody>
</table>

The other ‘remedy’ to decreased yield is improved price for ‘sweet’ fruit. Of course, the greatest value of thinning will be achieved when the mean of the population lies close to, but under, a quality control standard (i.e. greatest increase in the proportion of crop above a TSS standard). A simple Excel<sup>TM</sup> (Microsoft) model was developed, based on the production costs estimated by Lovatt <i>et al.</i> (1997) (Table 3.7). Model inputs include yield (trays/ha or kg/ha), the percentage of melons meeting a quality control grade (e.g. ‘sweet’ TSS ≥ 10%), and the price received for ‘sweet’ (e.g. $15 per 14 kg tray) and ‘non-sweet’ (e.g. $8 per 14 kg tray) melons. The extra pre-harvest thinning cost was assumed to be the same as for picking ($1047/ha).

Thinning imposes extra cost, and reduces harvested yield, which must be balanced by an improved price on the product. For example, when thinning was imposed, yield was approximately halved, although 81% of fruit had TSS above 10% (data of Kabra trial, Fig. 3.4B, Fig. 3.5). At that time the market price was $8.00 per 14 kg tray and a return of $19.00 per ‘sweet’ tray would have been necessary for gross margin figures...
to be equivalent for thinned production (approximately $5000/ha) (Fig 3.6).

Alternatively, 95% of fruit would need to be ‘sweet’, or the yield after thinning would need to be 16 t/ha, to match the gross margin of normal production (Fig 3.6).

**Table 3.7.** The calculation of gross margin for melon production using fruit thinning to increase the yield of fruit with TSS > 10%. Model assumptions (top panel) can be altered in the Excel spreadsheet version. Variable costs were taken from the QDPI Rockmelon and Honeydew information kit (1998), whilst the market prices of $8.00 and $15.00 per 14 kg tray were actual farm gate prices in June 2001.
**Figure 3.6.** The effect of changing each of three parameters: (A) farm gate price for fruit ≥ 10.0% TSS (‘sweet’ fruit), (B) the proportion of sweet fruit, and (C) yield of sweet fruit per hectare, on the thinning cost benefit model. Dotted line in B and C represent the gross margin of normal non-thinned production. Arrows indicate actual values from field thinning experiment (Fig. 3.4B, Fig. 3.5).

From a practical perspective, thinning may not be an appropriate pre-harvest technique if leaves (source organs) are damaged by trampling during the thinning treatments. However, a system involving workers (human or robotic, e.g. Edan and Miles, 1993) on a boom, allowing thinning without plant destruction, could be engineered.
4 Water relations

Abstract
For irrigation of melon in Australia, the current production practice is to allow soil moisture to decline during the period before and during harvest. Field irrigation experiments were conducted in Bourke, New South Wales, to test the effectiveness of this recommendation, in terms of edible flesh sugar content (indexed as total soluble solids, % TSS), individual fruit fresh weight (FW), and yield (t.ha⁻¹). There was no evidence that decreasing water availability to melon crops just before or during harvest enhanced TSS of fruit at harvest. Indeed this treatment reduced fruit TSS and FW (e.g. for 2001 trial: no stress 11.2% TSS and 1180 g FW cf. stress late in development 8.8% TSS and 990 g FW). Trickle irrigation is recommended, with irrigation to field capacity delivered to plants at sowing, and adequate yet lesser amounts of water delivered between sowing and flowering to promote the growth of the root system. Following fruit set, irrigation should be delivered to field capacity to provide deficit-free watering to maximize fruit cell division, cell expansion and fruit sugar accumulation.
**Introduction**

Maintenance of soil moisture at or close to field capacity is generally accepted as an optimum management practice for most crops, with the absence of water stress effecting maximal stomatal aperture and photosynthesis (Heermann et al., 1990). However, in Australia the current recommendation for melon is that soil moisture be allowed to deplete as harvest approaches (Lovatt et al., 1997; Hulme et al., 2002) (Fig. 4.1).

As observed for the impact of source-sink manipulations on melon quality and yield (El-Keblawy and Lovett-Doust, 1996; Valantin et al., 1998; Long et al., 2004) (Chapter 3), the impact of a source or sink manipulation (e.g. source destruction or stress) on melon fruit quality and harvestable yield will depend on the timing of the manipulation or stress with reference to the phenology of the crop. From this earlier work it is expected that (water) stress during vegetative growth will reduce the photosynthetic capacity of the plant, and thus potentially reduce harvestable fruit biomass, without affecting fruit TSS at harvest. Water stress at flowering may cause flower loss. Water stress during early fruit development may affect fruit cell number, and thus final fruit size (Higashi et al., 1999), but, again, may not affect fruit TSS at harvest. Importantly, stress imposed in the later part of fruit development, during the sugar accumulation phase following fruit cell expansion, is expected to have the greatest impact on fruit TSS relative to the impact on fruit biomass (Long et al., 2004).
The impact of plant water status during the life of melon crops on fruit quality and yield parameters has been variously reported in the literature. Wells and Nugent (1980) reported that rainfall in the final stages of fruit development can affect melon TSS either positively or negatively, depending on cultivar, and that TSS was most influenced by rainfall during the five days preceding harvest.

Phene et al. (1987) imposed different water deficit regimes during vegetative growth using several irrigation practices (sub-surface, high frequency surface, and low frequency surface trickle) and reported no effect on fruit TSS between treatments. Other quality factors such as ground spot and fruit rot were differentially affected by treatments.

In Iran (Alizadeh et al., 1999) marketable yield was similar for full and 25% soil moisture deficit for both furrow and trickle treatments (imposed from early vegetative stage), but water use efficiency was doubled using trickle irrigation. Fruit TSS did
not differ between furrow and trickle treatments for full and 25% deficit irrigation, but a 50% deficit treatment for furrow irrigation increased fruit sugars slightly.

The Queensland Department of Primary Industries’ (QDPI) melon grower’s guide (Lovatt et al., 1997), which represents common melon production practice recommendations in Australia, recommends that a mild water stress be imposed during plant establishment to stimulate roots to penetrate deeper into the soil. It is also advised that the soil should be allowed to dry out slightly during flowering, to continue deep root penetration and to stimulate flower set. Upon fruit set, it is recommended that soil moisture be maintained near field capacity to maximise cell elongation and to facilitate fruit sizing and filling, but moisture should be allowed to decline to the refill point as harvest approaches (Fig. 4.1).

As noted earlier from previous work (Long et al., 2004), it is expected that stress events early in fruit maturation may effect fruit biomass but not TSS, while a stress event close to harvest is predicted to negatively impact fruit TSS. However the effect of a dry-down of the soil profile and stressing of the melon plant before and during harvest, on TSS content, is not well documented. In this study, trials were established to verify whether the practice is of benefit or not to melon sweetness.

**Materials and methods**

*Plant culture*

Two experiments (2001, and 2002 harvest season) were conducted at a commercial farm near the town of Bourke, New South Wales, Australia (lat. 30°2’S long. 145°57’E). Single melon seedlings were transplanted at 40 cm spacing into single
200 m long row beds spaced 2 m apart. Beds were covered with black plastic mulch containing a trickle line buried at either 25 cm or 30 cm, for the 2001 and 2002 trials respectively. The field sites were uniform in soil type and slope. The soil was a heavy clay. The delivery of pre-plant base and fertigated mineral nutrition totalled 50 kg N/ha, 17 kg P/ha, 113 kg K/ha, and 15 kg Ca/ha.

**Experimental design**

Treatments were applied to single rows arranged in a completely randomised design. Fruit were sampled from 5 m plots from each row, with 4 and 6 replications for the 2001 and 2002 experiments, respectively. Guard rows were not employed, but we contend that in these heavy clay soils, the trickle irrigation is unlikely to affect the water status of plants in adjacent rows (2 m apart). Muldoon *et al.* (1999) reported that for melon plants propagated in clay soil (in Australia) with a trickle line at 10 cm depth, water extraction primarily occurred in a region 40 cm from the trickle line. This contention is supported by the observations of the irrigation front (soil moisture monitoring and visual observations, data not shown).

**2001 experiment**

Cultivar Dubloon melon seedlings were transplanted on 22 February 2001. Tensiometers were installed at a depth of 25 cm in each replicate to measure soil moisture tension. Treatments were: (i) ‘No stress’, which involved the delivery of adequate irrigation to plants from flowering to the end of harvest. Tensiometer readings remained between 10-15 kPa of soil tension. (ii) ‘Stress before harvest’ effected an irrigation deficit approximately 7 days before harvest (DBH) by
maintaining tensiometer readings at 40 kPa for this period only (reverting to 10-15 kPa during harvest). (iii) ‘Stress before and during harvest’ ensured soil moisture tension decreased (to 40 kPa) for the period starting 7 DBH and continuing through the harvest period.

2002 experiment

Cultivar Dubloon melon seedlings were transplanted on 30 November 2001. To monitor soil moisture content, capacitance-based Enviroscan sensors (Sentek Ltd.) were installed at depths of 10, 20 and 50 cm in one replicate plot per treatment. The field capacity of the clay soil was determined to be 50 mm of soil water per 10 cm soil depth. The refill point was determined when a marked decrease in the extraction rate was noted (in probes at 10 and 20 cm soil depth), and was approximately 15 mm of soil water per 10 cm soil depth. For the ‘No stress’ treatment, watering events were implemented regularly (every 1 to 3 days) such that soil moisture was brought to field capacity followed by extraction by the crop to the refill point (Fig. 4.2). ‘Stress’ treatments were defined as soil moisture at the refill point.

Irrigation treatments were as follows: (i) ‘No stress’, which involved the delivery of regular irrigation events from flowering to the end of harvest (Fig. 4.2A). (ii) ‘Stress before harvest’, in which a water deficit was imposed 10 DBH followed by normal irrigation during harvest (Enviroscan data not available). (iii) ‘Stress before and during harvest’, in which a water deficit was imposed 7-10 DBH, followed by a single irrigation event at the start of harvest, and than deficit during harvest (Fig. 4.2B). (iv)
‘Stress during harvest’, in which irrigation ceased when harvest commenced (Fig. 4.2C).

Figure 4.2. Soil moisture (mm H₂O per 10 cm soil depth) for a trial in Bourke, New South Wales (2002 harvest season) recorded by Enviroscan (Sentek Ltd) probes buried at 10, 20 and 50 cm, for deficit free irrigation from flowering through to the end of harvest (A); an irrigation deficit implemented 7-10 days before harvest with a single irrigation event delivered at the start of harvest, and then continued deficit during the harvest period (B); and for an irrigation deficit implemented at the start of harvest (C). Field capacity was determined to be 50 mm of soil water, and the refill point was 15 mm.
Fruit harvesting and processing. Fruit were considered mature and ready for harvest when the peduncle fully abscised (when fruit ‘slip’ occurred). Fruit were weighed fresh to an accuracy of one gram using digital scales. One 22 mm core of tissue was taken from one equatorially positioned side of each melon (180 degrees relative to the ground spot). The rind and approximately 5 to 10 mm of greener tissue and seeds were removed leaving the typical orange-coloured edible mesocarp. Each core was crushed in a garlic press and the resulting juice measured for % TSS (to one decimal place) on a Bellingham and Stanley temperature compensated RFM 320 digital refractometer.

Data analysis

The SAS 6.12 software package (Cary, NC, USA) was employed for ANOVA of data. Least significant difference (LSD$_{0.05}$) was calculated to facilitate means separation for ANOVA models that were significant (P < 0.05). Mean and standard error values are reported where the corresponding ANOVA model was not significant (P > 0.05).

Results and Discussion

Experiments were superimposed on a trickle-based cropping system because scheduling was more easily controlled at key plant development stages. Specifically for melon production, (in comparison to furrow) trickle irrigation has been reported to be more water use efficient, allows more control for the delivery of water and fertiliser, and has been associated with higher yields and better quality attributes.
(Phene et al., 1987; Camp, 1998; Ayars et al., 1999; Leskovar et al., 2001). However the Australian melon industry has traditionally employed furrow irrigation. Trickle is currently used for approximately 1% of all irrigated crop production worldwide, although it is slowly achieving wider adoption (Plusquillec and Ochs, 2003).

For the 2001 experiment, treatments in which an irrigation deficit was implemented immediately before and during harvest produced fruit significantly lower in TSS than those plants delivered adequate water during harvest (no stress 11.2% TSS, stress before harvest 8.8% TSS, stress before and during harvest 9.5% TSS) (Fig. 4.3). Tensiometers were maintained at 40 kPa during the ‘stress’ period, which was in accord with the QDPI recommended level of 35-60 kPa for clay soil 7 DBH and at harvest (Lovatt et al., 1997). This irrigation deficit also detrimentally affected fruit weight (no stress 1180 g fresh weight - FW, stress before harvest 990 g FW, stress before and during harvest 1070 g FW) and total yield, but the difference between treatments for the latter was not significant (Fig. 4.3). During the harvest period 14 mm of rain was recorded, and 22 mm of rain was recorded during the period prior to harvest (Fig. 4.4A). These rain events were thought to have negligibly impacted on crop production, because the soil moisture (tension) monitoring equipment recorded no measure of additional moisture.

For the 2002 experiment, the record of soil water content (Fig. 4.2) confirmed that the treatments impacted on available soil water for the melon crop. Similar effects on TSS, and FW were recorded as in the previous year’s experiment. When an irrigation deficit was applied during either the harvest period, before harvest, or during both, fruit had reduced TSS compared to plants maintained with adequate water (e.g. no
Figure 4.3. Irrigation deficit treatments imposed from 7 days before harvest (DBH) to the beginning of harvest (stress before harvest), and from 7 DBH including the harvest period (stress before and during harvest), for field grown ‘Dubloon’ melons in Bourke, New South Wales (2001 harvest season). Mean and least significant difference (LSD, 0.05) values are reported for fruit TSS and fresh weight (ANOVA P < 0.01), mean and corresponding SE values are reported for yield (ANOVA P = 0.42).

stress 10.6% TSS cf. 9.0% TSS for fruit from treatments with stress before and during harvest, Fig. 4.5). Fruit weight and total yield were also detrimentally affected (no stress fruit FW 1700 g, yield 31 t.ha⁻¹; stress before and during harvest fruit FW 1300 g, yield 25 t.ha⁻¹ - Fig. 4.5). Precipitation was recorded in negligible amounts (1 mm during harvest and 3 mm for the period prior to harvest - Fig. 4.4B).
Figure 4.4. Precipitation (mm) recorded by the Australian Bureau of Meteorology during the experimental periods in Bourke, New South Wales in 2001 (A) and in 2001/ 2002 (B). Horizontal dark lines indicate the harvest periods.

The practice of allowing soil moisture to deplete close to and during harvest (‘to promote ripening and sugar accumulation in melon fruit’), as recommended by Lovatt et al. (1997) and Hulme et al. (2002) was to the detriment of fruit quality in our experiments. Inadequate irrigation during this period will give rise to plant wilting which will impede photosynthesis and the delivery of assimilate to fruit, resulting in fruit abscission prior to maximal sugar accumulation. Thus a provisional recommendation would appear to be in order to ensure that after fruit set irrigation should be delivered optimally to provide deficit-free watering to maximise fruit cell division, cell expansion and fruit sugar accumulation (exemplified in Fig. 4.2A).

The common-place practice of reducing irrigation close to harvest may be an over-response to the observed negative effects of too much irrigation close to harvest. Lester et al. (1994) showed that additional water close to harvest produced fruit with lower TSS and greater volume; whilst Wells and Nugent (1980) demonstrated that rainfall events close to harvest detrimentally affected melon fruit TSS (depending on cultivar). Further, with excessive soil moisture and thus improved plant water

4. Water relations
potential, fruit storage cells may become hyperosmotic, leading to an uptake of water into these cells and the dilution of accumulated sugar.

**Figure 4.5.** Irrigation deficit treatments imposed at the start of harvest (stress during harvest), 10 days before harvest (DBH) with irrigation delivered during harvest (stress before harvest), and at 7-10 DBH including the harvest period (stress before and during harvest), for field grown ‘Dubloon’ melons in Bourke, New South Wales (2002 harvest season). Mean and least significant difference (0.05) values are reported for fruit TSS, fresh weight and yield (ANOVA P < 0.05) (data common to Fig. 4.2).

![Bar chart showing TSS, fresh weight, and yield for different irrigation treatments.](image)

Water-logging causes root anoxia and impedes root respiration (Barrett-Lennard, 2003) which in turn slows the uptake of water, causes stomata to close and ultimately retards photosynthesis (Lester *et al.*, 1994). Indeed Kroen *et al.* (1991) reported that melon plants subjected to root flooding for four days close to harvest showed
decreased root respiration (by 30%) and decreased sucrose accumulation in fruit (by 36% and 88% for inner and outer mesocarp tissue, respectively). The decrease in the rate of sugar accumulation in the fruit was attributed to an increase in the glycolytic activity of the anaerobic roots and the subsequent increased transfer of carbohydrates to the roots at the expense of the fruit (Kroen et al., 1991; Su et al., 1998).

Future work on irrigation scheduling should focus on the periods pre-harvest and during harvest and should include studies encompassing irrigation scheduling on different soil types. Additional work is recommended, particularly on newer techniques that may have potential to enhance melon fruit yield and quality and that generally increase the efficiency of water delivery. For example the use of partial root zone drying (Davies et al., 2000) may enhance water use efficiency. Partial root drying involves the simultaneous drying and wetting of different parts of the root zone of a given plant, and was reported to improve water use efficiency by up to 50% and to decrease vegetative vigour in grapes, while crop yield, berry size and TSS were not significantly reduced (Stoll et al., 2000). Those roots exposed to dry conditions produce abscisic acid, which stimulates a decrease in leaf stomatal conductance. Such a technique might be implemented in melon production using trickle irrigation lines located at depth between rows, or on each side of the row. Another technique worthy of consideration is aeration of water used for trickle irrigation. Bhattarai et al. (2004) showed that subsurface aerated irrigation in heavy clay soil increased soybean pod yield by 82-96%, and cotton lint yield was increased by 14-28% under aerated treatments.
Abstract
The imposition of a minimum total soluble solids (TSS) value as a quality standard for melon fruit requires either a batch sampling procedure (i.e. the estimation of the mean and SD of a population), or the individual assessment of fruit (e.g. near infrared, NIR non-destructive testing). The sample size needed to represent a population was estimated as 25 fruit, based on an uncertainty of 0.5% TSS and using an average SD value of 1.28% TSS (acquired from 22 separate populations). Due to the heterogeneous distribution of melon tissue TSS, it was expected that estimates of TSS would be affected by the sampling method. Outer mesocarp TSS was three TSS units higher at the stylar end of the fruit compared to the stem end, while the TSS of inner mesocarp was higher than outer tissue and more uniform across spatial positions. The linear relationship between the outer 1 cm and the subsequent inner 1 cm of tissue varied for mature fruit from different populations (cv. Eastern Star 2001 $r^2 = 0.88$, cv. Malibu 2001 $r^2 = 0.59$), which notably effected NIR calibration performance ($R^2 = 0.80$ and $R^2 = 0.41$, Eastern Star and Malibu, respectively). The linear relationship between outer and inner tissue varied for fruit of different maturities (42 DBH $r^2 = 0.8$, 13 DBH $r^2 = 0.4$, maturity $r^2 = 0.7$). The TSS of juice from a single 22 mm core method was not significantly different to that from four 8 mm cores ($P = 0.59$). Little difference was noted in NIR calibration performance between the stylar, ground spot and equatorial positions, and the cold storage of fruit (0, 7 and 14 d at 5 °C) did not impact on calibration performance.

Introduction

Currently, melon fruit supplied to consumers in Australia are routinely of an unacceptable sweetness level (see Chapter 1). A quality standard of 10% TSS has been determined by Mutton et al. (1981), and largely accepted, although not enforced, by retailers (Appendix 2). To impose a quality standard on production, there are two basic strategies that may be employed. A ‘batch’ strategy relies on assessment of the mean and standard deviation of the lot (in the case of melon production, the harvest of a given field). Lots can be accepted where the population is clearly above a quality standard (e.g. population mean greater than the quality standard by more than two standard deviations), and must be rejected when the population mean falls below this point. Acceptance rates can be improved by increasing the population mean, or by decreasing the population spread. Alternatively, the quality of every item (i.e. every piece of fruit) can be assessed. Of course both strategies suffer from the error of the measurement technique.

Melon flesh is heterogenous with respect to TSS. Peiris et al. (1999) reported that, for a single melon fruit of a single cultivar of a single harvest, TSS increased by three to four TSS units from proximal to distal (stem to stylar) ends and in the radial directional (from exocarp to seed cavity) orientations, and by one to two TSS units around the equator of the fruit. In that study, radial variation was assessed at a single location on the fruit. Guthrie et al. (1998) reported a poor correlation between outer (2-5 mm) and inner (8-20 mm) flesh of cultivar Eldorado fruit, although correlation statistics were not presented. Greensill and Walsh (2000) showed that TSS increased along the distal axis of the fruit by 1.9 and 2.5% TSS for outer and inner tissue

5. Determination of melon sweetness
respectively, with an average difference of 2.8% TSS between outer and inner tissue. The same authors reported a correlation of $r^2 = 0.16$ between outer and inner tissue.

Given the heterogeneity of melon tissue with respect to TSS, it is to be expected that estimates of fruit TSS will be sensitive to the sampling procedure used. For example, the standard retail procedure is to gently squeeze a longitudinally cut slice of melon, expressing juice onto a refractometer. This action will tend to sample the inner tissues. The sampling methods used by other researchers determining TSS and specific carbohydrates vary. Lester et al. (2001) reported that ‘middle mesocarp sections’ were sampled for sugar analysis, while Miccolis and Saltveit (1991) sampled TSS after ‘each fruit was cut in half’. Lester (1998b) acquired juice from ‘a 10 mm diameter equatorial plug of mesocarp tissue’, while Hubbard et al. (1989) used a cork borer (unspecified diameter) to sample from an equatorial location, with each core ‘sliced into sections approximately 1 to 2 cm wide from just beneath the exocarp to the innermost tissue’; they specified that small fruit mesocarp tissue was divided into three sections and larger fruit was divided into four sections.

A tissue sampling regime for TSS determination must be designed with knowledge of the variation in TSS within a fruit. The output of non-invasive methodologies used to assess TSS of intact fruit also need to be interpreted with respect to the volume of fruit ‘optically’ sampled by the technique. A number of authors have reported the use of near infrared spectroscopy to assess melon TSS. Dull et al. (1989) used two wavelengths (896 and 860nm) and reported an SEP of 2.18 and an $R^2 = 0.36$. Spectra were acquired from equatorial positions and a 20 mm core, less 12 mm of outer rind and tissue, was used for chemical analysis. Aoki et al. (1996) obtained transmittance
spectra (640-1075 nm) of melon and acknowledged that a TSS value was required to represent the whole fruit. However, the sampling methodology used was poorly documented, being described as ‘the fruit was sliced at a thickness of 2 cm, at the equator’. The authors reported a SEP of 0.40% TSS for whole melon (no other statistics presented). Ito et al. (2001) acquired reflectance spectra from the stylar position of fruit, and obtained juice by sampling a 40 mm diameter core, although the depth of sampled tissue was not specified. Calibrations using 906, 874, 830 and 856 nm yielded an SEC = 0.44 and $R^2 = 0.88$ (no prediction statistics given).

Spectral data collected in reflectance or partial transmittance modes will carry more information on the exocarp and outer mesocarp than on inner mesocarp tissue. Indeed Lammertyn et al. (2000) reported that for Jonagold apples using reflectance optics, the penetration depth was up to 4 mm and between 2-3 mm in the 700-900 and 900-1900 nm range respectively. For melon fruit, models based on TSS of internal mesocarp tissue and reflectance spectra of intact melon performed poorly ($R^2 = 0.36$) relative to models developed using spectra of melon flesh ($R^2 = 0.94$) (Guthrie et al., 1998). Correlations between NIR spectra and TSS of edible flesh (inner mesocarp) will be influenced by the relationship between outer and inner mesocarp TSS (Long et al., 2002). For example, Ito et al. (2000) reported NIR assessment based on the stylar end of the fruit, because “flesh in the blossom end of melon fruit is thinner than that in other parts”. This region may also have a higher correlation between inner and outer mesocarp TSS. The extent (thickness and uniformity) of lenticel formation in the exocarp is also different for various positions on a melon fruit and different between cultivar groups, and may also affect NIR calibration model performance. The ground
spot on a melon fruit has less developed netting than other parts and lacks chlorophyll in the outer mesocarp.

The robustness of calibration models for mesocarp TSS, developed on near infrared spectra of intact fruit, will be sensitive to a range of variables beyond the relationship between the TSS of inner and outer tissues. Issues related to the optical properties and chemistry (matrix effects) of fruit will influence model performance, which will be related to the cultivar of melon, the maturity of the fruit, and the post-harvest age of the fruit. For example, fruit spend up to 14 days in cool storage during transit to market, and then further time on the shelf. Although TSS does not change after fruit have been picked (Lester and Dunlap, 1985), flesh firmness declines (Miccolis and Saltveit, 1995; Wakabayashi, 2000).

