CHAPTER 1

THE MACADAMIA NUT
SUMMARY

Different aspects on the shelf life of macadamia nuts have been studied previously such as: the effect of moisture, light and heat in both raw and roasted nuts; the direct application of antioxidants on dry roasted nuts; vacuum packaging for raw and roasted nuts; the maximum safe storage moisture and relative humidity for macadamia nuts-in-shell; and the naturally present antioxidants in macadamia nuts of the variety Nelmar and Macadamia integrifolia of the "Yonik" variety. However, the use of an accelerated shelf life to predict rancidity, the measurement of rancidity using headspace gas chromatography, the determination of naturally present antioxidants and the effect of water activity on the texture of Macadamia integrifolia has not been studied. Studies such as the relationship between rancidity and water activity in raw and roasted kernels, an accelerated shelf life with the measure of hexanal at different temperatures and the amount of individual and total tocopherols present by methods such as GC and HPLC need to be investigated.
INTRODUCTION

The Macadamia or Queensland nut is a native of Australia which grows naturally in the coastal regions of Southern Queensland and Northern New South Wales. It is a new crop (domesticated for the first time in 1858 in Australia) when compared to crops such as corn, sweet potato, cacao and kidney beans which were cultivated about four thousand years ago (Rosengarten, 1984).

As reported in the Australian Macadamia Society press release (1990), Australia is the largest exporter of Macadamia nuts in the world and sells to about 25 countries. By the mid-nineties it hopes to be the largest producer. At present, the market base is being expanded due to the large production increase taking place. There is therefore, a need for the industry today to have a good understanding of the shelf life of this unique kernel.

In 1988 and 1989 growers received a high price for the nut in shell which gave them high expectations for the following year. The production for 1990 was high but quality was poor which slowed down processing and increased the costs of storage for the processors. There was fear in that year that the non-contracted nut-in-shell crop would be unsold.

In the past, the Australian Macadamia Society (AMS), aimed at funding the grower side of the industry (Industry Review Update, 1990). It was outlined in the AMS research priorities in the macadamia industry 1989-90 (1989), that funding priorities were given to topics such as sustainable Agriculture, root system physiology, mechanical harvesting methods, insect and vermin control, pesticides and chemical residues, botrytis, nematodes and rootstock and variety trials. With increased production, there was a definite change in the research priorities for 1990.
In the AMS Research priorities for the macadamia industry in 1991-1992 and published in 1990, the priorities were diversified to marketing, promotion, processing and storage. In one of the surveys conducted by Warfield (1993) it was found that the most positive attribute of macadamia nuts was taste and other attributes included crunchy texture.
THE MACADAMIA NUT

HISTORY AND DESCRIPTION OF THE NUT

Production of Macadamia nuts in Hawaii, from cultivated trees started much earlier than in any other country following its introduction in 1892 (Howes, 1961). Although the botanists in Australia named the tree over 125 years ago after John Macadam, a scientist, medical doctor, philosopher and politician (born in 1827 and died in 1865) the farmers in Australia only took advantage of cultivating the tree in 1950 (Shigeura and Ooka, 1984).

These nuts belong to the Family Proteaceae whose edible nuts are produced by two species: *M. integrifolia*; and *M. tetraphylla*. Both species reach a height of sixty feet and spread forty feet when well grown. Eight other wild species of Macadamia nuts exist which are small, bitter and unpalatable due to the presence of a cyanogenic glucoside (Rosengarten, 1984). Their characteristics have been described by Trouchoulias et al. (1989) as resolved by L.A. Johnson in 1954. *Macadamia tetraphylla* (rough shell type) usually has a pebbled surface. Mature plants have four leaves at the node while seedlings have two. Sometimes leaves have very short petioles or none at all. The margins on the leaf are serrated with spines along the edges.

The young leaves are either purple or reddish in colour and the flowers are pink. *Macadamia integrifolia* (smooth shell type) has a smooth surface. There are three leaves (10 to 30 cm each) at the node in mature plants. The margins on mature leaves have no spines. The young leaves are pale green or bronze in colour while the flowers are creamy white.
CULTIVARS AND AREAS OF PRODUCTION

Trochoulias et al (1990) indicated the most important characteristics sought in selecting a cultivar commercially as early and frequently bearing plants, high yield kernel recovery, all nuts falling off the trees at maturity, firm limb structure, plants not susceptible to insect and disease attack, and kernels with a high oil content.

Most of the varieties of macadamia nuts grown in the world were developed from Hawaiian cultivars namely: Keaouhou, HAES (Hawaiian Agricultural Experimental Station) 246; Kakea, HAES5508; Ikaika HAES333; Kau HAES 344; Keaau, HAES 660; Mauka, HAES741 and Makai HAES5800. The Australian cultivars include: Hinde, H2; Own Choice; A4 and A16.

Trochoulias et al (1990) reported on the production of Macadamia nuts in the different parts of the world. Production of Macadamia tetraphylla is from the coastal areas of Northern N.S.W and M.integripolia from South East Queensland. Both are in the latitude between 25°S and 31°S. Production has been reported in Hawaii. Intensive plantings exist in places such as Costa Rica, Malawi, South Africa, Guatemala and Kenya and minor cultivations are in Brazil, China, U.S.A, Thailand, NewZealand and Zimbabwe.

CHEMICAL COMPOSITION OF MACADAMIA NUTS

It is essential to know the composition of a particular food product as this helps in explaining the different scientific issues needed in maximising the quality of the end product. Improvement may be required in agronomic practices, post harvest handling, processing, storage, and distribution. Data on the composition of tree nuts in general varies widely. Beuchat and Worthington (1978), explained that this variation was due to factors such as lack of uniformity in analytical techniques used for quantification, changes in the agronomic practices and the introduction of new cultivars.
Trochoulias (1989), reported on the constituents of 100 g *Macadamia integrifolia* kernels as shown in Table 1-1. The highest constituent is fat (78%).

Prichavudhi and Yamamoto (1965) found total sugars as 4.57% and reducing sugars were 0.06% in fresh *Macadamia integrifolia*. They found that nuts with a low reducing sugar content (which was dependent on the moisture content) gave the best quality roasted nuts. Dela Cruz et al. (1966) obtained values of 4.98% total sugar and 0.07% reducing sugar in *Macadamia integrifolia* whose initial moisture content was 1.1%. Cavaletto et al. (1968), determined the average total sugar to be 5.1% and a reducing sugar of 0.04% on a dry weight basis with three varieties of *Macadamia integrifolia*. Macadamia nuts of the cultivar Nelmar were studied by Fourie and Basson (1990) and were found to have 0.11% reducing sugars (glucose and fructose) and 4.09% sucrose.

Saleeb et al. (1973) reported on the protein and the amino acid composition of the oil-free meal of Macadamia nuts. The protein content on a wet (N x 6.25) basis was 34.8% in F1 hybrid, 34.9% for *M. tetraphylla* and 39.8% in *M. integrifolia*. In *M. integrifolia*, *M. tetraphylla* and F1 hybrid, the total amino acids were 30.6%, 27.7% and 27.1% respectively as shown in Table 1-2.

Macfarlane and Harris (1981) reported on the difference in fatty acid composition due to different grades as shown in Table 1-3. The species nor cultivar were not indicated. The main fatty acid constituent is oleic acid (mono unsaturated) with a very small amount of linoleic and palmitoleic acid indicating that it is relatively stable to oxidative deterioration. Although, the fatty acid composition differed as presented in Table 1-4 by various researchers the major constituent in all the studies was oleic acid.
A difference in fatty acid composition has also been reported between species by Saleeb et al. (1973) as presented in Table 1-4. Significant differences in stearic, oleic, arachidic and eicosenoic acid between *Macadamia integrifolia* and *Macadamia tetraphylla* were found. In their study, palmitic, palmitoleic and oleic acid were reported to account for 90% of the fatty acids present.
PROCESSING OF MACADAMIA NUTS

USES OF MACADAMIA NUTS

Nuts are eaten raw or cooked in oil, roasted and salted as stated by Duke (1989). Trochoulias et al. (1990) indicated that the nuts are eaten dry roasted, lightly roasted in coconut oil or in a wide range of confectionery products. The husk is used as an under tree mulch and the shell is useful for fuel to dry nuts or as an extender in plastic manufacture. Husks proved to be good in potting mixes (Trochoulias, 1989).

HARVESTING

Prior to processing, the nuts are harvested and then husked. Harvesting is a vital step as the quality of the nut will greatly depend on it. Trochoulias et al. (1990) indicated that before the actual process of harvesting is begun, the ground is prepared by spraying herbicides to control grass and weeds. Any immature or left over nuts are removed from the ground. After the ground is cleared the mature nuts on the ground are brushed from under the trees into windrows using mechanical sweepers ready to be picked by harvesting machines. Ethephon can be applied to encourage obscission and finish the harvest more quickly. The use of mechanical harvesters is economical compared to manual harvesting as more nuts are collected. The disadvantage is that they cause damage to the soil and roots. Their use in the wet season can be hazardous and therefore are not used. The nuts on the rocky or steep ground can only be picked by hand.

Spooner (1987) stated the major advantages of both manual and mechanical harvesting. In manual harvesting the following are the advantages: most weather conditions are suitable to carry out harvesting; the mulch and top soil are undisturbed as the nuts are not swept or blown; no mechanical faults involved; there is no need for precleaning as the stones are not picked up; and steep slopes and corners can be reached.
In mechanical harvesting the advantages are that, one is assured of harvesting as labour shortage is not experienced, no paying casual workers is needed, and large farms have low cost operation.

HUSKING

The nuts are husked immediately after harvesting. Woodroof (1979) reported that when the nuts are freshly harvested (green) the moisture content is high and the shells fit well giving a good protection to the kernels. If they dry, the kernels shrink and the impact may possibly cause damage. Nuts left without husking for more than twenty four hours result in heat build up thus causing deterioration. It is best to leave the nuts on the trees if they are not to be not husked immediately (Trochoulias et al., 1990).

DRYING

The process of dehydration in nuts is vital as the moisture content initially will determine the shelf life of the nuts. Dela Cruz et al. (1966) found that kernels with low moisture content initially (1.1%) showed good stability during storage. If storage of nuts for long period is required before cracking, Cull (1984) indicated that a temperature of 52°C is used to bring the moisture content to 4.5% and a further 72°C to get to a moisture content of 1.5%.

Prichavudhi et al. (1965) discovered that the elevated temperatures used for drying fresh high moisture containing nuts resulted in dark centres when roasted due to high reducing sugar content which came about as a result of sugars being converted to reducing sugars. Enzymatic activity was favoured by the high moisture content. Cavaletto et al. (1968) studied the effects of in shell storage on the quality of roasted nuts and found that nuts stored at 1.2% kernel moisture in their shells for twelve months, were not different in quality after roasting over those that were freshly harvested.
The nuts are dried either in silos or sun dried to reduce the moisture content. In silos they are dried with unheated air. The nuts have to at least be 10% moisture in shell before selling them to the processor (Trochoulias et al., 1990).

CRACKING
Palipane (1992) explained that cracking of nuts below the moisture content of 11.5% resulted in the recovery of kernels with less damage as the space between the shell and the kernel becomes less prominent at such moisture levels.

The nuts are dried to 1.5% moisture, they are cracked with machines using compression force to obtain nuts with minimal damage (resulting in a high percentage of whole kernels) as chips and fines are regarded as low grade (Shigeura and Ooka, 1984).

SORTING
Screens and electronic colour sorters are used to remove the dark looking kernels and finally the sorting is carried out manually to remove any unwanted nuts or other impurities (Shigeura and Ooka, 1984). At this stage the raw kernels can be stored at a temperature of about -17.8°C for about sixteen months as recommended by Cavaletto et al. (1966) if required raw by consumers or processing later.

ROASTING
Woodroof (1979a) reported that the dried kernels are either dry roasted in the oven (at 135°C for 40 to 50 minutes) or roasted in deodorised coconut oil at 135°C for 12 to 15 minutes depending on the grade of nuts. Grade one has a high oil content and a low specific gravity. It can either be dry or oil roasted giving the same quality whereas grade two nuts become dull when dry roasted. For both grades oil roasting has been reported to be suitable.
Cooked kernels are removed from the oil, drained, centrifuged and cooled by air. When the nuts are lukewarm an adhesive oil is applied (for oven roasted kernels, a 15% water solution of gum Arabic and for oil roasted an oil which melts at 32.2°C is used) and the nuts are salted.

