

Avocado Postharvest Research: 1998/99: Seasonal Changes in Lipid Content and Fatty Acid Composition of 'Hass' Avocados

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June 1999

Report to the NZ Avocado Industry Council

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*HortResearch Client Report No. 2000/1
ContractNo.5262*

This report has been prepared by The Horticulture and Food Research Institute of New Zealand Ltd (HortResearch) which has its Head Office at Batchelar Research Centre, Private Bag 11 030, Palmerston North and has been approved by:

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EXECUTIVE SUMMARY

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Lipids are the most important component of avocado fruit. They comprise from 60 to 80% of the dry matter of the mesocarp, the edible portion of the fruit, and are responsible for the taste and mouth-feel that consumers demand in avocados. Not surprisingly, lipid content of mesocarp (% of total dry matter) may be used to define avocado fruit maturity. Although there has been some work relating lipid content to fruit maturity in NZ grown avocados, there has been no examination of the fatty acid makeup of the lipids, or how they might vary between regions. Nor is there any information on lipid content for NZ avocados later in a commercial season. Such information is important from fruit quality, health and marketing points of view.

The main objective of the work presented here was to determine the influence of time in the season and growing region on lipid content and composition of New Zealand 'Hass' avocados.

On seven occasions between September, 1998 and April, 1999 fruit from two orchards (one in Te Puke and one in the Far North) were harvested and analysed for dry matter, lipid content and fatty acid composition. Dry matter assessments were carried out using the commercial method and the total lipids were extracted from fresh tissue using a modification of the Bligh and Dyer technique. Later, the fatty acid composition of the lipid was determined by gas chromatographic analysis.

Average dry matter increased over the period of study (September to April). Dry matter for Te Puke fruit increased from 24.6% to 36.4%, while dry matter from the Far North fruit increased from 24.1% to 32.3% over the same period. Total lipid content increased from 17.2% to 31.3% (i.e. by 82%) in Te Puke and from 16.4% to 26.7% (by 62%) in the Far North from September to April.

We confirm that there is a high correlation between total lipids and dry matter content in avocados. During this study, fruit from Te Puke always showed higher lipid content (and dry matter content) than fruit from the Far North. At both sites, the beneficial monounsaturated oleic acid was the major fatty acid accumulated. However, fruit from Te Puke showed higher levels of oleic acid than fruit from the Far North. From a health and nutrition point of view, the ratios of monounsaturated (oleic and palmitoleic acid) to saturated fatty acids (palmitic acid) and of polyunsaturated (linoleic and linolenic acid) to saturated fatty acids found in NZ-grown avocados compare favourably with those of the recommended olive oil.

INTRODUCTION

Lipids are the major component in the flesh of avocado fruit. Research overseas has attributed flavour, texture and part of the nutritional characteristics of the fruit to the quantity and quality of lipids. A large number of overseas studies have reported changes in lipid content and composition during growth and maturation of the fruit. However, in New Zealand studies on this area are scarce.

The market success of traditional avocado producers and exporters such as California and South Africa is partly due to the continual acquisition of information concerning the quality of their fruit, which allows confidence in the planning of marketing activities. To maintain a differentiated place in the world market, the NZ avocado industry needs to continue obtaining thorough knowledge of the features of its product.

Postharvest research represents an important part of the development of consistently high-quality fruit delivered to the consumer. Among the different aspects which influence final quality is the issue of harvesting fruit at the right stage of maturity. Harvesting only mature avocados enhances good eating characteristics at the end of the ripening process (Lewis, 1978). Thus, greater understanding of maturity measures, such as dry matter, and how they relate to total lipid content is important to our success. Aside from the issue of minimum maturity, another important aspect is the quality of fruit late in the commercial season since there are allegations by international competitors of rancidity in “over mature” fruit.

With a fast growing export avocado industry, New Zealand needs continuing research examining seasonal changes in the components of the fruit for an accurate determination of quality standards to minimise the possibility of inferior fruit being placed on the market.

This work examines the effect of time in the season and growing region on the lipid content and composition of New Zealand avocados.

LITERATURE REVIEW

This review aims to provide some background to lipids (oils) in avocado, their chemistry and nomenclature, the importance of the area to the avocado industry, and how they can be measured.

1. Lipids and Fatty Acids Composition of Avocados

1.1. Lipids in Avocado

High lipid content is one of the distinguishing features of avocado fruit. The types of lipids which occur in the avocado include tri-, di- and mono-glycerides. In mature fruit, approximately, 85% of these lipids are triglycerides (Platt-Aloia and Thomson, 1981) and are

normally regarded as storage material to provide carbon and energy (e.g. to germinating seeds). The other glycerides are mainly structural lipids present in cellular membranes where they exist as a lipid bilayer (Stumpf and Conn, 1987). Structural lipids are present in tissues in relatively small amounts and are therefore consumed as part of the fruit. Many of the components of the structural lipids in foods will be incorporated into the structural lipids in the body of the consumer (Enser, 1995). Additionally, minor non-glyceride (non-saponifiable fraction) compounds are present in avocado lipids. These contain flavour components and nutritionally important vitamins. The triglycerides are present in the avocado mesocarp oil cells or idioblasts (Schroeder, 1953; Platt-Aloia et al., 1983).

The storage lipids are the triglycerides present in the avocado mesocarp fat cells or idioblasts (Schroeder, 1953; Platt-Aloia et al., 1983). More than 85% of the lipids in avocados are triacylglycerols (Platt-Aloia and Thomson, 1981). The lipids are synthesised only during growth and maturation of the fruit on tree and not during storage and ripening (Platt-Aloia and Thomson, 1981; Luza et al., 1990). The idioblasts are distinguished by their large size and complex three-layered cell walls (Platt-Aloia et al., 1983 and Kaiser et al., 1992). Dolendo (1966) reported that the fat cells are bound together by pectin substances of the middle lamella. Thus, although the total lipid content of the fruit is high at the moment of harvest, it is most difficult to extract then due to the high amounts of protopectin – the binding or cementing substances of the lipid cells (middle lamella) - which also contribute to the observed changes in the fruit texture. During the softening process a decrease in the degree of esterification of the pectin of the avocado fruit loosens the cells from each other and at that stage the cells may also be more easily ruptured, resulting in the release of the lipids. In mature fruit these cells are about 60 μm in diameter (Kaiser et al., 1992; Werman and Neeman, 1987). Ross et al. (1993) when studying the presence of oleosins proteins in avocado and olive seeds found that unlike olives the avocado seed is not a major site of storage lipid accumulation, containing only about 1% on a fresh weight basis (Biale and Young, 1969).

The lipid content and composition of avocados is affected by many factors such as fruit race, fruit position on the tree (Hatton et al, 1957) site within the fruit (Schroeder, 1987), maturity (Davenport and Ellis, 1959; Mazliak, 1965a; Kikuta and Erickson, 1968; Vakis et al., 1985; Eaks, 1990; Inoue and Tateishi, 1995), cultural practices (irrigation) (Lahav and Kalmar, 1977; Kruger and Claassens, 1996a), environmental conditions (rainfall, temperature) (Kaiser and Wolstenholme, 1994; Kruger and Claassens, 1996a, 1996b; Mc Onie and Wolstenholme, 1982) and post harvest handling (atmosphere storage composition) (Mazliak, 1965b).

1.2. Saturated and Unsaturated Fatty Acids

Lipids are chemical compounds containing one or more fatty acids. In general, it is the combination of the fatty acids which determine the physical and nutritional characteristics of the lipid.

Fatty acids are organic acids and as such are composed mostly of carbon and hydrogen atoms. The short chain organic acids, with less than ten carbon atoms, are all water-soluble. However, the long chain fatty acids are much less soluble in water due to the size of the hydrocarbon chain. The fatty acids found in the avocado mesocarp are long chain, with 16 carbon atoms or more (Kikuta and Erickson, 1968).

There are three main types of fatty acids: saturated, monounsaturated and polyunsaturated. A saturated fatty acid has the maximum possible number of hydrogen atoms attached to every carbon atom. It is therefore, said to be “saturated” with hydrogen atoms. Some fatty acids are missing one pair of hydrogen atoms in the middle of the molecule resulting in a carbon:carbon “double bond.”. As this hydrogen deficit results in one double bond, the fatty

acid is said to be “monounsaturated”. Fatty acids that are missing more than one pair of hydrogen atoms have more double bonds and are called “polyunsaturated” (Mayfield, 1994).

The usual shorthand nomenclature for fatty acids shows two numbers; the length of the chain and the number of double bonds. For example, “18:2” denotes a fatty acid of 18 carbon chain length and 2 double bonds eg linoleic acid. Similarly, a saturated fatty acid, such as palmitic acid will be shown as “16:0”. Usually saturated (S) fatty acids are found in foods of animal origin while monounsaturated (M) and polyunsaturated (P) fatty acids are mostly found in foods of plant origin.

Triacylglycerols may contain a wide variety of fatty acids, although only five or six are usually present in significant amounts in avocado fruit. These are the saturated fatty acids palmitic acid (16:0) and stearic acid (18:0), the monounsaturated fatty acids oleic acid (18:1) and palmitoleic acid (16:1) and the polyunsaturated fatty acids linoleic acid (18:2) and linolenic acid (18:3). Oleic acid is the predominant fatty acid, representing close to 60% of the total lipids (Mazliak, 1965a; Kikuta and Erickson, 1968; Luza et al., 1990). In general, the combination of fatty acids in the triacylglycerol fraction determine the physical and nutritional characteristics of the lipid. To date the changes in fatty acid makeup have not been studied in New Zealand since most maturity studies have concentrated on total lipid content.