In this study, the number of fruit required to facilitate the ‘batch’ sampling procedure was determined. Additionally, the variation of TSS within a fruit, between inner and outer mesocarp tissue, for fruit varying in maturity, and following postharvest storage, and the effect of these factors on NIR calibration model performance, was considered. Further, the effect of storage of whole fruit, fruit cores or juice on the assessment of TSS was examined.
Materials and methods

Batch sampling

The size of the required sample (n) can be estimated given the desired uncertainty (ε), the standard deviation of the population (s) and the students t statistic (t) for ∞ degrees of freedom (ν) (at 95% level of significance):

$$\varepsilon = t \cdot \frac{s}{\sqrt{n}}$$  \hspace{1cm} \text{eqn. 1}

Therefore, rearranging:

$$n = \left(\frac{t \cdot s}{\varepsilon}\right)^2, \quad \text{where} \ t = 1.96 \ (\alpha = 0.05, \ \nu = \infty)$$

TSS sampling - coring

Two melon tissue coring methods were compared, involving collection of either four 8 mm diameter cores, or a single 22 mm diameter core (Fig. 5.1). Cores were collected from the equatorial region of 10 melon fruit (cultivar unknown), and consisted of the tissue between the seed cavity and outside rind. The rind was removed, including approximately 5 mm of ‘green’ tissue, leaving the typical yellow-orange coloured flesh that is usually consumed (Fig. 5.2 D). Cores were crushed in a garlic press, the resulting juice collected in a 50 mL tube, and hand agitated for approximately 10 seconds. An aliquot of this juice was assessed for % TSS on a Bellingham and Stanley RFM 300 temperature compensated digital refractometer.

TSS sampling - location

Cores (22 mm diameter) were sampled from the stylar, an equatorial, and stem, positions (Fig. 5.2) of 10 melon fruit (cultivar Malibu). Rind tissue was removed, specifically only 1 to 2 mm, and the remaining mesocarp was cut into 10 mm sections (Fig. 5.2 E). TSS was determined for each 10 mm slice.
TSS sampling – juice and core storage

The effect of storage of tissue cores or juice on TSS determination was assessed using 60 cores of mesocarp tissue (22 mm diameter, with rind and ‘green’ tissue removed as described previously, Fig. 5.2 D) sampled from 15 melon fruit (cultivar unknown).

Five groups of 12 randomly chosen cores were designated to the following treatments: (i) immediate determination (control), (ii) refrigerated (4°C) storage of cores for 4 d, (iii) frozen storage (-20°C) for 6 d, (iv) refrigerated (4°C) storage of juice for 4 d, (v) frozen storage (-20°C) of juice for 6 d.

Figure 5.1. Coring apparatus employing (A) four 8 mm coring tubes and (B) a single 22 mm coring tube. To compare coring methods, cores were taken from an equatorial region on melon fruit as shown in the fruit image.
Figure 5.2. Melon fruit anatomy (A) and the detailed view of the stem/ peduncle (B) and blossom (C) positions. Mesocarp cores (22 mm) sampled from the ground spot and from one other position are shown (D), and the mesocarp tissue sampled for agronomic assessment (equatorial position only - dotted line) (D) and for NIR analysis (E) are shown.
**Figure 5.3.** 0° light source to collecting optic interactance arrangement used to collect spectra of whole melon fruit.  **(A)** A mirror within the lens barrel directs light to the receiving fibre optic for the Zeiss MMS1 spectrometer.  **(B)** Fruit were positioned such that the skin made full contact with the collecting probe.

**NIR instrumentation**

A prototype NIR spectrometer as described by Greensill and Walsh (2000) was used to acquire partial transmittance (interactance) spectra from whole melon fruit (Fig. 5.2, 5.3). The instrument consisted of a 100 W quartz halogen lamp in conjunction with a custom built collecting probe (0° collecting probe to light source angle) and a Zeiss
MMS1 (NIR enhanced) spectrometer (300-1150 nm). The spectrometer and lamp were warmed up for a minimum of two hours prior to use. Integration time was optimised to record maximum counts without saturation, for a sub sample of fruit prior to spectral acquisition of all fruit; an integration time of 20-50 msec was normal for melon fruit. White and dark references were recorded before the spectral collection of each fruit population. To record a dark reference, a double metal plate arrangement was positioned such that one plate covered the lamp and the additional plate covered the collecting probe. A single white Teflon plate was used as the white reference.

**NIR calibrations – cultivars**

The 0° interactance arrangement was used to collect spectra from the stylar, an equatorial, and stem, positions for: i) 48 cultivar Dubloon fruit from Kununurra W.A. in 2001. ii) 50 cultivar Eastern Star fruit from Kabra Qld in 2001. iii) 40 cultivar Malibu fruit from Kabra Qld in 2001 and iv) 30 cultivar Malibu fruit sourced from Kabra Qld in 2002. Following spectral acquisition, 22 mm cores were sampled from each spectral collection location, and following the removal of 1-2 mm of outer rind (Fig. 5.2E), TSS was determined for the outer 10 mm and subsequent next inner 10 mm of mesocarp tissue.

**NIR calibrations – post harvest cold storage**

128 cultivar Eldorado melons and 150 cultivar Eastern Star melons were sourced from Kabra Qld during 2001. Fruit were stored in commercial packing trays in a cold room (2-4°C, 85-90% RH) for up to 14 d. Melons were allowed to equilibrate overnight to
ambient temperature (approximately 24°C) prior to experimental work. Each population was divided into 3 groups, and spectra were collected from the stylar, an equatorial, and blossom, positions, for one group immediately following harvest, for a second group of fruit following 7 d cold storage, and for a third group subject to 14 d cold storage.

*NIR calibrations – fruit maturity*

Thirty melon fruit were sampled at 7 d intervals, starting approximately 7-10 days after anthesis, from a commercially grown cultivar Malibu planting in Kabra Qld during 2002. NIR spectra were collected from two equatorial positions for each fruit. Mesocarp cores were sampled and TSS determined for outer, middle and inner tissue samples (Fig. 5.2E).

*NIR chemometrics*

Raw spectra were transformed into absorbance (ABS) spectra (eqn. 2). The Unscrambler 7.6 (CAMO ASA, Norway) was employed to generate partial least squares 1 (PLS1) and principle component analysis (PCA) models utilising ABS spectra (783 to 955 nm).

\[
ABS = \log (\text{white} – \text{dark}) / (\text{sample} – \text{dark}) \quad \text{eqn. 2}
\]

NIR calibration statistics (taken and adapted from The Unscrambler) are presented:

Root mean standard error of cross validation/ prediction

\[
\text{RMSECV or RMSEP} = \sqrt{\sum (Y_p – Y_A)^2 / (n-1)} \quad \text{eqn. 3}
\]
Where $Y_P$ is the predicted value and $Y_A$ is the actual reference value and \( n \) is the number of samples in the calibration set.

Bias

$$Bias = \frac{\sum (Y_P - Y_A)}{n} \quad \text{eqn. 4}$$

Standard error of prediction (bias corrected)

$$\text{SECV or SEP} = \sqrt{\frac{\sum (Y_P - Y_A - \text{bias})^2}{n-1}} \quad \text{eqn. 5}$$

Coefficient of determination

$$R^2 = 1 - \left(\frac{\text{SEP}}{\text{SD}}\right)^2 \quad \text{eqn. 6}$$

This coefficient of determination is calculated for calibrations developed using separate prediction data (e.g. either via cross validation or a separate validation population). SD is that of the actual reference population.

Standard deviation residual

$$\text{SDR} = \frac{\text{SD}}{\text{RMSECV or RMSEP}} \quad \text{eqn. 7}$$

SD is that of the actual reference population.

General population statistics

The SAS 6.12 software package (Cary, NC, USA) was employed for ANOVA of data. Least significant difference (LSD\(_{0.05}\)) was calculated to facilitate means separation for ANOVA models that were significant (\( P < 0.05 \)). Mean and standard error values are reported where the corresponding ANOVA models were not significant (\( P > 0.05 \)). Microsoft Excel was employed for regression analysis.
Results and discussion

Batch sampling

The destructive batch sampling method would prove useful for a primary producer or marketer wishing to know an average TSS value for a specific population of melon fruit. However, the number of fruit required for sampling which would best represent a given population needs to be determined. Equation 1 allowed an ‘optimal’ number to be calculated in conjunction with those data collected for various melon populations sampled from 1998 to 2003 (Table 1.1). Using the lowest, the highest and the average SD values from those populations (SD 0.9, SD 2.0, average SD 1.28), the number of samples required to achieve an uncertainty of 0.5% TSS was calculated as 12, 62 and 25, respectively (eqn. 1).

TSS sampling – coring and tissue storage

The four core approach samples a larger area of tissue (25 cm²), and more closely represents the tissue ‘seen’ by the NIR equipment than the single core sample. However, there was no significant difference in mesocarp TSS assessed using either coring method (P = 0.59, Fig. 5.4). As the single core method was also easier and quicker to implement, this method was adopted in all subsequent work.

There was no significant difference (P = 0.47) in TSS between the various storage treatments (Fig. 5.5), indicating that either melon cores or juice can be stored for up to 6 d prior to TSS determination. This allows for extended sample processing following spectral acquisition of a large population.
Figure 5.4. Mean TSS and SE for coring method (A) and coring method (B) (in common with Fig. 5.1) for 10 melon fruit. ANOVA P = 0.59.

![Graph showing TSS for coring methods A and B](image)

Figure 5.5. The effect of storing 22 mm diameter mesocarp cores and the juice from cores, in refrigerated storage for four days and in freeze storage for 6 days, on juice TSS (n = 12). ANOVA P = 0.47.

![Graph showing TSS for different storage treatments](image)
TSS distribution

Outer mesocarp TSS was three TSS units higher at the stylar end of the fruit, and one unit higher than at the equator, than at the stem end, similar to the result of Peiris et al. (1999) (Fig. 5.6). The TSS of inner mesocarp tissue was higher than that of outer tissue, and more uniform across the spatial positions (Fig. 5.6). The linear relationship between outer and inner TSS varied for different populations. For equatorial samples, the relationship was strong for both cultivar Eastern Star 2001 and Dubloon 2001 populations ($r^2 = 0.88$ and 0.92 respectively), whilst coefficients of determination were lower for cultivar Malibu 2001 and 2002 populations ($r^2 = 0.59$ and 0.68, respectively) (Table 5.1).

In the 42 d preceding fruit harvest, outer mesocarp TSS tended to increase linearly from 4% to 6%, while middle and inner tissue increased from 4 to 8% and from 5 to 10%, respectively (Fig. 5.7). The linear relationship between outer tissue and middle tissue was strong ($r^2 = 0.8$) early in fruit development, poorer between 28 and 13 days before harvest (DBH), and stronger again for more mature fruit ($r^2 = 0.7$) (Fig. 5.7).

TSS significantly increased with time of cold storage ($P<0.001$, control 7.1% TSS, 7 days 7.6%, 14 days 7.7%, data not shown) in Eastern Star fruit, while no significant change was noted for Eldorado fruit ($P = 0.57$, control 7.9%, 7 days 7.9%, 14 days 8.0%). The increase in fruit TSS probably represents loss of water (transpiration) during storage (fruit weight was not assessed).
Figure 5.6. TSS of the outer, middle and inner 1 cm of mesocarp tissue (22 mm core) sampled at the stylar (●), an equatorial (○), and stem (▼) positions for 10 replicate fruit sampled from an initial harvest (A) and late harvest (nine days later, B) from a field grown cultivar Malibu planting. Note for the inner sample at the styler position, n = 2. Factorial ANOVA was significant for both populations (P < 0.05).
Table 5.1. Mean TSS and SD for the outer 1 cm and subsequent inner 1 cm of mesocarp tissue, sampled from the equatorial positions of n fruit for cultivar populations Eastern Star 2001, Dubloon 2001, Malibu 2001 and Malibu 2002. NIR calibration statistics are presented for PLS1 models developed on outer and inner tissue TSS (Zeiss MMS1). Outer and inner calibration performance was directly compared as the percentage of the inner SDR relative to the outer SDR (inner SDR/outer SDR x 100). The linear relationship ($r^2$) between outer mesocarp TSS and inner mesocarp TSS for each cultivar are reported.

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<td>n</td>
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<td>outer mean (± SD)</td>
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<td>7.0 (1.1)</td>
<td>8.1 (1.6)</td>
<td>6.7 (1.6)</td>
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<tr>
<td>inner mean (± SD)</td>
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<td>9.1 (1.4)</td>
<td>10.0 (1.1)</td>
<td>9.0 (1.5)</td>
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<tr>
<td></td>
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<tr>
<td>Coefficient of 1st 10 mm determination TSS vs. 2nd 10 mm TSS</td>
<td>0.88</td>
<td>0.92</td>
<td>0.59</td>
<td>0.68</td>
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</table>
Figure 5.7. Mean % TSS (A), SD (B), the linear relationship between outer and middle TSS and outer and inner TSS (C), and NIR calibration statistics RMSECV (D), SDR (E) and $R^2$ (F), for the outer 1 cm (---), middle 1 cm (--•--) and inner 1 cm (—-•-—) of 22 mm cores of mesocarp tissue sampled from two equatorial regions of 30 field grown cultivar Malibu melon fruit (n = 60).
NIR calibrations – effects of tissue sample, cultivar and maturity

Model performance varied between different cultivar populations (Eastern Star $R^2 = 0.80$, Dubloon $R^2 = 0.61$, Malibu 2001 $R^2 = 0.41$, Malibu 2002 $R^2 = 0.57$) for calibrations developed using inner mesocarp tissue, while calibrations using outer tissue performed better and were more uniform across cultivar populations ($R^2 = 0.8$ to 0.9) (Table 5.1). Model performance using inner mesocarp TSS data was related to the strength of the linear relationship between inner and outer mesocarp TSS (which varied from a $r^2$ 0.59 to 0.92 between populations). The light path through the fruit to the detector will be predominately through the outer mesocarp tissue, and thus calibrations for the tissue of interest (inner mesocarp) were expected to be stronger when a stronger relationship between outer and inner tissue is present.

The performance of calibration models developed on outer tissue was consistently higher for populations sampled between 42 and 21 DBH (RMSECV 0.3% TSS), relative to that for populations sampled closer to harvest (RMSECV 0.5) (Fig. 5.7).

Similar trends were evident for models based on middle and inner mesocarp tissue (Fig. 5.7). Fruit stop growing approximately two weeks prior to maturity, while sugar accumulation continues (see Chapter 3). Sugar accumulates first in the inner mesocarp, and later in the outer mesocarp. Thus the relationship between inner and outer mesocarp TSS is strong in immature fruit, poor in the later maturation period, and improves in mature fruit. Again, the performance of models based on outer mesocarp TSS was related to the strength of the relationship between outer and inner tissue TSS.
NIR calibrations – anatomical position

Principle component analysis (PCA) of spectra (783-955 nm) collected at various locations around the fruit (cultivars Eldorado and Eastern Star, Fig. 5.8C, D) indicated distinct differences between spectra from equatorial positions and those from other locations on the fruit, and some distinction between spectra acquired from the ground spot and stylar ends.

The ground spot possessed little or no chlorophyll in the outer mesocarp (Fig. 5.2D), and it was hypothesised that the TSS of inner and outer tissue might be more closely related at this point, and thus support improved calibrations models. This was not true (e.g. calibration on inner mesocarp tissue of Eastern Star 2001, ground spot $R^2 = 0.74$, equator $R^2 = 0.75$, stylar $R^2 = 0.71$) (Fig. 5.9).

The styler end calibration proved stronger for the Eldorado but not for the Eastern star population, evident by consistently higher $R^2$ and lower RMSECV values for inner and outer tissue across storage treatments (Fig. 5.9). Ito et al. (2000) recommended NIR assessment at the stylar end of intact melons, on the basis that the “flesh in the blossom end of melon fruit is thinner than other parts”. In the current study the thickness of the mesocarp from the stylar end was about 8 and 4 mm shorter than equatorial and stem ends, respectively, although the ‘green’ inedible mesocarp averaged 5 mm thickness for all three positions (Fig. 5.10). Stylar end mesocarp was consistently higher in TSS than other positions, and more uniform in TSS between inner and outer tissue (Fig. 5.6). Therefore, the recommendation of Ito et al. (2000) is supported.
**Figure 5.8.** Sample scores for principle components number 1 and number 2, generated from PCA models (783-955 nm) for cultivar Eldorado and cultivar Eastern Star 2001 populations. Scores are classified as cold storage day treatments (top panels A and B) 0 days (●), 7 days (○) and 14 days (▼), and fruit sample positions (lower panels C and D) stylar (■), equator (□) and ground spot (▲).
Figure 5.9. Calibration statistics for PLS1 models (783-955 nm) created using Zeiss MMS1 spectra collected from the ground spot (GS), equator (E) and the stylar (S) positions (ALL is combined position data), and the outer 1 cm (■) and inner 1 cm (□) fruit TSS, for a control treatment (0 days) and two cold storage treatments (7 days and 14 days) at 5 °C, 90% RH. Cultivar Eastern Star (total n = 150, n = 50 for storage treatments) and cultivar Eldorado (total n = 128, 0 days n = 31, 7 days n = 48, 14 days n = 49) melon fruit were harvested at maturity during the 2001 season at Kabra QLD.
Figure 5.10. The mean length of 22 mm diameter cores of mesocarp tissue sampled from the stylar, an equatorial, and stem positions for 10 mature cultivar Malibu fruit. Total length was fractioned into the edible mesocarp and outer non-edible ‘green’ mesocarp tissue. For total core ANOVA P < 0.01 (LSD reported). Green tissue means were not significant (error bars are SE).

NIR calibrations – effect of cold storage

In PCA, the spectra (783-955 nm) of Eastern Star fruit varied little with cold storage. For Eldorado fruit however, 14 d treatment spectra appeared different (Fig. 5.8). However, calibration models developed for fruit of a given time of cold storage predicted well against fruit of different storage periods for both cultivar populations (e.g. Eldorado 7 d model, 0 d RMSEP 0.51, 7 d RMSEP 0.38, 14 d RMSEP 0.45, combined days RMSEP 0.44), except for the 14 d Eldorado model, which predicted poorly against other data (against 14 day RMSEP 0.45, 7 d RMSEP 0.76, 14 d RMSEP 0.86, all days RMSEP 0.69), this exception consistent with the spectral difference noted in 14 d data detected by PCA (Fig. 5.8A). Combined day spectra calibrations predicted well against individual treatment days (e.g. Eldorado 0 days RMSEP 0.41, 7 days RMSEP 0.41, 14 d RMSEP 0.36 cf. self prediction RMSEP 0.40) (Table 5.2).
Table 5.2. Validation statistics for PLS1 models (783-955 nm) developed using Zeiss MMS1 spectra for outer 1 cm tissue TSS sampled from a combination of positions (ground spot, equator and blossom) from cultivar Eldorado and cultivar Eastern Star populations subject to cold storage treatments (control or 0 days, 7 days and 14 days) (data in common with Fig. 5.9).

<table>
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<tr>
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<td>0 days</td>
<td>7 days</td>
</tr>
<tr>
<td></td>
<td>0 days</td>
<td>7 days</td>
</tr>
<tr>
<td>Validating</td>
<td>0.94</td>
<td>0.94</td>
</tr>
<tr>
<td>population</td>
<td>0.91</td>
<td>0.97</td>
</tr>
<tr>
<td>7 days</td>
<td>0.96</td>
<td>0.94</td>
</tr>
<tr>
<td>14 days</td>
<td>0.92</td>
<td>0.95</td>
</tr>
<tr>
<td>all days</td>
<td>0.94</td>
<td>0.94</td>
</tr>
<tr>
<td>RMSEP</td>
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<td>RMSEP</td>
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</tr>
<tr>
<td></td>
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<td>7 days</td>
</tr>
<tr>
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<td>0.51</td>
</tr>
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<td>0.38</td>
</tr>
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<td>7 days</td>
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<tr>
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</tr>
<tr>
<td>SDR</td>
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<td>SDR</td>
</tr>
<tr>
<td></td>
<td>0 days</td>
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<tr>
<td></td>
<td>0 days</td>
<td>7 days</td>
</tr>
<tr>
<td>Validating</td>
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</tr>
<tr>
<td>population</td>
<td>3.39</td>
<td>5.45</td>
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<tr>
<td>7 days</td>
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<td>4.09</td>
</tr>
<tr>
<td>14 days</td>
<td>3.61</td>
<td>4.43</td>
</tr>
<tr>
<td>all days</td>
<td>3.61</td>
<td>4.43</td>
</tr>
<tr>
<td>BIAS</td>
<td>Prediction model</td>
<td>BIAS</td>
</tr>
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<td>7 days</td>
</tr>
<tr>
<td></td>
<td>0 days</td>
<td>7 days</td>
</tr>
<tr>
<td>Validating</td>
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<td>-0.04</td>
</tr>
<tr>
<td>population</td>
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<td>0.04</td>
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<tr>
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<td>0.01</td>
</tr>
<tr>
<td>all days</td>
<td>-0.07</td>
<td>0.01</td>
</tr>
</tbody>
</table>

5. Determination of melon sweetness
Conclusions

Soluble sugar distribution is indeed variable within melon fruit. For assessment of the eating quality of melon fruit, it is recommended that a core (22 mm diameter or greater) be sampled from a randomly selected equatorial position on the fruit, and the rind and green inedible tissue (5 mm thickness) and placenta and seeds removed. All remaining edible mesocarp tissue should be juiced. For calibration of NIRS operating in interactance or partial transmittance modes, it is recommended that TSS be assessed of the outer 10 mm of a core (22 mm diameter or greater) taken at the point of spectroscopic assessment, with only the rind (1-2 mm) removed. The relationship of this value to the eating quality of the fruit (i.e. the TSS of all mesocarp in a core) should be established for a given population (and will vary by, for example, fruit maturity).
Sugar ‘imaging’ of fruits using a low cost CCD camera

Abstract
Near infrared (NIR) hyperspectral imaging of cut melon tissue was attempted using a low cost 8-bit charge coupled device (CCD) camera and filters at 830, 850, 870, 905 and 930 nm, arranged in a transmittance configuration employing three 50 W halogen lamps. Approximately 60 and 20 mins from power up was required to achieve a relatively stable output for the CCD camera and lamps, respectively. Multiple linear regression (MLR) calibrations were developed based on absorbance data for all five wavelengths and sucrose concentration of solutions on a cellulose matrix. Calibration models performed poorly ($R^2 = 0.4$) when based on individual pixel data, performed well ($R^2 = 0.98$, RMSECV = 1.1) ($n = 20$, mean = 13.9% TSS, SD = 6.04) when based on an average of a 23x23 pixel block (i.e. 529 pixels). For a calibration based on melon tissue TSS, using spectral data averaged over groups of 529 pixels, results were poorer than expected ($R^2 = 0.4$, RMSEP = 1.74 ($n = 163$, mean = 9.45, SD = 2.07% TSS), with a false colour image of the cut surface of melon indicating sugar variation through the fruit. We conclude that this application requires a higher level of signal to noise platform (e.g. 10 bit CCD).
**Introduction**

Spectroscopic technologies utilising the short wave near infrared (NIR) region (750 – 1100 nm) have been used for measuring the external and internal quality of fresh fruit for the past 30 years. Early instrumentation was filter based, using specific wavelengths to measure the constituent of interest. For example, McClure *et al.* (1975) used 740 and 800 nm wavelengths to sort blueberries based on anthocyanin content. From the late 1970s full spectrum NIR analysis was facilitated via scanning grating spectrometers, and from the 1990s, diode array spectrometers in conjunction with chemometric analysis, have been used to measure fruit internal parameters such as the sugar content of oranges (Kawano *et al.*, 1992).

Instrumentation used for the assessment of total soluble sugars (TSS) in fruit has been characterised as requiring a wavelength range between 650 and 1050 nm, a high signal to noise ratio (s/n) and a related high analogue to digital conversion (ADC) resolution. Walsh *et al.* (2000) indicated the importance of a high s/n over wavelength resolution for calibration accuracy, and Greensill and Walsh (2000) showed that a s/n (mean to standard deviation) less than 5000:1 degraded model performance for the prediction of sucrose in a water-cellulose matrix, and recommended use of instrumentation with a s/n $\geq$ 5000 for the assessment of TSS of whole fruit.