Mason (1987) recommended roasting times and temperatures for freshly harvested *Macadamia integrifolia*. Temperatures between 115°C (minimum time of 19 minutes) to 125°C (10 minutes). With these temperatures the process of cooking is manageable as higher temperatures cause nut deterioration. Cavaletto et al. (1971) found that over a period of 13 weeks the oil was used for roasting and the use of oil continuously had no substantial effect on the shelf life or flavour of the nuts.

PACKAGING

Bowden and Reeves (1987) found that the most suitable film for packaging raw Macadamia nuts was a nylon/foil/polyethylene film under a -90 Kpa vacuum. Storage of these nuts for 18 months under ambient conditions maintained their quality. Cavaletto et al. (1971) studied the stability of vacuum packaging (0, -50.8 and -81.2 Kpa) and applications of antioxidants directly to dry roasted Macadamia nuts (about 16 ppm butylated hydroxyanisole and butylated hydroxytoluene). Antioxidant-treated kernels were more stable than untreated kernels regardless of the vacuum level. Vacuum packaging showed some beneficial effect on the untreated kernels but none on antioxidant treated kernels.
RANCIDITY

Rancidity is related to a distinguished, unpalatable odour and flavour of edible oils or fats caused either by oxidative or hydrolytic rancidity, Hamilton (1989). Another form is ketonic rancidity which is less well known (Rossell, 1989a). The rate of lipid oxidation is influenced by factors such as the fatty acid composition, oxygen concentration, temperature, concentration of free fatty acids versus those of corresponding acylglycerols, surface area, moisture and prooxidants (Nawar, 1985). Hydrolytic rancidity is influenced by a combination of micro-organisms and moisture (Rossell, 1989a).

It is one of the deterioration processes that occur during the shelf life of nuts (Burdon, 1989). Most tree nuts contain a high amount of fat, with the exception of chestnuts which makes them less susceptible to rancidity and so they can be stored at moderate temperatures (Woodroof, 1979a).

DIFFERENT FORMS OF RANCIDITY

Hydrolytic rancidity occurs as a result of free fatty acids (lauric and myristic) liberated during hydrolysis of lauric fat in the presence of water catalysed by lipolytic enzymes such as lipases (Barnes, 1982; Rossell, 1989a; and Rossell, 1989b). Rossell (1989a) mentioned the liberation of capric acid besides lauric and myristic during hydrolysis as well as mould and yeast being catalysts. This is encountered mainly in products that contain lauric oils such as palm kernel and coconut.

Free fatty acids (oleic, linolenic and linoleic) that arise as a result of hydrolysis can undergo a further autoxidation (Robards et al., 1988) as shown in Figure 1-1.
Hydrolytic rancidity was explained as not important in oils having more fatty acids of longer chains than those with short ones unless used in deep fat frying of snack foods. In order to minimize hydrolytic rancidity, good practices such as cold storage, good transportation, packaging and sterilisation are necessary (Hamilton, 1989). The enzyme and substrate can also be separated where possible (Barnes and Galliard, 1982).

A second type of rancidity is ketonic rancidity which occurs as a result of fungal attack on the food in the presence of a small amount of water and oxygen in which short chain saturated fatty acids are released. These are then attacked by β-oxidation producing homologous even carbon methyl ketones and aliphatic alcohols (Rossell, 1989a).

A third type of rancidity is oxidative rancidity which is a reaction between oxygen and unsaturated fatty acid moieties. Figure 1-1 shows the oxidative rancidity (autoxidation, photoxidation or enzymic) in path 'T' which is initiated by heat, light, prooxidants or some enzymes such as lipoxygenases. The three oxidation processes produce hydroperoxides with off flavours whose production has not yet been fully understood (Hamilton, 1989).

Lipoxygenase is specific on the substrate and prefers certain positions in the carbon chain as stated by Hamilton (1989). Enzymatic rancidity is not common in snack foods due to the high temperatures used in deep frying but present in raw unprocessed nuts (Robards, 1988). From a food scientist's point of view, in lipid oxidation it is not the fat content that is important but the amount of unsaturated fatty acids such as oleic, linoleic and linolenic acid (Labuza, 1971). Linoleic acid is oxidised 64 times faster than oleic acid and linolenic acid 100 times faster than oleic acid (Hamilton, 1989). Non enzymic oxidation takes place through a free radical chain mechanism as established by Bateman (1954) and cited by many researchers, some of whom include Gray (1978); Chan et al. (1982), Gunstone and Norris (1983a), Robards et al. (1988) and Hamilton (1989).
This involves initiation, propagation and termination as formulated:

**Initiation:**

\[ RH + O_2 \rightarrow R^\cdot + \cdot OH \]

**Propagation:**

\[ R^\cdot + O_2 \rightarrow ROO^\cdot \]
\[ ROO^\cdot + RH \rightarrow ROOH + R^\cdot \]

**Termination:**

\[ R^\cdot + R \rightarrow R-R \]
\[ R^\cdot + ROO \rightarrow ROOR \]
\[ ROO^\cdot + ROO^\cdot \rightarrow ROOR + O_2 \]

\( RH \) = unsaturated fatty acid in which the H is on a carbon adjacent to a double bond.

\( R^\cdot \) = free radical formed

\( ROO^\cdot \) = lipid peroxy radical

\( ROOH \) = hydroperoxides

During initiation, a catalyst such as light, heat, high energy radiation, metal ion or metalloprotein (haem) is involved and a free radical is formed through the interaction of an unsaturated fat with oxygen (Hamilton, 1989). A lipid peroxy radical (ROO-) is formed from the reaction of 'R·' with \( O_2 \) from which a hydroperoxide is formed as well as a free radical 'R·' which makes it a chain process as the presence of a catalyst initiates the reaction. The process is terminated in the last step where two radicals are combined.
The cyclic nature of this process is shown in Figure 1-2. The lipid hydroperoxides formed are very unstable and thus further secondary reaction products are produced, as indicated in Figure 1-3 and these may be responsible for the off flavours. Oxidative rancidity produces toxic components such as lipid peroxides, hydroxy fatty acids, carbonyl compounds - malonaldehyde, cyclic monomers, dimers and polymers, polycyclic aromatic hydrocarbons and oxidised sterols (Sanders, 1989). Chan (1987) described autoxidation as one of the few "natural" chemical reactions that take place on the earth's surface. It is not normally stopped by lowering the temperature due to its low activation energy, 4-5 Kcal mole\(^{-1}\) for the first step and 6-14 Kcal mole\(^{-1}\) for the second step (Hamilton, 1989).

Different ways of preventing oxidative rancidity include avoiding contact with oxygen, reduction of unsaturation in the product and avoiding conditions such as light, high temperatures and pro-oxidants which favour oxidation (Gunstone and Norris, 1983b).
STORAGE LIFE STUDIES IN MACADAMIA AND PECAN NUTS

Storage life studies have been carried out by some researchers. Although the fatty acid composition between pecans and macadamia nuts is not the same, the fat content of both nuts is above 70% as reported by Woodroof (1979b). A brief literature review was done on pecan nuts as a guide due to the limited literature available on macadamia nuts.

The storage of pecans has been reported by Senter et al. (1984) as difficult due to the high degree of unsaturation. They subjected pecan kernels to dielectric heating at different times (1, 2 and 2.5 minutes) and kernels which were exposed to atmospheric steam for 4 minutes in shell were evaluated during a 16 week period at accelerated temperature of 21°C and 65% RH. The flavour quality stabilised during storage. For nuts exposed to high intensity, the peroxide value was high initially but subsided at the end of storage.

Abd El-Wahab et al (1984), in their evaluation on three cultivars, found that heating of the nut prior to storage at 180°C for 15 minutes and storage at 5°C provided a stable product. Subjective and objective measurement of pecan kernels at 21°C, 65% RH were evaluated. The parameters determined by GLC were the most effective objective indicators of quality as they correlated well with sensory analyses (Forbus et al., 1980).

Pecan nuts with a small amount of total tocopherol consisting mainly of γ-tocopherol went rancid after 4 months of storage (Fourie and Basson, 1989).

One of the major criteria used in evaluating quality of pecans is that of colour. During a 16 week storage at 32°C, 50% RH the colour change of testa was found to correlate well with peroxide indicating that it could be used as an index for pecan quality evaluation (Senter and Forbus, 1978). Phenolic acid contents in the testa of pecans decreased during
a 12 week accelerated storage of kernels at 21°C, 65% RH. A high correlation ($r = 0.95-0.97$) was found between sensory quality and dihydroxy and trihydroxy benzoic acid (Senter et al., 1980). Rancidity is slowed down by storage at refrigerated temperatures such as 0°C at 70-75% relative humidity for a year (Woodroof, 1979c).

Cavaletto et al. (1966) carried out work on the stability of raw macadamia kernels and observed that stability decreased with an increase in temperature and moisture. At 1.4% moisture and temperatures of -17.8°C, 1.7°C, ambient and 37.8°C good stability with little change in chemical composition after storage for 16 months was observed. For kernels having a moisture content of 2.3 and 4.3% a low temperature of -17.8°C was recommended. Light was found to have no effect on the stability of raw nuts. Dela Cruz et al. (1966) reported good stability of roasted nuts at 1.1% moisture unlike those at higher levels. Light had no effect on roasted nuts.

The roasting quality and shelf life of macadamia nuts stored in shell for 12 months at 1.2% moisture were not different from those prepared from freshly harvested nuts (Cavaletto, 1968). Hansen and Gough (1977) found the maximum for safe storage for *Macadamia tetraphylla* as 11.8% moisture at a relative humidity of 70%. Changes in peroxide value were useful in predicting the onset of rancidity without the involvement of subjective evaluation in nuts according to a study carried out by Fourie and Basson (1989) on macadamia, pecans and almonds. Spoilage of nuts during longer term storage was due to lipolytic decomposition (Rosenthal et al., 1984).
SOME WAYS OF MEASURING RANCIDITY IN SNACK FOODS

Gray (1978), described sensory evaluation as a sensitive method but impractical and unreproducible. Hydrolytic rancidity can be measured either by factors causing it (the enzyme lipase or the moisture) or the end products of the process, free fatty acids (Rossell, 1989a). Free fatty acids are measured as determined by the AOAC method 940.28 (Helrich, 1990a) by titrating the extracted fat with an alkali.

Rossell (1989a), mentioned ketonic rancidity as closely related to hydrolytic rancidity because its first products are fatty acids with short chains. Moisture, microbial activity and free fatty acids are measured. Volatiles are distilled off and methyl ketones and aliphatic alcohols having odd-numbered carbon chains are identified.

Gray (1978) presented four useful questions in selecting a useful test procedure to measure lipid oxidation as follows:

a) would there be any other deterioration process responsible for the property to be measured other than oxidation?

b) are all oxidising systems capable of having the property being measured?

c) is the particular property specifically measured by that method?

d) is the extent of oxidation being adequately represented by the property that is determined?
One of the methods used in lipid oxidation is peroxide value which in itself is a better indicator of the initial stages of oxidation as the peroxides formed are likely to undergo thermal decomposition and/or further oxidation as in Figure 1-3. This would give an incomplete history of the oxidation that takes place (Gray, 1978).

Peroxide value is measured by titrating the mixture of fat, chloroform-acetic acid and saturated potassium iodide solution with sodium thiosulfate solution as in AOAC method 965.33 (Helrich, 1990b).

Robards et al. (1988) outlined the equation that takes place by the reaction of iodine with peroxides when titrated with thiosulphate solution as follows:

\[ \text{ROOH} + 2\text{H}^+ + 2\text{I}^- \rightarrow \text{I}_2 + \text{ROH} + \text{H}_2\text{O} \]

where

ROOH is a lipid hydroperoxide

\[ \text{I}_2 + 2\text{S}_2\text{O}_3^{2-} \rightarrow \text{S}_4\text{O}_6^{2-} + 2\text{I}^- \]

Another method is that by headspace gas chromatography analysis in which the product is heated and the volatiles collected in the headspace and analysed by the GC. One of the problems which may arise with this method is that further decomposition of compounds could occur when the sample is heated at high temperatures which may not drive the volatiles into the headspace (Rossell, 1989a).
ANTIOXIDANTS

As defined by Gunstone and Norris (1983b), antioxidants are substances which in small amounts interfere with the oxidation process in the oils and fats by delaying the production of noticeable off-flavour and/or odours as shown in Figure 1-4.