2. The Importance of Lipids to the Avocado Industry

There is a range of reasons to examine avocado lipid content and its quality, referred to its fatty acid composition, in New Zealand.

2.1. Maturity indices; Dry Matter and Lipid Content

Although total lipid content in avocados is the base for determining maturity parameters, the most common parameter used for the world trade industries is the percent dry weight of the fruit. The rationale behind this is that the percent dry matter of the flesh correlates well with lipid content and represents a simpler and cheaper method for maturity evaluations (Stahl, 1933; Biale and Young, 1969; Appleman, 1969; Kikuta and Erickson, 1968; Lee et al., 1983; Ranney et al., 1992). It has been shown that percent lipid (on a fresh weight) and percent moisture content are reciprocal and with slight variations generally sum to a constant value. For instance for 'Hass' avocados, Swarts (1976 cited by Kruger et al., 1995) found this constant value to be $\cong 87.8\%$. Similarly, Kruger et al. (1995) found it to be approximately 86.5%, while Hopkirk (1989) and Pearson (1975) found it to be approximately 88% and 91% respectively. A relationship between lipid and water content enables the lipid content to be easily calculated from water content.

There is relatively limited research examining avocado lipids in New Zealand. Lawes (1980) demonstrated the relationship between lipid and moisture content for Fuerte and Zutano cultivars and analysed the fatty acid composition of 'Hass' fruit twice during fruit development. Hopkirk (1989) measured lipid levels in 'Hass' fruit during four successive seasons from 1981 to 1985, and the relationship between lipid content and both maturity and consumer acceptability. The research found that as the season progressed from September to February the dry matter content of avocados from Kaitaia (in the Far North) increased from about 31% to 35% while lipid content increased from about 18% to 23%. In the same period, the mesocarp of avocados from the Bay of Plenty increased in dry matter from 28% to 36% and in lipid content from 16% to 23%. However due to the great fruit variability and technical difficulties encountered with the tasting procedures, it could be established only that total lipid content and dry matter content increased concomitantly as the fruit matured. Hopkirk concluded there was a strong relationship between dry matter and lipid content and that this

relationship may vary across regions. Only a weak relationship between total lipid content or dry matter, and taste acceptability could be found. No examination of the lipid composition (fatty acids) was carried out.

2.2. Commercial Extraction of Avocado Oil

Certainly, the most interesting characteristic of the avocado fruit is its high lipid content. In fact, of all fruits, only the olive (*Olea europea*) and the oil palm fruit (*Eleaëis guineensis*) can rival the avocado in oil content (Lewis, 1978). This characteristic encouraged producers overseas to press the avocado to extract the lipids. Avocado oil is widely used in the cosmetic industry due to its properties as a natural antioxidant and to its ability to penetrate the skin (comparable to lanolin) (Human, 1987). Moreover, since avocado oil composition compares favourably with olive oil in its high oleic acid content, it can also be promoted as cholesterol reducing. Thus in the future, the extraction of avocado oil may represent an alternative for adding value to the industry in New Zealand.

2.3. Taste and Rancidity Issues

Kaiser et al. (1992) reported that the lipid component in avocado fruit, among others, provides a unique and desirable texture and taste to the fruit. After harvest, avocados ripen and soften, changing to a soft buttery flesh depending on the amount of lipid in the fruit at the point of harvest. It should be remembered that the triglycerides of avocados are synthesised only during growth and development on the tree and not during storage or ripening (Platt-Aloia and Thomson, 1981).

Differences in oil content of olive cultivars has been observed in different regions, and quantitative differences in flavour compounds were also observed (Lercker et al., 1973; Montedoro et al., 1978). Thus, New Zealand fruit may have a characteristic flavour that could be used for produce differentiation. In this respect, flavour and rancidity issues have been claimed to be threatening the New Zealand avocado industry. The issue of rancidity will not be examined this season, but the relationship of fatty acids to the development of flavour and off-flavours in avocados represents a major research area as it is most applicable to consumer appreciation of the fruit.

2.4. Chilling Injury Disorders and Health Aspects

Lipids are a major component of the human diet, contributing up to 40% of the calories in the diet of developed countries (Enser, 1995). For many years, the nutritional benefits of such a high consumption has been questioned, particularly the consumption of a high proportion of saturated (S) fatty acids (cholesterol-promoting fatty acids) and lower proportions of unsaturated (U) fatty acids, specially monounsaturated (M) fatty acids (cholesterol-reducing fatty acids) (WHO, 1982; COMA, 1984). Polyunsaturated and monounsaturated fatty acids do not promote the formation of artery-clogging fatty deposits (the phenomenon is known as atherosclerosis and causes coronary heart disease (CHD)) the way saturated fatty acids do (Mayfield, 1994). However it has been reported recently that diets high in polyunsaturated fatty acids may also be promoters of cholesterol raising and therefore increase the risk of CHD. It appears that it is the high levels of monounsaturated fatty acids that aid in the prevention of accumulation of cholesterol in the aorta (Howard and Savage, 1994). Therefore, the dietary ratio of polyunsaturated fatty acids to saturated fatty acids (*P:S*) may no longer be appropriate (Ulbricht and Southgate, 1991). Slater et al. (1975) reported for 'Hass' avocados an average *P:S* ratio of 0.75 from September to November (Southern Hemisphere equivalent). Nutrition results have shown that diets high in monounsaturated fatty acids (of which oleic acid has been the only fatty acid so far investigated) may be more favourable to health imparting resistance to atherosclerosis (Christakis, 1980).

Chilling injury sensitivity of plant materials has been associated with the ratio of saturated fatty acids (S) to unsaturated (U) fatty acids in the cell membrane (Lyon, 1973; Wang, 1982; Eaks, 1990). Lipids constitute an important part of the cell membrane and are actively involved in membrane exchange processes.

The ratio of unsaturated fatty acids to saturated fatty acids in cell membrane glycerolipids of avocado fruit could be influenced by ambient temperatures during fruit development (Moreton, 1988 cited by Kaiser and Wolstenholme, 1994). The functioning of the cell membrane may be affected by the viscosity of its lipid phase. Unlike saturated fatty acids, unsaturated fatty acids are usually liquid at cool temperatures. Moreover, an increase in temperature results in increased kinetic movement thus increasing membrane fluidity (Stryer, 1988). It was suggested that at low (environmental) temperatures the plant membrane should contain higher levels of unsaturated fatty acids in order to perform properly (Moreton, 1988). Thus, it has been suggested that avocados grown under cooler conditions tend to accumulate more unsaturated fatty acids during development than fruit produced under warm conditions. The latter needs to be further studied in New Zealand where 'Hass' avocados are successfully grown in a range of climatically different regions. Results could suggest significant health advantages that could be exploited from the marketing strategy point of view.

3. Measurement of Lipids and Fatty Acids in Avocados

3.1. Determination of Lipid Content

Determination of lipid content is time consuming and expensive and a range of techniques have been employed to date. Refractometric index (RI) method developed by Leslie and Christie (1929) using Halowax oil as a solvent (monochloronaphtalene), was officially used for measurement of percent of total lipid in avocado in California. However, due to its inconsistency of readings which are easily influenced by temperature, and equipment costs, this method was considered inconvenient for growers. RI methods are also of questionable accuracy especially when testing ripe fruit. In addition, Halowax is a suspected carcinogen and is no longer available (Lee, 1981).

Soxhlet technique using petroleum ether (non-polar solvent) is the standard method for analysing lipid content in foods. In the Soxhlet extraction the tissue has to be previously dried, therefore extending the time of assessment. This method can take up to 12 hours and automated systems usually only run eight samples at one time. Thus, this technique is considered too slow to be used as a routine test for the industry.

An adaptation of the Gerber method originally developed for the dairy industry showed accuracy in the determination of total lipids in avocados (Rosenthal et al., 1985). However, it not only uses a combination of flammable and dangerous solvents, but as it is an adaptation, it uses equipment used for assessing fat levels in dairy products that are not always available in the horticulture industry. Nuclear Magnetic Resonance (NMR), although having advantages such as accuracy, simplicity and fast determination involves very high equipment costs (Barry et al., 1983; Bergh et al., 1989). It is important to note that extraction technique employed will influence the apparent amount of total lipid in a sample.

Several lipid extraction methods originally developed for animal produce have been used as such for determination of total lipid in avocados with relative success. This is the case with the methods developed by Folch et al. (1957) and Bligh and Dyer (1959). However, large sample size and large volumes of solvent, a relatively high level of difficulty, and slow techniques are the main inconveniences found.

The present study attempts to develop a rapid technique for assisting researchers and the industry in the determination of total lipid in avocado. The technique developed represents a modification of the Bligh and Dyer method.

3.2. Determination of Fatty Acid Composition

Gas chromatography is now the standard technique for detailed analysis of fatty acid (as fatty acid methyl esters) composition in avocados (Mazliak, 1965a; Kikuta and Erickson, 1968; Eaks, 1990; Luza et al., 1990; Kaiser and Wolstenholme, 1994; Inoue and Tateishi, 1995). In this work, the fatty acid composition of the extracted lipids will be determined by gas chromatographic analysis of the derivative fatty acids methyl esters.