These applications involve the collection of diffusely reflected or transmitted light as a single measurement for each sample. However, attributes such as soluble sugar and dry matter content vary within a given piece of fruit (Peiris *et al.*, 1999), and this distribution often contains information pertinent to an understanding of the fruit
development and ripening process. Two dimensional or hyper-spectral imaging allows the measurement of a constituent over the surface of the sample. Such technology typically employs a 2 D detector array, using either a line scan method (step wise assessment of the item, with the second dimension of the detector used for spectral information) or a filter-based method (with sequential images of the item taken using different wavelengths). These methods suffer from the usual problems of transferring calibrations between spectrometers, exacerbated in that every pixel acts as a distinct spectrometer. To avoid this problem, Burling-Claridge and Dodd (2004) proposed that a Hadamard coding scheme be used to illuminate a sequence of ‘pixels’, with signal strength recorded using a scanning grating and single detector. Spectra are then deconvolved to yield individual pixel spectra. The method improves s/n, speed of capture and calibration transfer. However, implementation has been limited by switching speed and reliability, spectral non-linearities of optical elements and cost.

Several ‘high end’ (expensive) commercial NIR imaging systems are now available, primarily used for assessing the homogeneity of pharmaceutical products. These units employ low noise components (typically cooled detectors) and boast high ADC resolution. For example, Spectral Dimensions Inc (Olney, MD, USA) produce an InGaAs detector array and acousto-optical tunable filter based imaging microscope, with a spectral response of 950-1720 nm and 14 bit ADC. This instrument is valued at US $170,000. Sensors Unlimited Inc (Princeton, NJ, USA) produce a temperature stabilised InGaAs array camera with a spectral response of 900-1700 nm with 12 bit digital output (dynamic range >2000:1, noise (RMS) < 1000 electrons, noise equivalent irradiance < 5x10⁹ photons cm⁻² s⁻¹). This camera was employed by Peirs
et al. (2003) in conjunction with a holographic transmission grating, to make line scan measurements of the spatial distribution of starch over the surface of cut apples. Their system was estimated to cost approximately US $40,000. Bellon-Maurel et al. (1999) also used a Sensors Unlimited camera to identify internal disorders such as apple ‘bitterpit’ and to discern physical features such as the stalk or stem in apple and green grapes.

Hyperspectral imaging of fruit TSS should be possible using a low cost Si CCD array (operating up to about 1050 nm), as long as signal to noise levels are kept reasonably high. Indeed, Martinsen and Schaare (1996) reported the design of an instrument which measured TSS across the cut surface of kiwifruit with a prediction error of 1.2% TSS. This apparatus consisted of a slit, collimating lens and mirror mounted on a translation stage, which was scanned past the object using a linear stepping motor. Line scanned images were reflected onto a diffraction grating and collected with a CCD camera (753 x 244 pixels, RMS noise 140 electrons per pixel, s/n not reported). The set up was reported to cost less than US $30,000. Spectral images of a 12 mm slice of kiwifruit were acquired in reflectance mode and the TSS of cores of flesh were determined and calibrated with single pixel spectral data acquired from with in the area of the image representing the core (Martinsen and Schaare, 1998). More recently, Sugiyama (1999) and Tsuta et al. (2002) used a 16 bit peltier cooled CCD camera designed for normal visual spectrum imaging. Reflectance images of cut rockmelons were collected using four filters (846, 874, 902 and 930 nm). To calibrate the instrument, 25 mm diameter cores were presented for spectral acquisition. The average intensity of a pixel block representing each core was used to calculate second derivative absorbances at 874 and 902 nm, and a MLR model developed against TSS
determined of a 1 mm thick slice of each core. This calibration model was applied to individual pixels.

Low noise cameras of the type used by Sugiyama (1999) are readily available commercially, however cost is an impediment to their uptake within the horticultural field. For example, Dalsa (Waterloo, ON, Canada) offers a 12 bit camera with a dynamic range of 500:1 for US $30,000. The Cooke Corporation (Auburn Hills, MI, USA) produces a 14 bit cooled camera featuring a dynamic range of 65 dB, a readout noise of 12 electrons and a dark charge < 3 electrons/ pixel-sec (at 20°C), which sells for approximately US $40,000. Cheaper cameras are available, albeit with lower s/n and ADC. Allied Vision Technology produce a 10 bit CCD camera that costs approximately US $2783, in comparison to a cost of <US $2000 for more common 8 bit CCD cameras.

In the current study we review the design criteria for a fruit sugar ‘imager’. Such an instrument could enable researchers to observe the sugar accumulating behaviour of fruit, e.g. in the breeding of new melon cultivars, or the use of genetic manipulation to increase sugar accumulation. The instrument was deigned in terms of wavelength and signal to noise requirements, and then the lowest cost components available to meet these requirements, were selected. The total cost was less than AU $5000 (US $3700).

Materials and methods

*Acquisition of fruit spectra using a Zeiss MMS1 (Jena, Germany)*
Cultivar Eastern Star melon fruit were sourced from a commercial farm in Kabra, Queensland. Spectra were acquired from stationary fruit using a Zeiss MMS1 silicon photodiode array (15 bit ADC, 400-1100 nm) integrated into a custom built instrument in which the detector assembly was placed in front of an illumination source consisting of a 100W lamp and a parabolic reflector (as described by Greensill and Walsh, 2000). Fruit were presented such that the detector assembly made contact with the fruit (i.e. interactance mode). A 22 mm diameter core of mesocarp tissue was then extracted, the rind removed, and juice extracted from the outer 10 mm of tissue using a hand operated garlic press. The % TSS of the resulting juice was determined using a Bellingham and Stanley digital refractometer (model RFM 320).

**Imaging - equipment set-up**

Optical components were mounted on a Thorlabs TMC optical bread board (120 x 60 cm) (Fig. 6.1). A Basler A301f monochrome digital camera (Turnkey Solutions, Australia) employing a Sony ICX074AL/AK – ½ inch HAD interline transfer progressive scan CCD, featuring 8-bit digitisation and a quantum efficiency of 31% (at 500 nm) and 5% (at 850 nm), was used in conjunction with an 8 mm Avenir CCTV F1.3 lens with field of view angles D 53°, H 43°, V 63° and a minimum focal distance of 22 cm. A CVI 8100 filter wheel (Laser Electronics, Australia) facilitated the use of five one inch CVI optical filters. Components were purchased for a total of AU $4131 (US $3064) (AU $1270 for filters and holder plus AU $2861 for camera and lens). Filters had centre wavelengths of 830, 850, 870, 905, and 930 nm. Each filter had a 15 nm full width at half maximum height (Fig. 6.2) and an out of band rejection of four optical density units. The filter wheel was positioned in front of the camera such that the camera lens was in alignment with one filter aperture.
A metal frame with a 100 mm diameter hole was used as a sample holder. The frame was positioned 22 cm from the camera lens. A teflon diffuser sheet (120 x 120 x 10 mm) was positioned 14 cm behind the sample holder, which was in close proximity to three Philips 50 W halogen reflector lamps (model 6439, Germany).

Figure 6.1. Component arrangement for the imaging of sucrose in a cellulose matrix and for 6 mm thick slices of melon tissue. An 8 bit camera acquired images at five wavelengths (830, 850, 870, 905, 930 nm). The light source consisted of three 50 W lamps directed at a teflon diffuser.
**Figure 6.2.** The ratio of raw ADC count data of spectra acquired using each of five wavelength filters, to that of an unfiltered spectrum. Spectra of a white Teflon tile, illuminated by a 50 W halogen lamp, were acquired using a Zeiss MMS1 linear photodiode array spectrometer (400 to 1100 nm).

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**Imaging - instrument characterisation**

With both the camera and light source powered on, without filters, the system was pre-optimised to acquire an image of 10 sheets of 125 mm diameter Whatman number 1 filter paper, such that the count level was close to 256 (max counts) without saturation. System components were assessed separately for ‘warm-up’ characteristics. Firstly, the light source was powered on for at least two hours, and the camera ‘warm-up’ was assessed by acquiring images from the camera at initial power-up (time zero) and during a following two-hour period. Secondly, with a warm camera (on for at least two hours), the light ‘warm-up’ was assessed by acquiring images at the same time intervals during a two-hour period.

Ten sheets of filter paper were soaked in water for at least 30 seconds and allowed to drip drain for approximately 10 seconds and placed in the sample holder. Twenty
images were acquired using each of the five filters and without a filter (6 spectral windows). Additionally 20 replicate collections of approximately 6000 images (120,000 images) were collected of a white Teflon reference (without use of a filter).

Signal precision was calculated as mean count divided by the standard deviation of counts. This measurement was assumed to be an index of, and is thus referred to herein as, signal to noise (s/n).

*Imaging - calibration development*

From an initial 50% (w/w) sucrose solution, nine additional solutions were created in 5% descending increments. The ten sugar solutions plus a distilled water solution were measured for % TSS as described previously. Filter paper stacks (9 pieces to a stack) were soaked in each sugar solution for at least 30 seconds, and allowed to drain for about 10 seconds before being presented for image acquisition.

To minimise noise, detector count levels of approximately 200 to 250 were achieved by optimising light intensity via integration time and camera aperture, and not via the camera electronic gain setting. One spectrum was taken for each of the five filters for each of five re-pack presentations for each filter-paper stack (5 filters x 5 re-packs x 11 sucrose samples = 275 images acquired). Spectral sampling was repeated the following day.

Images were converted into absorbance (ABS) to account for lamp inconsistency, where ABS = log (reference/sample), with the reference being water (0.0% TSS) on
filter paper. Second derivative \((d^2\text{ABS})\) was calculated as a Norris derivative (for wavelengths \(A, B, C\), \(d^2\text{ABS}_B = \text{ABS}_C - 2 \times \text{ABS}_B + \text{ABS}_A\)).

**Imaging – melon tissue calibrations**

Each raw image \((658 \times 494, 325052 \text{ pixels})\) was de-resolved to 588 \((28 \times 21)\) pseudo-pixels using amplitude averaging over single groups of 529 pixels \((23 \times 23 \text{ pixel blocks})\). Melon tissue slices \((6 \text{ mm thick})\) were created using an industrial meat slicer \((\text{model 250G})\), and positioned between two 2 mm thick 120 x 120 mm glass plates, marked with a 6 x 6 mm grid pattern. A reference sample consisted of five sheets of Whatman number 1 filter paper. A single pseudo-pixel \((\text{block of 529 pixels})\) was designated as the ‘calibration’ block and a separate pseudo-pixel was designated as the ‘validation’ block. To acquire calibration and validation spectral information, melon slices were presented such that individual 6 mm square samples were presented sequentially to both the calibration and validation pseudopixels. 163 melon tissue blocks, from melon slices from six different fruit, were acquired. Following spectral acquisition, the melon tissue was sliced into cubes \((6 \text{ mm sides})\) using a grid pattern as a cutting reference. Tissue cubes were squeezed and TSS of juice measured on a refractometer.

An additional melon slice was used to provide a validation image. This cross sectional slice clearly represented the important anatomical features \((\text{exocarp, mesocarp, placenta and seeds})\) of a fruit. The image was reduced into 23 x 23 \((529)\) pixel blocks and TSS was predicted for each average block \((\text{over the entire image})\).
Data manipulation, chemometrics and statistics

Script written in Matlab 6.5.1 was employed to manipulate CCD image data (e.g. average blocks of 529 pixels). Stepwise MLR models were developed in Win ISI and The Unscrambler 7.6 chemometric packages, The Unscrambler 7.6 was used to generate PLS1 models. Model calibration results are presented in terms of the coefficient of determination ($R^2$), the root mean squared error of cross validation (RMSECV) or root mean squared error of calibration (RMSEC) and the slope of the regression relationship. Prediction results are reported in terms of the root mean squared error of prediction (RMSEP) and the standard deviation of population divided by RMSEP (SDR).

MLR model equations were used to calculate predicted TSS from block averaged images using a Matlab script and Sigma Plot 8.0 was employed to display a two dimensional coloured contour graph of the predicted TSS data for the validation melon image.

Results and discussion

Estimation of required signal resolution

PLS1 calibration models were developed using spectra acquired with the Zeiss MMS1 arrangement and the TSS of equatorially positioned outer mesocarp tissue samples ($n = 149$, mean = 5.95% TSS, $SD = 1.77$). Original raw count data (max counts 32768 = $2^{15}$ ADC resolving levels) were converted into absorbance (ABS) for chemometric analysis (Table 6.1). To artificially degrade raw count data, the 15 bit spectra were divided by 32768 and then multiplied by the number of resolving levels required for the desired ADC bit emulation. These data were then rounded to integer values, and
calibrations developed. For example, to convert 15 bit data into 14 bit data, 15 bit data were divided by 32768 and then multiplied by 16384 ($2^{14}$) and then rounded to integer values. Spectra data were degraded in one-bit steps to 6 bit ADC.

The calibration based on 15 bit data supported a model with $R^2 = 0.92$ and RMSECV = 0.51. Calibration models developed with sequentially degraded data exhibited a lesser number of principle components (15, 14 and 13 bit had 9 PC’s; 12, 11, 10, 9 and 8 bit had either 7 or 8 PC’s; 7 and 6 bit had 1 PC), which can be explained as a loss of spectral information or ‘features’. Calibration models based on 8 bit data had lost information, but were still potentially useful ($R^2 = 0.76$, RMSECV = 0.87) (Table 6.1). Calibration model performance was markedly decreased when based on 7 and 6 bit data ($R^2 = 0.54$ and RMSECV = 1.20).

Table 6.1. Chemometric statistics for PLS 1 calibrations (783-955 nm) developed on spectra of intact melons acquired using a Zeiss MMS1, and TSS values of juice from the outer 1 cm of mesocarp (n = 149, mean = 5.95, SD = 1.77% TSS). Spectral raw count data were artificially degraded from the original 15 bit ADC resolution to lower ADC resolution levels.

<table>
<thead>
<tr>
<th>ADC resolution (bits)</th>
<th>Resolving levels</th>
<th>$R^2$</th>
<th>RMSECV</th>
<th>SDR</th>
<th>PLS factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original 15</td>
<td>32768</td>
<td>0.92</td>
<td>0.51</td>
<td>3.47</td>
<td>9</td>
</tr>
<tr>
<td>15 (control)</td>
<td>32768</td>
<td>0.91</td>
<td>0.52</td>
<td>3.40</td>
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</tr>
<tr>
<td>14</td>
<td>16384</td>
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<td>0.53</td>
<td>3.34</td>
<td>9</td>
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<td>13</td>
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<td>3.28</td>
<td>9</td>
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<tr>
<td>12</td>
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<td>0.87</td>
<td>0.63</td>
<td>2.81</td>
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<td>11</td>
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<tr>
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<td>0.87</td>
<td>2.03</td>
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<td>1.20</td>
<td>1.48</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>64</td>
<td>0.54</td>
<td>1.20</td>
<td>1.48</td>
<td>1</td>
</tr>
</tbody>
</table>
Wavelength selection

The same data set as utilised for the data degrade exercise was used for the development of MLR calibrations using a combination of wavelengths. Spectral count data was converted to absorbance and second derivative of absorbance (based on slope calculated from a linear fit across 9 data points, or 25 nm). A Win ISI stepwise MLR model was optimised at four wavelengths (869, 882, 905 and 915 nm), and achieved a result equivalent to the original PLS1 model ($R^2 = 0.92$) (Table 6.2). A MLR model developed using The Unscrambler using those wavelengths selected by ISI performed equivalently ($R^2 = 0.91$), but calibration performance was less successful when second derivative was used, and when a lesser number of wavelengths were employed (Table 6.2).

MLR wavelengths were in partial agreement with those wavelengths recommended by Sugiyama (1999) (846, 874, 902 and 930 nm). Filter purchases were constrained by commercial availability (830, 850, 869, 905 and 930 nm). MLR models based on these five wavelengths and the Zeiss spectral data set yielded better statistics than for three wavelengths or when second derivative ($d^2\text{ABS}$) spectral data were used. Calibration performance was markedly worse using 8-bit compared with 15-bit data (five wavelengths 15 bit $R^2 = 0.87$ vs. 8 bit $R^2 = 0.58$) (Table 6.3).

The band assignments of the major water and sugar (OH and CH) vibrations within the SW-NIR (i.e. 750 to 1000 nm) include second and third overtones of OH stretching at 970 and 740 nm, a OH combination band at 840 nm, the third overtone of a CH stretch at 910 and a CH$_2$ stretch at 930 nm (Golic et al., 2003). However, as the chemical environment of each OH and CH bond in a sugar molecule is different,
Table 6.2. Calibration statistics of intact melon tissue TSS (data set in common with Table 1) for a PLS model (783-955 nm) and ISI stepwise MLR models (with and without second derivative – d2Abs), and The Unscrambler MLR models based on the four wavelengths selected by ISI and calibrations developed with two and three wavelength combinations.

<table>
<thead>
<tr>
<th>Package</th>
<th>Model - details</th>
<th>Spectra</th>
<th>Wavelengths/ window (nm)</th>
<th>$R^2$</th>
<th>RMSECV (SEC*)</th>
<th>SDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unscrambler</td>
<td>PLS1 original model</td>
<td>Abs</td>
<td>783-955 9 PC's</td>
<td>0.92</td>
<td>0.51</td>
<td>3.47</td>
</tr>
<tr>
<td>ISI</td>
<td>MLR stepwise λ selection</td>
<td>Abs</td>
<td>869, 882, 905, 915</td>
<td>0.92</td>
<td>0.51*</td>
<td>3.47</td>
</tr>
<tr>
<td>ISI</td>
<td>MLR stepwise λ selection</td>
<td>d^2Abs</td>
<td>721, 843, 862, 875, 882, 898, 915, 931</td>
<td>0.91</td>
<td>0.54*</td>
<td>3.28</td>
</tr>
<tr>
<td>Unscrambler</td>
<td>MLR using ISI λ</td>
<td>Abs</td>
<td>869, 882, 905, 915</td>
<td>0.91</td>
<td>0.52</td>
<td>3.4</td>
</tr>
<tr>
<td>Unscrambler</td>
<td>MLR using ISI λ</td>
<td>d^2Abs</td>
<td>869, 882, 905, 915</td>
<td>0.82</td>
<td>0.76</td>
<td>2.33</td>
</tr>
<tr>
<td>Unscrambler</td>
<td>MLR using ISI λ less one</td>
<td>Abs</td>
<td>869, 905, 915</td>
<td>0.61</td>
<td>1.1</td>
<td>1.61</td>
</tr>
<tr>
<td>Unscrambler</td>
<td>MLR using ISI λ less alternate</td>
<td>Abs</td>
<td>869, 905, 882</td>
<td>0.66</td>
<td>1.04</td>
<td>1.7</td>
</tr>
<tr>
<td>Unscrambler</td>
<td>MLR alternate two λ's</td>
<td>Abs</td>
<td>882, 915</td>
<td>0.54</td>
<td>1.2</td>
<td>1.48</td>
</tr>
</tbody>
</table>
Table 6.3. MLR calibration models developed in The Unscrambler for intact melon spectra (Zeiss MMS1) and outer mesocarp tissue TSS, at specific wavelengths representing the five filters intended for use for CCD imaging, for both full 15 bit and degraded 8 bit spectral data. Data set in common with Table 6.1.

<table>
<thead>
<tr>
<th>ADC resolution</th>
<th>Wavelengths (nm)</th>
<th>Spectra</th>
<th>$R^2$</th>
<th>RMSECV</th>
<th>SDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>830, 850, 869, 905, 930</td>
<td>Abs</td>
<td>0.87</td>
<td>0.65</td>
<td>2.72</td>
</tr>
<tr>
<td>15</td>
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<tr>
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<td>830, 850, 869, 905, 930</td>
<td>Abs</td>
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<td>1.54</td>
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<tr>
<td>8</td>
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<td>1.48</td>
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<tr>
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<td>$d^2$Abs</td>
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<td>1.29</td>
<td>1.37</td>
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</table>

the effective absorption bands are wide, especially at the second and third overtones.

Relating such assignments to the wavelength weighting of a PLS or MLR calibration on sugar content is notoriously difficult, with shoulder areas rather than absorption ‘peaks’ weighted by the regression. Nonetheless the selection of an area around 910 nm in all regressions is logically attributed to the third overtone of the CH stretch.

Instrument characterisation

Prior to calibration development, the CCD camera and light source were tested for ‘warm up’ characteristics by following the response of 5 individual pixels, chosen randomly from across the image plane, over time. The camera was arranged to view the filter paper target, without intervening filters. Pixel output decreased by 10% for the first 10 min following power up of the light source (with the detector powered up for more than 2 hours), and was relatively stable after 20 min (Fig. 6.3A). Following CCD power up (with the light source powered up for at least 2 hours), counts increased over 60 min (by approximately 5%) and stabilised at between 60 to 120 min (Fig. 6.3B). Walsh et al. (2000) reported the warm up time for a 50 W quartz halogen lamp to be 30 min, and that a (non-cooled) linear CCD array instrument (Ocean Optics S2000) failed to stabilise after 90 min while the silicon photodiode based Zeiss
MMS1 was stable within 60 min from start up. A ‘warm up’ period of 120 min was imposed in all other data collection exercises in this study.

Figure 6.3. Detector response without filters for five equally distributed single pixels during (A) the ‘warm up’ of a light source bank consisting of three 50 W halogen lamps (with a warm detector), and during (B) the ‘warm up’ period of the CCD detector (with warm lamps). Response is presented as the difference to counts at time zero.

![Graph showing detector response](image)

The response of the five pixels differed by between 10 and 20 counts (Fig. 6.4A). Count level also varied for the different wavelength filters, with raw counts for pixel 1 at 850, 870, 905 and 930 nm being 90, 87, 61 and 54% respectively, relative to the count level at 830 nm (Fig. 6.4A). This result is consistent with that expected for the use of a halogen light source and a Si CCD detector.
Signal to noise (s/n), estimated as mean / standard deviation of count data (n = 20), varied between pixels (from 100:1 to 200:1) (Fig. 6.4A). S/n was improved through the use of pixel averaging, either temporally (Fig. 6.5) or spatially (e.g. block of 5551 pixel data, 3800:1) (Fig. 6.4B). This improvement was proportional to the square root of the number of averages up to approximately 256 averages for images collected over time (Fig. 6.5) and up to 1024 pixels for spatial averaging (Fig. 6.6B). An acquisition time of approximately two hours was required to collect the 20 replicate groups of about 6000 images to facilitate the temporal averaging exercise, and it is likely that lamp and detector fluctuations may have confounded the pixel averaging effect. Spatial averaging was exempt from any temporal fluctuations because all pixel data were collected at the same time. However, illumination was not uniform over the whole field of view. Thus the decrease in the s/n statistic occurred because the size of the pixel block exceeded the area of uniform illumination of the sample, and mean count decreased and the SD increased.

Calibrations
Calibration models were developed based on absorbance at five wavelengths using sucrose solutions (TSS range of 0 to 50%). Calibration model performance increased with the size of the pixel block averaged (i.e. with higher s/n), with a dramatic increase noted on increasing pixel block area from 1 to 64 (improving R^2 from 0.39 to 0.95) (Fig. 6.6 C). Subsequent imaging work was based on a block of 529 pixels as a compromise between optimising s/n and calibration performance, and image resolution (with the 23 x 23 (529 pixels) pixel block equivalent to a 6 mm square of the image plane). A cube with 6 mm sides was also the minimum amount of melon tissue capable of yielding sufficient juice for a refractometer reading.
Figure 6.4. (A) Raw count intensity for five single pixels from the CCD camera (common to Fig. 3), and the mean and SD for a group of 5551 pixels for each of five wavelength filters (830, 850, 870, 905, 930 nm). (B) Estimate of signal to noise (mean/SD) for 20 replicate measurements is presented for the five single pixels and the 5551 pixel group at 830 nm.

Figure 6.5. Estimate of signal to noise (mean count / SD) of 20 replicates for groups of measurements varying in the number of averages for ‘pixel 1’ (●) and ‘pixel 5’ (○) (common to Fig. 6.3) acquired from CCD camera images of a white Teflon standard (no filter).
Figure 6.6. (A and B) Estimate of signal to noise (mean / SD) for 20 replicate images (CCD camera no filtration) and (C) calibration statistics for a MLR model of sucrose in a cellulose matrix using five wavelength filters (n = 50, mean = 28.2, SD = 13.8% TSS), using the average of a block of CCD camera pixel data (1 pixel to 5551 pixels).

Calibration models developed using absorbance data at all five wavelengths or 870, 905 and 930 were comparable in their performance (e.g. $R^2 > 0.97$), and these models were marginally superior to those developed using second derivative data at two or three wavelengths ($R^2 = 0.91$) (Table 6.4). Use of absorbance data at fewer than three wavelengths gave poor results. The absorbance at three or five wavelength models validated well on an independent set of spectra (collected from the same set of filter papers, but on a different day), with an SEP of 1.4 and a SDR (standard deviation of population divided by RMSEP) of greater than 4 recorded. For subsequent imaging work of melon slices, models were based on absorbance at all five wavelengths.
Table 6.4. Calibration and validation statistics for MLR models developed for sucrose solution in a cellulose matrix (n = 20, mean = 13.85% TSS, SD = 6.04), using a CCD camera and five wavelength filters, for the average intensity from 23 x 23 (529) pixel block data.