They are free radical acceptors (primary antioxidants) which terminate the initiation step in oxidation by forming a stable compound which is not oxidised in autoxidation where a free radical (R) is attacked by oxygen forming peroxides and hydroperoxides. Secondary antioxidants such as thiodipropionic acid and thiodipropionate are capable of decomposing hydrogen peroxide produced during oxidation into stable compounds (Dziezak, 1986).

Some antioxidants act as reducing agents or "oxygen scavengers" such as ascorbyl palmitate, sulfites, ascorbic acid, glucose oxidase and erythorbic acid (Dziezak, 1986). Synergists include citric acid, polyphosphates and ethylenediaminetetraacetic acid (EDTA) but are not antioxidants. Antioxidants used in foods should be safe to use, impart no colour or odour, used effectively at low concentration, easy to add to the food, withstand high cooking temperatures and sold at low cost (Coppen, 1989).

According to the National Food Authority (1992), antioxidants permitted in walnut and pecan kernels are butylated hydroxy-toluene (BHT) at 70 mg/kg and ascorbyl palmitate with no limit imposed. They did not mention the use of these synthetic antioxidants in macadamia nuts. Hoover and Nathan (1981) concluded that the addition of tertiary butylhydroquinone (0.02% as allowed by FDA) to granulated roasted peanuts provided an effective antioxidant which suppressed the formation of carbonyl compounds (20%) as compared to that without the antioxidant whose carbonyl content was increased by 110%.
Cavaletto and Yamamoto (1971) found that the stability of antioxidant (76 ppm butylated hydroxyanisole and butylated hydroxy toluene) treated macadamia kernels was more than that of kernels without antioxidant regardless of the vacuum level. On the other hand antioxidants are present naturally in most raw foods and it has been pointed out that crude vegetable oils which have their own natural antioxidants are more stable than those that are refined (Barnes and Galliard, 1982). Other things being equal, vegetable oils resist oxidative rancidity better than animal fat due to the presence of natural antioxidants (Gunstone and Norris, 1983).

Different findings have been reported on naturally present antioxidants in macadamia nuts. Fourie and Basson (1989) found 2.5 mg/100 g α-tocopherol and trace amounts of γ-tocopherol in macadamia nuts of the variety Nelmar. On the other hand, Rosenthal et al. (1984) revealed that studies on Macadamia integrifolia of the "Yonik" variety showed a high resistance to thermal oxidation but no known natural antioxidants were found.
TEXTURE AND WATER ACTIVITY IN SNACK FOODS

Texture is an important food attribute whose loss leads to consumer rejection of food. A considerable amount of research has been carried out to describe the relationship between water activity and microbial growth and not texture.

There has been no known research carried out to describe the relationship between texture and macadamia nuts. However some researchers have carried out work on snack foods. Katz and Labuza (1981) found that, as the water activity decreased, the sensory acceptability increased in four snack foods (potato chips, popcorn, puffed corn curls and saltines). The correlations for low quality flour biscuits was $r = -0.89$ and $r = -0.94$ for high quality flour biscuits between crispness and relative humidity as was examined by Zabik (1979). Seymour and Hamann (1988) found sensory crispness decreased as the water activity increased in five low moisture foods. Strong correlations ($r>0.90$) were found between sensory crispness and crunchiness and mechanical force and work.

Crunchiness has been described by Szczesniak (1990) as an important universally accepted textural attribute whose loss could result in consumer rejection of the product.
CONCLUSION

Development of many new markets for bulk raw macadamia nuts in different grades and other packs, including high quality products, points to a great need for the understanding of the product in terms of its shelf life. The use of an accelerated shelf life study as presented in Chapter 2 would help understand the stability of the raw kernels. This knowledge would in turn apply to the understanding of the shelf life of new products on the market. With an increased focus on the export markets of macadamia nuts, there is a need for a sensitive, reliable, fast and quantitative method of evaluating the nuts from the time of harvest until they reach their final consumers, local or overseas.

The headspace gas chromatography study in Chapter 3 was conducted to further look into the production of oxidative volatiles relative to storage stability of the nuts. In the same chapter the presence of natural antioxidants in the nuts were likewise determined to help understand the relative stability of the nuts in Chapter 3. There was need to understand at what water activity the product would be rejected by the consumer as this type of deterioration process has never been investigated in these nuts as outlined in Chapter 4.
REFERENCES


Table 1-1.
Constituents of 100 g *Macadamia integrifolia* kernel

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<tr>
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<th>grams</th>
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<th>milligrams</th>
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<tr>
<td>Fat</td>
<td>78.6</td>
<td>Riboflavin mg</td>
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<td>Protein</td>
<td>9.3</td>
<td>Niacin mg</td>
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<td>Carbohydrates</td>
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Adapted from Trochoulias et al. (1989).
Table 1-2.
Amino acid composition of macadamia nut oil free meal of *M. tetraphylla*,
*M. integrifolia* and their F<sub>1</sub> hybrid

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th><em>M. tetraphylla</em></th>
<th><em>M. integrifolia</em></th>
<th>F&lt;sub&gt;1&lt;/sub&gt; hybrid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>1153 (4.2)</td>
<td>1313 (4.3)</td>
<td>1175 (4.3)</td>
</tr>
<tr>
<td>Histidine</td>
<td>599 (2.2)</td>
<td>679 (2.2)</td>
<td>606 (2.2)</td>
</tr>
<tr>
<td>Ammonia</td>
<td>453 (1.6)</td>
<td>475 (1.6)</td>
<td>452 (1.7)</td>
</tr>
<tr>
<td>Arginine</td>
<td>3259 (11.8)</td>
<td>3571 (11.7)</td>
<td>3318 (12.2)</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>3020 (10.9)</td>
<td>3278 (10.7)</td>
<td>2919 (10.8)</td>
</tr>
<tr>
<td>Threonine</td>
<td>943 (3.4)</td>
<td>1058 (3.5)</td>
<td>882 (3.3)</td>
</tr>
<tr>
<td>Serine</td>
<td>1257 (4.5)</td>
<td>1444 (4.7)</td>
<td>1213 (4.5)</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>6586 (23.8)</td>
<td>6974 (22.8)</td>
<td>6557 (24.1)</td>
</tr>
<tr>
<td>Proline</td>
<td>1263 (4.6)</td>
<td>1803 (5.8)</td>
<td>1250 (4.6)</td>
</tr>
<tr>
<td>Glycine</td>
<td>1308 (4.7)</td>
<td>1529 (5.0)</td>
<td>1271 (4.7)</td>
</tr>
<tr>
<td>Alanine</td>
<td>1246 (4.5)</td>
<td>1247 (4.1)</td>
<td>1153 (4.3)</td>
</tr>
<tr>
<td>Cystine</td>
<td>378 (1.3)</td>
<td>371 (1.2)</td>
<td>498 (1.8)</td>
</tr>
<tr>
<td>Valine</td>
<td>1177 (4.2)</td>
<td>1282 (4.2)</td>
<td>1134 (4.2)</td>
</tr>
<tr>
<td>Methionine</td>
<td>337 (1.2)</td>
<td>356 (1.2)</td>
<td>318 (1.2)</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>861 (3.1)</td>
<td>1016 (3.3)</td>
<td>787 (2.9)</td>
</tr>
<tr>
<td>Leucine</td>
<td>1662 (6.0)</td>
<td>1887 (6.1)</td>
<td>1574 (5.8)</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>1238 (4.5)</td>
<td>1315 (4.3)</td>
<td>1124 (4.1)</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>971 (3.5)</td>
<td>1002 (3.3)</td>
<td>887 (3.3)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>27,711 (100)</strong></td>
<td><strong>30,600 (100)</strong></td>
<td><strong>27,118 (100)</strong></td>
</tr>
</tbody>
</table>

Adapted from Saleeb et al. (1973).

<sup>1</sup>Amino acids expressed as percentage of total amino acids recovered.
Table 1-3.
Fatty acid composition of three grades of Macadamia kernel oil (g/100 g of total fatty acid).

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Grade I</th>
<th>Grade II</th>
<th>Grade III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lauric (12:0)</td>
<td>1.4</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Myristic (14:0)</td>
<td>0.8</td>
<td>0.7</td>
<td>0.8</td>
</tr>
<tr>
<td>Palmitic (16:0)</td>
<td>8.0</td>
<td>9.2</td>
<td>8.5</td>
</tr>
<tr>
<td>Palmitoleic (16:1)</td>
<td>15.6</td>
<td>21.6</td>
<td>24.6</td>
</tr>
<tr>
<td>Stearic (18:0)</td>
<td>3.4</td>
<td>3.4</td>
<td>3.3</td>
</tr>
<tr>
<td>Oleic (18:1)</td>
<td>64.2</td>
<td>57.0</td>
<td>54.8</td>
</tr>
<tr>
<td>Linoleic (18:2)</td>
<td>1.6</td>
<td>1.5</td>
<td>1.9</td>
</tr>
<tr>
<td>Arachidic (20:0)</td>
<td>2.4</td>
<td>2.7</td>
<td>2.6</td>
</tr>
<tr>
<td>Eicosenoic (20:1)</td>
<td>2.1</td>
<td>3.1</td>
<td>3.1</td>
</tr>
<tr>
<td>Behenic (22:0)</td>
<td>0.5</td>
<td>0.7</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Adapted from Macfarlane and Harris (1981)
Table 1-4.
Fatty acid composition of Macadamia nuts as presented by four researchers.

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Fatty acid content (g /100 g of total fatty acid )(^a) of Macadamia:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>integrifoli(^1)</td>
</tr>
<tr>
<td>12:0</td>
<td>0.62</td>
</tr>
<tr>
<td>14:0</td>
<td>0.75</td>
</tr>
<tr>
<td>16:0</td>
<td>6.15</td>
</tr>
<tr>
<td>16:1</td>
<td>19.11</td>
</tr>
<tr>
<td>17:0</td>
<td>1.64</td>
</tr>
<tr>
<td>17:1</td>
<td>1.34</td>
</tr>
<tr>
<td>18:0</td>
<td>1.50</td>
</tr>
<tr>
<td>18:1</td>
<td>1.74</td>
</tr>
<tr>
<td>22:0</td>
<td>0.79</td>
</tr>
</tbody>
</table>

1. Adapted from Dela Cruz et al. (1966).
2. According to Saleeb et al. (1973)
3. Adapted from Beuchat and Worthington (1978)
4. According to Fourie and Basson (1990)
\(^a\) Standard deviations in parenthesis
\(^*\) Trace
FIGURES
Figure 1-1.

Reaction scheme for (I) oxidative and (II) hydrolytic rancidity (Robards et al, 1988).
Triglycerides

Oxidation (autoxidation, photooxidation or enzymic)

Hydrolysis (hydrothermal or enzymic)

Triglyceride hydroperoxides
Mono-, di-, epoxy and cyclic peroxides

Free fatty acids + glycerol

Hydrolysis

Oxidation

Free fatty acid hydroperoxides

Secondary and tertiary products
saturated, unsaturated, di- and epoxyaldehydes
ketones
lactones
furans
monobasic, dibasic, oxo and hydroxy acids
saturated and unsaturated hydrocarbons etc.
Figure 1-2.

The cyclic chain of autoxidation (Robards et al, 1988).
Some paths of decomposition of fat hydroperoxide (Gray, 1978).
Figure 1-4.