Gas chromatography results will also show the time in the season where the nutritional quality (in terms of fatty acid composition) of the fruit is at a maximum. These results are of importance as they could be used by marketing experts to compare New Zealand-grown avocados with avocados from other countries from a health perspective.

Avocado is an unusual fruit because its characteristic composition could vary dramatically with the cultivar, time in the season and environment. Thus, better knowledge of the variations in total lipid level and composition in the fruit during the season and its relationship with dry matter content is important for New Zealand.

The main objective of the present report is to determine the influence of time in the season and regional changes on content and composition of lipid in New Zealand avocados. This will assist growers in selecting fruit with a minimum desired total lipid content. Furthermore, any additional information about lipids in New Zealand grown avocados will be helpful.

MATERIALS AND METHODS

Sample Collection

Orchard Location

Avocado fruit (*Persea americana* cv. 'Hass') were obtained from two orchards (one situated in the Far North and one in Te Puke), selected on the basis of different climatic conditions. The warmer site was in the Far North at Awanui located at Lat. S. 35° 03'; Long 173 ° 16'. The cooler site was in the Bay of Plenty at Te Puke, located at Lat.S. 37° 47'; Long. 176° 20'.

Avocado Tree Characteristics

On twenty ten-year old 'Hass' avocado trees, clusters of 10-20 fruit in the East-Northeast quadrant of the tree at 1-2 m height, were tagged in both orchards. At the time of tagging, Sapac dataloggers (Sapac TempRecord, Argus Distributors Ltd, Auckland) were placed in the tree canopy (shaded by a plastic container) to log the air temperature every hour.

Fruit Collection

Fruit was harvested on seven occasions between September 1998 (beginning of the harvest season) to April 1999. These harvests cover the main commercial harvest season in New Zealand. From each tree, the two largest fruit were selected, clip-picked and one fruit placed into each of two trays; one for measurement of dry matter/lipids, and the other for determination of weight loss. The tray of fruit for dry matter/lipids assessments was wrapped in a plastic bag with 3 small holes to minimise water loss during transport. Fruit were green and did not show purple/black discoloration caused by excessive sunlight.

Unless otherwise stated, the avocados were picked early in the morning (thus avoiding water stress) and transported overnight by courier to HortResearch Auckland, arriving at the laboratory within 30 hours of harvest.

Fruit Assessments

On arrival in the laboratory, fruit were weighed and firmness measured using the Anderson Firmometer.

Dry Matter

The fruit in one tray from each orchard were divided into four replicates of five fruit. Two types of dry matter test were carried out.

Commercial Dry Matter

This is the method routinely used by the industry. A quarter of each fruit (sliced vertically) was peeled, seed coat removed and the flesh grated in a food processor. A subsample of 20 grams was then taken and dried in a petri dish for 36 hours at 60°C (until constant weight) and then re-weighed.

Plug Dry Matter

A plug from the equatorial part of each fruit was taken using a brass cork borer (5mm internal diameter) and cut longitudinally. The plugs were dried in a petri dish for 36 hours at 60°C (until constant weight) after which they were re-weighed.

Preparation of the Sample for Total Lipid Extraction

For the determination of total lipid concentration a second plug, adjacent to the plug taken for dry matter, was taken from each fruit. The plugs were sliced thinly (approximately 5mm diameter, 0.5-1.0 mm width) then weighed in tared KMax glass test tubes. Ten mL of a chloroform-methanol solution (1:1 v/v) was added to each tube, which were then vortexed for 10 seconds to ensure that slices were fully immersed in the solvent. The homogenates were left at room temperature for 36 hours with occasional shaking. After this time the slices of tissue were clear and had sunk to the bottom of the tube (the water in the tissue had been replaced by the methanol). Samples were then stored at -20°C until required for quantitative lipid extraction and fatty acid analysis.

Firmness and Weight Loss

Weight Loss

For some harvests, weight loss was measured during 14 days of ripening at 15°C.

Firmness Assessments

Fruit firmness was determined using an Anderson Firmometer as described by Woolf et al., (1997). The Firmometer measures the resistance offered by the fruit to a compression force of a 300g weight through a 17mm diameter convex button over a 10-second period. The reading (mm displacement) is multiplied by 10 to give the Firmometer value. Immediately after harvest Firmometer values are typically 10 to 15, depending on the time of the season. The Firmometer value increases to a maximum of 110 as fruit soften.

Quantitative Determination of Total Lipids

The technique used for quantification of the total lipid content in the samples is a modification of the Bligh and Dyer method (1959) for total lipid extraction. Lipids were extracted with a mixture of chloroform, methanol and water = 1:1:0.9 v/v/v. Following thorough mixing and brief centrifugation, two clear layers were resolved. The lower layer was predominantly chloroform and contained the lipids from the original tissue while the upper layer was composed of methanol and water and contained water soluble material from the original extract. Thus, when the chloroform layer was isolated, a purified lipid extract was obtained.

For simplicity and convenience, we had initially hoped to be able to extract total lipids from thin slices of mesocarp into chloroform/methanol without resorting to homogenisation. However, although slices of ripe mesocarp were extracted adequately, slices of unripe (hard) tissues apparently were not (Appendix 1). Therefore, different methods of extracting total lipids from hard mesocarp into chloroform/methanol were tested.

Results showed that when using hard unripe avocados, grinding using an overhead blender (Polytron) achieved the highest lipid extraction efficiency (see Appendix 1 for results showing lipid extraction using three different methods). In addition, lipid content of avocados remained constant during ripening from harvest (day 0) through ripening (at 15°C) until achieving soft- eating ripeness (day 8 after harvest) as previously reported by Platt-Aloia and Thomson (1981) and Luza et al. (1990) (see Appendix 1 for results). The difficulties in maximising extraction may be due to the complex three-layered cell walls surrounding the idioblasts mentioned by Platt-Aloia et al. (1983) and Kaiser et al. (1992). This grinding method was reasonably practical with no major difficulties and was used for the samples taken throughout the season.

While the above extraction trials were being carried out, the avocado samples collected during the season were sliced, weighed and immersed in 10 volumes of chloroform-methanol solution and stored at -20°C until the most efficient technique was developed. The final developed technique used for the avocado samples collected during the season is as follows:

The frozen sliced samples in chloroform-methanol were thawed for at least one hour at room temperature. Following addition of 5 mL of (1:1 v/v) chloroform/methanol solution, samples were homogenised using a Polytron (model CH- 6010 Kinematica Kriens-Lu, PT 10-35 cm., head diameter 1.5 cm., with a universal speed controller) for approximately 30 seconds. After standing for 15 minutes the samples were vortexed and immediately filtered through Miracloth. The remaining fruit tissue in the filter paper was rinsed with 5 mL of chloroform/methanol solution and pressure applied by squeezing to ensure maximum solvent recovery.

Ten mL of the filtrate was transferred to a new KMax test tube, 4.2 mL of 1% v/v NaCl solution added and then centrifuged (2500 rpm for 30 seconds). The salt solution was added for partitioning, and to correct the proportion of water in the system. The final system should contain chloroform:methanol:water in 1:1:0.9 proportions in order to form the biphasic system. The mixture was vigorously shaken and centrifuged to allow better separation and clarification of the lipid-containing chloroform layer, which was then aspirated with a glass syringe. A small volume of the chloroform layer was left behind to avoid removal of the methanol-water layer. The remaining mixture was re-extracted by adding 2.5 mL of petroleum ether (boiling point 40-60°C) vortexed and centrifuged. The lipid-containing petroleum ether layer was aspirated and combined with the first chloroform extract. The solvents were evaporated at 40°C under a continuous stream of oxygen-free nitrogen (to prevent oxidation of the fatty acids in the sample) to a constant weight. The weight of the dry lipid fraction was recorded. On one occasion, the Soxhlet method using petroleum ether was carried out manually and the results of the two methods compared.

Fatty Acid Analysis of Lipids

Conversion of Triglycerides to Fatty Acid Methyl Ester (FAME)

The weighed lipids were immediately resuspended in 5 mL of chloroform and stored at -20°C . To determine its fatty acid composition, a 50 μL subsample of the lipid-in- chloroform was treated with 100 μL of 0.5 N sodium methoxide in methanol (prepared with a solution of dimethoxypropane and methanol (95:5, v/v)). Transesterification of fatty acids to fatty acid methyl esters (FAME) was complete after standing at room temperature for 15 minutes. Sulfuric acid (400 μL of 0.125 N) was added and fatty acid methyl esters were recovered in 7.5 mL of petroleum ether (boiling point $60-80^{\circ}\text{C}$).

Gas Chromatography Analysis

One μL of fatty acid methyl esters in petroleum ether was injected into the gas chromatograph (Hewlett Packard model 5890A), equipped with a Supelco fused silica capillary column No. 11484-02A, catalogue No. 2-4019 (30 m x 0.25mm ID x 0.2 μm film Mfg.) and a flame ionisation detector (FID). The temperature was 100°C initially, then increased by 15°C per minute to 190°C and held at 190°C for 25 minutes. Injector and detector temperatures were at 200 and 220°C respectively. The carrier gas was nitrogen, flowing at 22 cm per second. For this study, an extra fatty acid component, stearic acid methyl ester (18:0) was added to each sample as an internal standard immediately prior to injection. The detector response was calibrated with a standard fatty acid methyl ester mixture (supplied by Sigma-Aldrich) containing five fatty acids which commonly occur in significant concentrations in avocado fruit; palmitic acid (16:0), palmitoleic acid (16:1), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3) and the stearic acid (18: 0). The fatty acid peaks in lipid samples were identified by comparison with the retention times of fatty acids in the standard mixture, and the amount calculated as a percentage of the total lipids.