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Spectra</th>
<th>calibration</th>
<th>validation</th>
</tr>
</thead>
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<td></td>
<td></td>
<td>R²</td>
<td>RMSECV</td>
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<td>1.7</td>
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<td>870, 905</td>
<td>ABS</td>
<td>0.04</td>
<td>6.0</td>
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<td>d²ABS</td>
<td>0.91</td>
<td>1.7</td>
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<tr>
<td>870, 905, 930</td>
<td>ABS</td>
<td>0.97</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Imaging of TSS in melon tissue

While the model was useful for predicting sugar concentration across the range utilised in the sugar-on-cellulose matrix exercise (with SD = 6.04% TSS) (Table 6.4), the lowest RMSEP achieved was 1.02% TSS (Table 6.4). This level of uncertainty is limiting for application to melon. The SD of the set of melon samples was only 2.1% TSS. As $R^2$ is related to RMSECV and SD (eqn. 1), it was expected that an $R^2$ of around 0.5 could be achieved.

\[ R^2 = 1 - (\text{RMSECV/SD})^2 \quad \text{eqn. 1} \]

In practice, a MLR calibration model developed based on melon tissue (6 mm cube side) and absorbance data of the 529 pixel image block supported a RMSECV = 1.56 and a $R^2 = 0.43$ (Fig. 6.7). The decrease in performance compared to that seen for the sugar-cellulose series is likely to result from non-uniformity in sugar distribution in the melon tissue samples. Application of the model to data acquired using a different block of 529 pixels gave poorer results again, with an SEP = 1.74 and a $R^2 = 0.30$ (Fig. 6.7). The model was applied across 529 pixel blocks of a validation image to yield a contour map of TSS across the fruit (Fig. 6.8). Exocarp, outer mesocarp
and seeds registered 5% TSS, while the mesocarp and endocarp tissue were predicted high in TSS content (10 and 15% TSS respectively) (Fig. 6.9).

**Figure 6.7.** Validation of a MLR model for melon fruit TSS based on a 23x23 pixel block (average of 529 pixels) using five wavelength filters (830, 850, 870, 905 and 930 nm). Tissue samples were cubes (with 6 mm sides) (n = 163, mean = 9.45, SD = 2.07). Calibration statistics are also displayed.

The previous reports of hyper-spectral prediction of TSS across cut fruit achieved more accurate results (Tsuta *et al.*, 2002, reported an SEC = 1.09% TSS and R² = 0.79 for melon flesh, no SEP reported, while Martinsen and Schaare, 1998, reported an SEP = 1.2% TSS and R² = 0.73 for kiwifruit flesh). This is attributable to several factors. Earlier studies employed a calibration populations with a higher SD than that used in the current study, which will improve the R² statistic for the former studies. Sugiyama’s group used reflectance geometry but calibrated on 2 mm thick slices of melon. In the current study, a transmission geometry using a 6 mm thick slice was adopted. This protocol has the disadvantage that light will scatter in passage through
the slice, so the light seen by the detector as emerging from a given 6 mm square
region of sample may have travelled through a wider zone in transiting the slice (i.e.
the tissue volume ‘optically’ may not match that for which juice was extracted for
refractometer measurement).

Figure 6.8. Flow chart detailing the prediction of TSS across a 6 mm slice of melon
tissue, using a MLR model based on five filtered images (common with Fig. 4) that
had been sequentially reduced into 23x23 (529) pixel blocks. Final image data were
displayed using a colour contour graph generated in Sigma Plot.

For each of the five wavelength filters (830, 850, 870, 905 and 930 nm) images were
acquired of the validation melon sample and the reference sample.

Data sets were reduced by sequentially averag-
ing entire images into 23 x 23 pixel blocks (i.e.
average of 529 pixels). Spectral images were
converted to absorbance to account for lamp
inconsistency:

Abs = log(reference/sample)

TSS was predicted across the entire image
using the MLR equation:

TSS = 15.93 - 111.44 (A830) + 75.08 (A850) -
47.47 (A870) + 270.02 (A905) - 191.45 (A930)

Colour contour graph for % TSS, developed in
Sigma plot.
Figure 6.9. Photograph of a melon fruit sample detailing the location of the seeds, and the endocarp, mesocarp and exocarp tissue (A), and a colour contour map depicting predicted TSS across the surface of a 6 mm slice of melon tissue (B).

Previous work has also been based on the use of much more expensive imaging systems. We recommend further work considering the use of image averaging, rather than pixel block averaging, to reduce s/n. However, with an integration time of 10 ms, averaging of 500 images for each of 5 filters would impose a time penalty of approximately 7 min, and introduce the disadvantage of a temperature variation of the sample during the acquisition time in addition to any instrument fluctuations confounding the averaging effect. Therefore a combination of pixel block averaging
and spectral averaging may be appropriate. Alternatively, future work could be based on intermediate quality camera equipment, appropriate for 10 bit A/D.
Abstract
Agronomic or breeding programs aiming to improve the eating quality of melons rely on total soluble solids (TSS) assessment of large numbers of fruit. Rapid assessment is important to such programs. Several NIR instruments and optical designs were assessed in this respect. Best results were obtained by using a $0^\circ$ light-sample interactance configuration, in which the detector sleeve penetrated the fruit (for outer melon tissue: $0^\circ$ non-invasive $R^2 = 0.7$ RMSECV = 0.7 cf. $0^\circ$ invasive $R^2 = 0.8$ RMSECV = 0.5). This arrangement ensured that detected light travelled through more fruit mesocarp. Although invasive, the technique may be utilised as a more rapid replacement to traditional destructive refractometer TSS determination.
Introduction

The assessment of fruit total soluble solids (TSS) has traditionally involved destructive sampling, with measurement of the refractive index of juice extracted from a tissue sample as an index of tissue soluble sugar content. However, the implementation of large scale breeding or agronomic programs focused on the improvement of melon fruit TSS rely on the rapid assessment of this attribute. Ideally, an assessment methodology is required that allows repetitive assessment of the one piece of fruit during its growth in the field.

Near infrared (NIR) technology has been used as a replacement for the destructive method across a wide range of thin skinned fruit such as nectarine, apple and kiwifruit (Lammertyn et al., 1998; McGlone and Kawano, 1998; Costa et al., 1999). However, the performance of NIR technology with respect to the TSS of intact melon fruit is compromised by the variation of TSS within the fruit (Long et al., 2002) and by the presence of a ‘skin’ layer (e.g. chlorenchyma, lenticels). For an interactance optical geometry, (Greensill and Walsh, 2000) suggested that the detected light will have primarily originated within the upper layer (first 10 mm depth) of fruit, noting that light intensity exponentially decreased with increasing thickness of melon fruit tissue. Thus calibration models using spectra acquired via this geometry were superior in performance when based on the TSS of the outer 10 mm of mesocarp tissue (rather than on inner tissue) of melon fruit (Malibu 2001 outer tissue $R^2$ 0.9, inner tissue $R^2$ 0.4; Chapter 5). Thus this technology will more closely predict inner (edible) tissue TSS when there is a strong relationship between outer and inner tissue TSS.
Ito et al. (2001) employed a portable NIR instrument manufactured by Kubota Corporation to measure the TSS of melon fruit (in situ) at different stages of maturity during glasshouse cultivation. They utilised a 40 mm diameter reflectance probe to acquire spectra of the stylar end of fruit in a non-contact mode (acquisition time not reported). The stylar end was designated because of more uniform lenticel development in that region, and the presence of a thinner ‘skin’. Calibration results were reported to have an RMSEC of 0.4 and RMSEP of 0.8% TSS, although the calibration and validation populations were small (16 and 5, respectively) and population statistics (mean and SD) were not reported.

Other geometries may be more appropriate to the assessment of the TSS of a whole melon fruit. A full or partial transmission method may be more suitable, despite the optical thickness of a whole melon and the consequent need for a specialised system (increased light intensity, increased integration time or increased spectrometer aperture/detector size). For example Aoki et al. (1996) employed a partial transmittance arrangement using 16 lamps (specifications not reported) positioned in a circle around the equator of the fruit, at 90° relative to the collecting optic (642 – 1072 nm wavelength range). The system was reported to predict melon TSS with an SEP of 0.4% TSS, although the acquisition time totalled 10 sec. However, Walsh et al. (2000) were unable to demonstrate a significant advantage of a 45° (light source – fruit – detector) geometry over a 0° (interactance) geometry.

The presence of a ‘skin’ on the melon fruit is a major limitation to non-invasive assessment. Krivoshiev et al. (2000) devised an approach to ‘virtually peel’ potato, based on the simultaneous measurement of reflectance and transmission spectra. This
approach could be applied to melon, although the fruit is thicker than potato, necessitating longer integration times. However, the technique would be cumbersome to implement, particularly in a portable form.

In this chapter alternative geometries / sample presentation strategies are considered to improve the performance of the NIR technique for assessment of TSS of intact melon fruit, and that are potentially adaptable to in-field use (i.e. portable instrumentation). Such strategies are designed to replace the more time consuming traditional refractometry based method.

Materials and methods

Non-invasive sampling

Spectra were collected from whole fruit using the interactance arrangement described in chapter 5 (Fig. 5.3) (method 1). This optical arrangement consisted of a detector probe at 0° to, and partially occluding, the light source, such that the probe viewed a shaded area of the sample.

Spectra were also acquired using two portable instruments. The first unit was manufactured by NIR Technology Australia (Bankstown, Australia) (Fig. 7.1) (method 2). This instrument (model 128 FOP) employed an ‘interactance’ probe (15 mm diameter) consisting of randomly positioned illuminating and collecting fibre optics, a 12 V (20 W) halogen lamp, and a silicon photodiode array detector (900-1100 nm). The second unit (prototype, no model number), manufactured by Integrated Spectronics (Sydney, Australia), utilised a Zeiss MMS1 spectrometer and a
15 W halogen lamp operating in the 0° interactance geometry referred to earlier (method 3).

**Figure 7.1.** Interactance probe employed by the NIR Technology Australia portable instrument. Images show the randomly assigned light source and collecting fibre optics (A) and the spectral collection method (B).

Spectra were also acquired using a transmittance arrangement involving a 100 W halogen lamp and a single 500 µm fibre optic cable connected to a Zeiss MMS1 spectrometer (method 4). Spectra were collected at equatorial positions on fruit at 180° relative to the light source, with the fibre optic probe making contact with the fruit surface. An aluminium foil shroud was used to create a light tight seal between the fruit and light source.

**Invasive sampling**

An Ocean Optics R400 bifurcated fibre optic probe (Fig. 7.2A) was used in conjunction with an Ocean Optics 3100 K tungsten halogen portable light source and a Zeiss MMS1 photodiode array spectrometer. The probe was used to collect spectra following insertion into melon tissue via either two methods: (i) The ‘whole fruit’ method, which consisted of inserting the probe 10 mm deep into the flesh of whole fruit following the removal of the rind and mesocarp tissue (the total amount removed varied between 0 –30 mm, Fig. 7.2B) (method 5). (ii) The ‘core’ method, in which the probe was inserted 10 mm deep into 22 mm diameter cores of mesocarp tissue (already extracted and in sample tubes) with the outer 5-10 mm of rind and green tissue and seeds removed (Fig. 5.2D – chapter 5) (method 6).
**Figure 7.2.** Ocean Optics R400 fibre optic probe. Light source and collecting optics totalled 1.3 mm in diameter (A). The probe was inserted into cut melon tissue (B).

In a separate exercise, the $0^\circ$ interactance arrangement (Fig. 5.3) was modified to allow the collection of diffusely reflected light from deeper within the tissue (method 7). Three metal tubes (15 mm diameter; 10 mm, 20 mm and 30 mm in length) were fabricated such that they could be attached to the collecting probe (Fig. 7.3A). The 10 mm long tube was employed as a spacer between the probe lens and the fruit surface (control configuration). The 20 mm long tube was inserted into the surface of the fruit to 10 mm depth prior to spectral acquisition (‘10 mm invasive’ configuration). The 30 mm tube was inserted into the fruit to a depth of 20 mm (‘20 mm invasive’ configuration) before spectra were collected. In all three configurations, a distance of 10 mm was maintained between the surface of the fruit and the collecting lens.

**Figure 7.3.** Modified $0^\circ$ interactance arrangement designed to acquire spectra of melon mesocarp using a metal tube extension (A) that was inserted into the fruit (B). Dotted line represents insertion of tube to a depth of 20 mm.
Melon populations

Five experiments were conducted using separate populations of melon fruit.

1) Forty eight melons (cultivar, cv. Dubloon) were sourced from Kununurra WA. Spectra were acquired from two equatorial positions for each intact fruit using the 0° interactance arrangement and using the NIR Technology Australia portable instrument (methods 1 and 2). For all experiments the integration time was optimised by pre sampling fruit so that spectral raw counts were maximised while avoiding detector response saturation. The 0° arrangement (method 1) employed an integration time of 20 msec. The portable instrument (NIR Technology Australia – method 2) possessed a nonadjustable automated integration time (time unspecified by the manufacturer). TSS was determined for the outer 10 mm and subsequent inner 10 mm of mesocarp tissue, as per the method described in chapter 5.

2) Forty four melons (cv. Dubloon) were sourced from Kununurra WA. Spectra were acquired from the stylar, ground spot and two equatorial positions of intact fruit employing the 0° interactance arrangement (integration time: 25 msec) (method 1). Spectra of cores of tissue of the same positions were also acquired using the Ocean Optics probe (integration time: 350 msec) (method 6). TSS was determined for each whole mesocarp core via the method described in chapter 5 (n = 176, mean = 11.1% TSS, SD = 1.14).

3) Ten fruit (cv. unknown) were sourced from a commercial retail outlet. Spectra were acquired via the ‘whole fruit’ Ocean Optics probe (method 5) with an integration
time of 400 msec. TSS was determined for a cube of tissue (with 5 mm sides) within
the region of spectral acquisition (n = 50, mean = 8.4, SD = 1.67).

4) Ten melons (cv. Eastern Star) were sourced from Kabra QLD. Approximately five
spectra were collected from each fruit using the Ocean Optics probe (‘whole fruit’
method 5) with an integration time of 300 msec. Spectra were collected from
approximately five randomly selected locations on the fruit. For each location, a 22
mm core was sampled and trimmed (10 mm sides and 20 mm in length) and TSS was
determined of juice extracted from the outer (closest to the probe) 10 mm cube. Juice
from the subsequent inner 10 mm of tissue was then added to the juice from the outer
10 mm sample and TSS was remeasured (n = 56; outer tissue: mean = 7.1, SD = 1.02;
outer plus inner tissue: mean = 7.6, SD = 1.02% TSS).

5) Forty one sutured fruit (cv. unknown) were sourced from a commercial outlet. A
63 mm diameter template was marked at three positions per fruit, one near the stylar
end, at an equatorial position and for one position near the stem end. Spectra were
collected at the centre of each location template via the Integrated Spectronics
portable instrument (method 3), and via the modified interactance arrangement at each
of the three configurations (control, 10 mm invasive and 20 mm invasive; method 7).
Integration time for the portable instrument automatically readjusted for each sample
and thus is not documented. Integration time was manually optimised for each 0°
configuration (control 30 msec, 10 mm invasive 450 msec, 20 mm invasive 3 sec).
Spectra were also collected (one per equatorial position) via a 180° lamp – fruit
-detector (transmittance) arrangement, using an integration time of 4 sec (method 4).
Subsequently, a sharp knife was used to remove approximately 2 mm of rind from the
three 63 mm diameter regions per fruit, and spectra were re-collected via the 20 mm invasive configuration and via the transmittance arrangement. A 22 mm core of flesh was acquired from the centre of each location on fruit. Each core was cut in half longitudinally and TSS was determined for one half. The other half was divided into subsequent 10 mm sections starting from the outer surface and TSS was determined for each section. TSS was also determined for an equatorial slice for each melon. The slice was 63 mm wide (the width of the equatorially positioned ‘template’ previously described) and was homogeneously liquefied using a Panasonic MJ 66PR juice extractor, and TSS was determined for a representative aliquot of juice as previously described.

Results and discussion

0° configurations

The TSS of melon tissue is heterogeneously distributed which detrimentally affects the ability of NIR to measure TSS that is representative of the entire portion of edible tissue, a requirement for agronomic assessment. Calibration model performance was superior when developed on outer tissue compared to inner tissues (0° non-invasive configuration; outer $R^2$ 0.70, RMSECV 0.66; middle $R^2$ 0.42, RMSECV 0.93; inner $R^2$ 0.03, RMSECV 1.22) (method 7, Table 7.1). Calibration model performance was poor when based on the ‘whole core’ section of mesocarp, but reasonable when based on the average of outer and middle tissue (for non-invasive 0° configuration, $R^2$ 0.20, RMSECV 0.98, and $R^2$ 0.59, RMSECV 0.75, respectively) (Table 7.1). These results parallel the linear relationship between TSS of the outer and inner tissues (e.g. outer vs. middle $r^2$ 0.79, outer vs. inner $r^2$ 0.35, outer vs. whole core $r^2$ 0.44) (Table 7.2).
Lammertyn et al. (2000) reported that the meaningful penetration depth in apples was 4 mm (700-900 nm) when using a diffuse reflectance geometry (i.e. detector optic viewing an illuminated region). In the 0° interactance geometry employed in the current experiment, light is ‘forced’ to travel some distance between the illuminated and detected regions, with that distance set by the thickness of the probe wall and by the diameter of the detected area.

Transmittance measurements

Transmittance (180°) calibrations failed when using either the whole melon equatorial slice or the whole 22 mm core TSS sample (skin on: whole fruit $R^2$ 0.02, whole core $R^2$ 0.13; skin off: whole fruit $R^2$ 0.07, whole core $R^2$ 0.11) (method 4, Table 7.1). The 4.0 sec integration time was the maximum time offered by the acquisition software, such that the acquired count level was approximately 5% of detector saturation, thus resulting in a low s/n and poor calibration performance.

To index light penetration through a melon, raw A to D counts were recorded while changing the detector/ sample/ light angle. At 45° the count level was 10.0 % of saturation, while from 90° to 180° count level was stable at only 0.3 % of saturation (Fig. 7.4).
Table 7.1. NIR calibration statistics for PLS models (783-955 nm) developed on melon fruit spectra and fruit TSS. Spectra were collected using a Zeiss MMS1 spectrometer employing different optical configurations (non-invasive and invasive 0° interactance, bifurcated Ocean Optics probe, portable), and a portable instrument manufactured by NIR Technology Australia.

<table>
<thead>
<tr>
<th>Cultivar / Instrument</th>
<th>Optical configuration Method</th>
<th>Tissue sample</th>
<th>n</th>
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<th>RMSECV</th>
<th>SDR</th>
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<tr>
<td>Zeiss MMS1</td>
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<td>6 mm cube</td>
<td>50</td>
<td>8.4</td>
<td>1.67</td>
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<tr>
<td>Zeiss MMS1</td>
<td>Ocean Optics probe (invasive)</td>
<td>10 mm cube</td>
<td>56</td>
<td>7.1</td>
<td>1.02</td>
<td>0.46</td>
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<td></td>
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<td>20 mm cube</td>
<td>56</td>
<td>7.6</td>
<td>1.02</td>
<td>0.53</td>
<td>0.73</td>
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</tr>
<tr>
<td>sutured unknown cultivar (Zeiss MMS 1)</td>
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<td>Integrated Spectronics</td>
<td>Interactance 3</td>
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<td>123</td>
<td>6.2</td>
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<td>1.73</td>
<td>0.71</td>
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<tr>
<td></td>
<td></td>
<td>middle 10 mm</td>
<td>123</td>
<td>7.6</td>
<td>1.21</td>
<td>0.44</td>
<td>0.92</td>
<td>1.32</td>
<td>0.53</td>
<td>9</td>
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<tr>
<td></td>
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<td>average outer middle</td>
<td>123</td>
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<td>1.18</td>
<td>0.56</td>
<td>0.78</td>
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<td>0.63</td>
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<td></td>
<td>inner 10 mm</td>
<td>94</td>
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<td>1.03</td>
<td>0.11</td>
<td>5</td>
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<td></td>
<td></td>
<td>whole mesocarp</td>
<td>123</td>
<td>8.0</td>
<td>1.09</td>
<td>0.27</td>
<td>0.93</td>
<td>1.17</td>
<td>0.33</td>
<td>7</td>
</tr>
<tr>
<td><strong>Zeiss MMS1</strong></td>
<td>0° Interactance 7</td>
<td>outer 10 mm</td>
<td>0.70</td>
<td>0.66</td>
<td>1.83</td>
<td>0.73</td>
<td>7</td>
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<td></td>
<td></td>
<td>middle 10 mm</td>
<td>0.42</td>
<td>0.93</td>
<td>1.30</td>
<td>0.46</td>
<td>7</td>
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<td>average outer middle</td>
<td>0.59</td>
<td>0.75</td>
<td>1.57</td>
<td>0.63</td>
<td>7</td>
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<td></td>
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<td>inner 10 mm</td>
<td>0.03</td>
<td>1.22</td>
<td>0.98</td>
<td>-0.02</td>
<td>0</td>
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<td>whole mesocarp</td>
<td>0.20</td>
<td>0.98</td>
<td>1.11</td>
<td>0.25</td>
<td>6</td>
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<td><strong>Zeiss MMS1</strong></td>
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<td>outer 10 mm</td>
<td>0.82</td>
<td>0.51</td>
<td>2.37</td>
<td>0.86</td>
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<td></td>
<td></td>
<td>middle 10 mm</td>
<td>0.51</td>
<td>0.85</td>
<td>1.42</td>
<td>0.56</td>
<td>8</td>
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<td></td>
<td></td>
<td>average outer middle</td>
<td>0.71</td>
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<td>1.87</td>
<td>0.77</td>
<td>9</td>
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<td></td>
<td></td>
<td>inner 10 mm</td>
<td>0.05</td>
<td>1.19</td>
<td>1.00</td>
<td>0.05</td>
<td>2</td>
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<tr>
<td></td>
<td></td>
<td>whole mesocarp</td>
<td>0.20</td>
<td>0.99</td>
<td>1.10</td>
<td>0.27</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Zeiss MMS1</strong></td>
<td>0° Interactance (20 mm invasive)</td>
<td>outer 10 mm</td>
<td>0.45</td>
<td>0.89</td>
<td>1.36</td>
<td>0.51</td>
<td>6</td>
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<tr>
<td></td>
<td></td>
<td>middle 10 mm</td>
<td>0.31</td>
<td>1.01</td>
<td>1.20</td>
<td>0.34</td>
<td>5</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>average outer middle</td>
<td>0.42</td>
<td>0.89</td>
<td>1.33</td>
<td>0.46</td>
<td>5</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>inner 10 mm</td>
<td>0.08</td>
<td>1.15</td>
<td>1.04</td>
<td>0.10</td>
<td>2</td>
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<tr>
<td></td>
<td></td>
<td>whole mesocarp</td>
<td>0.19</td>
<td>0.98</td>
<td>1.11</td>
<td>0.21</td>
<td>2</td>
<td></td>
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<tr>
<td><strong>Zeiss MMS1</strong></td>
<td>0° Interactance (20 mm invasive skin off)</td>
<td>outer 10 mm</td>
<td>0.65</td>
<td>0.72</td>
<td>1.68</td>
<td>0.71</td>
<td>10</td>
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<tr>
<td></td>
<td></td>
<td>middle 10 mm</td>
<td>0.64</td>
<td>0.74</td>
<td>1.64</td>
<td>0.70</td>
<td>10</td>
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<tr>
<td></td>
<td></td>
<td>average outer middle</td>
<td>0.69</td>
<td>0.66</td>
<td>1.79</td>
<td>0.75</td>
<td>10</td>
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<td></td>
<td></td>
<td>inner 10 mm</td>
<td>0.36</td>
<td>1.01</td>
<td>1.19</td>
<td>0.51</td>
<td>10</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>whole mesocarp</td>
<td>0.31</td>
<td>0.93</td>
<td>1.17</td>
<td>0.41</td>
<td>10</td>
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<tr>
<td><strong>Transmittance</strong></td>
<td>(skin on) 4</td>
<td>Whole fruit</td>
<td>33</td>
<td>7.1</td>
<td>0.77</td>
<td>0.02</td>
<td>0.77</td>
<td>1.00</td>
<td>0.04</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>(skin on)</td>
<td>Whole mesocarp</td>
<td>33</td>
<td>8.1</td>
<td>1.00</td>
<td>0.13</td>
<td>1.05</td>
<td>0.95</td>
<td>-0.06</td>
<td>0</td>
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<tr>
<td><strong>Transmittance</strong></td>
<td>(skin off) 7</td>
<td>Whole fruit</td>
<td>41</td>
<td>7.1</td>
<td>0.73</td>
<td>0.07</td>
<td>0.74</td>
<td>0.99</td>
<td>-0.03</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(skin off)</td>
<td>Whole mesocarp</td>
<td>41</td>
<td>8.1</td>
<td>0.98</td>
<td>0.11</td>
<td>1.01</td>
<td>0.97</td>
<td>-0.04</td>
<td>0</td>
</tr>
</tbody>
</table>
Transmission has been successfully used to predict TSS in melons, using a combination of high illumination and long integration times (Aoki et al., 1996). This geometry is therefore conceded to be inappropriate for high speed sorting (Chen and Nattuvetty, 1980). The same limitations apply to applications for rapid bench top or field assessment. Even if a high illumination/long integration time system could be employed, this arrangement would essentially be destructive (with heat damage to fruit).