Inhibition of peroxide decomposition by an antioxidant (Gunstone and Norris, 1983b).
RH + H → R' + O₂ → ROO' → RH → ROOH + R

phenolic antioxidant

hydroperoxide

relatively stable antioxidant radicals
CHAPTER 2

ACCELERATED SHELF LIFE STUDIES ON MACADAMIA NUTS
SUMMARY

Raw *Macadamia integrifolia* kernels were stored for eleven weeks at four accelerated temperatures (30°C, 35°C, 40°C and 45°C) and a relative humidity of approximately 50%. Rancidity was determined subjectively using trained panellists and objectively using peroxide value and % free fatty acid (% oleic acid). Analyses were done subjectively at 14, 35 and 56 days and objectively at 0, 14, 35, 56 and 77 days. Hydrolytic rancidity as determined by % free fatty acids followed zero order kinetics. Oxidative rancidity as determined by peroxide values followed a zero of reaction order kinetics at temperatures 30°C and 35°C and a first order at 40°C and 45°C. Hydrolytic rancidity at low temperature (25°C) was predicted using an Arrhenius plot of log K versus reciprocal (absolute) temperature (1/T). Correlations were found between subjective and objective measurements (r > 0.65 p > 0.05).
INTRODUCTION

SHELF LIFE OF MACADAMIA NUTS

The stability of macadamia nuts has been studied under certain conditions. Cavaletto et al. (1966) observed little change in chemical composition of raw kernels stored for 16 months at 1.4% moisture and temperatures of -17.8°C, 1.7°C, ambient and 37.8°C. Dela Cruz et al. (1966) reported good stability of roasted nuts stored for 16 months at -17.8°C, 1.7°C, at ambient temperature and 37.8°C. The quality of roasted Macadamia kernels from nuts stored in shell at 1.2% moisture for 12 months was not different from those prepared from freshly harvested nuts (Cavaletto et al 1968). Hansen and Gough (1977) found the maximum safe storage for whole nut Macadamia tetraphylla as 11.8% moisture and 70% relative humidity. An accelerated shelf life test has not been reported before for macadamia nut kernels.

Foods containing unsaturated fats are subject to attack by oxygen resulting in rancidity. According to Labuza (1971), the development of most rancid flavours in foods is due to the degree of unsaturated fatty acids present in a food.

Macadamia nuts have been reported as having a high percentage of unsaturated fatty acids. Macadamia integrifolia, were found to contain 80-90% unsaturated fatty acids of the fatty acids present as reported by Cavaletto et al (1966), Dela Cruz et al. (1966) and Saleeb et al. (1973). Fourie and Basson (1990) found 64.29% in Macadamia nelson.

ACCELERATED SHELF LIFE STUDIES

Accelerated shelf life studies enable the food scientist to determine the shelf life of a product without actual long-term storage studies.
As reported by Labuza (1984), information such as the major mode of deterioration, environmental conditions (such as temperature, relative humidity and light), factors controlling initial quality or nutritional value during manufacture, packaging material and the kinetics involved in the loss of quality are necessary to make useful predictions on the shelf life of a food.

Paul and Roylance (1962) and Pohle et al. (1964) found that the use of one temperature in accelerated shelf life test of edible oils and fats was not reliable for shelf life predictions at room temperature. This is due to the fact that other reactions such as melting of fat (liquid fat could be a reaction medium) and an increase in water activity may occur at higher temperatures and not at room temperature (Labuza, 1984). This increase in water activity may accelerate the reaction and thus lead to predictions of overlong shelf life at room temperature.

TWO ORDERS OF REACTIONS

Labuza (1984) stated that most literature data in food quality are based either on chemical reaction, microbial growth, death, or sensory value follow a zero or first order reaction (shown in Figure 2-1). A zero and first order reaction for quality change has been described by Labuza (1982a).

The basic kinetic equation can be written as follows:

\[ \frac{dA}{d\theta} = kA^n \quad (1) \]

where

- $A$ = the quality factor being measured
- $k$ = rate constant which depends on temperature and water activity
- $\theta$ = time
- $n$ = a power factor called the order of the reaction
\[ \frac{dA}{d\theta} = \text{the rate of change of the quality factor } A \text{ with time.} \]

For a zero order reaction the loss of food quality based on sensory evaluation, chemical reaction, microbial growth or death is constant at constant temperature and water activity as in equation (2):

\[ -\frac{dA}{d\theta} = k \]  \hspace{1cm} (2)

\[ \frac{A_e}{A_0} = \int_{0}^{\theta} k \, d\theta \]  \hspace{1cm} (3)

The rearrangement and integration of equation (2) would give

\[ A = A_0 - k\theta \]  \hspace{1cm} (4)

\[ A_e = A_0 - k\theta_s \]  \hspace{1cm} (5)

where \( A_0 \) = initial quality value

\( A \) = amount remaining after time \( \theta \)

\( A_e \) = amount of \( A \) at end of shelf-life

\( \theta_s \) = shelf life

\( k \) = rate constant which depends on temperature and water activity

For a first order reaction the rate of loss is given by:

\[ \frac{dA}{d\theta} = kA^1 \]  \hspace{1cm} (6)

Rearrangement of (6) gives (7) and integration gives (8):
\[ \int_{A_0}^{A} \frac{dA}{A} = -\int_{0}^{\theta} k d\theta \quad (7) \]

\[ \ln \frac{A}{A_0} = -k \theta \quad (8) \]

or

\[ \ln \frac{A_E}{A_s} = -k \theta_s \quad (9) \]

where \( A \) = Amount left after time \( \theta \)
\( A_0 \) = Initial quality value
\( A_E \) = Amount left at end of shelf life \( \theta_s \)
\( k \) = rate constant in units of reciprocal time
\( \theta_s \) = shelf life

**ACCELERATION OF TEMPERATURE FOR SHELF LIFE TESTING OF A FOOD: ARRHENIUS MODEL**

As described by Labuza (1984) the Arrhenius model is used to predict how fast a reaction will occur at a certain temperature. For a zero or first order equation, theoretically \( k \) obeys the Arrhenius model in the form:

\[ k = k_0 e^{-E_A/RT} \quad (10) \]

where \( k_0 \) = a pre-exponent constant
\( E_A \) = the activation energy in cal/mole
\[ R = \text{the gas constant in cal/mole K} = 1.986 \text{ or } 8.314 \text{ JK}^{-1}\text{mol}^{-1} \]

\[ T = \text{temperature in K (°C} + 273) \]

On semi-log paper the rate constant plotted against reciprocal (absolute) temperature (1/T) gives a straight line as shown in Figure 2-2.

**THE Q_{10} MODEL**

The Q_{10} model is the change in the rate of reactions that occurs when the temperature is changed by 10 celsius degrees (Richardson and Hyslop, 1985).

If the temperature accelerating factor (Q_{10}) is known at higher temperature, then the expected shelf life at lower temperatures can be determined by extrapolation. Labuza and Schmidl (1985) described the Q_{10} factor as follows:

\[ Q = \frac{\text{rate at temperature } (T+10)}{\text{rate at temperature } T} \quad (11) \]

\[ = \frac{\text{shelf life at temperature } T}{\text{shelf life at temperature } (T+10)} \quad (12) \]

\[ = \frac{t_s(T)}{t_s(T+10)} \quad (13) \]

where \( T = \text{temperature in °C} \)

\( t_s = \text{the shelf life at the indicated temperatures if the temperature difference (Δ) is not } 10\text{°C then this changes to:} \)
If $Q_{10}$ is not provided then the mathematical expression as illustrated in equation (13) could be used in which the reaction follows the zero or first order mathematical expression. An Arrhenius plot as shown in Figure 2-2 could be used to establish the $Q_{10}$ by simply getting the ratio of the shelf life at two temperatures with a difference of 10°C.

SOME DISADVANTAGES OF USING ACCELERATED SHELF LIFE

The disadvantages in using accelerated shelf life have been outlined below (Labuza, 1984, 1982b and Labuza and Schmidl, 1985).

1. Sometimes one is unable to identify the quality index related to the mode of deterioration and the analytical errors involved.

2. When the product is frozen, such as controls, the reactants are in the unfrozen liquid which upon thawing would have a higher rate of quality loss but are not accounted for in the typical kinetic reaction.

3. In a food, there are reactions that take place which cause deterioration. It is possible that above some temperature a reaction with a high activation energy will prevail where as below that temperature, a different reaction would lead to loss of shelf life. Therefore the use of only one reaction will lead to wrong predictions of shelf life.

4. At high temperature, the carbohydrates crystallize which would react at lower temperature in the amorphous state.

5. The solubility of a gas decreases with an increase in temperature thus oxygen becomes limited for the reaction to take place.

6. Products stored in a permeable or semipermeable pouch will have increased moisture loss at high temperature and low humidity compared to one with no moisture change resulting in underprediction at low temperature.
7. At high temperatures proteins become denatured and these could lead to an increase or decrease in the amino acids resulting in the underprediction or overprediction of the reaction.

The objectives of the present study were:

1. to evaluate the effect of temperature on the rate of oxidative and hydrolytic rancidity of raw macadamia nuts (at temperatures, 30°C, 35°C, 40°C and 45°C) in order to predict product storage quality at temperatures below these (25°C was evaluated); and

2. to correlate the sensory and chemical analyses in the prediction of rancidity.
MATERIALS AND METHODS

MATERIALS

Whole shelled first grade *Macadamia integrifolia* of the cultivar Keauhou (Hawaii Agricultural Experimental Station, cultivar 246) were purchased from Macadamia Plantations of Australia. At the factory the nuts were harvested and dehusked. They were placed in the silo at 10% moisture and subjected to the following conditions in order to obtain a final moisture content of 1.5%: 38°C for 48 hours; followed by 48 hours at 45°C; and finally at 60°C for 48 hours. Tests were done on moulds, aflatoxins, coliforms and salmonella. The nuts were then vacuum packed in nylon/foil/polyethylene film and flushed with nitrogen at -15°C. Samples were analysed immediately upon receipt in all the experiments.

STORAGE CONDITIONS

Upon receipt the kernels were divided into four duplicate 2 kg lots of samples and placed in sealed vessels over a saturated solution of magnesium nitrate having a relative humidity of about 50% at four different temperatures of 30°C, 35°C, 40°C and 45°C. The incubators used were light-proof with a good seal. Eight 500 g samples were vacuum packaged and stored at -18°C as standards for sensory and chemical analyses.

CHEMICAL ANALYSES

Initially fat was determined using the soxhlet method described by Pearson (1981). Samples were evaluated objectively at 0, 14, 35, 56, and 77 days of storage. A 250 g sample of nuts was obtained from each treatment (-18°C, 30°C, 35°C, 40°C and 45°C) and the fat was extracted using the Bligh and Dyer method modified by Hanson and Olley (1963). Analyses for peroxide value (PV) method 965.33 and free fatty acids (FFA) method 940.28 were carried out according to the AOAC (Helrich, 1990) within 24 hours of extraction.
DETERMINATION OF WATER ACTIVITY

The nuts were crushed to about 1 mm particle size using a mortar and pestle for measuring the water activity. Water activity at each day of sampling was determined at 20°C using the Novasina Water Activity meter type TH2.

SENSORY ANALYSES

Samples were evaluated for aroma and flavour by fourteen trained panelists at 14, 35 and 56 days of storage. The panelists were selected using the triangle test. Multiple comparison tests were used for further training using nuts with different levels of rancidity by evaluating the aroma and the flavour. For the training of panellists, rancid nuts were used. These nuts had become rancid as a result of exposure to different extreme storage conditions. During sampling 200 g of nuts from each temperature were chopped to about 2 mm size. A sample of 15 g was placed in a plastic cup sealed with aluminium foil and left for about an hour to let the volatiles in the headspace equilibrate and bring the samples to ambient temperatures. The panelists were presented with a reference sample marked 'R' (from -18°C storage) and the other four samples (from the four different temperatures) were each coded with three digits presented in a random order with ambient water for rinsing to help clear the flavour in between testing.

This was carried out in a quiet and comfortable area having individual sensory evaluation booths. The panelists were instructed to evaluate the reference sample first for aroma and flavour followed by comparison of each sample to the reference sample 'R' (aroma was evaluated first and then flavour). The panelists recorded their responses on a score sheet using the hedonic scale ranging from 1 (extremely inferior) to 9 (extremely superior) where 5 indicated no difference in comparison with the reference sample marked 'R'.
STATISTICAL ANALYSIS

Correlation coefficients were determined using the BMDP, a computer statistical package. Significant differences of treatments were tested by Duncan's multiple range test (Duncan, 1955) and analysis of variance at 5%. Assessment of the confidence level of the reaction rate value (true slope) and the goodness of fit of the Arrhenius relation was carried out as described by Labuza (1983a) using computer packages Lotus and Least Squares.
RESULTS AND DISCUSSION

SENSORY EVALUATION

Good correlations were found between subjective and objective analyses. In Table 2-1 the correlation between peroxide value and flavour for both trial 1 and 2 was -0.753 and -0.706 respectively. While that of aroma and peroxide value was -0.867 and -0.806. In trial 1 the correlation for both aroma, flavour and free fatty acids was -0.856 and -0.906. In trial 2 correlations between flavour and free fatty acids was -0.741. On the whole the correlations in both trials were good except for aroma and free fatty acids in the second trial which was observed as -0.655. This value was quite low compared to the others. In view of the generally high correlations, kinetic analyses were performed using values obtained from free fatty acids and peroxide values.