RESULTS

Fruit Firmness and Weight Loss

During the season, fruit from the Te Puke orchard had an average weight of 252.1 g and an average Firmometer value of 14.3, while fruit from the Far North had an average weight of 261.7 g and an average Firmometer value of 13.9.

Far North and Te Puke fruit harvested in November and January lost approximately 2.7% and 3.8% of their weight respectively during 14 days of ripening at 15°C . The rate of weight loss during ripening was fairly constant such that it equated to approximately 0.2%/day for Far North and 0.3%/day for Te Puke. The differences in weight loss may be due to differences in fruit size from the orchards.

Dry Matter and Lipids

Average dry matter increased over the period of study. Dry matter for Te Puke fruit increased from 24.6% to 36.4%, while dry matter for Far North fruit increased from 24.1% to 32.3% over the same period. Along with the standard commercial technique for determining dry matter, we also examined the use of a single small plug sample. We found a good correlation between results from the commercial dry matter and the plug dry matter tests ($R^2 = 0.92$), Appendix 2. The determination of plug dry matter was significantly quicker than the commercial dry matter technique, however, there was more variability in the results obtained.

Considering the time savings possible using this technique, we suggest that further trials be carried out.

The percentage dry matter and total lipid content during the season for fruit from the two regions is plotted in Figure 1. Over the period of study, dry matter value was higher for Te Puke than for the Far North. Although percentage dry matter in both orchards started at a similar value in September ($\cong 24\%$), dry matter of Te Puke fruit increased at a faster rate than that of the Far North fruit. For Te Puke fruit, dry matter content increased rapidly from September (24.6%) to a peak in mid January (35.2%) where it levelled off and later showed a tendency to increase slightly through March and April. For the Far North, dry matter showed rapid increase from 24.1% in September to a peak of 33.5% at the end of February, almost a month later than Te Puke, and then decreased slightly to plateau at 32.4% during April.

Total lipid content (% fresh wt) measured in September was on average 17.2% in Te Puke and 16.4% in the Far North. Lipid content of Te Puke fruit remained higher over the season, increasing rapidly to peak at 29.6% in January and then increasing slowly to 31.3% during April. The Far North showed a rapid increase to a peak of 26.7% in March, two months later than Te Puke, and then appeared to level off at approximately 26% through April. Total lipids in the Far North peaked a month later than its corresponding dry matter value (percent dry matter peaked in February and percent lipids peaked in March).

The relationship between lipid and dry matter content for fruit from the two regions is shown in Figure 2. Linear regression analysis between percent dry matter and percent total lipids showed that there is a close relationship between these factors in fruit from both locations. The correlations resulted in an R^2 for Te Puke of 0.99, and of 0.9 for the Far North. In addition, the sum of total lipids and water content of the fruit for each region remained constant through the season at about 94% for Te Puke and 93% for the Far North.

Analysis of the extracted lipids by gas chromatography showed similar fatty acid composition for both regions. Fatty acids identified using commercial standards showed that the lipids were predominately composed of oleic (18:1), palmitic (16:0), linoleic (18:2), palmitoleic (16:1) and linolenic acid (18:3). The monounsaturated oleic acid was the most abundant fatty acid at both sites.

At Te Puke, oleic acid (18:1) concentration rose from 67% in September to 71% of the total lipids during October. It then decreased slowly and finally plateaued at 62% in April (Figure 3a). Palmitic acid (16:0) was the second most abundant fatty acid, remaining fairly constant at about 15% of the total lipids. Concentrations of linoleic acid (18:2) remained constant at 11% until around January when it slowly increased to 15% in April. Concentrations of palmitoleic acid (16:1) remained fairly constant at about 6% while linolenic acid (18:3) increased slightly from 0% in September to 1.4% in March and then decreased to 0.9% through April.

In the Far North, oleic acid also increased slightly from 62% of the total lipids in September to 63% in November after which it decreased slowly and plateaued at 57% through March and April (Figure 3b). As in Te Puke, palmitic acid (16:0) was the second major fatty acid present and remained fairly constant at about 18%. Linoleic acid (18:2), remained fairly constant at about 13% until January when it increased slightly to 16% in April. Palmitoleic acid (16:1) remained fairly constant at about 6% until mid January where it showed a slight increase to 9% at the end of February. Linolenic acid (18:3) increased slightly from 0% in September to 1.2% in March where it plateaued.

On average oleic acid (18:1) levels were 10% lower in the Far North (60%) than in Te Puke (66%), while palmitoleic acid (16:1) was 17% higher in the Far North (7%) than in Te Puke

(6%). Palmitic acid (16:0) was 20% higher in fruit from the Far North (18%) than in Te Puke (15%). Linoleic acid (18:2) was 17% higher in the Far North (14%) than in Te Puke (12%). In both regions, linolenic acid (18:3) on average, remained constant for most of the season at about 0.8%.

Air Temperature

The mean daily temperatures for Te Puke and the Far North are shown in Figure 4 (see Appendix 3 for detailed temperature records). From the figure it is clear that the average and minimum temperatures were generally higher for the Far North than for Te Puke. However, the maximum temperature was similar for both orchards. Temperature may not be the only factor to determine the rate of lipid synthesis.

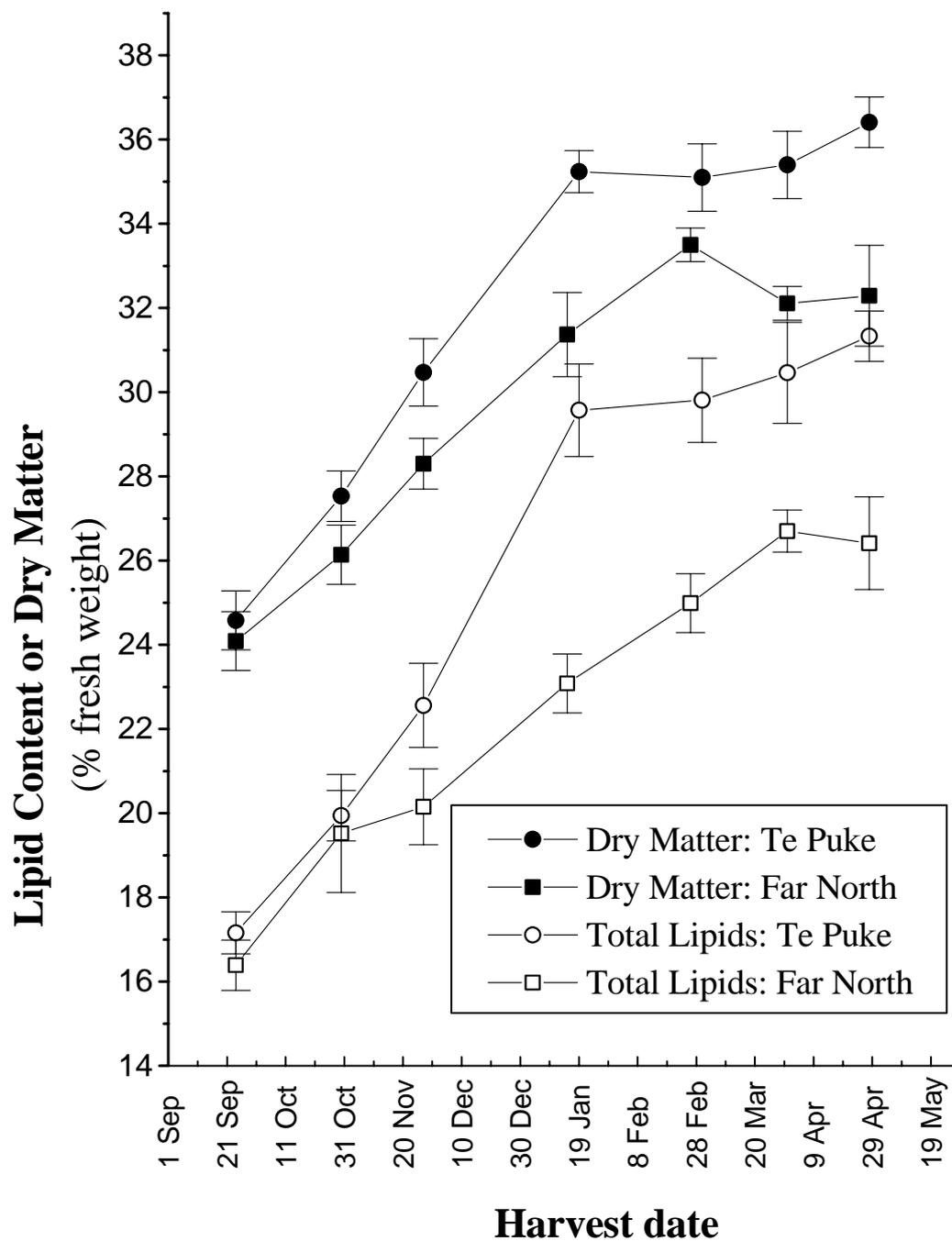


Figure 1. Mean lipid content and dry matter of 'Hass' avocado fruit harvested from Te Puke and the Far North from September to April. Each point is the average of four replicates of five fruit. Vertical bars = SEM.