Table 7.2. Linear relationship ($r^2$) between melon fruit TSS sampled from three locations on 41 sutured melon fruit (cultivar unknown).

<table>
<thead>
<tr>
<th></th>
<th>Outer</th>
<th>Middle</th>
<th>Inner</th>
<th>Average out &amp; mid</th>
<th>Whole fruit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole core</td>
<td>0.44</td>
<td>0.67</td>
<td>0.77</td>
<td>0.58</td>
<td>0.57</td>
</tr>
<tr>
<td>Outer</td>
<td>-</td>
<td>0.79</td>
<td>0.35</td>
<td>0.94</td>
<td>0.51</td>
</tr>
<tr>
<td>Middle</td>
<td>-</td>
<td>0.78</td>
<td></td>
<td>0.95</td>
<td>0.74</td>
</tr>
<tr>
<td>Inner</td>
<td>-</td>
<td></td>
<td>0.60</td>
<td></td>
<td>0.71</td>
</tr>
<tr>
<td>Average out &amp; mid</td>
<td></td>
<td></td>
<td></td>
<td>0.71</td>
<td></td>
</tr>
</tbody>
</table>

Figure 7.4. Raw count data ($\log_{10}$) at 870 nm (●) and 905 nm (○) acquired from one melon fruit via a single collecting fibre optic and Zeiss MMS1 spectrometer. The fibre optic made contact with the fruit surface and measurements were made across the equatorial surface of the fruit at varying angles relative to the light source, from $45^\circ$ to $180^\circ$. Integration time was 4 seconds per acquisition.
Portable instrumentation

The Integrated Spectronics portable instrument yielded similar results to the bench top 0° configuration for the different tissue samples (e.g. outer: portable $R^2$ 0.66 vs. bench top $R^2$ 0.70) (Table 7.1). This result was not surprising as both units operate using a similar optical geometry and both employ a Zeiss MMS 1 spectrometer, which has been reported to feature a signal to standard deviation of approximately 5700:1 (at 735 nm) (Walsh et al., 2000).

The portable instrument manufactured by NIR Technology Australia performed poorly compared to the Zeiss based unit (on outer melon tissue: NIR Technology $R^2$ 0.26 RMSECV 1.00, bench top Zeiss unit $R^2$ 0.82 RMSECV 0.48) (methods 2 and 1 respectively, Table 7.1). The signal to standard deviation of the NIR Technology unit was assessed at only 320:1 (mean/ SD of 50 repeated readings of a white Teflon tile, data not shown), which is much lower than the recommended minimum of 5000:1 reported by Greensill and Walsh (2000) for the application of sugar level assessment. Further, the probe for the NIR Technology instrument was the same diameter (15 mm) as the reflectance arrangement (Fig. 7.1, 7.3A) but consisted of randomly allocated light source and collecting fibre optics, such that the system would effectively have operated in a diffuse reflectance mode. The 0° configuration arrangement involved a short path-length partial transmittance mode.

Invasive probe

The Ocean Optics bifurcated probe/ Zeiss MMS1 arrangement consisted of an interactance probe 6 mm in diameter, with the light source and collecting optic
totalling 1.3 mm in diameter. This unit was used to collect spectra of cut melon tissue, with calibration performance superior when based on the TSS derived from smaller pieces of tissue in comparison to tissue samples representing the whole edible mesocarp (e.g. 6 mm tissue cube $R^2$ 0.81 RMSECV 0.71, whole mesocarp core $R^2$ 0.24 RMSECV 1.00) (Table 7.1). This result is consistent with a shallow optical sampling volume for this optical configuration.

Extension of the detector probe into the fruit ensures that the detected light has travelled further through fruit tissue. Indeed, the 10 mm invasive $0^\circ$ interactance arrangement yielded better calibrations (for outer, middle and the average of these positions) than the non-invasive $0^\circ$ interactance arrangement (e.g. for outer: non-invasive $R^2$ 0.70 RMSECV 0.66 vs. invasive 10 mm $R^2$ 0.82 RMSECV 0.51) (method 7, Table 7.1). However, calibrations developed on inner tissue and whole core TSS were equally poor when using the 10 mm invasive probe (inner $R^2$ 0.03 RMSECV 1.19, whole core $R^2$ 0.2 RMSECV 0.99) (method 7, Table 7.1). Using the deeper 20 mm invasive arrangement, calibration performance was not improved and was worse than the non-invasive arrangement for all tissue samples (for outer tissue: 20 mm invasive $R^2$ 0.45 RMSECV 0.89, 10 mm $R^2$ 0.82, non-invasive $R^2$ 0.70). Integration time was increased to maintain signal level with the 20 mm invasive probe, so the decrease in performance may be attributable either to tissue damage or to optical sampling of an inappropriate volume of the fruit (e.g. seed cavity).
Removal of the melon skin improved the performance of the 20 mm invasive arrangement (e.g. outer tissue $R^2$ 0.65 RMSECV 0.72, middle tissue $R^2$ 0.64 RMSECV 0.74), but not to the level reported for thin skinned fruit (e.g. $R^2>0.9$ Walsh et al., 2004). This indicates that while the rind of the melon is an impediment to calibration performance for the 20 mm invasive arrangement, other issues contribute, such as heterogeneity of TSS within fruit (Long et al., 2002).
Melon eating quality in Australia is generally substandard, with the TSS of fruit flesh often being below the accepted minimum standard of 10%. This situation is finally becoming recognised throughout the supply chain, from farmer to retailer (the consumer was always aware of the problem!), and there appears to be a new will to change practices.

The work of this thesis was part of a national Horticulture Australia Limited (HAL) project: ‘Development of a Crop Management Programme to Improve the Sugar Content and Quality of Rockmelons’ number VX00019. While the thesis studies focused on two components of the project, source sink manipulation and irrigation scheduling, the larger project also included nutrition and cultivar selection studies. In this thesis, source sink in-field work was augmented by more in-depth glasshouse and additional field experimentation, while irrigation scheduling work was supported by soil moisture monitoring technology. The nutrition and cultivar trials included in the larger project provided useful demonstrative outcomes for primary producers, however these trials suffer limitations in experimental design. Future work on cultivar selection specific to particular growing regions, with development of separate irrigation and nutrition management guidelines for each cultivar holds the potential to greatly improve the standard of melon production in Australia. Further, the breeding of new cultivars that produce sweeter fruit, either by traditional methods or by using recombinant DNA technology, will also improve production. Future nutrition work
should focus on consideration of the timing of delivery of nutrients with respect to crop development.

Manipulating either the source or sink early in fruit development affected final fruit fresh weight in preference to sugar accumulation. When source or sink perturbations were implemented late in fruit development (typically after 21 DBH) an impact on fruit TSS in preference to weight was noted. Thinning late in fruit development had the greatest positive impact on fruit TSS, although yield was halved. Thus a cost benefit model showed that the price for ‘sweet fruit’ (greater than 10% TSS) would need to increase by 33% (assuming that 80% of the thinned population were sweet fruit). However, thinning may be impractical because of damage to plant foliage. Future work could examine the viability of robotic harvesting to implement thinning (additional to other field practices). There is also scope for studies on the impact of netting covers (reduced solar radiation), used for insect protection in organic production, on melon fruit TSS.

The current recommendation for irrigation scheduling is to allow soil moisture to decrease prior to harvest. This practice was shown to be to the detriment of fruit TSS, because a decline in soil moisture is thought to initiate a stress response and climacteric and thus fruit abscission prior to complete sugar accumulation. Some primary producers utilise this response to control early fruit abscission to meet market demands. Maintaining soil water close to field capacity prior to and during harvest prevents early abscission of fruit. Future work should consider the use of partial root zone drying and oxygenation of irrigation water in conjunction with subsurface drip irrigation (on heavier soils), with respect to fruit quality.
NIR sorting technology has been successfully used to grade thin skinned fruit based on quality attributes. For melons, NIR can be used to measure flesh TSS non-invasively, however the performance of the technology is limited by the heterogeneity of melon fruit flesh TSS, and the presence of the outer rind. TSS was shown to be higher at the stylar end compared to the stem end, and was higher for tissue sampled toward the centre of fruit. NIR calibration performance was more successful when based on the TSS of tissue sampled from the outer 10 mm than on inner tissue, although the linear relationship between TSS for discrete 10 mm tissue samples (from outer to inner) varied between cultivars and during the development period for fruit. Notably, the linear relationship between outer and inner tissue was high during early fruit development, weakened during later development (lowest relationship two weeks before harvest) and than strengthened at fruit maturity.

Thus NIR calibration performance based on inner tissue TSS was reflected by the strength of the linear relationship between outer and inner tissue TSS. Poor agronomic management (e.g. decreasing soil moisture) is likely to promote the abscission of fruit prior to complete sugar accumulation, and the level of sugar accumulation is likely to be related to the uniformity of the gradation in TSS from outer to inner mesocarp (i.e. fruit maturity).

Extending the acquisition barrel on the NIR instrument and inserting this ‘front end’ into fruit tissue enabled the acquisition of spectral information pertaining more to inner tissue and thus improved calibration results. Such an arrangement could be
engineered onto a portable NIR instrument and be used in-field as a more rapid, yet still destructive, method to replace refractometry sampling.

Two dimensional spectral imaging enabled the TSS of melon flesh to be visualised across the cut surface of fruit. The use of a low cost 8-bit CCD camera limited the accuracy to predict TSS in melon flesh, although an averaging technique was employed to increase signal to noise. A camera with a greater signal to noise ratio is required to efficiently measure TSS in cut melon fruit. Such an instrument should provide a useful tool for future studies of sugar accumulation in fruit, e.g. for attempts to increase fruit TSS through genetic manipulation of the regulation of enzymes such as sucrose phosphate synthase and acid invertase.
Appendix 1

Source-sink manipulation to increase stonefruit biomass and soluble sugar content.

Abstract

Tree thinning protocols were assessed for their effect on fruit fresh weight and sugar content (indexed as total soluble solids, %TSS) for peach, nectarine and plum grown in Ballandean, Queensland. Thinning to 1 fruit.cm\(^{-2}\) branch cross-sectional area increased fruit TSS by approximately 2%TSS for peach and nectarine, and by 0.5%TSS for plum. Thinning increased fruit fresh weight by an average of 10g for plum, 30g for nectarine and 40g for peach. Fruit harvested from the upper tree canopy (>3m altitude) were higher in TSS compared to lower (0-1.5m) canopy fruit (%TSS increase: 1.5% plum, 2% nectarine, 3% peach). For plum, fruit harvested from the east side of the ‘Tatura Y’ trellis were on average 10g greater in fresh weight compared to the west side. The trade off between reduced yield and improved fruit quality, and the influence of canopy architecture and timing of thinning is discussed. A novelty of the work was the use of a commercially available on-line sweetness and weight grader, used to assess the fresh weight and TSS content of every piece of fruit from each tree.
Introduction

Agronomic programs have often focussed on improving harvest index (i.e. biomass of fruit) rather than fruit eating quality (e.g. TSS). This tendency reflects the relative difficulty of assessing large numbers of fruit for quality attributes such as TSS. The advent of non-invasive methods (e.g. NIR spectroscopy) for assessment of fruit TSS, and the application of this technology within in-line grading units, changes this situation. This thesis was originally conceived around the notion of using NIR technology within large scale agronomic trials designed to produce sweeter rockmelons. However, the error of prediction (as discussed in Chapter 5) of the technique with respect to prediction of melon TSS prevented the use of this strategy.

NIRS calibration model performance is, however, better with thin-skinned fruit such as stonefruit and apples, in comparison to melons. Typical calibration statistics of $R^2 = 0.9$, $\text{RMSECV} = 0.30$ are reported for nectarines, in comparison to $R^2 = 0.75$, $\text{RMSECV} = 0.90$ for melon (Walsh et al., 2004). Therefore, as a parallel exercise to the melon research undertaken in this thesis, agronomic manipulation of nectarine and plum TSS was attempted, with assessment of all fruit using NIR based inline sorting technology.

In an earlier chapter (Chapter 3), the effect of fruit thinning on fruit weight and TSS content was reported for melon, a plant which sets only a few fruit per plant. This issue is also pertinent, but more complicated to address, for fruit trees, which bear many fruit per plant. A number of studies in the ‘old’ western agricultural literature may be found that address the issue of agronomic treatment, canopy position and fruit TSS, however such studies have been ‘out of vogue’ in recent decades, presumably
due to the cost of labour to implement such studies, and lack of market reward for improvement in fruit TSS, as opposed to fruit weight. For example, Reitz and Sites (1948) mapped the TSS of all (1,800) fruit from a ‘Valencia orange tree which was considered representative of the variety and bearing an average crop’ in relation to position within the canopy. A set of recommendations were given for ‘spot picking’ of the outer canopy to increase quality (TSS) of the harvested fruit. The advent of NIR technology for TSS assessment enables such studies to be easily implemented.

Manipulating the source to sink ratio has been shown to effect stonefruit yield and quality. Ballinger et al. (1963) in Lill et al. (1989) demonstrated that when heavy fruit pruning was implemented in peach, remaining fruit exhibited decreased fruit colour, an increase in resistance to internal browning, and had higher titratable acidity. George et al. (1990) reported that cincturing of peach branches, implemented after the stone hardening period (‘just prior to harvest’), had a desirable effect on fruit TSS (cinctured 10.6% TSS cf. control fruit 7.4%). George et al. (2001) also reported that for low-chill stone fruit in Australia, early fruit growth depleted starch reserves in the tree trunk, and that ‘early’ fruit thinning from trees had the potential to increase the available source reserves relative to remaining fruit, stimulating the production of larger fruit.

Chemical spray applications have been used to affect flowering, fruit load, fruit development time, and fruit quality. For example, Lill et al. (1989) demonstrated that ethephon spray resulted in reduced crop load, early maturity and enhanced colour, and that applications 3-4 weeks prior to harvest for Early Amber peach produced fruit with a higher TSS:acid ratio that were preferred by a sensory panel. Byers et al.
(2003) also reported that ethephon promoted flower and fruit thinning, with the drawbacks of unwanted leaf loss, and the loss of the ability to thin to a specific size class of fruit. George and Nissen (2003) showed that, in combination with a high N rate, applying the growth retardant paclobutrazol increased peach fruit TSS by approximately 1% TSS.

These previous studies have relied on the tedious and destructive traditional measure of fruit TSS using refractometric juice analysis. This has limited experimentation. For example, George et al. (1990) assessed TSS of only 20 fruit per thinning treatment. Given the variation in TSS possible per tree, such sampling rates may be problematic. In the current study commercial NIR sorting equipment was used to assess the TSS of every fruit from treated plum, nectarine and peach trees, to facilitate the examination of the distribution of fruit TSS within the tree canopy, and the effects of hand thinning regimes on fruit TSS.

**Methods and materials**

*Plant material and treatments*

Experiments were conducted on plum (cultivar, cv. Autumn Giant), nectarine (cv. Summerbrite) and peach (cv. O’Henry) plantings located at a commercial orchard in Ballandean, QLD (28° 48’ S, 151° 50’ E). These plantings consisted of mature trees (20, 9 and 10 years old for plum, nectarine and peach trees, respectively) at a density of 2470 trees/ha (plant spacing of 1 m and a row spacing of 5 m). The plantings were on a sandy loam granite soil. Irrigation was delivered by micro-sprinklers (one per tree) at the rate of 4 l.hour⁻¹, and delivered by the primary producer to meet the requirements of each crop. Fertiliser was delivered via fertigation throughout the crop.
season at a normal rate of: 203 kg N/ha, 7 kg P/ha, 99 kg K/ha, 85 kg Ca/ha and 8.2 kg S/ha.

Individual trees were the experimental units for thinning treatments. Thinning treatments were made based on the diameter of the branch (fruit.cm\(^{-2}\) branch cross sectional area). An initial count of fruit per branch was made, and the number of fruit required to be removed to achieve a given load calculated. Thinning was implemented in several passes over the branch (removing one fruit per branch) to ensure a uniform fruit load was achieved.

Thinning treatments were 1, 2, 3 and 5 fruit cm\(^{-2}\) for plum, and 1, 2, 2.5, 3, 4 and 5 fruit cm\(^{-2}\) for nectarine and peach. Treatments were replicated six times (24 plum trees and 36 peach and nectarine trees) as a completely randomised design. Plum trees were hand thinned on the 17 December 2001, 90 days before harvest (beginning 18 March 2002). Nectarine fruit were hand thinned on the 15 November 2002, 50 days before harvest (beginning 4 January 2003). Peach fruit were hand thinned between 12 to 14 November 2002, 57 days before harvest (beginning 8 January, 2003).

*Fruit harvest*

Fruit were harvested at maturity over four days for peach and two days for plum and nectarine. All fruit from trees were harvested: 7458 plums, 8028 nectarines and 9613 peaches. Harvested fruit were sorted by tree and altitude within the canopy (0-1.5m, 1.5-3m and >3m). For plum, fruit were also categorised into those harvested from the
East and West sides of the Tatura Y trellis system. Fruit were stored in paper bags within polypropylene boxes, and transported from the field to the pack-house.

**TSS and fresh weight assessment**

A commercial packline (Colour Vision Systems, Bacchus Marsh, Victoria) installed in the packhouse was used to assess all fruit. Bags of fruit were tipped onto the singulator unit and hand arranged to travel ‘blush side up’ on the conveyor. The fruit were conveyed at a speed of 0.5 m/s over a weight cell (with weight assessed to an accuracy of 1 g) and then under a NIR spectroscopic system (Colour Vision Systems ‘InSight’ system). The InSight system viewed the top half of the fruit (the blush side of the fruit in this case). Weight and TSS results were automatically saved to text files in this system.

For TSS assessment using NIR spectra, chemometric (partial least squares) calibration models were developed using UnScrambler v7.6 (Camo, Norway) software, based on the spectra and associated, destructive, refractometric assessment of juice TSS. The model developed for plum fruit was based on 600 fruit (mean 12.6% TSS, SD 1.7% TSS), and possessed a $R^2$ 0.92. Second derivative (25 nm gap) spectra were used, over the wavelength range 735 to 932 nm. In validation of an independent set of fruit (i.e. from a different harvest day to those in the calibration set), a $R^2$ of 0.89 was recorded (on a population SD of 2.1%TSS). A separate model was developed for peach and nectarine fruit (combined), based on 831 fruit (after removal of 3 outliers from the set, leaving a population with mean 11.0% TSS, SD 2.3% TSS), and again using second derivative (25 nm gap) spectra over the wavelength range 735 to 932 nm ($R^2$ 0.90, RMSECV 0.68). In validation of an independent set of fruit (i.e. from a
different harvest day to those in the calibration set), a $R^2$ of 0.88 was recorded (on a population SD of 1.9% TSS).

**Statistics**

The SAS 6.12 package (Cary, NC, USA) was used for ANOVA of data. For significant results (P<0.05) LSD$_{0.05}$ was employed for means separation. Mean and SE values are reported where the corresponding ANOVA models were not significant (P>0.05).

**Results and discussion**

**Harvest date**

For all populations, there was a significant difference (P<0.05) between harvest days for both fruit TSS and fresh weight (FW). For peach, average TSS trended higher with successive harvest days (day 1 11.4%, day 2 12.5%, day 3 12.9%, day 4 13.6%), whilst fruit fresh weight was higher in the first two days of harvest (day 1 116g, day 2 135g, day 3 106g, day 4 107g). For nectarine the first harvest yielded fruit higher in TSS and weight (day 1 TSS 12.2%, FW 79g; day 2 TSS 11.3%, FW 77g). Plum fruit TSS and weight was marginally different for the two harvest days (day 1 TSS 12.4%, FW 79g; day 2 TSS 12.2%, FW 77g).

**Canopy position influences**

Total soluble solids was consistently higher for fruit harvested from higher canopy altitudes (average %TSS increase for plum 1.5%, for nectarine 2% and for peach 3%) (Fig. 1, 3b, 4b). This result is consistent with greater availability of photo-assimilate at the higher canopy positions, because of higher irradiation levels. The lack of
response of fruit weight to altitude (for plum and nectarine) may reflect a change in canopy leaf area index during the crop development cycle, with more uniform lighting through the canopy earlier in the season. For peach, fruit harvested from higher in the canopy yielded greater fresh weight (Fig. 4c).

Plum fruit were propagated in a ‘Tatura Y’ trellis arrangement with distinct east and west orientations. There was a negligible effect of this orientation on fruit TSS, although fruit harvested from the east were higher in fresh weight (average increase of 5g) than those harvested from the west (Fig. 1).

**Thinning**

For plum, fruit thinning from 5 to 1 fruit.cm\(^{-2}\) of branch cross-sectional area resulted in a small but significant increase in fruit sugar content (0.5% TSS), but this effect was prominent in high altitude fruit only (Fig. 1). Thinning also yielded fruit greater in fresh weight (10g increase) (Fig. 1). For nectarine and peach, fruit thinning from 5 to 1 fruit.cm\(^{-2}\) yielded fruit with greater TSS and fresh weight (increasing by: 2%TSS and 30g FW for nectarine; 3%TSS and 40g FW for peach) (Fig. 3b,c; 4b,c). These results are attributed to the increased availability of photoassimilate made to fruit early in fruit development. Stone fruit, unlike melon, have an ability to utilise additional assimilate for both increased biomass and soluble sugar content; increased melon TSS was only augmented when fruit thinning was implemented late in fruit development (Chapter 3).
Agronomy – practice and adoption

Improving fruit TSS and size generally improves the value of the crop. Certainly, increasing TSS improves the eating quality of stonefruit, with a minimum standard of 11 or 12% TSS generally recognised (McGlasson, 2001; Kader, 2002). Other agronomic manipulations may also serve to improve fruit TSS. For example, water status/ scheduling is another tool (e.g. Chapter 4, melons). Indeed, for part of the season for the Autumn Giant plum crop, irrigation supply was limited due to severe drought conditions. The primary producer believed that the average fresh weight of plums (75g) was smaller than for plantings propagated with adequate water. The dry conditions are also likely to have affected fruit TSS. Canopy architecture may also be altered to improve light distribution; the Tatura ‘Y’ shape (Van den Ende et al., 1987) is likely to support development of higher fruit TSS.

While the positive effect of thinning on the attributes of TSS and weight is obvious, the loss of yield due to thinning is also pertinent. Thinning to 1 fruit.cm⁻² reduced yield by 23%, 60% and 37% for plum, nectarine and peach respectively (Fig. 2, 3a, 4a). The thinning operation will add to the cost of production in terms of the labour requirement of thinning, but there will be savings in reduced picking, packing and transport costs. However, overall the grower is likely to require an additional financial incentive to implement thinning.

Thus unless a supply chain can clearly differentiate the high TSS fruit to the consumer, allowing consumer demand to be measured against the requirement for higher prices, there is little chance that growers will focus on the product of superior eating quality (higher TSS) fruit. The case for management of fruit TSS would
change dramatically, however, if retail chains began to consistently enforce their own quality standards. For example, the Coles website (http://www.coles.com.au) provides product specifications for all fruit and vegetable commodities, listing the minimum TSS standards of 12%, 10% and 11% for plums, nectarines and peaches respectively (Appendix 2).
Figure 1. Second order factorial effects of fruit thinning, canopy altitude (0-1.5m ●; 1.5-3m ○; >3m ▼) and branch orientation (east ■; west □) on fruit TSS and fruit fresh weight, for cv. Autumn Giant plum grown in Ballandean, QLD in 2002. $P$ values for ANOVA models are <0.05, except for thin x orientation FW $P = 0.15$.