Determination of the order of reaction was done using values obtained objectively because the sampling times were more (0, 14, 35, 56 and 77th day) than those of sensory evaluation (14, 35 and 56th day). Subjective analyses were performed only up to the 56th day because on the 77th day of sampling the nuts were unacceptably rancid and the experiment was stopped.

The differences due to temperature and time effects on aroma and flavour are shown in Figure 2-3 and 2-4 as well as Tables 2-2 and 2-3. Figure 2-3, whose corresponding values are in Table 2-2 shows no significant differences at any temperature for both trial 1 and 2 on the 14th day of sampling. On the 35th day of sampling there is no significant difference between temperatures 30°C and 35°C as well as 40°C and 45°C in trial 1. On the same day in trial 2, the values are significantly different from each other except at temperatures 40°C and 45°C. On the 56th day of sampling, the results are the same in both trials and are similar to those obtained on the 35th day in trial 1.
The significant differences due to temperature and time effect on the flavour are shown in Figure 2-4 and Table 2-3. On the 14th day at all temperatures, there is no significant difference in both trials except at 45°C in trial 1. On the 35th day, there is no significant difference between temperatures 30°C and 35°C as well as 40°C and 45°C in trial 1. In trial 2 there is a significant difference at all temperatures on the 35th day. On the 56th day the results for both trials are the same showing significant differences at 30°C and 35°C but not at 40°C and 45°C.

The average scores for both aroma and flavour at the lowest temperature of 30°C, in both trial 1 and 2 show a rating between slightly (4) and moderately (3) inferior after 56 days of storage. The peroxide value at this time was 0.395 meq/kg in trial 1 and 0.694 meq/kg in trial 2. This is similar to the findings reported in chapter 3 in which the panellists detected rancidity at two months of storage with a peroxide value of 0.367 meq/kg. The panellists used in this work detected rancidity at a lower peroxide value compared to those reported by Fourie and Basson (1989) who detected rancidity by sensory means in macadamia nuts with a PV between 1.5 and 1.6 meq/kg at two months of storage.

Samples with the worst sensory quality as detected by both aroma and flavour were those at 40°C and 45°C. They were found to be statistically significant throughout the storage in both trials. On the 56th day of sampling the panellists detected the sample as between moderately inferior and much inferior.

Generally the trend was that the longer the product was kept at all temperatures and the higher the temperature at each time, the lower was the score for both aroma and flavour. These results agree with those of Cavaletto et al. (1966 and 1968) who found that the storage stability of macadamia nuts is dependent upon the time and temperature of storage.
ORDER OF REACTION

Figure 2-5 shows a plot of peroxide values against time. The peroxide values are between 0 and 12 meq/kg. At temperatures 40°C and 45°C there is a linear plot of PV versus time up to about 35 days and then a bimolecular curve is experienced after this. Labuza and Bergquist (1983b) in their study on oxidation of potato chips used data only up to a peroxide value of 10, above which a bimolecular exponential curve was observed.

The number of samples in their study was 81, 71 and 32 at temperatures 30°C, 37°C and 45°C respectively. If more sampling times had been used in this study, data up to 35 days of storage at all four temperatures could have been used in order to determine the order of reaction. It was found that at 40°C and 45°C a bimolecular curve was experienced after these periods.

The coefficient of determination ($r^2$) and rate constants of peroxide values are shown in Table 2-4. Trial 1 and 2 exhibited a much higher $r^2$ at temperatures 40°C and 45°C for a first order assumption as compared to a zero order. At the lower temperatures (30°C and 35°C) however, the $r^2$ in both trials was higher for a zero order than a first order assumption except at 30°C in the second trial. However, the confidence interval for the zero order was better than that of the the first order. A zero order assumption was made for temperatures 30°C and 35°C and a first order for temperatures 40°C and 45°C.

Determination of the order of reaction for free fatty acids as % oleic acid is shown in Table 2-5 and Figures 2-6 to 2-9. Clearly at all temperatures $r^2$ best fits a zero order with confidence limits lower than the first order.

Labuza (1984) indicated that for data up to approximately 50% loss in food quality it was difficult to distinguish between a zero and first order reaction (Figure 2-1).
He mentioned most foods being unacceptable with 20-30% change from the initial value suggesting that a zero order model could be selected. The more data points used in regression the more confidence one has in using the r² value as a statistical tool which assists in deciding upon a zero or first order rate.

TEMPERATURE DEPENDENCE OF RATE OF DETERIORATION

Determination of the Arrhenius plot for peroxide value was not carried out for the reason that at low temperatures (30°C and 35°C) a zero order reaction was observed whilst a first order reaction was experienced at the high temperatures (40°C and 45°C). Therefore having only two temperatures at each reaction order, an Arrhenius plot was not carried out. Labuza and Bergquist, (1983) mentioned that it is obvious that if only two temperatures are used a straight line would be found and confidence limits can not be calculated.

In order to determine the Arrhenius plot from the results of free fatty acids, the rate constant results obtained from the zero order plot were investigated in order to determine the goodness of fit.

A regression was done on the upper and lower confidence limits of the rate constants shown in Table 2-5 in order to obtain the activation energy values for free fatty acids and the pre-exponential constant. The Arrhenius kinetic values are shown in Table 2-7. It was observed that the zero order rate constant had a better goodness of fit ($r^2 = 0.796$ and 0.499 for trial 1 and 2 respectively), standard deviation and confidence interval than the first order rate plot for the first and second trial (0.175 and 0.119 respectively). From these results in which the plot of ln K versus 1/T is not a good fit, errors could be obtained during extrapolation.
REACTION ORDER AND EQUILIBRICATION

The puzzling aspects of the rates of increase in peroxide value and free fatty acid content at the four storage temperatures may be related to the delay in equilibrating the water activities of the stored nuts to the relative humidity set by the saturated magnesium nitrate solution in the storage chambers.

As shown in Table 2-6, at higher temperatures (40°C and 45°C) the water activity was higher than at the lower temperatures on both the 56th and 77th day. Water activity also varied with time with nuts held at at least three temperatures not reaching equilibrium by the 77th day.

It would appear that the increase in free fatty acid content may be zero order within the water activity range 0.3 to 0.5 and that variations within this range have little impact on the order of the reaction. On the other hand the finding of apparent zero and first order reaction for development of peroxide value at 30 and 35°C as against 40 and 45°C respectively may indicate that variations in water activity in the range 0.3 to 0.5 have a marked influence on the development of peroxide value.

Perhaps a vacuum could have been introduced initially in order to facilitate equilibrium as was demonstrated by Veerraju et al. (1978) in the study on walnuts. There was no available dessicator to which a vacuum could have been applied. About twenty four dessicators were required at the time of experiment, eight for this particular experiment and another sixteen for the experiment in Chapter 4 (the effect of water activity on the texture of Macadamia nuts). The cost of this number of dessicators was too high. At the same time larger incubators for this particular experiment were required which was beyond our budget.
Thus future studies of the development of oxidative rancidity in raw Macadamia kernels should explore the effects of water activity and temperature on equilibrated nuts. Hopefully this would clarify the factors controlling the reaction and deliver more accurate and precise shelf life predictions. While hydrolytic rancidity may not be as susceptible to fluctuations in water activity as oxidative rancidity further investigations with equilibrated kernels may yield more precise shelf life predictions.

PREDICTION OF SHELF LIFE AT 25°C

According to the Arrhenius plots in Figures 2-10 and 2-11, the rate constant indicates that it took a longer time of 61.8 and 79 days in trial 1 and 2 compared to 56 days to reach FFA values of 0.108 and 0.125% (obtained from the actual experimental values) 30°C. It could therefore be said that there is some error in the interpretation of shelf life at the lower temperatures such as that of 25°C as was selected. Table 2-8 shows the days it took as calculated from the Arrhenius plots to reach an FFA of 0.108 and 0.125% oleic acid at five temperatures (25°C, 30°C, 35°C, 40°C and 45°C). At 25°C it took a longer time (83.0 and 91.9 days in trial 1 and 2 respectively) compared to the other four higher temperatures (30°C, 35°C, 40°C and 45°C) to reach an FFA of 0.108 and 0.125 % oleic acid. It is difficult to compare these results with those by other researchers such as Cavaletto et al. (1966) who subjected the kernels to a vacuum and Fourie and Basson (1989) who did not look at the FFA although the storage conditions of were the same.

Cavaletto et al. (1966), vacuum sealed samples of macadamia kernels in jars at -94.8 Kpa for storage and observed that the stability decreased with an increase in temperature and moisture. At 1.4% moisture and temperatures of -17.8°C, 1.7°C, ambient and 37°C good stability with little change in chemical composition after storage for 16 months was experienced. They recommended a low temperature of -17.8°C for kernels having a moisture content of 2.3% and 4.3%. Fourie and Basson
(1989), reported on rancidity detected by panellists after two months of storage with a peroxide value between 1.5 meq/kg and 1.6 meq/kg.

The \( E_A \) for hydrolytic rancidity (Table 8) measured by FFA was 10 ± 3.25 Kcal/mol for trial 1 and 6.47 ± 4.59 Kcal/mol for trial 2. The activation energy determined for hydrolytic rancidity, could not be compared with those of literature as there was none available for nuts of any type. There has been only two studies reported in literature describing the shelf life of raw macadamia kernels.

The *Macadamia integrifolia* nuts were found to contain about 75% fat as determined initially, a level at which reactions such as those described by Labuza and Riboh (1982) could have occurred. Temperature rise causes solid fat to change to liquid. There is a possibility of organic reactants being more mobile in the liquid state than the solid resulting in underprediction of shelf life at lower temperature.

Labuza (1982a) indicated that for a food having two modes of deterioration with different \( E_A \) values at a higher temperature, the one with a high activation energy will predominate. Error may occur in the extrapolation of the shelf life. This may have occurred in the study for free fatty acids. Although not proven, it is possible that such a reaction might have taken place.
CONCLUSION

Subjective and objective measurements were correlated ($r>0.65$). The nuts were organoleptically acceptable for 56 days at 30°C and a relative humidity of 50%. Hydrolytic rancidity followed apparent zero order kinetics as determined by free fatty acid. Oxidative rancidity as determined by peroxide values followed apparent zero order kinetics at temperatures 30°C and 35°C and apparent first order at 40°C and 45°C. However the poor precision of some of the data, especially at temperatures of 40 and 45°C leads to ambiguity in the conclusions about the order of the reactions. The failure to equilibrate the nuts with respect to water activity before starting the storage trial appears to have clouded the results. Future studies should clarify more definitively the impact of temperature and water activity on the development of free fatty acids and peroxide value and elucidate the relationship between these objective measures and subjective measures of quality. To do this a more intense sampling will be required in which the times of sampling at both high and low temperatures should be more frequent to have a better confidence interval in order to carry out an Arrhenius plot on the peroxide values.
REFERENCES


Table 2-1.
Correlation coefficients between sensory (aroma and flavour) and chemical analysis (peroxide value and free fatty acids).

<table>
<thead>
<tr>
<th></th>
<th>PV</th>
<th>FFA</th>
<th>A</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trial 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peroxide value (PV)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free fatty acids (FFA)</td>
<td>0.727</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aroma (A)</td>
<td>-0.867</td>
<td>-0.856</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Flavour (F)</td>
<td>-0.753</td>
<td>-0.906</td>
<td>0.931</td>
<td>1</td>
</tr>
<tr>
<td><strong>Trial 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peroxide value (PV)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free fatty acids (FFA)</td>
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<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aroma (A)</td>
<td>-0.806</td>
<td>-0.655</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Flavour (F)</td>
<td>-0.706</td>
<td>-0.741</td>
<td>0.938</td>
<td>1</td>
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</table>

* p<0.05
Table 2-2.
Average scores of aroma for both trial 1 and 2.