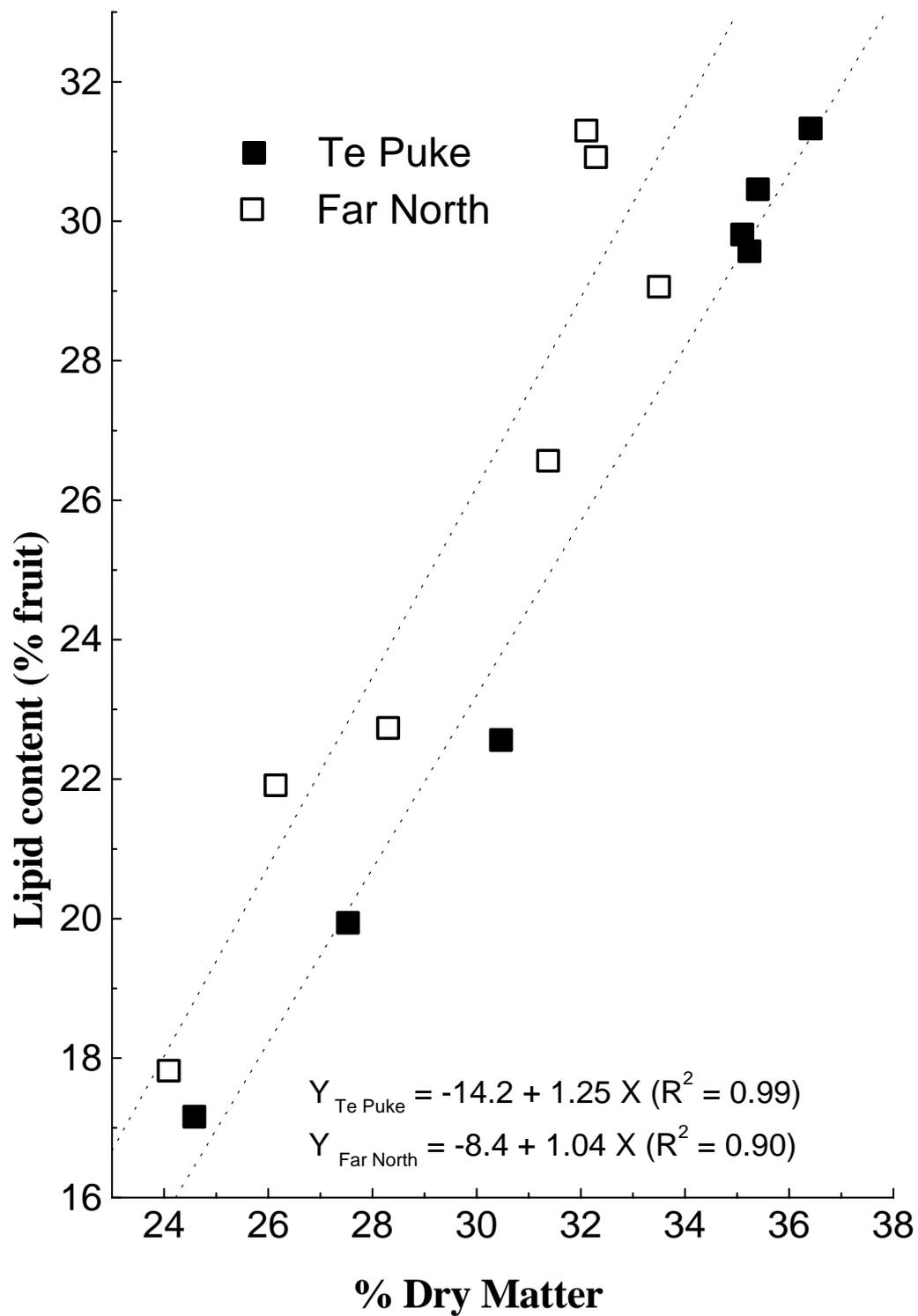


Figure 2. Correlation between mean lipid content and mean dry matter of 'Hass' avocado fruit harvested from Te Puke and the Far North from September to April. Each point is the average of four replicates of five fruit.

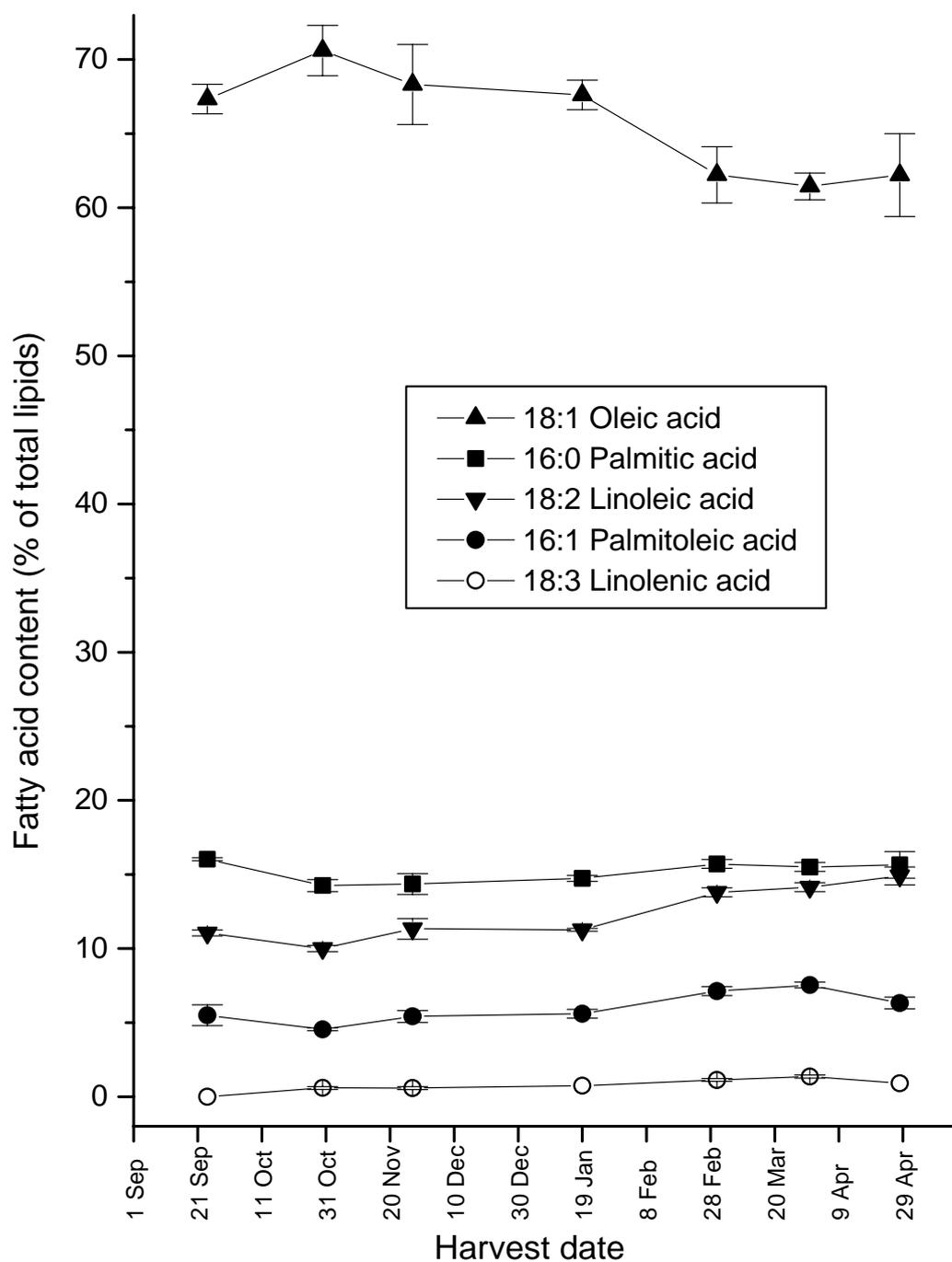


Figure 3a. Te Puke fatty acid content as a percentage of total lipids for 'Hass' avocado fruit harvested from September to April. Each point is the average of four replicates of five fruit. Vertical bars = SEM.