Figure 2. The effect of fruit thinning on yield (bar) and fruit number per plant (line ●) for cv. Autumn Giant plum grown in Ballandean, QLD in 2002 (data common to Fig. 1). ANOVA $P$ for yield 0.13, number 0.01.
Figure 3. The effect of fruit thinning on (a) yield (bar) and fruit number per plant (line ■); and the factorial combined effects of fruit thinning and canopy altitude (0-1.5m ●; 1.5-3m ○; >3m ▼) on (b) fruit TSS and (c) fruit fresh weight, for cv. Summerbrite nectarine in Ballandean, QLD in 2003. Note, P values for ANOVA models were <0.01.
Figure 4. The effect of fruit thinning on (a) yield (bar) and fruit number per plant (line ■); and the factorial combined effects of fruit thinning and canopy altitude (0-1.5m ●; 1.5-3m ○; >3m ▼) on (b) fruit TSS and (c) fruit fresh weight, for cv. O’Henry peach in Ballandean, QLD in 2003. Note, P values for ANOVA models were <0.05.
Appendix 2

Defining assessable eating quality standards for fruit

Abstract

The eating quality of a given fruit type can be correlated to a number of variables, including sugar content, acid content, dry matter content, juiciness, texture, firmness and volatiles content. The relative importance of the different attributes is different between fruit types, which can be effectively utilised as standards for eating quality. Achieving these standards has traditionally involved destructive testing of a relatively small proportion of the fruit harvested, and, as such, these standards have generally been poorly implemented. New non-invasive technologies, such as near infrared spectroscopy, which allow assessment of every item of fruit for certain attributes should allow the adoption of uniform quality standards. We review the quality standards that have been set by researchers, regulatory boards and retailers for each fruit type to deliver product of acceptable or high eating quality to the consumer, with a focus on physiological factors (such as sugar and acid content) rather than post-harvest disease issues.
Introduction

Fresh fruit can deliver a powerful sensory experience, and everyone can relate to the pleasure derived from a strawberry, peach, melon or other fruit of excellent eating quality. The eating experience of a piece of fruit reflects physical characters such as texture, and chemical characters such as sweetness, acidity, and volatiles composition. The setting of standards can be difficult, due to the complexity involved in defining some characters, and the interaction of characters, varieties and growing conditions.

The industry, however, currently grades fruit primarily on size, shape, colour and other external appearances (e.g. blemish). These attributes are a poor guide to the eating quality of the fruit, and consumers are often disappointed after purchase. The fruit industry is well aware of this issue. For example, Shewfelt and Bruckner (2000) reported consumer survey work in which the most important criteria of fruit and vegetable quality were (in order) freshness, low chemical residues, good taste, and firm crisp texture. Damage and price were rated as lower priorities, while size and uniformity of size were rated least important.

Harker et al. (2002) reinforced this point, reporting a UK survey in which 80% of consumers considered quality more important than price, and a study which showed that consumers are willing to pay higher prices for quality when they trust the product to deliver on taste. It is also generally reported that while consumers do not return product when eating quality is not delivered, repeat sales suffer. Harker et al. (2003) summarised a survey of Australian consumers following a bad apple eating experience, in which 58%
changed cultivars, 31% purchased fewer apples, 24% switched to other types of fruit, 17% stopped buying for a while, 10% changed to higher priced apples, and <1% changed to lower priced fruit.

Setting a quality standard to deliver eating quality in a consistent fashion requires the use of quantitative (but easily measurable) characters that can be correlated to eating quality. Curiously, internal eating quality is often not considered within discussions of ‘fruit quality’. For example, the text “Fruit and Vegetable Quality” (2000), edited by Shewfelt and Bruckner, details a range of concepts, from the breeding of cultivars to the economics of quality, but does not address the issue of measuring internal eating quality or the setting of minimum standards. Similarly the text, “Quality Factors of Fruits and Vegetables” (1989), edited by Jen, features a broad range of topics relating to the processing of fruit and vegetables, but offers little on quantitative levels for eating experience components, and does not report any minimum standards. Additionally, in the specific context of citrus, Fellers (1985) concedes that the words ‘flavour’ and ‘flavour quality’ often appear in the literature, without sensory ratings for quality or palatability existing.

Traditionally, fruit has been inspected visually for a range of characters. As fruit vary both between plants and within a plant, a non-destructive assessment procedure capable of checking every item of fruit with respect to the desired characters would be ideal. In-line electronic grading technology has been increasingly adopted through the latter part of the 20th century for sorting of fruit on the basis of external characters such as weight, size,
colour and external blemish. The relative lack of technologies for sorting of fruit on internal attributes has meant that, while a range of studies have been published on internal quality, few standards have been adopted and enforced within the industry.

Several technologies for inline grading of internal quality attributes of fruit are, however, now available. The legendary focus of the Japanese consumer on food quality, and willingness to pay a premium for such products, supported the adoption of ‘sweetness sorting’ near infrared (NIR) technology within Japan from the 1980s. In the last few years, however, a range of manufacturers have been offering this and other technology (e.g. fruit firmness testing) in a ‘pilot commercial’ fashion to western packing houses. Most large pack-houses are now at the stage where they do not wish to commit to the technology (with attendant cost and disruption of production system), but require an upgrade path should an appropriate market opportunity arise, or should retailers begin to enforce their standards (e.g. on fruit sweetness). Accordingly, the major manufacturers of fruit grading equipment must either offer such capabilities directly, or indirectly through compatibility with another supplier of the technology.

Grade standards can improve marketing efficiency by improving the understanding of the product to both sellers and buyers, but the use of grade standards is generally voluntary in fresh produce marketing, e.g. in the USA (Florkowski, 2000). This situation is likely to change as consumers become more discerning, and as retailers seek to work with a product supply chain that allows the supply of differentiated products (price/quality) to meet market demands. As these market forces come into play, and as the availability of
technology allows the adoption of standards relating to internal eating quality, the criteria collated in this review should find application in quality control manuals across the fresh fruit industry.

Food sensory experience

Before embarking on a review of fruit eating quality criteria, it is useful to briefly review the physiology of taste and smell, summarising Marieb (1995), Key (1999) and Bartoshuk (1999).

The sensory experience derived from eating fruit involves the trigeminal (feel), gustatory (taste), and olfactory (smell) sensory systems. Different branches of the trigeminal, or fifth cranial nerve, reach the tongue and floor of mouth, the hard and soft palates, and the cheek mucosa, respectively. Trigeminal nerves are also present in the olfactory neuroepithelium. The trigeminal system involves the sensation of heat and cold, texture, and shape (‘mouth-feel’), and also the detection of pungency and astringency. Sodium chloride and food acids can also produce irritation, detected by the trigeminal nerve.

For example, fat molecules produce tactile sensations in the mouth, mediated by the trigeminal neurons. Trigeminal nerves terminate under the epithelium, but can respond to membrane permeable chemicals. These nerves are therefore responsible for the detection of the pungency of an odorant, typically involving a slow onset but a persistence of sensation. Thus the description of food (e.g. mustard, horseradish, chilli, pepper, ginger,
carbon dioxide) as pungent, tangy, sharp, tingling, biting, or spicy typically refers to stimulation of the trigeminal nerve in nose or mouth.

The gustatory (taste) sensory cells are not neurons, as in the olfactory system, but rather are specialised epithelial cells innervated by primary sensory axons from the three peripheral nerves. The system involves approximately 2-5,000 taste buds (spherical clusters of cells, 70 mm height, 40 mm diameter) that are scattered across the tongue, with much smaller numbers present on the soft palate, inner cheek and epiglottis. On the tongue, taste buds are localised to fungiform (4 buds/papilla), vallate (250 buds/papilla) and foliate (600 buds/papilla) papillae (folds in the tongue epithelium). Filiform and fungiform (ca 200) papilla occur on the anterior two thirds of tongue (primarily tip and sides), vallate (ca 10) in the middle area, and foliate (2-9 ridges) on the back portion of the tongue. Each taste bud contains about 40 sensory epithelia cells.

Given this multiple innervation, complete loss of taste sensation is very rare. Responsiveness is lost in localised regions with age, but the ‘whole of mouth’ response is considered to be similar across age, except for bitterness. Perception may also change with certain physiological conditions (e.g. heightened during pregnancy). Comparisons across population groups (e.g. age, racial group) are, however, usually non-significant, due to the wide variation in number of buds between individuals. Individuals with a high density of taste buds are termed ‘super tasters’.
There are, however, only five functional types of taste sensory cells. These cells respond to ‘salt’ (inorganic metal ions, with sodium chloride eliciting the greatest response), ‘sweet’ (sugars, also saccharin, alcohols, some amino acids, some lead salts), ‘bitter’ (alkaloids; e.g. quinine, caffeine), ‘acid’ (H+ ions) or ‘umami’ (or ‘delicious taste’, recognised as a primary taste sensation elicited by monosodium glutamate). One type of taste cell usually dominates in a given taste bud, although a mix of different types is present. The taste cells have a life span of about 10 d, with basal cells acting as stem cells, but it is not known if the replacement cells are true to type. The response of the five sensory cell types is not simply linear. For example, the addition of citric acid to sucrose solution increases overall taste without increasing sourness, due to suppression of the sensation of sourness by sweetness (MacBride and Finlay, 1990).

The total soluble solids (TSS or the ‘Brix’ refractometric measurement) relative to acid (TSS:acid ratio) is commonly used as a measure of maturity and palatability for citrus fruit, although it has been reported that this index is a poorer measure of eating quality than previously thought (Fellars, 1991; Jordon et al., 2001). Certainly fruit may have the same TSS to acid ratio, but taste different because of differences in the actual amounts of either sugar or acid. Jordan et al. (2001) proposed an alternative in which the brix reading is modified to account for the sweetness reducing effects of acid. The index is called BrimA, and is based on the linear weighted difference between Brix and total acid rather than the ratio of the two.
The olfactory (smell) system has hundreds of distinct receptor proteins and function types of sensory cells (in contrast to the taste system, with only five receptor types). This diversity of sensory cells allows for greater distinction between foods. Thus a melon with a TSS of 12%, a titratable acidity of 3.4 meq / 100 mL and a firmness of 1.1 kg force does not ‘taste’ the same as a peach of similar TSS, acid and firmness.

The olfactory receptor system is linked directly to the central nervous system, in contrast to the indirect link for gustatory sensory receptor cells. As with taste sensory cells, olfactory sensory cells are directly exposed to the external environment. External changes result in cell death, and these cells are continually replaced. Olfactory neurons have a life of approximately 30 d. Nevertheless, the ability to detect odour decreases with age, particularly past the age of 50 years.

Determinants of Taste

The primary measurable attributes of a fruit that can be related to taste are texture, TSS, type of sugar present (fructose elicits a greater sweetness sensation than sucrose), and in certain fruit, acidity (sourness sensation). Volatiles and semi-volatile organic compounds can also impact the flavour and aroma of foods. However, analysis of volatiles can be a “daunting task, and obtaining useful information from such measurements can be even more challenging” (Marsili, 1997). In some fruit starch is accumulated during maturation, with conversion to sugar during ripening, and for these fruit, starch (or dry matter content, DM) at fruit maturity is a useful guide to fruit sugar content at ripeness,
and thus to potential eating quality. In other fruit, other parameters are of importance (e.g. oil content of avocado fruit).

The importance of these various parameters will vary by fruit commodity, but, to generalise, the TSS/DM content is arguably the most important criterion, with other characters developing in relation to this parameter. As easily measured attributes, TSS/DM is therefore the logical criterion on which to establish a quality control (QC) criterion.

Texture

The physical structure or texture of fruit is generally related to the maturity or ripeness of the fruit, and thus indirectly to chemical composition. Fruit texture can also be impacted by storage conditions. Texture can also vary within a maturity/ripeness level, and will affect the mouth feel of the product. For example, apples can be described as crisp or floury, stone fruit as stringy or non-stringy, and bananas as soft or dry and crisp. The texture can also affect the amount of juice released on a single bite, and thus the apparent taste of the fruit.

Harker et al. (1997) reported that the gross texture of fruit primarily depends on the size, cell wall thickness, strength, and turgor pressure of parenchyma cells. Collenchyma, phloem and xylem elements and epidermal cells were noted to be localised to specialised parts of the fruit such as the skin, seeds and vasculature, but these could influence fruit texture in some fruits. For example, some skins are simple, comprised of tightly packed
epidermal cells covered with wax, and can be eaten with the mesocarp (e.g. pome and stone fruit), enhancing the eating experience. In other fruit, the skin is inedible, being composed of thicker and or unpalatable structures such as trichomes and lenticels (e.g. melon and kiwi fruit). Vascular tissue can produce a fibrous characteristic (e.g. giving a ‘stringy’ sensation in stone fruit). Seeds are not usually consumed, although the seeds in some fruit (e.g. strawberry and kiwi) are eaten, contributing to the overall texture of the food.

Most fruit soften as they ripen, as a result of degradation and change in parenchymal cell walls. These cell wall changes cause a loss of cellular turgidity. This softening may enhance eating experience to a degree, with tissue becoming more juicy. Eventually fruit senescence will prevail, rendering fruit inedible and potentially a danger to eat. Most consumers would describe the texture of an over ripe or rotten fruit, excluding the reaction of other senses (smell and taste), in a negative manner.

Measuring fruit firmness or pressure is a quantitative measure which best defines the general soft or hard texture of the fruit. The penetrometer measures the resistance of the flesh to the penetration of a standard probe or plunger (Witherspoon and Jackson, 1995).

Who enforces Quality Standards?
Of course, growers-packers differ in their consistency in maintaining a quality standard. Individual discipline has, however, traditionally a means of achieving product reliability and recognition.
The advent of the central marketing system allowed imposition of a more formal (often government sponsored) regulatory body. For example Smith (1988) reported that early in the pineapple industry, Queensland government inspectors attempted to enforce a minimum flesh TSS standard of 12% for summer fruit and 10% for winter fruit, through random inspections of fruit in the Brisbane Central Market. Similarly, Greer (1990) detailed Queensland’s then current legal requirement for Lychee fruit, being a minimum TSS:acid ratio of 35:1. He reported that fruit could be destroyed if they did not meet the grade. However, current Queensland department of primary industries (QDPI) recommendations (Menzel et al., 2001) exclude internal quality grade standards, and only describe visual characteristics.

The emergence of ‘super’ retailers, undertaking direct purchasing, has worked to weaken the central market system and associated broad regulatory structures. Each of these retailers, however, has developed a quality control system. For fruit, this has extended to the setting of standards.

Quality Standards by Commodity

In the following section, for fresh fruit commodities common to the Australian market, the fundamental aspects of fruit anatomy and physiology as related to internal quality are described, followed by a review of the published criteria for assessing fruit eating quality (Table 1), criteria formulated by official/ government regulatory bodies (Table 2) and by retail chains (Table 3 and 4).
A typical product specification (e.g. for stonefruit) used in the retail trade covers a range of features. For example, a product specification for peach and nectarine might cover size, pack count per box, pressure (8 mm probe), sugar (% TSS), temperature, blemish level, colour tolerance, shrivelling, split stones, flesh colour, and foreign taints or odours. Of these 11 features, only two relate directly to internal eating quality (pressure and % TSS). For this review, only those standards and recommendations directly describing, or those closely correlated with, internal eating quality, are described.

For the official regulatory standards presented in this review (Table 2), recommendations were from readily available and well-known sources. The Australian United Fresh Fruit and Vegetable Association (AUF) published a national product description language (Story and Martin, 1996), which was created not as a product specification, but as ‘a language – a tool to enable the producer/ wholesaler/ retailer to standardise the method of description at all levels of distribution’. In this review, AUF standards are reproduced as published – categories and classification levels. The Food and Agricultural Organisation (FAO) of the United Nations (UN) and World Health Organisation (WHO) created the Codex Alimentarius Commission in 1963. The main purpose of the program was to protect the health of consumers and to promote fair trade practices by augmenting the coordination of all food standards. The United Nations Economic Commission for Europe (UNECE) acknowledges that ‘quality is the key to international food markets’ and have documented standards intended for application at the point of export/ dispatching control.
### Table 1. Eating quality standards for fresh fruit as published in the scientific literature.

Values are minimum recommended levels unless otherwise stated. Units for common standards are: total soluble solids (TSS) %, dry matter (DM) %, juice content %, firmness kgf (plunger diameter reported if available), titratable acidity (TA) % or units as published. Other standards and associated units are reported as published.

<table>
<thead>
<tr>
<th>Fruit</th>
<th>Climacteric</th>
<th>Attribute</th>
<th>Level</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avocado</td>
<td>+</td>
<td>DM (at harvest) oil</td>
<td>21</td>
<td>Whiley et al (2001)</td>
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<tr>
<td></td>
<td></td>
<td>8% FW</td>
<td></td>
<td>Seymour et al. (1993)</td>
</tr>
<tr>
<td>Banana</td>
<td>+</td>
<td>TSS</td>
<td>6.7-12.7 (unripe) 23.0-31.0 (ripe)</td>
<td>Choon and Choo (1972)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>fullness index variable per cv.</td>
<td></td>
<td>Samson (1989)</td>
</tr>
<tr>
<td>Citrus</td>
<td>-</td>
<td>TSS:acid limonin</td>
<td>8:1-10:1</td>
<td>Baldwin (1993)</td>
</tr>
<tr>
<td>(grapefruit)</td>
<td></td>
<td>TSS:acid</td>
<td>6:1</td>
<td>Kader (2002)</td>
</tr>
<tr>
<td>(lemon)</td>
<td></td>
<td>TSS:acid</td>
<td>8:1</td>
<td>Kader (2002)</td>
</tr>
<tr>
<td>(mandarin)</td>
<td></td>
<td>TSS:acid</td>
<td>8:1</td>
<td>Kader (2002)</td>
</tr>
<tr>
<td>(orange)</td>
<td></td>
<td>TSS:acid</td>
<td>8:1</td>
<td>Kader (2002)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>juice content</td>
<td>50% FW</td>
<td>Samson (1989)</td>
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<td>Custard Apple</td>
<td>+</td>
<td>TSS</td>
<td>-</td>
<td>Weaver (1976)</td>
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<td>Grape - table</td>
<td>-</td>
<td>TSS</td>
<td>-</td>
<td>Weaver (1976)</td>
</tr>
<tr>
<td>Kiwifruit</td>
<td>+</td>
<td>TSS (at harvest)</td>
<td>6.2</td>
<td>Given (1993)</td>
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<td></td>
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<td>Kader (2002)</td>
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<td></td>
<td></td>
<td>15</td>
<td></td>
<td>Mitchell et al. (1991)</td>
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<td></td>
<td></td>
<td>TSS (for long-term storage)</td>
<td>7-9</td>
<td>Sale (1985)</td>
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<td></td>
<td></td>
<td>firmness (8mm probe)</td>
<td>0.71</td>
<td>Cheah and Irving (1997)</td>
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<td>Lychee</td>
<td>-</td>
<td>TSS:acid</td>
<td>35:1</td>
<td>Greer (1990)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.4cmolH⁺/Kg</td>
<td></td>
<td>Batten (1989)</td>
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<tr>
<td>Mango</td>
<td>+</td>
<td>TSS</td>
<td>15</td>
<td>Yamashita (2000)</td>
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<td></td>
<td></td>
<td>DM (at harvest)</td>
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<td>Satyan and Chaplin (1986)</td>
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<td></td>
<td></td>
<td>specific gravity</td>
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<td>Level</td>
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<td>11.5</td>
<td>Sankat and Maharaj (2001)</td>
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<td>14</td>
<td>Smith (1988)</td>
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<td>12</td>
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<td>optimum 50-60%</td>
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<td>Jonathan 11; Delicious and Red Delicious 10</td>
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<td>12-14 (ripe)</td>
<td>Harker (2001)</td>
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<td>Truter and Hurndall (1988)</td>
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<td>pH</td>
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<td>highly liked at 0.6-1.5; optimum at sale 1.3-1.5</td>
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**Table 1. continued**
Table 1. continued

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<td>0.9-1.4</td>
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<td>internal breakdown / TSS amber jewel ≥17% (reduces internal breakdown)</td>
<td>11</td>
<td>Kader (2002)</td>
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<td>Ward and Melvin-Carter (2001)</td>
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<td>TSS</td>
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<td>moisture content</td>
<td>94-94.5% for good quality</td>
<td>Hobson and Davies (1971)</td>
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<td>1.0-1.5 (8mm probe)</td>
<td>Kader and Morris (1976)</td>
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Table 2. Eating quality standards and classifications for fresh fruit published by government and official regulatory bodies. Values are minimum recommended levels unless otherwise stated. Units for standards are: total soluble solids (TSS) %, dry matter (DM) %, juice content %, and firmness kgf (plunger diameter reported if available).

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<td>&lt;30; ≥30</td>
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<td>(lime) juice content</td>
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<td>42</td>
<td>-</td>
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<td>-</td>
<td>8:1</td>
<td>6.5:1</td>
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<td>33</td>
<td>-</td>
<td>28</td>
<td>-</td>
</tr>
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<td></td>
<td>(orange) TSS</td>
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<td>-</td>
<td>7-9; 10-11; ≥11</td>
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</tr>
<tr>
<td></td>
<td>TSS:acid</td>
<td>-</td>
<td>-</td>
<td>Navel &lt;8:1; other ≥8:1; &gt;10:1</td>
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<td></td>
<td>(seeded) TSS</td>
<td>13 (12 some cv.)</td>
<td>-</td>
<td>≤14; 15; ≥16; &gt;18</td>
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<td>14; 15; ≥16; &gt;18</td>
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<td>-</td>
<td>≤6; &gt;6; ≥6</td>
<td>6.5</td>
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<tr>
<td></td>
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<td>-</td>
<td>-</td>
<td>1.0; 1.5; 2.0; 2.5</td>
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<tr>
<td>Lychee</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>Mango</td>
<td>DM</td>
<td>-</td>
<td>-</td>
<td>&lt;14; &gt;14</td>
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Appendix 2. Fruit quality standards 168
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<td>Melon</td>
<td>TSS</td>
<td>10 Charentais; 8 other -</td>
<td></td>
<td>honeydew ≤10; 10-12; &gt;12</td>
<td>cantaloupe &gt;8.0, *≥9.0</td>
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<td>rockmelon ≤9; 9-12; &gt;12</td>
<td>honeydew 10</td>
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<td>12</td>
<td>&lt;10; &lt;12; &gt;12</td>
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<td>-</td>
<td>green ≤10; 11; 12; Jonathan 12 ≥13</td>
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<tr>
<td></td>
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<td>-</td>
<td>-</td>
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<td>-</td>
<td>green ≤5.5; 6.0; ≥6.5 Jonathan 8.6</td>
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<td>-</td>
<td>-</td>
<td>≥14-16 dep cv.</td>
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<td>Tomato</td>
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Table 3. Eating quality standards for fresh fruit set by three major Australian retail outlets and for two European retail outlets. Values are minimum required levels unless otherwise stated. Units for standards are: total soluble solids (TSS) %, dry matter (DM) %, juice content %, and firmness kgf (plunger diameter reported if available).

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<th>Australia iii.</th>
<th>Europe i.</th>
<th>Europe ii.</th>
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<td>Hass 22-26; Shepard 23</td>
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<td>firmness</td>
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<td>1.8-4.1; Hass 1.4-3.6</td>
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<td>10.0</td>
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<td>6.5</td>
<td>-</td>
<td>-</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Tomato</td>
<td>TSS</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>na</td>
<td>na</td>
</tr>
</tbody>
</table>

Appendix 2. Fruit quality standards 171
Table 4. Eating quality standards for Pome fruit set by three major Australian retail outlets. Values are minimum required levels unless otherwise stated. Units for standards are: total soluble solids (TSS) %, and firmness kgf. Retail Australia 2 specified standards in season and for controlled atmosphere storage (CA).

<table>
<thead>
<tr>
<th>TSS (%)</th>
<th>Firmness (kgf)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Australia i.</td>
</tr>
<tr>
<td></td>
<td>Coles/ Bi-Lo</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Apple</td>
<td></td>
</tr>
<tr>
<td>Abas</td>
<td>-</td>
</tr>
<tr>
<td>Akane</td>
<td>11.5</td>
</tr>
<tr>
<td>Bonza</td>
<td>12.6</td>
</tr>
<tr>
<td>Braeburn</td>
<td>14.0</td>
</tr>
<tr>
<td>Cameo</td>
<td>12.0</td>
</tr>
<tr>
<td>Cox Orange</td>
<td>-</td>
</tr>
<tr>
<td>Crofton</td>
<td>-</td>
</tr>
<tr>
<td>Firmgold</td>
<td>-</td>
</tr>
<tr>
<td>Fuji</td>
<td>13.0</td>
</tr>
<tr>
<td>Golden</td>
<td>12.5</td>
</tr>
<tr>
<td>Golden delicious</td>
<td>-</td>
</tr>
<tr>
<td>Granny Smith</td>
<td>-</td>
</tr>
<tr>
<td>Gravensten</td>
<td>-</td>
</tr>
<tr>
<td>Johnagold</td>
<td>13.6</td>
</tr>
<tr>
<td>Johnathan</td>
<td>12.6</td>
</tr>
<tr>
<td>Lady William</td>
<td>14.0</td>
</tr>
<tr>
<td>Matsu</td>
<td>-</td>
</tr>
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</table>
Table 4. continued

<table>
<thead>
<tr>
<th></th>
<th>TSS (%)</th>
<th></th>
<th>Firmness (kgf)</th>
</tr>
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<tr>
<td></td>
<td></td>
<td>Australia i.</td>
<td>Australia ii.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Coles/ Bi-Lo</td>
<td>Coles/ Bi-Lo</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(in season) (CA)</td>
<td>(in season) (CA)</td>
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<td></td>
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<td></td>
<td>Australia i.</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Coles/ Bi-Lo</td>
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<td></td>
<td></td>
<td>(in season) (CA)</td>
<td>(in season) (CA)</td>
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<tr>
<td></td>
<td>Apple</td>
<td></td>
<td></td>
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<tr>
<td>Pink Lady</td>
<td>14.0</td>
<td>13.5</td>
<td>14.0</td>
</tr>
<tr>
<td>Red Delicious</td>
<td>12.0</td>
<td>12.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Royal Gala</td>
<td>12.6</td>
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<td>12.0</td>
</tr>
<tr>
<td>Stark Blushing Gold</td>
<td>-</td>
<td>12.0</td>
<td>-</td>
</tr>
<tr>
<td>Sundowner</td>
<td>13.0</td>
<td>12.8</td>
<td>14.5</td>
</tr>
<tr>
<td>Toffee Apple</td>
<td>-</td>
<td>11.0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Pear</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buerre Bosc</td>
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<td>12.0</td>
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<tr>
<td>Packham</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Packham Ripe and Ready</td>
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<td>12.0</td>
<td>-</td>
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<tr>
<td>Red Sensation</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sensation</td>
<td>-</td>
<td>11.0</td>
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</tr>
<tr>
<td>Sirrera</td>
<td>-</td>
<td>12.0</td>
<td>-</td>
</tr>
<tr>
<td>Sophia Pride</td>
<td>-</td>
<td>11.0</td>
<td>12.0</td>
</tr>
<tr>
<td>William</td>
<td>11.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ya</td>
<td>9.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Other</td>
<td>-</td>
<td>11.0</td>
<td>12.0</td>
</tr>
</tbody>
</table>
Avocado

Avocado (Persea americana, family Lauraceae) fruit range from 75 to 300 g FW and are comprised of an exocarp with a smooth waxy cuticle and an edible fleshy mesocarp surrounding a large seed. The fleshy mesocarp is made up of parenchyma cells and specialised oil-containing cells called idioblasts (Jacob and Young, 1971). Avocado fruit readily accumulate lipids in the edible flesh, which may represent 50-75% of the dry weight and from 4-20% of the fresh weight of the fruit (Jacob and Young, 1971).