<table>
<thead>
<tr>
<th>DAYS</th>
<th>30°C</th>
<th>35°C</th>
<th>40°C</th>
<th>45°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trial 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>4.786a</td>
<td>3.929a</td>
<td>4.286a</td>
<td>3.786a</td>
</tr>
<tr>
<td>35</td>
<td>3.929a</td>
<td>4.000a</td>
<td>3.286b</td>
<td>3.071b</td>
</tr>
<tr>
<td>56</td>
<td>3.538a</td>
<td>3.615a</td>
<td>2.692b</td>
<td>2.308b</td>
</tr>
<tr>
<td>Trial 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>4.857a</td>
<td>4.429a</td>
<td>4.357a</td>
<td>4.929a</td>
</tr>
<tr>
<td>35</td>
<td>4.786a</td>
<td>4.143b</td>
<td>3.571c</td>
<td>3.571c</td>
</tr>
<tr>
<td>56</td>
<td>4.000a</td>
<td>3.692a</td>
<td>2.692b</td>
<td>2.769b</td>
</tr>
</tbody>
</table>

Means followed by the same letters at each day are not significantly different from each other (p <0.05).
Table 2-3.
Average scores of flavour for both trial 1 and 2.

<table>
<thead>
<tr>
<th></th>
<th>30°C</th>
<th>35°C</th>
<th>40°C</th>
<th>45°C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trial 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>4.429a</td>
<td>3.929a</td>
<td>4.500a</td>
<td>3.286b</td>
</tr>
<tr>
<td>35</td>
<td>3.786a</td>
<td>3.500a</td>
<td>2.714b</td>
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</tr>
<tr>
<td>56</td>
<td>3.538a</td>
<td>3.000b</td>
<td>2.308c</td>
<td>2.385c</td>
</tr>
<tr>
<td><strong>Trial 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>4.571a</td>
<td>4.500a</td>
<td>4.000a</td>
<td>4.143a</td>
</tr>
<tr>
<td>35</td>
<td>4.643a</td>
<td>3.786b</td>
<td>2.929c</td>
<td>2.429d</td>
</tr>
<tr>
<td>56</td>
<td>3.615a</td>
<td>3.154b</td>
<td>2.308c</td>
<td>2.385c</td>
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</tbody>
</table>

Means followed by the same letters at each day are not significantly different from each other (p<0.05).
Table 2-4.
Comparison of coefficient of determination ($r^2$) and rate constants
($k \pm 95\%$ confidence interval) of peroxide values obtained by
linear regression for the first and second trial.

<table>
<thead>
<tr>
<th>Temp.</th>
<th>Zero $r^2$</th>
<th>Rate Constant k (PV/day)</th>
<th>$n^*$</th>
<th>First $r^2$</th>
<th>Rate Constant k (PV/day)</th>
<th>$n^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30°C</td>
<td>0.919</td>
<td>0.009</td>
<td>15</td>
<td>0.822</td>
<td>0.016</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>±0.0016</td>
<td>±0.0029</td>
<td></td>
<td></td>
<td>±0.0068</td>
<td></td>
</tr>
<tr>
<td>35°C</td>
<td>0.933</td>
<td>0.018</td>
<td>15</td>
<td>0.923</td>
<td>0.026</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>±0.0029</td>
<td>±0.0066</td>
<td></td>
<td></td>
<td>±0.0066</td>
<td></td>
</tr>
<tr>
<td>40°C</td>
<td>0.669</td>
<td>0.128</td>
<td>15</td>
<td>0.937</td>
<td>0.055</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>±0.0539</td>
<td>±0.0100</td>
<td></td>
<td></td>
<td>±0.0100</td>
<td></td>
</tr>
<tr>
<td>45°C</td>
<td>0.733</td>
<td>0.122</td>
<td>15</td>
<td>0.964</td>
<td>0.053</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>±0.0440</td>
<td>±0.0071</td>
<td></td>
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<td>±0.0071</td>
<td></td>
</tr>
<tr>
<td>Trial 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30°C</td>
<td>0.905</td>
<td>0.016</td>
<td>15</td>
<td>0.934</td>
<td>0.032</td>
<td>12</td>
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<tr>
<td></td>
<td>±0.0031</td>
<td>±0.0059</td>
<td></td>
<td></td>
<td>±0.0059</td>
<td></td>
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<td>35°C</td>
<td>0.880</td>
<td>0.016</td>
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<td>0.750</td>
<td>0.017</td>
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<tr>
<td></td>
<td>±0.0034</td>
<td>±0.0071</td>
<td></td>
<td></td>
<td>±0.0071</td>
<td></td>
</tr>
<tr>
<td>40°C</td>
<td>0.850</td>
<td>0.154</td>
<td>15</td>
<td>0.979</td>
<td>0.048</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>±0.0388</td>
<td>±0.0050</td>
<td></td>
<td></td>
<td>±0.0050</td>
<td></td>
</tr>
<tr>
<td>45°C</td>
<td>0.724</td>
<td>0.138</td>
<td>15</td>
<td>0.866</td>
<td>0.043</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>±0.0509</td>
<td>±0.0121</td>
<td></td>
<td></td>
<td>±0.0121</td>
<td></td>
</tr>
</tbody>
</table>

*n* is the number of peroxide value samples used in the regression at each temperature.
Table 2-5.
Comparison of coefficient of determination ($r^2$) and rate constants
($k \pm 95\%$ confidence interval) of free fatty acid values (% oleic acid) obtained by
linear regression for the first and second trial.

<table>
<thead>
<tr>
<th>Temp</th>
<th>Zero $r^2$</th>
<th>Rate Constant $k$ (FFA/d)</th>
<th>$n^*$</th>
<th>First $r^2$</th>
<th>Rate Constant $k$ (FFA/d)</th>
<th>$n^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trial 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30°C</td>
<td>0.848</td>
<td>0.002 ±0.0004</td>
<td>15</td>
<td>0.763</td>
<td>0.014 ±0.0055</td>
<td>12</td>
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<tr>
<td>35°C</td>
<td>0.900</td>
<td>0.002 ±0.0005</td>
<td>15</td>
<td>0.800</td>
<td>0.020 ±0.0070</td>
<td>12</td>
</tr>
<tr>
<td>40°C</td>
<td>0.911</td>
<td>0.003 ±0.0006</td>
<td>15</td>
<td>0.860</td>
<td>0.015 ±0.0043</td>
<td>12</td>
</tr>
<tr>
<td>45°C</td>
<td>0.918</td>
<td>0.003 ±0.0006</td>
<td>15</td>
<td>0.870</td>
<td>0.016 ±0.0043</td>
<td>12</td>
</tr>
<tr>
<td><strong>Trial 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30°C</td>
<td>0.876</td>
<td>0.002 ±0.0004</td>
<td>15</td>
<td>0.863</td>
<td>0.015 ±0.0036</td>
<td>15</td>
</tr>
<tr>
<td>35°C</td>
<td>0.884</td>
<td>0.002 ±0.0003</td>
<td>15</td>
<td>0.837</td>
<td>0.015 ±0.0039</td>
<td>15</td>
</tr>
<tr>
<td>40°C</td>
<td>0.848</td>
<td>0.003 ±0.0008</td>
<td>15</td>
<td>0.718</td>
<td>0.020 ±0.0075</td>
<td>15</td>
</tr>
<tr>
<td>45°C</td>
<td>0.733</td>
<td>0.002 ±0.0009</td>
<td>15</td>
<td>0.612</td>
<td>0.017 ±0.0082</td>
<td>15</td>
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</tbody>
</table>

$n^*$ is the number of free fatty acid samples used in the regression at each temperature.
Table 2-6.
Water activity readings each day of sampling taken at 20°C.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Trial 1</th>
<th></th>
<th></th>
<th>Trial 2</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14 days</td>
<td>35 days</td>
<td>56 days</td>
<td>77 days</td>
<td>14 days</td>
<td>35 days</td>
</tr>
<tr>
<td>30°C</td>
<td>0.332</td>
<td>0.340</td>
<td>0.470</td>
<td>0.470</td>
<td>0.258</td>
<td>0.430</td>
</tr>
<tr>
<td>35°C</td>
<td>0.350</td>
<td>0.350</td>
<td>0.456</td>
<td>0.480</td>
<td>0.292</td>
<td>0.404</td>
</tr>
<tr>
<td>40°C</td>
<td>0.366</td>
<td>0.480</td>
<td>0.480</td>
<td>0.490</td>
<td>0.303</td>
<td>0.518</td>
</tr>
<tr>
<td>45°C</td>
<td>0.294</td>
<td>0.410</td>
<td>0.490</td>
<td>0.500</td>
<td>0.294</td>
<td>0.400</td>
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Table 2-7.
Arrhenius kinetic figures for free fatty acids derived from end points of 95% confidence limits for zero and first order rate constants for the two trials.

<table>
<thead>
<tr>
<th></th>
<th>EA (kcal/mol)</th>
<th>KJ/mol</th>
<th>lnk0</th>
<th>r²</th>
<th>sd**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero order T1</td>
<td>10±3.25*</td>
<td>41±13.58</td>
<td>10.99±5.27</td>
<td>0.796</td>
<td>0.267</td>
</tr>
<tr>
<td>Zero order T2</td>
<td>6.47±4.59</td>
<td>27.04±19.19</td>
<td>4.30±7.45</td>
<td>0.499</td>
<td>0.378</td>
</tr>
<tr>
<td>First order T1</td>
<td>2.12±4.86</td>
<td>8.86±20.31</td>
<td>-0.72±7.89</td>
<td>0.175</td>
<td>0.400</td>
</tr>
<tr>
<td>First order T2</td>
<td>1.59±5.40</td>
<td>6.65±22.57</td>
<td>-1.58±8.76</td>
<td>0.119</td>
<td>0.445</td>
</tr>
</tbody>
</table>

* 95% confidence limit  
** standard deviation of the points
Table 2-8.
Number of days it took to reach an FFA of 0.108 and 0.125 as % oleic acid in trial 1 and 2 respectively.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>FFA trial 1</th>
<th>FFA trial 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>25°C</td>
<td>83.0</td>
<td>91.9</td>
</tr>
<tr>
<td>30°C</td>
<td>61.8</td>
<td>79.1</td>
</tr>
<tr>
<td>35°C</td>
<td>48.1</td>
<td>66.0</td>
</tr>
<tr>
<td>40°C</td>
<td>36.0</td>
<td>56.3</td>
</tr>
<tr>
<td>45°C</td>
<td>28.1</td>
<td>47.5</td>
</tr>
</tbody>
</table>
FIGURES
Figure 2-1.

Food quality loss for a zero and first order reaction. The end of shelf life ($t_s$) occurs when quality gets to a maximum allowable loss value ($A_s$) (Labuza, 1984).
Figure 2-2.

Arrhenius plot of log k versus inverse absolute temperature showing projections to a lower temperature (Labuza, 1984).
Figure 2-3.
Sensory evaluation differences observed due to temperature and time effect for aroma in trial 1 and 2.
Figure 2-4.

Sensory evaluation differences observed due to temperature and time effect for flavour in trial 1 and 2.
Figure 2-5.

The variation of peroxide value with time of storage of macadamia nuts at various temperatures in trials 1 and 2.

(Data points are means of at least duplicate samples at each period of reaction).
Figure 2-6.

Zero order plot of free fatty acids for trial 1.

(Data points are means of at least duplicate samples at each period of reaction).
Figure 2-7.

Zero order plot of free fatty acids for trial 2.

(Data points are means of at least duplicate samples at each period of the reaction).
Figure 2-8.

First order plot of free fatty acids for trial 1.

(Data points are means of at least duplicate samples at each period of the reaction).
Figure 2-9.

First order plot of free fatty acids for trial 2.

(Data points are means of at least duplicate samples at each period of the reaction).
Figure 2-10.