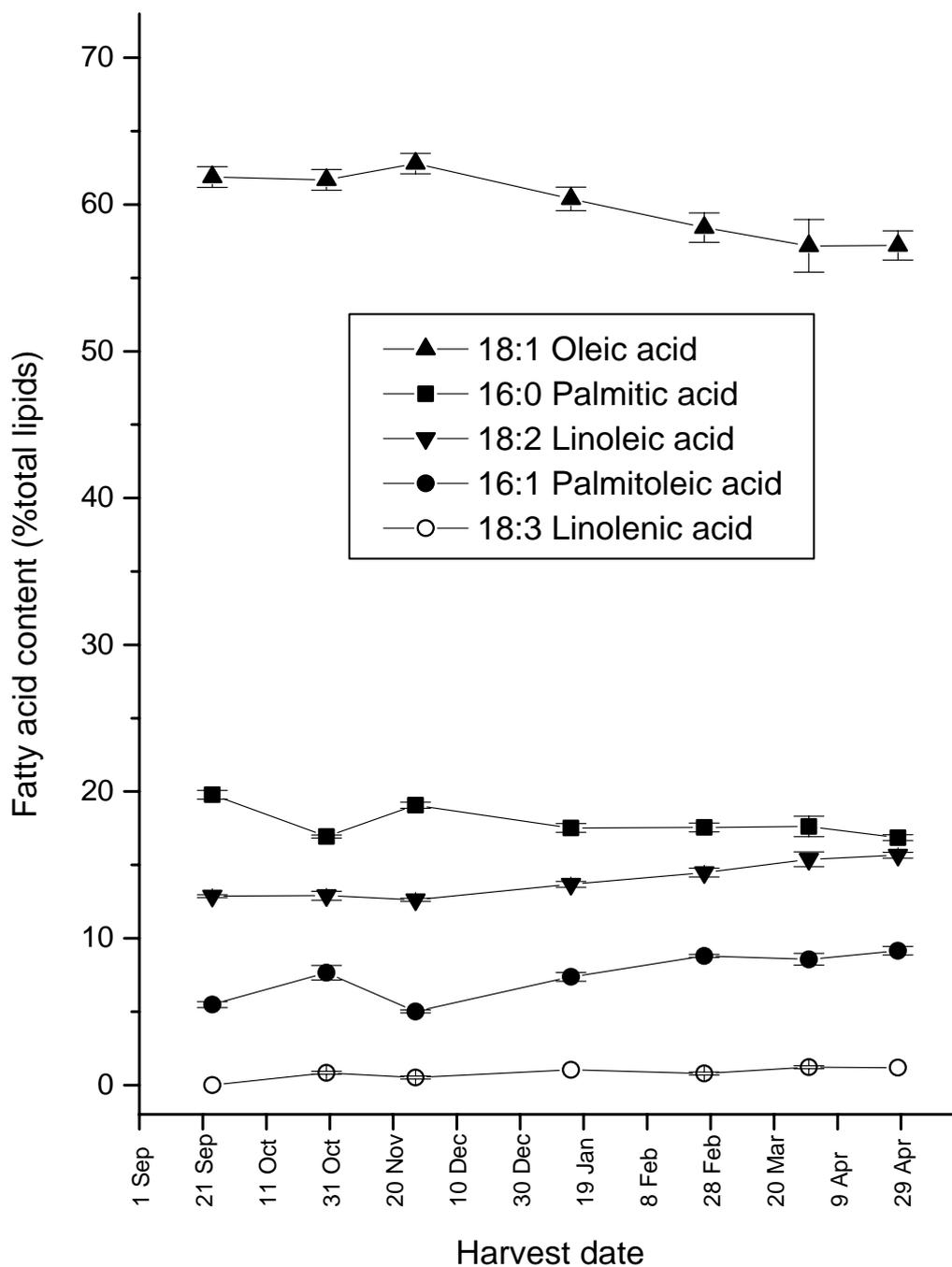


Figure 3b. Far North fatty acid content as a percentage of total lipids for 'Hass' avocado fruit harvested from September to April. Each point is the average of four replicates of five fruit. Vertical bars = SEM.

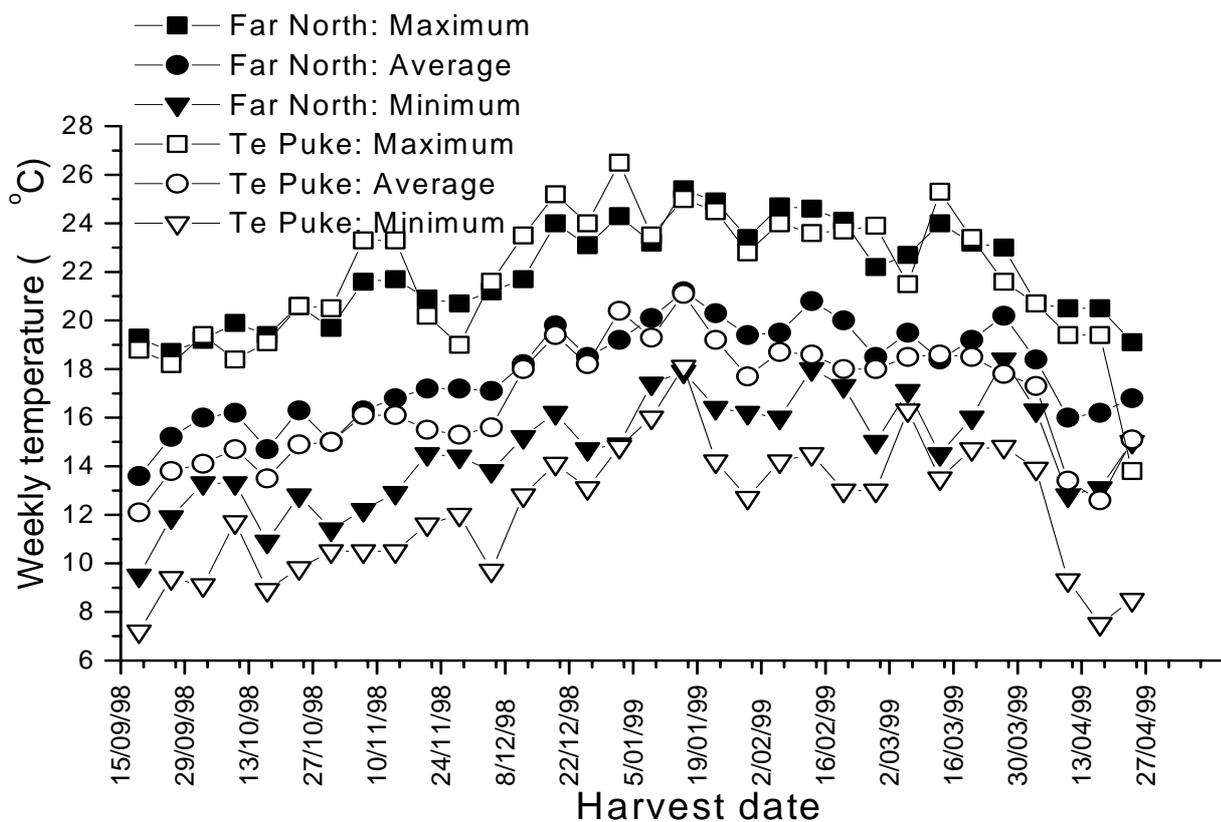


Figure 4. Average weekly temperatures (minimum, average and maximum) for Far North and Te Puke orchards over the sampling period. Temperature was logged every hour (see Appendices 3 for raw data).

DISCUSSION

We have confirmed the close relationship between lipid content and dry matter in avocado fruit previously reported for NZ-grown avocado fruit by Lawes (1980) and Hopkirk (1989), and in overseas studies by Stahl (1933), Biale and Young (1969), Appleman (1969), Kikuta and Erickson (1968), Lee et al., (1983), and Ranney et al. (1992). Thus, for Te Puke and the Far North, fruit water content steadily decreased as lipid content increased ($R^2 = 0.96$) during the time of assessments. The correlation equations calculated in Figure 2 mean that we can predict the percentage of lipids in the fruit at a given a percentage dry matter or vice-versa (although with higher accuracy for fruit from Te Puke than for the Far North).

In comparison to previously reported methods for the determination of lipids in avocados, this optimised technique offers advantages such as small sample size, low expense of operation, simplicity, accuracy and relatively fast determination (particularly for large number of samples). This new technique was able to extract on average 5% more lipids than the extraction using the Soxhlet technique. Lewis et al. (1978) also demonstrated that polar solvents such as chloroform/ methanol, extracted lipids on average 5-8% higher than the Soxhlet method (using petroleum ether). They suggested that chloroform/ methanol is polar enough to release some protein-bound lipids, probably phospholipids and glycolipids. In this study both techniques were found to be positively correlated ($R^2 = 0.7$), but it is recommended that the two techniques be compared further using the Soxhlet technique at least twice in the season. Different lipid extraction methods and conditions can give different results. Care should therefore be taken in making comparisons between results reported here and elsewhere.

Overall, two stages of lipid accumulation could be distinguished. An initial stage of rapid increase in levels, occurring from September until January for Te Puke, and from September until March for the Far North, was followed by a second stage of slow increase in lipid content for the rest of the season. Eaks (1990) reported that a reason for the decrease in total lipids content after December (Southern Hemisphere equivalent) could be that lipid synthesis has slowed or stopped but the fruit are still increasing in weight. In addition, the late peak in lipids in the Far North may be due to a change in climate temperatures. Kaiser et al., (1992) suggested that lipids may have been respired due to high temperatures thus, delaying its synthesis. Orchard temperature records during this period (Appendix 3) show that the maximum temperatures of the two regions are similar, but that Te Puke has lower average and minimum temperatures. Our results suggest that fruit from the Far North may take longer to achieve maturity. However further research, examining for instance changes in a wider range of environments, is necessary to correlate these results found with climatic temperatures.

The Far North region recorded higher average temperatures than Te Puke region. Nevertheless, fruit from Te Puke always showed higher values for dry matter (and lipid content). Lawes (1980) found similar values for lipid content (19.5% in early November and 22.9% mid January) to those found in this work for the Far North (19.5% end of October and 23% in mid January). However, Lawes only studied fruit from one area in New Zealand (Gisborne) and on just two occasions during the season. Conversely, Hopkirk (1989) found higher values of dry matter and lipid content for fruit from Kaitaia (in the Far North) than for fruit from the Bay of Plenty. In Kaitaia, dry matter content increased from 31% to 35% and lipids increased from 18% to 23% from September to February. In the Bay of Plenty, dry matter increased from 28% to 36% while lipids increased from 16% to 23%. Hopkirk's results agree with Kaiser and Wolstenholme (1994) in South Africa who also found higher lipid content in fruit from the warmer site in comparison to that from the cooler site during part of

the development period they were studying. These contrasting results may imply that the temperature of the growing region is not the only factor that affects the maturity of avocados. Therefore further research, including fruit from more regions and more orchards within a region, is necessary to assess the different factors that may affect the maturation of the fruit.