Avocado fruit do not ripen on the tree, and appropriately mature fruit need to be harvested so that they ripen with acceptable flavour and texture (Seymour and Tucker, 1993). Fruit are climacteric, and ripening is associated with a typical increase in respiration and ethylene production.

Seymour and Tucker (1993) reported that in California, a minimum oil content of 8%, based on the fresh weight of the edible mesocarp, was a standard implemented for all varieties, although dry weight or dry matter (because of ease of measurement and good correlation with oil content) was also reported to be a useful standard. Hoffman et al. (2000) warned that oil content and dry matter content, were not reliable late-maturity standards, because body rot (caused by Colletotrichum sp.) and the flesh disorders grey pulp and vascular browning, increased during the harvest period. Thus, internal disorders were accounted as the main determinants of latest acceptable harvest, rather than eating quality.
A minimum harvest maturity standard of 21% dry matter (DM) was recommended for marketing avocados in Australia (Newett et al., 2001). Newett et al. (2001) also noted that a more palatable flavour could be achieved if DM levels reached 3 to 5% more than the 21% level.

The UNECE suggested that avocados should reach a physiological stage to ensure a continuation of the ripening process, and set a minimum DM content of 21% for Hass and 19% for other varieties. The AUF product description language defined three maturity/ripeness rankings based on DM content, which were: below 21%, 21-23%, and above 23%, however no minimum standard was recommended. Kader (2002) reported that minimum standards for avocado in California ranged from 18.4-21.9% DM depending on cultivar.

The Coles/ Bi-Lo retail outlet (Australia 1) published a maturity standard for Hass avocado of 22-26% DM and 23% for the Shepard cultivar. Another large Australian retailer (Australia 2) set a general standard for avocados at 21-35% DM. The European retailers made no minimum recommendations for DM, although one (Europe 2) set a firmness standard of 1.4-3.6 kgf for Hass and 1.8-4.1 kgf for other cultivars.

Banana

Banana (Musa sp.) belongs to the family Musaceae. Fruit are berries, containing many ovules but no seeds, and develop via parthenocarpy (Samson, 1989). Banana fruit are climacteric fruit, exhibiting a respiratory peak and an increase in the production of
ethylene during ripening. Typically, the respiration rate rises from 20 mg CO₂/Kg/h in hard green fruit, to 125 mg CO₂/Kg/h at the peak of the climacteric. Fruit accumulate starch as the main carbohydrate store, which may form up to 20-25% of the fresh weight of unripe pulp (Marriott et al., 1981; Seymour, 1993). Apart from a loss of water, the most significant chemical change is the hydrolysis of starch to soluble sugars, with total sugars increasing from 1-2% in green fruit to 15-20% in ripe pulp, of which 66% is sucrose, 14% fructose and 20% glucose (Kotecha and Desai, 1995). The same authors reported for fully mature (not yet ripened) Dwarf Cavendish banana, a fruit firmness of 2.34 kg/cm², a titratable acidity of 3.89 mg/100 g (fresh weight of pulp), a total sugar content of 6.34% (pulp fresh weight), and a starch content of 7.58% (pulp fresh weight).

Tezenas and Montcel (1987) conceded that it is difficult to know when to harvest bananas, and suggested that growers measure maturity based on a subjective judgement of the ‘fullness’ of the fruit, which is a reflection of the angularity of the fingers, with rounder fingers being more mature (fuller) than angular fingers. They also stressed that bunch cutting time will vary due to the cultivar and also due to its intended use. Lindsay et al. (1998) similarly detailed a ‘finger fullness’ assessment method but did not detail specific methods of measurement or any grade standards. Samson (1989) also described a ‘fullness index’, which is the weight divided by the length of an internal fruit of the first or second hand, however it was reported that values would vary across cultivars, and possibly across growing localities; no recommended minimum index values were reported. Dadzie and Orchard (1997) reported that peel colour had been previously used to gauge fruit maturity, although they conceded that high temperatures and low relative
humidity could cause fruit to retain a green colour of the peel, whilst internally the fruit was mature.

Choon and Choo (1972) reported good correlations between taste panel scores for flavour and sweetness, and TSS and TSS:acid ratio. TSS:acid ranged between 20-180 and best explained flavour ratings (correlation coefficient 0.79), whilst a correlation coefficient of 0.93 strongly linked sweetness ratings and TSS (TSS ranged between 7-32). It was reported that TSS ranged from 6.7-12.7% for unripe fruit, and from 23.0-31.0% for ripe fruit. It was suggested that TSS and TSS:acid ratio are good predictors of ripe banana eating quality, however would not be appropriate to judge fruit maturity or harvest time because commercially produced fruit are picked green. Presumably starch content (DM content) could be used to index fruit maturity.

The AUF product description does not detail internal measures of eating quality, but rankings based on finger size. No minimum internal quality standards were reported by the UNECE, FAO codex, California/ USA, or any of the retail outlets considered.

*Citrus*

The *Citrus* genus belongs to the family Rutaceae. Three species make up the most popular citrus fruit: *C. sinensis* (sweet orange), *C. reticulata* (mandarin), and *C. limon* (lemon). The fruit are classified as berries (hesperidium), characterised by a juicy pulp made of vesicles within segments (Baldwin, 1993).
Citrus fruit cannot be picked immature for after-ripening, because the fruit contain little starch and are non-climacteric (Samson, 1989; Baldwin, 1993). Baldwin (1993) reported that colour was a poor indictor of quality, and suggested a TSS:acid ratio of 8-10 was generally accepted as a measure of minimum maturity. Samson (1989) reported that TSS, the percentage of ‘water free citric acid’, and juice content, were good measures of maturity. It was suggested that fruit should have a juiciness of about 50%.

Kimball (1991) extensively detailed, and emphasised the importance of, TSS, acid, and the TSS:acid ratio, as measures of sensory quality, although primarily in the context of citrus juice processing. He suggested, that in citrus products the sourness of the organic acids and the sweetness of the sugars compete for the same receptor sites on the tongue, with the actual amount of sugar or acid being less important to the taste of the product than the ratio of the two. Indeed, as detailed earlier, Jordan et al. (2001) reported that ratio had come into disfavour and proposed the BrimA scale as a replacement.

Davies (1986) reported that limonin causes bitterness in citrus fruit. Fruit containing 9 ppm limonin were regarded as bitter, while fruit with < 6 ppm were reported to be acceptable.

*Citrus - grape fruit*

Kader (2002) suggested a minimum TSS:acid content of 6:1, whilst the FAO/ WHO codex standard was a minimum 35% juice content. Both Coles/ Bi-Lo and Australia 2 suggested a minimum of 9% TSS, and both set minimum juice content standards (35 and
33%, respectively). Australia 2 additionally recommended a minimum 4.8:1 TSS:acid ratio.

_Citrus - lemon_

A minimum juice content of 25% was recommended by the UNECE. The AUF described two grades based on juice content: 1) 30% or more, and 2) less than 30%. California set a minimum of 30% juice content for all cultivars, and a US standard set a minimum juice content of 28-30% (depending on cultivar).

Coles/ Bi-Lo requires a minimum juice content of 30%, whilst Australia 2 set a minimum of only 10% juice content. Other retailers reported did not set any standards for lemon fruit.

_Citrus - lime_

Little published material was sourced detailing quality standards for lime fruit, although of the regulatory bodies reported, the FAO/ WHO codex recommended a minimum juice content of 42%.

Coles/ Bi-Lo recommended 20% juice content, while Australia 2 designated 10% juice as a minimum standard.

_Citrus - mandarin_

UNECE published one minimum requirement of 33% juice content for all cultivars. The AUF product description reported three minimum maturity grades: 1) 8% TSS, 2) TSS:acid ratio of 8:1, 3) 28% moisture content. For California, a minimum TSS:acid ratio of 6.5:1 was reported.

The Coles/ Bi-Lo chain published a minimum juice content for all cultivars of 33% and specific standards for cultivar Ellendales (8% TSS, 7:1 TSS:acid) and cultivars Honey Murcott and Imperial (10% TSS, 10:1 TSS:acid). Australia 2 reported the minimum TSS, TSS:acid and juice content standards of 9%, 8:1 and 33%, respectively, for all cultivars. Specifically for cultivar Clemantine, a TSS:acid of 7:1 was designated. For Australia 3, a single minimum TSS standard of 10% was set for all cultivars. No standards were either set or made available from the European retailers.

_Citrus - orange_

Consumer research reported by the NSW citrus industry (Anonymous, 2002) documents juiciness and sweetness as the most important eating quality attributes (50% of interviewees), followed by colour and then size. Consumer dissatisfaction was caused by fruit being dry and juiceless (28%), being sour or not sweet (19%), having little taste or flavour (14%), or that were too expensive (4%).

Samson (1989) recommended that orange fruit should have a TSS:acid ratio of between 10 and 16 to satisfy most consumers, and that fruit left on the tree for too long will over
ripen and become unpleasantly sweet (with a TSS:acid ratio of 20 or higher). A juiciness of about 50% was also recommended. Davies (1986) considered that a TSS:acid ratio ranging between 7.5:1 to 9:1 indicated maturity in navel oranges (varying by growing region). Davies (1986) also detailed fruit dryness or granulation, as a major internal disorder; larger, faster growing fruit were thought to be more susceptible, and the condition is related to low TSS and acid.

UNECE maturity requirements, specifically for the Israeli market, were a minimum juice content of 30% for Navel cultivar fruit and 35% for other cultivars and a minimum TSS:acid ratio of 5.5:1 for pigmented cultivars and 6.0:1 for other cultivars. A minimum juice content of 38% and TSS:acid ratio of 6.5:1 were general standards for European markets. FAO codex set no minimum standards. The AUF product description language detailed three TSS categories (7-9%, 10-11%, > 11% TSS) and three TSS:acid ratio categories (< 8:1 for Navel, > 8:1 for other cultivars, > 10:1 for all variety). One category recommended a minimum juice content of 30% for Navels and 33% for other varieties. For California, a minimum TSS:acid ratio of 8:1 was the minimum standard (Kader, 2002).

Coles/ Bi-Lo set minimum TSS and TSS:acid levels of 11% and 8:1 for Navel, and 7% and 7.5:1 for Valencia fruit. A minimum juice content of 33% was designated for all cultivars. Australia 2 reported a minimum TSS of 9% for all cultivars, TSS:acid ratios of 7:1 and 8:1 for Valencia and other cultivars, respectively, and a minimum juice content of 25% for Navelina and Sevile cultivars, and 33% for other cultivars. Australia 3 reported
a minimum of 10% TSS for all cultivars with no recommendations for either TSS:acid or moisture content. Neither of the European retail outlets reported any standards.

Custard Apple

Custard apple (Annona squamosa, family Annonaceae) fruit are compound structures, formed by the fusion of many single-seeded fruitlets with the receptacle. The hard seeds are surrounded by an opaque white edible mesocarp (Samson, 1989). The fruit are climacteric, experiencing a respiratory peak three to seven days after harvest (Brown et al., 1988; Pal and Kumar, 1995). During this period, fruit TSS level increases from 20.4% to 24.2% over 3 days (Brown et al., 1988). Fruit TSS is subsequently constant, but taste acceptability declines after 4 d from the first detectable softening. Forced ethylene ripening (24-36 h) can be used to produce fruit of excellent eating quality (Brown et al., 1988). However, harvest of immature fruit results in poor eating quality (Pal and Kumar 1995).

George et al. (1999) detailed external features used in harvest maturity assessment (change in skin colour from dark to light green, widening of the grooves between the carpels, carpels shape change, becoming more rounded, particularly at the base of the fruit). However, Pal and Kumar (1995) reported that maturity assessment using physical characters is generally unreliable. These authors also considered that there was no information available on maturity standards based on internal factors. Babu et al. (2002) stressed the economic importance of grading fruit by size, but internal quality grading was not mentioned. The AUF also do not document grade rankings for maturity based on
internal quality factors, using only descriptive terms relating to fruit firmness (firm, softening and soft). Neither of the other regulatory bodies and retail outlets sampled in this study gave any maturity or ripeness standards.

_Grapes (table)_

Varieties of _Vitis vinifera_ (family Ampelidaceae) contribute to over 90% of the world table grape production (Weaver, 1976). Grape berries consist of a thin exocarp, often with a visible waxy coat covering the surface, and an edible fleshy mesocarp (pulp), which surrounds 0-4 seeds (depending on variety). Table grapes need to be of good eating quality, of uniform size, and to have firm pulp and a tough skin to facilitate transport and distribution to the consumer. Grapes produce a very small amount of ethylene during development (up to 0.4 mL/L), and are therefore classified as non-climacteric (Kanellis and Roubelakis-Angelakis, 1993). Glucose and fructose are the dominant sugars stored in the fruit, with less than 0.1% of the fresh weight of ripe grapes being sucrose; tartaric acid is the predominating acid (Peynaud and Ribereau-Gayon, 1971).

Weaver (1976) considered that sugar and acid content were the best indices of palatability, and that the TSS:acid ratio was a better measurement than either attribute alone. The minimum TSS:acid ratios for Thompson seedless, Malaga and Ribier varieties was suggested to be 25:1, while that for Ohanez, Cornichon, Muscat and Emperor varieties was set at 30:1, and Tokay, Olivette Blanche and Red Malaga, 35:1. It
was also reported that Ribier, Red Malaga and Emperor should have a TSS of at least 16%, whilst most other varieties should have a minimum TSS of 17%.

The UNECE specified a minimum TSS level of 13% for seeded varieties and 14% for seedless. In addition to these standards “varieties must have satisfactory sugar/acid ratio levels”, although no recommended TSS:acid ratio values were published. FAO codex reported no standards, but the AUF product language, published five maturity rankings based on TSS for seeded grapes (14% or less, 15%, 16%, 18% and >18%) and six rankings for seedless grapes (14% TSS or less, 15%, 16%, 17%, 18% or more, and TSS:acid ratio of 18:1). Kader (2002) detailed a US standard of a minimum TSS of 14-17.5% (depending on cultivar) and a TSS:acid of ≥ 20:1, whilst California was reported to have TSS standards for specific cultivars (e.g. Muscat 17.5%, all other cultivars 16.5%).

Coles/Bi-Lo reported general and cultivar specific TSS grades (e.g. for Thompson 18%, Sweet White seedless 15.5%, Muscatel White 20%, other cultivars 16%). Additionally, it was specified that for fruit that met a minimum TSS:acid ratio but possessed low TSS, would still be accepted. Australia 2 detailed cultivar specific TSS and TSS:acid grades similar to Coles/Bi-Lo, however Australia 3 specified only a single general quality criterion of 15% TSS, and neither of the European retailers detailed any standards.
Kiwifruit

The kiwifruit (Actinidia sp.) is a berry, which develops from a superior multicarpellate ovary borne on a 3 to 4 cm pedicel (Given, 1993), and is considered to be climacteric. The fruit require approximately 20 weeks of on-vine maturation, with accumulation of starch in the fruit. Harvest occurs when fruit flesh TSS reaches a minimum level of 6.2% (Sale, 1985), which corresponds to the initial hydrolysis of stored starch to soluble sugars. Soluble sugars continue to be produced for another 20 weeks in fruit stored at 0°C, with starch content declining from 40% dry weight to approximately 2% and TSS increasing to 13 or 14% at full ripeness (Hopkirk et al., 1986 in Given, 1993).

Kader (2002) recommended a minimum of 14.0% TSS for mature fruit. For long term storage, Sale (1985) suggested that fruit should have TSS ranging between 7.0-9.0%, whilst Mitchell et al. (1991) suggested a higher minimum of 15%. However, Paterson et al. (1991) reported that ripe flavour correlated with “low” TSS, starch and sucrose, and that sweetness did not correlate with TSS. Sweetness and ripe flavour were reported to be correlated with low fruit firmness and high ester levels, while “tangy/acid (citrate) and unripe flavours” were correlated with firm fruit, low levels of esters and “high” TSS. Tangy/acid flavour also correlated with high citrate and unripe correlated with high sucrose.

Jordan et al. (2000) examined the possibility of using density measurements of unripe kiwifruit, to predict future eating quality parameters. Strong linear relationships were noted between density and dry matter ($r^2 = 0.83$), and with SSC measured after ripening...
It was proposed that kiwifruit at harvest might be sweetness graded by floating off low DM fruit in prepared salt solutions. NIR technology is also being used to non-destructively measure DM in kiwifruit (Jordan et al., 2000).

Harman (1981) in Given (1993) noted that firmness was not a good indicator of maturity, because it did not correlate with eating quality after storage, although Cheah and Irving (1997) suggested that fruit were the most suitable for eating when they soften to around 0.7 kgf.

The UNECE and Californian minimum standard was 6.2% TSS at harvest. The AUF national product description has two grades for unripe fruit based on TSS (< 6% TSS, > 6% TSS) and four grades based on firmness (1.0, 1.5, 2.0 and 2.5 kgf).

Coles/ Bi-Lo published a minimum requirement of 8% TSS and a firmness of between 0.8-1.0 kgf, assumed to be for unripe fruit at wholesale. Australia 2 specified a minimum of 6% TSS as a general standard and 10% for cultivar Kiwigold, and also recommended a minimum of 16% DM. Australia 3 designated (for ripe fruit) 10% TSS for Haywood and 12% for Gold. Europe 1 assigned minimum TSS requirements for ripe fruit of 13% for cultivars Baby and Gold and 11% TSS for Green cultivar, and also set an optimal firmness level between 0.45-2.26 kgf (8 mm probe) for Gold and Green fruit. Europe 2 recommended a minimum of 10% TSS (target 12%) for cultivars Hayward and Gold, and firmness ratings of between 0.9-2.3 and 0.5-1.8 kgf for each cultivar, respectively.
Lychee

The lychee (*Litchi chinensis*) (family Sapindaceae) is a subtropical evergreen producing fruit of about 40 mm in diameter with a rough tubercle covered red skin. The edible translucent flesh is an aril, surrounding a single seed (Gaur and Bajpai, 1978; Paull *et al*., 1984; Taylor, 1993). The aril can accumulate up to 24% TSS (Samson, 1989). Salunkhe and Kadam (1995) reported for a fruit exhibiting a TSS of 15.3%, 81.7% was reducing sugars and 18.3% was sucrose; fruit do not accumulate starch. Fruit do produce ethylene during ripening, but appear not to have a distinct climacteric peak, and are classified as non-climacteric (Seymour *et al*. 1993). Tree-ripening is advised to maximise post harvest quality (Menzel *et al*., 2001).

The relationship between TSS and eating quality has been reported to be highly variable (Underhill *et al*., 2001). Indeed Batten (1989) reported that TSS alone was not a suitable maturity indicator, and suggested either a titratable acidity (TA) level of 4.4 cmolH⁻¹kg⁻¹ or a TSS:acid ratio of 4.3 as maturity indices. Underhill and Wong (1989) reported a correlation between TSS:acid and eating quality of $R^2$ 0.75 and recommended a TSS:acid ratio of 30-40 (calculated to give EQ 5 to 6).

Greer (1990) detailed Queensland’s then current legal requirement of a minimum TSS:acid ratio of 35:1. Fruit with a ratio level lower than this “may be seized in the market and destroyed at the growers expense”. To ensure fruit were appropriately mature, it was suggested that fruit should be harvested when full in colour with no green patches, and sweet with no sourness to taste. Current DPI recommendations
(Menzel et al., 2001) exclude internal quality grade standards, and only describe visual characteristics, including that fruit should be mature and firm with good colour of either bright red or pink.

No quality standards were published by any of the regulatory agencies reported in this study, and although the scientific literature has consistently reported little correlation between TSS and internal eating quality, Australia retail 2 set a minimum TSS of 16%, while Europe 1 set a minimum standard of 14% TSS. None of the other retail outlets reported any internal quality standards.

*Mango*

*Mangifera indica* (family Anacardiaceae) produce a laterally compressed fleshy dupe fruit. Fruit grow on the tree for approximately 90 d, and during this time, starch accumulates (increasing from 1 to 13%) (Popenoe et al., 1957; Hulme, 1971). Fruit are climacteric and after picking starch is hydrolysed into sucrose, fructose and glucose within about eight days. Hulme (1971) reported that total acidity of the fruit peaked early in the fruit development and then fell until the fruit had tree-ripened. Fruit are typically harvested at the ‘mature green stage’. Grading of fruit is practiced based on weight and size, and fruit grading has been proposed on the basis of specific gravity, although no systematic effort has been implemented (Negi, 2000). Growers generally rely upon the ability of pickers to assess fruit maturity, although some larger producers sample orchard blocks for fruit dry matter at three, two and one week before picking (Kulkarni and Landon-Lane, 1990). In practice, many orchards fail to
sample fruit correctly and pick the crop too early, and thus pack a considerable amount of immature fruit (Kulkarni and Landon-Lane, 1990); the result of such practice is fruit entering markets lacking in quality uniformity (Medlicott, 1988).

Satyan et al. (1986) reported that cultivars of acceptable eating quality had TSS levels not less than 16%, although the cultivar Banganpalli, with a TSS of 19%, was rated lowest for overall acceptability. It was suggested that neither TSS or total sugar content (fructose + glucose + sucrose) were sensitive indices of eating quality \((r^2 = 0.27)\), and that total sugars combined with total acidity and pH was more closely related with acceptability \((r^2 0.51)\). Baker and Scholefield (1986) found no relationship between taste panel scores and TSS, acidity, and TSS:acid ratio, and reported that the lack of these relationships indicated the complexities of taste acceptance, and that the simple measures traditionally used to describe quality in the past may not be as appropriate as once thought. They suggested that other flavour compounds needed to be studied.

In Japan premium quality Irwin mangos are cultivated in green houses, with only fruit with a TSS greater than 15% harvested (Yamashita, 2000). Such mangos are ‘very expensive’, but satisfy urban consumers in place of the lower quality imports. Bally et al. (2000) reported that the Australian domestic market requires fruit with a minimum dry matter content of 14%, and with an internal colour corresponding to number 27 Hunter b scale or stage 3 of the mango picking guide. This picking guide for Kensington Pride mangos (Holmes et al., 1990) consists of three colour plates.
with a corresponding physical description for each photo (white flesh with fruit starting to fill out around the beak; flesh colour mostly white with some yellowing near the skin and filling out around the beak; flesh colour almost yellow with the fruit filled out around the beak – ready to pick). Mitra and Baldwin (2001) generalised that fruit intended for local markets or shipments by air (three day market window) are harvested after the colour break, whilst fruit destined for longer storage (8-10 days) should be harvested firm and green, but physiologically mature. Samson (1989) suggested three objective picking standards: 1) TSS of at least 12%, 2) Specific gravity of 1.01-1.02, and 3) The ability to withstand 1.75-2 Kg/cm² pressure.

Two AUF rankings were based on dry matter: 1) less than 14% DM, and 2) More than 14% DM. Of the retail standards reported, Coles/ Bi-Lo recommended a minimum TSS of 14% specifically for Kensington Pride, Australia 2 gave a minimum DM of 14% for any cultivar, and Europe 1 specified a minimum of 10% TSS for all cultivars.