Arrhenius plot for zero order rate of hydrolytic rancidity in FFA/day versus inverse absolute temperature for trial 1 showing projections to a lower temperature of 25°C.
Figure 2-11.
Arrhenius plot for zero order rate of hydrolytic rancidity in FFA/day versus inverse absolute temperature for trial 2 showing projections to a lower temperature of 25°C.
CHAPTER 3

MEASUREMENT OF RANCIDITY USING THE HEADSPACE SAMPLER AND THE DETERMINATION OF NATURALLY PRESENT ANTIOXIDANTS IN MACADAMIA NUTS
SUMMARY

Volatile\s formed in the oxidative deterioration of Macadamia integrifolia kernels were identified using headspace analysis by gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS) and comparison with authentic external compounds. Aldehydes, alcohols, alkanes and a ketone were identified. The development of these volatiles was examined during a four month storage at 30°C and a relative humidity of 50%. Hexanal was shown to have potential as an index for measurement of rancidity. Panellists observed rancidity at two months of storage at which the amount of hexanal was 0.39 ppm with a peroxide value of 0.37 meq/kg. In order to help explain the shelf life of macadamia nuts, naturally present antioxidants were separated by TLC on silica gel and identified after extraction. The antioxidants present were α-tocopherol and α-tocotrienol identified by comparison with R_f values obtained by previous workers. In the case of α-tocopherol this was supported by using an authentic standard.
INTRODUCTION

Other methods have been reported to measure rancidity in nuts but not the use of headspace-gas chromatography. This method has been used extensively in distinguishing flavour and odour as well as a reference for quality in other foods. The initiative was therefore taken to explore the possibility of using this method. No one has ever reported on the different oxidative volatile compounds formed in the deterioration of Macadamia nuts. Work on the separation and identification of naturally present antioxidants was necessary in order to provide a better understanding of the shelf life.

HEADSPACE VOLATILE ANALYSIS OF EDIBLE KERNELS, OIL SEEDS, OILS AND FATS DUE TO RANCIDITY

In order to determine rancidity, headspace gas chromatography has been used in different types of foods. Singleton and Pattee (1980) found the volatiles produced from hydroperoxides in both peanut and soybean homogenates by concentrating headspace volatiles on a porous polymer before injecting into the gas chromatography. The volatiles present in both homogenates were pentane, pentanal and hexanal.

In potato chips, n-pentanal and n-hexanal were found to be good indices for measuring flavour quality using the headspace (Jeon and Bassette, 1984). In their study both n-hexanal and n-pentanal were related to the development of off flavours. Snyder et al. (1985) identified 34 volatile compounds (from autoxidation of unsaturated fatty acids) on the basis of relative retention time of reference compounds and gas chromatography - mass spectrometry from eight different oils. Volatile compounds of the oils increased with oxidation levels as carried out by peroxide value.

Snyder et al. (1986) found that hydrogenated oils produced less volatiles than unhydrogenated ones after long hours of heating and bread frying.
Volatile compounds such as 2,4-heptadienal (in non hydrogenated oil) and 2-nonanal (in hydrogenated oil) were identified. A decrease in 2-heptanal (in nickel hydrogenated oil) and hexanal (in all hydrogenated oils) was observed. It was also demonstrated by Frankel et al. (1987) that the use of headspace gas chromatography of both ground beans and crude oils (from soybeans) was more sensitive to evaluate oxidative deterioration based on hexanal and total volatiles rather than the use of near infrared spectrometry fluorescence measurements and silicic acid chromatography. However hydrolytic deterioration in stored soybean was analysed suitably and rapidly by the use of near-infrared spectrometry.

Selke and Frankel (1987) analysed quantitatively the major volatile products of oxidation in soybean oils. It was found that the volatiles were significantly affected by temperature or presence or absence of citric acid used before storage. About 13 volatiles were identified at peroxide value of 2 and said to probably be representative of those present in oils when being tested. Robards et al. (1988), found a suitable procedure which was capable of detecting concentrations of pentanal and hexanal below 1 ppm which is below the level where rancidity based on sensory testing would be noticed in corn chips.

A rapid, sensitive and convenient capillary gas chromatographic headspace method to determine hexanal (a decomposition product of hydroperoxides from n-6 polyunsaturated fatty acids in rat liver samples) as well as total volatiles due to lipid peroxidation was developed by Frankel et al., 1989.

GAS CHROMATOGRAPHY ANALYSIS OF VOLATILES DUE TO RANCIDITY IN EDIBLE KERNELS, OIL SEEDS, OILS AND FATS.
Scholz and Ptak (1966) studied a gas chromatographic method for measuring the degree of rancidity in cotton seed oil and other vegetable oils.
In this study n-pentane, was used as an internal standard and its quantity related well with organoleptic panel test results. The use of gas chromatography has also been used as an objective measurement of volatiles produced due to rancidity. Hovart and Senter (1979) identified the volatile products of mildly oxidised pecan oil by gas liquid chromatography-mass spectrometry. The compounds identified were: acetaldehyde; pentane; acetone; hexane; 1-hexene; butanol; 2-butenal; pentanal; pentanol; hexanal; toluene; n-propylbenzene; ethylphenylketone; heptane; benzaldehyde; octane; 2-heptanone; heptanal; octanal; 1-dodecene; decanal; and 1-tetradecene.

Flavour quality and degree of oxidation of soybean oil, hydrogenated soybean oil and corn oil (exposed to light over different periods of time) was measured using gas chromatographic analyses by Min (1981). As the amount of isomers of 2,4-decadienal increased in all three oils, the scores for flavour decreased and the correlation between the two was high. Kinderlerer and Johnson (1992), found that the use of volatile aldehydes such as hexanal (from oxidation of linoleic acid) and octanal (from oxidation of oleic acid) could be used to assess rancidity in hazel nuts rather than the non specific tests such as peroxide value and iodine value.
ANTIOXIDANTS

Tocopherols are natural antioxidants of vegetable oils whose content and relationship with oil stability is quite complicated (Peers, 1981). He described vegetables, cereal products, oilseeds and nuts as rich sources of tocopherols.

Rammell and Hoogenboom (1985) described tocols as structurally related compounds which are named according to whether the phytol side chain to the chroman ring is a single bond (tocopherols), one double bond (tocomonoenols) or three double bonds (tocotrienols). Figure 3-5 shows the structure of tocols adapted from Schuler (1990).

They are further classified by their methyl position on the phenolic ring: 5,7,8-trimethyltocol (α-tocopherol); 5,8-dimethyltocol (β-tocopherol); 7,8-dimethyltocol (γ-tocopherol) and 8-methyltocol (δ-tocopherol). Gunstone and Norris (1983) described antioxidants as free radical acceptors which terminate the initiation step in oxidation. They do so by forming a stable compound which is not oxidised in autoxidation where a free radical is attacked by oxygen forming peroxides and hydroperoxides. Generally the antioxidant activity decreases from δ to γ to β to α tocopherol as cited by Gunstone and Norris (1983) from Evans et al. (1971).

Yamaoka et al. (1991) studied the differences in the antioxidative activities between α-tocopherol (α-Toc) and α-tocotrienol (α-Toc3), and between γ-Toc3 and γ-Toc. It was found that Toc3 added to the liposome solution was consumed more than Toc during the induction period. When the two were incorporated their activity was the same.
Cillard et al. (1980) found that the prooxidant effect of α-tocopherol during autoxidation of linoleic acid occurred when its concentration was equal to or greater than $5 \times 10$ mol α-Toc/1 mol of linoleic acid. The pro-oxidant effect took place only in an aqueous medium of water and not in nonpolar solvents such as ethanol, dimethylsulfoxide, acetonitrile, hexane and petroleum. The pro-oxidant effect varied in an inverse ratio to the initial hydroperoxide level.

The only published work on the presence of natural antioxidants in macadamia nuts is that by Fourie and Basson (1989) who found 2.5 mg/100 g α-tocopherol and trace amounts of γ-tocopherol in the variety "Nelmar". However, Rosenthal et al (1984) found none in Macadamia integrifolia of the "Yonik" variety.

The objectives of the present study were to:

1. compare rancidity using the headspace gas chromatography, peroxide value and sensory evaluation;
2. identify a few compounds such as hexanal or octanal which could be used as an index for quality in terms of rancidity;
3. identify some products of oxidised macadamia nut kernels by gas chromatography-mass spectrometry; and
4. to separate and identify the antioxidants present by TLC
MATERIALS AND METHODS

MATERIALS

For headspace analyses, whole shelled first grade *Macadamia integrifolia* of the cultivar Keauhou (Hawaii Agricultural Experimental Station, cultivar 246) were purchased from Macadamia Plantations of Australia. The samples were treated before receipt as described in Chapter 2.

STORAGE CONDITIONS

Two 3 kg lots of macadamia nut kernels were placed in a canister over a saturated solution of magnesium nitrate (to maintain an equilibrium relative humidity of 50%) at a temperature of 30°C. The incubators used were light proof with a good seal. Samples (100 g) for the analysis of headspace were collected monthly from each canister, vacuum packaged and stored at -70°C and then at -30°C until ready for analysis as there were technical problems associated with the Headspace gas chromatography system.

For the determination of antioxidants of *Macadamia integrifolia* nut in shell of the cultivar keauhou was used.

HEADSPACE GAS CHROMATOGRAPHY

Each 100 g sample was crushed into a butter using a mortar and pestle. Three 7 g samples were placed in a 20 mL headspace vial and covered with a rubber septum followed by an aluminium cap. The aluminium cap was (drilled on the centre with a hole of 9 mm in diameter) crimped tightly on the bottle top using a clamp. The sample was placed in an oil bath at 110°C for 42 minutes. The headspace sampler was a Hewlett Packard model 19395A purge which was attached to the gas chromatograph (GC) Hewlett Packard model 5890.
External standards from BDH laboratory were placed in the vials as described for macadamia samples.

A μL of each authentic standard of alcohol (butanol, pentanol, hexanol, heptanol, and octanol), hydrocarbons (pentane, hexane, heptane, octane, nonane, decane and undecane) and aldehyde (propanal, pentanal and hexanal) was made up to 5 mL with water.

A J and W DB1 (30 m x 0.32 mm x 0.32 mm) column (non-polar) was used in the GC. A flame ionisation detector was used. The zone inlet temperature was 200°C and the detector temperature was 190°C. The oven program was as follows: initial temperature = 45°C; initial time = 1 minute; the rate was 5°C/minute; final temperature was 200°C; final time of 10 minutes; and a next run time of 42 minutes.

GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)

A 1μL sample of the headspace from a vial containing macadamia nuts at four months of storage (sample was subjected to the same conditions as those described in the headspace gas chromatography section) was injected into the GC-MS using a gas tight syringe. A gas chromatograph (Hewlett Packard 5890 series II) fitted with a J and W DB-1, 1.0 μm film thickness, 30 m x 0.25 mm capillary column connected to a Hewlett Packard mass selective detector (5971) was used to identify some of the compounds of oxidative rancidity. Data was acquired under the conditions; initial temperature: -5°C, 1 min, program rate: 50°C/min, hold temperature 1:60°C, 2 min, program rate 5°C/min, final temperature: 240°C, final time: 10 min, total time: 55 min, injector temperature; 220°C, transfer line temperature: 280°C, carrier gas: He at 22 cm/s, split ratio: 1:40. The column was terminated at an Hewlett-Packard Mass Selective Detector (MSD)(HP 5971A). The ion source was run in the EI mode at 90°C using an ionisation energy of 70 eV. The scan rate was 0.9 scans/sec. Data from the MSD was
stored and processed using a Hewlett-Packard Vectra QS20 computer installed with Mustang software and the volatiles were identified from the Wiley Mass Spectral Library.

CHEMICAL ANALYSES

A sample of 200 g was obtained from storage at 1, 2, 3 and 4 months of storage and extracted using the Bligh and Dyer method modified by Hanson and Olley (1963). Analyses for peroxide value method 965.33 were carried out according to the AOAC method (Helrich, 1990) within 24 hours of extraction.

SENSORY EVALUATION

Aroma and flavour was evaluated by twelve trained panellists at 1, 2, 3, and 4 months of storage. The panellists were selected after training using the triangle test followed by a multiple comparison test with nuts having different levels of rancidity. Rancid nuts due to different extreme storage temperatures as described in Chapter 2 were used for training panellists. Nuts from 30°C and -18°C (reference) storage were taken at sampling (200 g) and chopped to 2 mm size. A 15 g sample was placed in a plastic cup sealed with aluminium foil and left to equilibrate and bring the samples to ambient temperature. The tests were carried out in a quiet and comfortable area with individual sensory evaluation booths. Panellists were presented with two samples (one from -18°C and 30°C) each coded with three digits and ambient water for rinsing in between tests. They were requested to evaluate the samples for rancidity by aroma first followed by flavour. They were instructed to write the sample number and record their responses on a score sheet with a number line for each sample. The line ranged as "not at all rancid", "slightly rancid", "moderately rancid" and "extremely rancid" for both aroma and flavour.
IDENTIFICATION OF TOCOLS

The method used in the identification of tocols was adopted from Contreras-Guzmán et al. (1982b).