The sum of lipids and water content remained fairly constant for each region over the period of study (September until April). This implies that the rate of increase in the percentage of lipids is the same as the rate of decrease in the water content in the fruit. Although there may be some slight variation between growing regions and cultivars, this constancy has also been reported before in overseas research (Pearson, 1975; Swarts, 1976; Kruger et al., 1995) and in New Zealand by Lawes (1980). Hopkirk (1989) also suggested this relationship, however the researcher's approach was that, due to the close relationship between dry matter and lipid content, the non-lipid dry matter fraction remains constant. Thus, for instance for fruit in the Bay of Plenty, the non-lipid dry matter value was calculated to be 10.5%. Although Stahl (1933) considered that water content could vary with rainfall, this calculation of water plus lipid content or non-lipid dry matter (which should remain fairly constant during maturation), is easy and practical to be used by growers and packers.

Oleic acid was the main fatty acid being synthesised and deposited as tryglicerides in the mesocarp tissue of the fruit. Thus, the increase in total lipids is due primarily to the synthesis of oleic acid. These results are in accordance with Eaks (1990). In this study the five fatty acids that occurred in significant amounts were oleic (18:1), palmitic (16:0), linoleic (18:2), palmitoleic (16:1) and linolenic acid (18:3). These fatty acids were also identified in similar amounts for part of the maturation period in California by Eaks (1990), in Chile by Luza et al., (1990), in South Africa by Kaiser and Wolstenholme (1994), and in Japan by Inoue and Tateishi (1995).

The levels of oleic acid, the cholesterol-reducing fatty acid, were 10% lower in the Far North than in Te Puke. In addition, the saturated palmitic acid, was 20% higher in the Far North than in Te Puke. On average, the sum of beneficial monounsaturates (oleic and palmitoleic acid) measured was about 7.5% higher in fruit from Te Puke (72%) than in fruit from the Far North (67%). Similar effects of climate on fatty acids were found by Kaiser and Wolstenholme (1994) in their study. They found oleic acid was approximately 20% lower in the warmer site than in the cooler site, palmitic acid was 16% higher in the warmer site than in the cooler site, and the sum of monounsaturates was about 10% higher in the cooler site than in the warmer site. Nevertheless, Far North avocados had lower levels of monounsaturated oleic acid than did Te Puke fruit. The levels of oleic acid (60%) and palmitoleic acid (7%) compare favourably to those of the recommended olive oil (56- 83% oleic acid and 0.3-3.5% palmitoleic acid) (IOOC, 1984 summarised table by Kiritsakis, 1990). The nutritional properties of olive oil as a cholesterol-reducing food are well known and are shown by the low indexes of coronary diseases in countries with high consumption rates of olive oil (Andrikopolous, 1989). In addition, the polyunsaturated to saturated ratio ($P:S$) has been suggested as an indicator or measure of whether the diet promotes coronary heart disease or not (known as index of atherogenecity). Over the period of study (September to April) $P:S$ varied from 0.7 to 1 in both regions. It can be seen that the ratio increases with maturity. However the averages for the season in both sites are about the same, 0.9 for Te Puke and 0.8 for the Far North. In California Slater et al., (1975) reported for 'Hass' avocados an average $P:S$ ratio of 0.75 from September to November (Southern Hemisphere equivalent). Olive oil $P:S$ ratio is in the range of 0.14-1.19 depending on the growing region (IOOC, 1984 summarised table by Kiritsakis, 1990). However, recent developments in nutrition reported better health benefits from a high dietary ratio monounsaturated (especially oleic acid) to saturated fatty acids ($M:S$) than from a diet with a high $P:S$ ratio. For instance for olive oil

M:S ratio is in the range of 3.1- 9.2 depending on the growing region. We found an *M:S* average ratio of 4.7 for Te Puke fruit and 3.8 for the Far North.

CONCLUSIONS

This work confirmed the significant and positive relationship that exists between total lipids and dry matter content in avocados. Over the harvest period, fruit from Te Puke had a consistently higher lipid content (and dry matter content) than fruit from the Far North. At both sites, the beneficial monounsaturated oleic acid was the major fatty acid synthesised. However, fruit from Te Puke showed higher levels of oleic acid than fruit from the Far North. This suggests that there may be a region-temperature effect influencing the synthesis and composition of lipids in the fruit. Further research is necessary to determine the extent of this effect. In addition, the ratio of monounsaturated (oleic and palmitoleic acid) to saturated fatty acids (palmitic acid) and the ratio of polyunsaturated (linoleic and linolenic acid) to saturated fatty acids found for the Far North and Te Puke regions compare favourably with those of the recommended olive oil.

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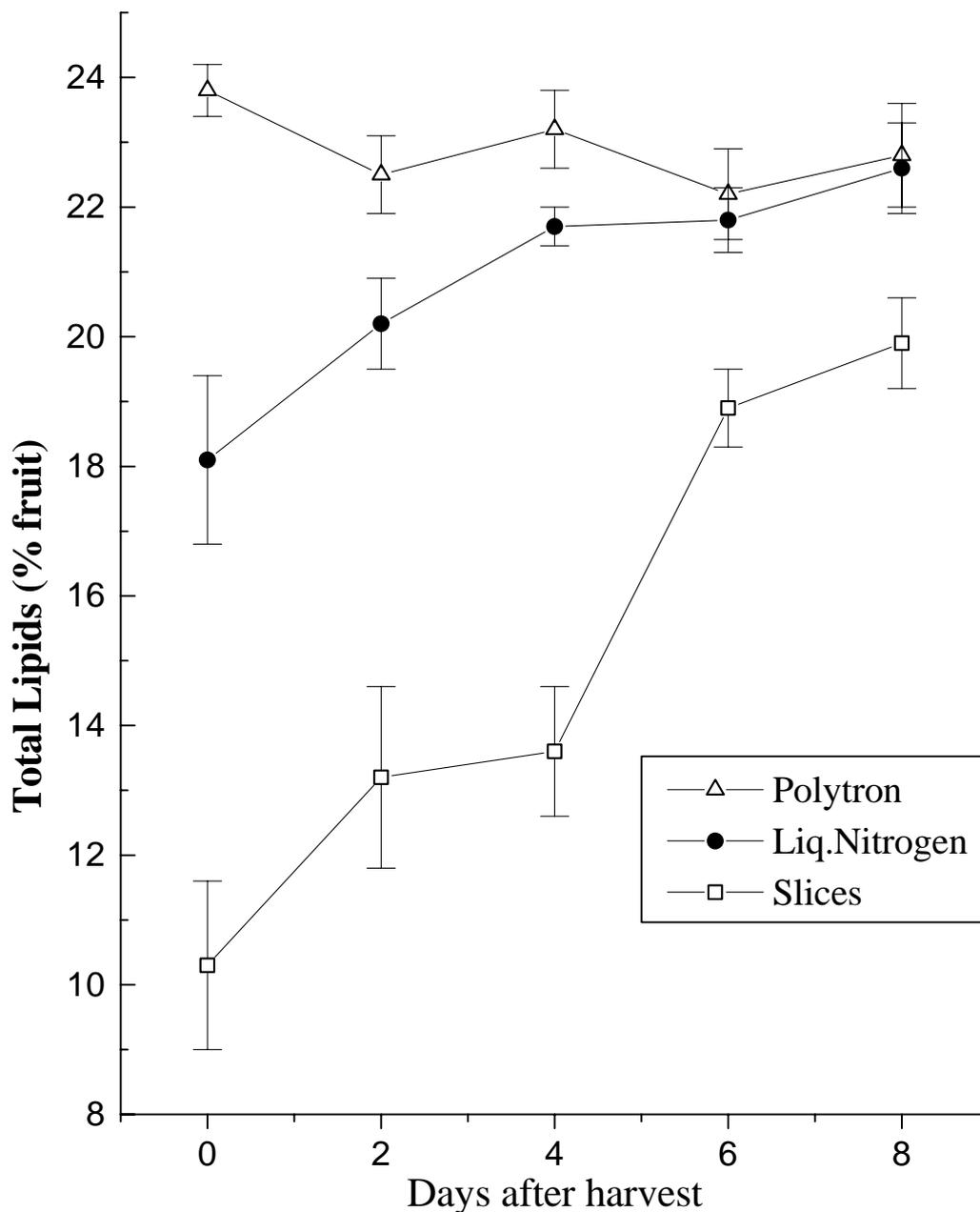
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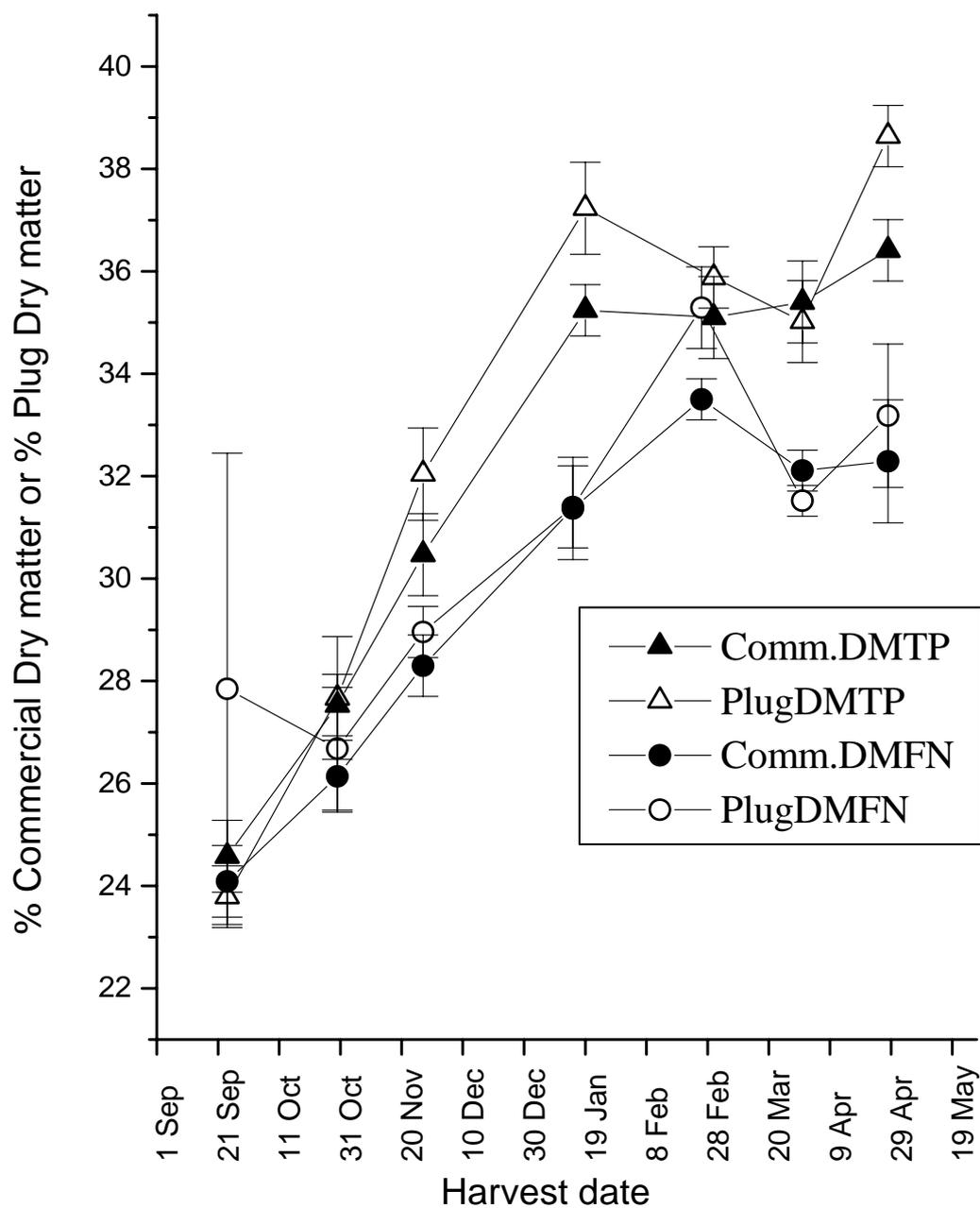
ACKNOWLEDGEMENTS