**Melon**

Rockmelon and honeydew fruit (*Cucumis melo* L) (family Cucurbitaceae) are berries and accumulate glucose and fructose early in development but sucrose during the final stages of development; fruit do not accumulate starch. Melon are climacteric and have a distinct ethylene dependent respiration phase (Seymour and McGlasson, 1993). Netted rockmelons or cantaloupe fruit (reticulatus group) ‘slip’ (abscise) from...
the vine when mature, but honeydew fruit (inodorus group) do not and are cut from
the vine during harvest (Lester and Shellie, 1992).

Mutton *et al.* (1981) concluded that, of 12 factors considered, tissue TSS and
penetrometer estimates of flesh firmness were the most useful predictors of melon
eating quality. A minimum of 10% TSS and 1-2 kg force flesh firmness were
recommended. Yamaguchi *et al.* (1977) reported that eating quality was highly
correlated to sweetness, with aroma and texture the second and third most important
characters, respectively. Yamaguchi *et al.* (1977) noted that TSS was not a measure
of quality in all instances, with immature fruit bearing high TSS being less palatable.
Lovatt *et al.* (1997) defined fruit maturity in terms of net development, the
development of a dark yellow colour in the skin between the netting, and a tissue TSS
of at least 8%.

Ozer *et al.* (1995) developed a neural network fusion classification of rockmelon
harvest maturity based on colour, fresh weight, fruit diameter and flesh firmness, with
prediction of 6 stages of fruit ripeness as determined by human graders. However,
although the study purported to consider fruit quality, quantitative measures of
internal sweetness were neglected as one of the model reference measurements.
Lester and Shellie (1992) correlated the whiteness of epidermal tissue of honeydew
melons with overall fruit preference (r = 0.52) in an attempt to define pre-harvest
indicators for honeydew maturity.
UNECE recommend a minimum flesh TSS of 10% for Charentais melons, and a minimum of 8% for other melons. The AUF gave three TSS based maturity rankings for honeydew melons (up to 10%, 10-12%, >12%) and seven for rockmelon (full slip, three quarter slip, half slip, ripped buttons, up to 9% TSS, 9-12% TSS and >12% TSS). The California state standard published a minimum of 8% TSS for cantaloupe and 10% TSS for honeydew, whilst a US standard specified 9% TSS for cantaloupe.

Australia 1 (Coles/ Bi-Lo) gave a general minimum of 10% for melons, but specified a minimum of 12% for rockmelon and honeydew. Australia 2 published 10% TSS minimum for all melon fruit.

_Papaya_

Fruit of the papaya or pawpaw (_Carica papaya_ L.) (family Caricaceae) are berries, and are climacteric, displaying increases in respiration and ethylene production during ripening (Paull, 1993). The climacteric peak occurs between 120 and 150 days after anthesis (depending on cultivar) and coincides with a change in external colour from green to yellow. The principle carbohydrates are glucose, fructose, and sucrose, with sucrose dominating during the latter part of fruit development. Some starch is accumulated in papaya, but makes little contribution to the quality of harvested fruit. Selvaraj and Pal (1982) in Sankat and Maharaj (2001) reported that ‘Thailand’ papaya exhibited a decrease in starch content from 0.11 to 0.09% between 140 and 160 days after anthesis. Citric and malic acid contribute to between 80-90% of the total acids, with levels reaching a minimum at the ripe stage and increase slightly with further
Appendix 2. Fruit quality standards 193

ripening (Paull, 1993). Numerous volatiles contribute to the aroma and flavour of papaya. Methyl butanoate is responsible for the ‘sweetish’ odour emitted by the fruit.

For local consumption, fruit are picked when the green colour changes into yellow halfway up the fruit, while for the export market fruit are picked sooner (blossom end changes colour) (Samson 1989). Sankat and Maharaj (2001) conceded that there were inadequate measures of optimum harvest maturity. Softness to touch, a change of latex colour from white to watery, and the amount of colouring in fruit has been reported as indices of harvest maturity (Ross et al., 2000).

Sankat and Maharaj (2001) detailed Hawaii’s minimum standard of 11.5% TSS with at least 6% surface colouration at the blossom end region. None of the government or international organizations detailed internal quality standards, although the AUF product description subjectively described fruit firmness levels (firm, soft, softening and over-ripe) as ranks of maturity. Coles/Bi-Lo and Australia 2 set a minimum TSS level of 10%, whilst Europe 1 designated 12% TSS and Europe 2 designated 9%.

Pineapple

The pineapple (*Ananas comosus*) (family Liliopsidae) is an herbaceous perennial producing a single multiple fruit as the product of the terminal inflorescence. Upon maturation of the first fruit, the plant will develop new shoots from axillary buds, and further produce another fruit (Coppens d’Eeckenbrugge and Leal, 2003). Primary sugars in pineapple are sucrose, glucose and fructose (70, 20 and 10% of total sugars,
respectively) (Guthrie and Walsh, 1997). Fruit do not accumulate starch (Paull, 2001).

The linear relationship of TSS to pineapple eating quality ($R^2 = 0.7$) was reported to be a reliable index of fruit quality for cultivar Smooth Cayenne, better than specific gravity, titratable acidity, TSS:acid or juice pH, and recommended a minimum of 14% TSS for fresh fruit (Smith, 1988). Paull and Chen (2003) noted that TSS determined by refractive index should be adjusted for acidity to estimate sugar percentage, and the formula \([\text{sugar} (%) = \text{TSS} - 0.192 (\text{mL of 1M NaOH to neutralise 100 mL of juice})]\) was proposed to suit all except very translucent or overripe fruit. A ‘rule-of-thumb’ for obtaining actual sugar content (multiplying the refractive index value by 0.85) was also proposed. The same authors reported that in Hawaii a minimum TSS of 12% was required for fresh fruit, and noted that older literature recommended a sugar:acid ratio of 20:40; Soler (1992) recommended a ratio of 14:20.

Smith (1984) correlated smooth cayenne fruit specific gravity with taste panel preference ratings and reported that fruit near maximal eating quality could be differentiated by choosing fruit with a specific gravity of 0.960-1.004.

Skin colour was previously used in Australia by growers and consumers as a non-destructive index of maturity and eating quality, but the attribute was perceived as a poor index, exemplified by reports that summer fruit were very palatable even when...
the flesh was hard, white and opaque and the skin dark blue-green (particularly summer fruit from Yeppoon 23° S) (Smith, 1988).

The UNECE and FAO/WHO codex proposed a maturity requirement of 12% TSS for juice extracted from tissue representing the whole fruit. Subjective standards based on appearance, size, and disease were also documented. AUF rankings for maturity gave three classifications for fruit TSS level (< 10%, < 12%, > 12%).

For the retail chains, Coles/ Bi-Lo set a minimum standard of 12% TSS, while Australia 3 set 10% and the European retailer 1 gave a minimum of 13% TSS with a target value of 16%.

_Pome Fruit_

Pome fruit include the apple and pear as members of the family Rosaceae. Pome fruit consist of the fused base of the calyx, corolla and stamens. Fruit will mature on the tree for a period between 105-140 days (depending on cultivar) (Hulme and Rhodes, 1971). As fruit grow, both reducing sugars and sucrose increase, along with a rise and peak of starch content close to fruit harvest. Malate accumulates during the early stages of fruit growth, and then slowly declines in the later stages. Fruit are climacteric, eventually expressing a distinct peak in respiration and ethylene production. Fruit can ripen on the tree, although pears may become “mealy” (Hulme and Rhodes, 1971). After harvest, remaining starch will be converted into sugar.
Kingston (1992) reviewed maturity indices for apples and pears, reporting that no single test could describe fruit eating quality. Firmness, TSS, and TA, were suggested to be good indicators of internal quality when fruit are to be consumed immediately. Additionally, it was suggested that fruit produced for prolonged storage, should have a higher starch content and be producing lesser amounts of ethylene compared to fruit destined for immediate consumption.

_Pome fruit - apple_

Yahia (1994) suggested that sweetness and acidity are dominant taste attributes of dessert apples, and that apple taste was related to the balance between sugars and acids. Sweetness in apples is caused by sucrose, glucose and fructose, with 50% of the sugar being fructose. Malic acid is responsible for the sour and acid taste (Yahia, 1994). Goodenough and Atkin (1981) recommended that high quality dessert apples should have a pH ranging 3.2-3.5 and high TSS (14-16%), good cooking apples should have high acidity (pH 2.8-3.2) and moderate sugar content (11-13%), and Delicious and Spartan cultivars ought to have low acid (pH 3.5-3.7) and low sugar (9-11%).

Harker _et al._ (2002) reported that apple crispness and juiciness are key attributes that determine consumer preferences, and that penetrometer measurements are good predictors of such textural perceptions. In a corresponding paper (Harker, 2001) TSS, total acidity, and starch pattern index, were described as apple maturity indices. He also reported that sensory studies had shown a close relationship between TA and
acid taste in apples, although the relationship between TA and consumer acceptability was cultivar specific and thus there was insufficient information to make recommendations. He also believed that although TSS has been known to be a good sweetness indicator for juices and other fruits, it was not so for apple; sensory research has shown that the relationship between sweetness and TSS is poorer than the relationships between texture and puncture force, or “acid taste” and TA, possibly due to the presence or absence of flavour volatiles that enhance the perception of sweetness. TSS can still be a valid measure of quality, because fruit with high TSS probably accumulated high levels of starch and other nutrients, and will be a better fruit in terms of having a better physiological ability to produce volatiles, additional to having high levels of sugars and acids. The minimum suggested TSS for apples ranged between 12-14%.

Fruit TSS, TA and firmness of flesh are important internal quality factors for apple (Chen and De Baerdemaeker, 1993; Lammertyn et al., 1998; Steinmetz et al., 1999). The Australian Horticultural Corporation (AHC) (1993) listed firmness, TSS, and starch, as the three criteria for measuring apple maturity. An 11 mm plunger was suggested for penetrometer readings, and apples with a flesh firmness less than 5.5 kg at the point of sale, was deemed unacceptable. A firmness no less than 6.5 kg was recommended for apples for long term storage. For fruit at the point of sale, minimum TSS levels were recommended for Jonathan (11%), and Delicious and Red Delicious (10%). For long term controlled atmosphere storage, TSS values were: Delicious >10%, Bonza >13%, Golden Delicious >12%, Gala >12.5%, Granny Smith
>12%, Fuji >13%, Pink Lady >15%, Sundowner >14.5%, and Lady Williams >14.5%. Starch testing provides a good guide for determining maturity. Starch hydrolysis into sugar initialises at the core and progresses out toward the skin. The iodine starch test facilitates a blue/ black visual on apple tissue containing starch, with clear tissue indicating no starch. AHC (1993) produced six starch distribution reference photographs: 1) Immature: all blue, 2) Harvest for controlled atmosphere (CA) storage: clear core, 3) Harvest for storage or fresh market: more core/ tissue clear, 4) harvest for fresh market: 50% clear, and 5) and 6) being between 90-100% clear: past harvest stage.

Middleton and McWaters (2001) in their final report detailing results of trials designed to increase the yield and quality of apples throughout Australia, used fruit firmness (penetrometer with 11mm probe), TSS, and the iodine starch test, as indicators of fruit maturity and quality.

Harker (2002) described mealiness as a textual disorder occurring when individual cells in the fruit flesh separate from each other, rather than breaking open and releasing their contents. Flesh has a floury juiceless feel, often with little taste. It was suggested that apples with a firmness level <5.0 kgf (11 mm probe), are more susceptible to mealiness, and should be checked. The disorder is very rare in fruit with a firmness value greater than 7 kgf.
Appendix 2. Fruit quality standards

No standards were described by the UNECE or FAO/WHO codex. The AUF defined a number of maturity rankings which use TSS and firmness as criteria. For green and gold apples, four rankings were based on TSS: 10% or less, 11%, 12% and 13% or more. Three firmness grades were: 5.5 kgf or less, 6.0 kgf and 6.5 kgf or greater. For red cultivars, TSS rankings were: < 10%, 10%, 11% and ≥12%, and firmness rankings were: ≤ 5.5 kgf, 6 kgf, ≥6.5 kgf. California detailed minimum standards for Jonathan (TSS 12%, firmness 8.6 kgf) and Red Delicious (TSS 11%, firmness 8.2 kgf).

The three Australian retail stores gave extensive internal quality standards for over 20 cultivars of apples, which was by far the most comprehensively differentiated fresh fruit group appraised in this study (Table 4). No standards were known to exist for the two European retail chains.

*Pome fruit - pear*

Zebrini (2002) summarised that pears with good eating quality attributes, exhibit an appropriate tissue texture with a balanced sweet and sour taste. The effects of storage time on fruit quality parameters, and other quality issues, such as juiciness and volatiles, were also discussed, although no minimum standards were recommended.

Harker *et al.* (2002) reported that consumers feel inconvenienced when pears are bought too hard, because consumers tend to forget to eat the fruit at an optimum firmness, and allow them to over-soften. It was concluded that the firmest fruit (4.5-
5.5 kgf) weren’t readily accepted by consumers, whilst softer fruit (0.6-1.5 kgf) at the point of sale were more desirable, with optimum firmness reported at 1.3-1.5 kgf. Kappel et al. (1995) in Harker et al. (2002) described TSS, TA, and TSS:acid that pertained to fruit of optimal eating quality. The study was done over three years, and the results were: 1991 TSS 14.8%, TA (mg malic acid/100 mL juice) 270, TSS:acid 2.87; 1992 TSS 17.2%, TA 93.3, TSS:acid 3.31; 1993 TSS 13.6%, TA 200, TSS:acid 2.85. Harker (2002) suggested that fruit with a TSS level of 12% should gain good preference scores if eaten at optimal firmness. However, he also reported that there was no data suggesting minimum requirements to gain consumer acceptance, in terms of TSS, TA, or TSS:acid ratio.

Neither the UNECE, FAO codex or AUF reported any internal attribute-based standards for pear fruit, although a California standard reported minimum TSS of 13% and minimum firmness of 10.4 kgf. Like apples, the three Australian retail chains surveyed in this study reported extensive minimum TSS and firmness standards for different cultivars, with some inconsistencies evident (e.g. for TSS, cultivar Buerre Bosc: Coles 13%, Aus retail 2 11% and 12% for controlled atmosphere).

**Stonefruit**

Stone fruit are classified within the Rosacea family, occurring as species of the *Prunus* genus. Important stone fruit crops are: *P. persica* (peach and nectarine), *P. domestica* (plum), *P. armeniaca* (apricot), and *P. avium* (cherry). Fruit are drupes,
and are derived from a superior ovary (Romani and Jennings, 1971). Peaches, nectarines, plums and apricots are climacteric, exhibiting a rise in respiratory activity and a surge of ethylene production at ripening (Lill et al., 1989; Seymour et al., 1993). Cherries are not considered climacteric (Brady, 1993).

The predominant organic acid in peaches and nectarines is malic acid, with lower levels of citric, fumaric, succinic, and quinic acids (Lill et al., 1989). Romani and Jennings (1971) reported that citric acid was the dominant acid in apricots, whilst malic acid predominated in cherries and plums. Sucrose was reported to be the dominant sugar (compared to glucose and fructose) in peach, nectarine and plum fruit (Lill et al., 1989) accounting for at least 80% of total sugars (Kawano et al., 1989). Cherries also accumulate sucrose, while apricots were reported to be low in sucrose and high in fructosans (ketose oligosaccharides) (Brady, 1993).

Crisosto (1994) reported that for peach, nectarine and plum, flesh firmness was the best indicator of ripening. Fruit with a firmness rating of 2.7-3.6 kgf were “ready to buy”, whilst it was suggested that fruit were “ready to eat” when having a flesh firmness of 0.9-1.4 kgf. Visagie (1984) in Lill et al. (1989) suggested that flesh firmness in conjunction with background colour was a reliable indicator of the picking maturity for peaches and nectarines, and recommended a firmness of 5-7 kgf (11mm plunger). McGlasson (2001) recommended a minimum of 11% TSS for peach, nectarine and plum fruit produced in Australia, whilst Kader (2002) suggested
a minimum TSS of 10% for apricot and peach, 14-16% (depending on cultivar) for cherry, and 12% for plum fruit.

Internal breakdown is a physiological disorder of stone fruit resulting from the abnormal ripening and early senescence of the fruit, with symptoms usually occurring during cold storage or during ripening after cold storage. Ward and Melvin-Carter (2001) reported that symptoms in plums appear as internal browning and gel breakdown. They found that for Amber Jewel plums, incidents of internal breakdown were minimised if the fruit were packed and appropriately cooled on the day of harvest. Additionally, fruit with TSS ≥ 17% had a significantly reduced risk of developing internal breakdown symptoms. Lill et al. (1989) found that the internal browning potential of peaches depends on the total amount phenolic compounds present in the fruit and the level of activity of polyphenol oxidase (PPO) enzyme. When fruit are bruised, PPO has access to the phenolic compounds, and this causes internal browning.

The UNECE published no quantitative standards for internal eating quality factors, only subjective notes, including “they must be sufficiently developed and display satisfactory ripeness”. Similarly, AUF maturity grades were based on firmness descriptors (‘hard’, ‘firm’ etc), with no mention of internal quantitative measures or recommendations.
Coles/ Bi-Lo provided comprehensive TSS and firmness standards compared to the other retailers, differentiating between flesh colour and fruit cultivar. For example, a minimum TSS was recommended for yellow flesh (10% TSS) and white flesh (12% TSS) nectarine, with a general firmness standard of 5.2 kgf. Australia 2 gave a single minimum TSS of 10% and a firmness of 4 kgf, while Australia 3 gave no standards for any stone fruit. Of the two European retailers surveyed, for nectarine, Europe 2 published a minimum standard of 9% and firmness of 1.4-3.5 kgf. Europe 2 was the only commercial outlet that provided grades for plums based on colour (black plum: 12% TSS and 1.8-3.6 kgf; red: 10% TSS and 1.4-2.3 kgf; yellow 14% TSS and 1.0-1.8 kgf).

**Strawberry**

Strawberry (*Fragaria sp.*) fruit are known as a false or aggregate fruit, because the true fruiting bodies are made up of achenes (or seeds) that develop from multiple ovaries on a common receptacle; each ovary contains a seed (Sistrunk and Morris, 1985). Strawberry fruit are considered to be non-climacteric, and the industry has placed emphasis on colour related maturity grades; fruit are divided into four ripeness stages: green, white, pink (or turning), and red. Fruit take about 21 days to reach the white stage, and turn red (are fully mature) within 30-40 days (Perkins-Veazie, 1995). Fruit predominately accumulate sucrose and the prevailing acid is citric acid (Green, 1971). TSS, particularly when expressed relative to TA, often determines consumer perception of fruit flavour, with TSS of ripe berries varying between 4-11%, and TA ranging from 0.45-1.81% (Perkins-Veazie, 1995).
Haffner and Vestrheim (1997) reported that in Norway, a ‘sweet-sour’ taste is important for strawberries produced for the fresh market, whilst firmness and storability were becoming more important factors, because of longer transportation distances. They measured quality indices for 15 local strawberry cultivars, and reported TSS, acid, and TSS:acid ratio, as reliable measures of internal eating quality. All cultivars had TSS levels above 9.0% (average 10.4%), an average TA of 1.18% FW, and an average TSS:acid ratio of 9.0. They conceded that there were few publications defining minimum eating standards, although they cited work by Naumann and Seipp (1989), which detailed positive correlations between flavour scores and high TSS, and high TSS:acid ratio. Green (1971) reported a TSS:TA ratio of 5.3 for ‘mature’ fruit, and a TA (% citric acid) for ‘typical ripe’ fruit of 0.92. Kader (2002) proposed, for acceptable flavour of ripe fruit, a minimum TSS of 7.0, and a maximum TA of 0.8%.

Flavour, sweetness, and juiciness were the attributes which tasters believed to contribute the most to strawberry eating quality (Ford et al., 1997). They also reported that good correlations (correlation coefficients greater than 0.9) were only achieved when sensory colour and appearance, overall acceptability and flavour intensity, juiciness or sweetness, and sweetness and flavour, scores, were included in the linear model. Objective measurements alone, such as TSS, were reported to be unreliable indicators of sensory quality.
UNECE gave no minimum internal grade levels, only subjective descriptors relating to appearance and disease. Similarly, none of the other regulatory bodies detailed any standards. Of the retail chains reviewed, Coles/ Bi-Lo gave a minimum standard of 6.5% TSS; the other retail chains reported no quality grades.

**Tomato**

Tomato (*Lycopersicon esculentum*) (family Solanaceae) fruit are fleshy berries in which the entire pericarp, consisting of outer, radial and inner walls, along with seeds embedded in a jelly-like parenchymatous tissue within the locular cavities, is consumed (Hobson and Davies, 1971). Tomatoes are typical climacteric fruit, displaying an increase in ethylene production and a marked increase in respiration. Ripening is typically represented by a ‘colour break’, whereby fruit change from green to pink/ red, occurring one or two days after the ethylene increase. Fruit accumulate starch during development (between 1.0-1.2% FW), which is rapidly hydrolysed to soluble sugars during ripening (Hobson and Grierson, 1993). In ripe fruit, soluble carbohydrates constitute 1.5-4.5% of fruit FW, with glucose and fructose dominating and occurring in equal proportions, and sucrose occurring in lesser amounts (less than 0.1% FW) (Hobson and Davies, 1971). Citric and malic acid are the predominating accumulating acids (Young *et al.*, 1993), and Hagg *et al* (1999) reported that fruit typically accumulate between 4.0-5.5 g of citric acid per litre, and that fruit are high in vitamin C (15-25mg/100g), lycopene (1.7-5.9mg/100g) and beta-carotene (0.35-0.67mg/100g). Hobson and Davies (1971) reported that the
moisture content of tomato increases from 91% in immature green fruit to 93% as fruit develop, with good quality tomato fruit having a moisture content of 94-94.5%.

TSS and acidity are considered to be good indicators of fruit eating quality, although Winsor et al. (1962) warned that sugars account for only 60-80% of measured TSS content (the remainder representing other soluble components). Pattee (1985) noted that flavour was related to high sugars and high acids in a proper balance, and criticized the use of the simple sugar:acid ratio. Young et al., (1993) advocated the use of TSS for fresh market tomato production, because of the large contribution sugars and acids make to tomato TSS, but advocated the use of both a TSS and a TSS:acid ratio standard in the production of good quality processing tomato.

Allowing fruit to fully mature and ripen on the vine allows the accumulation of a ‘full flavour spectrum’, including the complex volatile components which contribute to fruit aroma (Stevens, 1979; Hobson and Grierson, 1993).

In the U.S. fruit are typically picked at a ‘mature-green’ or ‘breaker-stage’, because these fruit transport better than fully vine-ripened fruit. However such immature fruit are lower in sugars, and typically contain higher concentrations of off-flavour tasting volatiles such as 2-methyl-2-butenal (Stevens, 1979). Kader and Morris (1976) suggested that fruit should have a well formed jelly-like matrix in the locules, and that when the fruit is sliced, the seeds should be pushed aside and not cut. Kader and Morris (1976) described ‘class 4 – fairly firm’, with a designated firmness value of
1.0-1.5 Kg (8mm probe) as an optimum firmness for tomato, ‘below which, red-ripe fruits would not be acceptable to most consumers.’

UNECE standards detailed no measurable internal quality factors, only descriptive minimum requirements such as ‘intact’, ‘clean’, ‘free from pests’ etc. Neither of the other regulatory or government agencies or any of the retail outlets gave any minimum grade standards.

**Moving Forward - Conclusion**

An array of quantitative standards are appropriate to the grading of fruit for eating quality, as indicated above. Such standards have been adopted, to a degree, into the quality control specifications used by packing houses, wholesalers and retailers. Indeed some commodities are more differentiated than others, for example there are standards offered for a large number of apple varieties (Table 4), whilst for banana few standards are published in the scientific literature and none exist in the retail chains. Standards for a specific commodity may also vary between retail chains and within the literature. For example the minimum TSS standard required by Coles/BiLo for Akane apples is 11.5%, yet is 13% for Australian retailer 2 whilst Australia 3 has no standard (Table 4). Similarly, for Mango, Yamashita (2000) recommended a minimum TSS of 15%, whilst Samson (1989) recommended 12% (Table 1).

With the advent of appropriate sorting technologies, there exists an opportunity to more comprehensively create new, refine and adopt old, and enforce, such
specifications. For example, near-infrared spectroscopy has been applied to the in-line grading of fruit for internal attributes such as sugar and dry matter content, and various methodologies (accelerometer, sonic) have been applied to the sorting of fruit on firmness. Other technologies, based on X-ray imaging, magnetic resonance, chlorophyll fluorescence, ultrasonics, and electrical impedance may be appropriate for assessment of other internal attributes. The availability of consistently improved eating quality fruit should allow for product specialisation, increased consumer confidence and increased consumption of fresh fruit.
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