The authors gratefully acknowledge the assistance of Jim Clark and Ron Bailey for harvesting and sending fruit.

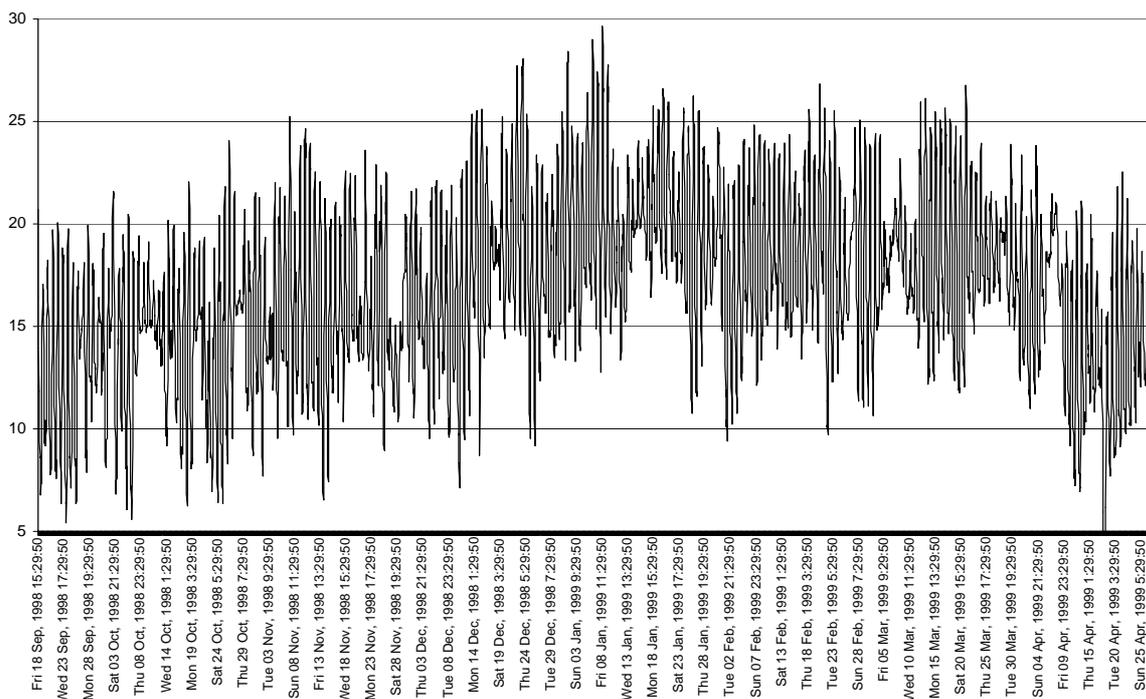
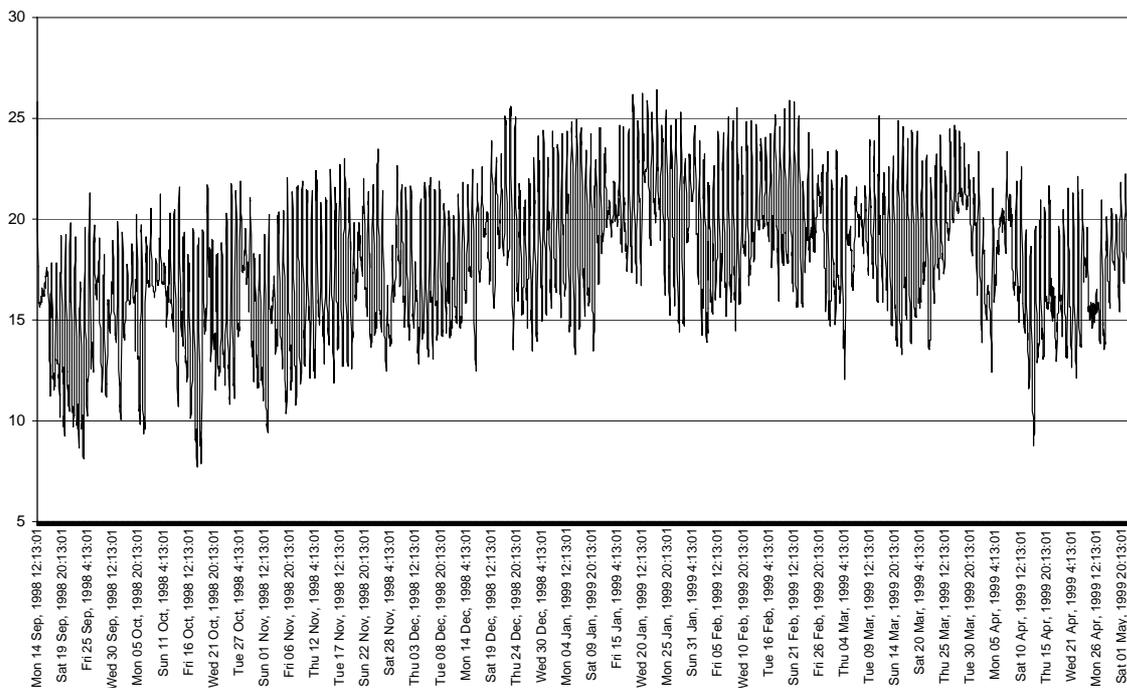
APPENDIX



Appendix 1. Mean percentage of lipids extracted from 'Hass' avocado fruit immediately after harvest, and after 2, 4, 6 and 8 days (8 = ripe soft) after harvest . Fruit were ethylene treated at 17 °C for 48 hours. Each point is the average of six replicates of three fruit. Samples were extracted using three different methods. Vertical bars = SEM. Initial average dry matter = 35%, n= 18.



Appendix 2. Mean commercial dry matter and plug dry matter of 'Hass' avocado fruit harvested from Te Puke and the Far North from September to April. Each point is the average of four replicates of five fruit. Vertical bars = SEM



Appendix 3. Temperatures of Far North (top) and Te Puke (bottom) orchards as recorded on an hourly basis.