REAGENTS

Standards of (±)-α-tocopherol (approximately 95%), (+)-δ-tocopherol (approximately 90%) and (+)-γ- tocopherol; 2,2'-biquinoline; and 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline (bathocuproine) were obtained from Sigma. Hexane, toluene and ethanol were treated as explained by Contreras-Guzmán et al. (1982a). Plates for TLC were aluminium sheets 20 x 20 cm, pre-coated with silica gel 60 F_{254} having a thickness of 0.2 mm obtained from Alltech.

SOLUTIONS

The solutions prepared were as follows: 0.50% Cu(NO_3)_2·3H_2O in distilled water (I); 0.10% Cu(NO_3)_2·3H_2O in absolute ethanol (II); 0.50% 2,2'-biquinoline in toluene (III); 0.50% bathocuproine in toluene (IV); 2.5% urea in absolute ethanol (V); 10 mL of (I) and 20 mL of (III) diluted to 50 mL with (V) was used for spraying the plate after development to produce purple spots (VI). Solution (VII) was a standard mixture of 0.4 mg of α-tocopherol, 1.6 mg of γ- tocopherol and 0.2 mg of δ-tocopherol per mL. Individual standards having the same concentration as in the standard mixture were prepared in hexane. Solutions II to IV were kept in the refrigerator. Solution VI was prepared just prior to use.

DIRECT SAPONIFICATION TO EXTRACT TOCOPHEROLS

A 500 g sample was ground in order to pass through a sieve with 0.25 mm openings. Five 3.5 g samples were each placed in a 125 mL round bottomed flask (to two of them, 100 μg α-tocopherol was added). Solid ascorbic acid (200 -250 mg) and 20 mL of absolute ethanol were added. A condenser was connected to the flask and heated on a boiling water bath.
Once boiling commenced, 5% potassium hydroxide (w/w) was added through the condenser and refluxed for 30 minutes. This was cooled and transferred to a 100 mL glass graduated cylinder and rinsing the flask with 2.5% sodium hydroxide and diluting the total volume to 75 mL. Extraction of the unsaponifiable material was done by shaking with 15-, 15-, 10-, and 10 mL solutions of hexane while transferring each extract to a funnel. Distilled water was used for washing hexane and anhydrous sodium sulphate for drying. This was then transferred quantitatively to a 125 mL round bottomed flask and the solvent was evaporated using a rotary evaporator (model WB 2000) at 40°C. The residue was made up to 10 mL with hexane.

ONE DIMENSIONAL SEPARATION OF SAMPLES OF TOCOPHEROL AND TOCOTRIENOLS BY TLC

The silica gel plate was divided as shown in Figure 3-6. Samples of 5 mL of hexane from the unsaponifiable, the standard mixture (VII), individual standards of α-tocopherol (20 μg), δ-tocopherol (10 μg) and γ-tocopherol (80 μg) were evaporated to dryness using nitrogen. Petroleum ether (30 μL) was added and a micropipette used to apply the samples on the silica gel plate. The method used was that cited by Contreras-Guzmán (1982b) from Muller Mulot (1976). This was a double development of 16 cm in the same direction using hexane-ethyl acetate, 92.5:7.5 v/v. The chromatograms were sprayed using solution (VI) producing purple spots on the separated components.

STATISTICAL ANALYSIS

Significant differences at 5% were tested using CoStat statistical package. Determination of the upper and lower confidence interval was done at 5% significance level of confidence based on Steel and Torrie (1980).
RESULTS AND DISCUSSION

SENSORY EVALUATION

Volatile produced from oxidation of unsaturated lipids are undesirable and can cause consumer rejection of the product. Values in Table 3-1 and Figure 3-1 show that, there was no discernible rancidity in the reference sample as measured by both aroma and flavour because the panellists observed the sample to be near the "not at all rancid" score of eight. For the sample at 30°C of storage, in the first and second month, there is a significant difference in aroma and flavour while in the third and fourth month there is no significant difference. The fact that the reference sample had no difference throughout the test, shows that any rancidity present was not detected by the panellists. As demonstrated in Figure 3-1 in the first month, the panellists observed the sample to be between the not at all rancid and slightly rancid region. In the second month the sample was in the slightly rancid and moderately rancid region. The third and fourth month was in the moderately and very rancid region. The sample was considered rancid after two months of storage.

HEAD SPACE GAS CHROMATOGRAPHY VOLATILES, PEROXIDE VALUE AND SENSORY ANALYSES WITH STORAGE TIME

Figure 3-2 shows the effect of sampling time on the chromatograms obtained with macadamia nuts at three different times. It is observed that there are marked differences with the fresh sample, sample at 2 and 4 months of storage. The major volatiles found with time of storage are shown in Table 3-3. No oxidative volatile decomposition products could be detected in the fresh sample just as the panellists discerned no rancidity in this sample. Volatiles increased as time progressed. In the second month the major volatile products observed were: pentanal; 4-methylpentan-2-one; pentanol; hexanal; and octane.
In the third and fourth month the volatiles were; pentanal; heptane; 4-methylpentan-2-one; pentanol; hexanal; octane; hexanol; heptanal; heptanol; octanal; octanol; and nonanal. Flavour deterioration in macadamia nuts at 30°C occurred in the first month of storage from the sensory evaluation results.

As shown in Table 3-3 the peroxide value at this stage was 0.393 meq/kg with a hexanal content of 0.393 ppm. The nuts were analysed at different times. The amount of hexanal increased as time progressed. Macadamia nuts contain 55 g per 100 g oleic acid and 1.9 g per 100 g linoleic acid per 100 g fatty acid as shown in Table 1-3 of Chapter 1. Frankel, 1982 reported on the products of oxidation of oleate being octanal and nonanal while hexanal is from linoleate. Octanal and nonanal were not detected at two months of storage as was hexanal in the first month of storage. Perhaps this was due to the fact that the temperature used was not high enough to detect low concentrations of this product. The boiling point of hexanal is 131°C and octanal is 163.4°C at 760 mm of Hg (Stecher et al, 1968). Selke and Frankel (1987) reported that the interpretation of dynamic headspace GC volatile analysis in terms of flavour significance is dependent on the temperature.

Aside from the difference in the boiling point between hexanal and octanal, the other reason for having detected hexanal earlier than octanal could be the kinetics involved with linoleate (from which hexanal is a product of oxidation) and oleate (from which octanal is a product of oxidation). Labuza (1971) stated that linoleate oxidises ten times faster than oleate. Hexanal being a product of linoleate was therefore detected much earlier than octanal and nonanal. Kinderlerer and Johnson (1992) found that hexanal and octanal could be used to assess rancidity in hazel nuts rather than the non specific tests such as peroxide value. Similarly hazel nuts contain 75 g per 100 g oleic acid and 9 g per 100 g linoleic acid. The GC headspace was a sensitive method to evaluate oxidative deterioration of the nuts and hexanal was shown to have potential as an indicator of rancidity.
COMPOUNDS IDENTIFIED BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS) AND AUTHENTIC COMPOUNDS

Identification of the major volatile compounds from stored Macadamia nuts was accomplished using Gas Chromatography-mass spectrometry (GC-MS) and the use of authentic standards shown in Table 3-2 and Figures 3-3 and 3-4. In Figure 3-3, the compounds (eleven) identified as shown in the chromatogram from the headspace gas chromatography were fewer than those in the chromatogram (Figure 3-4) from the GC-MS (sixteen). This was due to the difference in sensitivity in the two instruments. Pentanal, hexanal, heptane, octane, pentanol, hexanol and octanol were identified by both GC-MS and authentic standards. Heptanal, octanal, nonanal, 4-methyl-2-pentanone, 2-methylpropanol, 2-methylbutanal, 3-methylbutanal and methylbenzene were identified by GC-MS only.

Frankel (1982) showed the major volatile carbonyls derived from the hydroperoxides of oleate were octanal and nonanal with minor amounts of pentanal, hexanal, heptanal, octanal, heptane and octane. Volatile decomposition products from oxidised linoleate gave hexanal, as the major compound with minor ones of pentanal, heptanal, and pentanol. As mentioned earlier, Tables 3-3 and 3-4 in chapter 1 show the fatty acid composition of Macadamia nuts which consist of oleic and linoleic acid whose corresponding fatty acid esters are responsible for the mentioned volatiles. The type of products obtained from unsaturated lipid oxidation are determined by the chemical composition of the oil.

THE SEPARATION AND IDENTIFICATION OF ANTIOXIDANTS

The TLC plate was developed as shown in Figure 3-6. Individual standards, mixture of standards, the actual sample and the sample spiked with α-tocopherol were well separated. From the TLC plate, the $R_f$ values were calculated. The $R_f$ values are shown in Table 3-4.
In the current experiment the Rf values for α-tocopherol standard was 0.52 whilst that of α-tocopherol from the mixture of the three standards (α-T, δ-T and γ-T) was 0.54 which was the same for the sample of macadamia nuts spiked with α-T. The Rf for the individual standard of γ-T was 0.38 and that from the mixture of standards was 0.37. Both the δ-T from the individual and the mixture standard had an Rf of 0.26 which agrees with that by Müller Mulot (1976) as cited by Contreras-Guzmán et al. in (1982b).

The results in Table 3-4 include those cited by Contreras-Guzmán et al. (1982b) from Müller Mulot (1976). The two used the same method as that used in this experiment to develop the plate. Müller Mulot as cited by Contreras-Guzmán et al. (1982b), found Rf values for α-T, α-T3, γ-T, γ-T3 and δ-T were 0.50, 0.43, 0.34, 0.29 and 0.26 respectively. In the same order Contreras-Guzmán et al. (1982) observed the Rf values as 0.50, 0.44, 0.34, 0.28 and 0.25. It is observed that the differences between the Rf values of the specific tocopherols obtained in the sample and those reported by the two authors in Table 3-4 are quite small or none at all.

Therefore from the comparison of Rf values from authentic standards and the sample, α-T was definitely present while the presence of α-T3 was confirmed by comparison with the Rf values already established by the other two authors in Table 3-4. The spot for the determined tocols shows a more intense colour for α-T3 than that of α-T. This probably suggests the presence of more α-T3 than α-T. This is in agreement with the personal communication letter from Catherine Cavalletto (1988) to Mr Ian McConachie which indicated that trace amounts of α-T and 1.33 to 2.79 mg/g of oil of α-T3. Neither the species nor the variety is given.

Fourie and Bason (1989) found α-T and α-T3 in macadamia "Nelmar" while Rosenthal (1984) found no antioxidants in Macadamia integrifolia of the "Yonik" variety.
CONCLUSION

Oxidative volatile compounds identified were, 2-methylpropanol, 3-methylbutanal, 2-methylbutanal, pentanal, heptane, 4-methylpentan-2-one, pentanol, methyl benzene, hexanal, octane, hexanol, heptanal, heptanol, octanal, octanol and nonanal. Panellists observed rancidity in the first month of storage. Hexanal was shown to have potential as an index for rancidity measurement. At two months of storage the headspace concentration of hexanal was 0.39 ppm with a peroxide value of 0.37 meq/kg. The use of headspace analysis appeared to be a precise and sensitive method which could be used in place of panellists. Very low flavour thresholds related to rancidity could be detected. If confirmed by further work including statistical correlation between sensory score, oxidative volatile compounds like hexanal and/or peroxide value, the headspace gas chromatography could be used to measure the degree of oxidation of macadamia nuts. The use of a different range of temperature and the oxidative products still need to be investigated.

Tocopherols present in *Macadamia integrifolia* of the cultivar keauhou were α-tocopherol and α-tocotrienol as determined by TLC. The presence of these antioxidants could contribute to the stable shelf life of the nuts provided the conditions in the environment such as relative humidity and temperature are controlled from the time the nuts are fresh. There is a need to carry out further work on the amount of individual and total tocopherols present in this variety. The amount of tocopherols present in the different cultivars could be analysed. Other methods which have been used for the determination of antioxidants such as the use of HPLC and GC could be compared.
REFERENCES


Table 3-1.
Average scores for aroma and flavour for the reference at -18°C and sample stored at 30°C.

<table>
<thead>
<tr>
<th>Months</th>
<th>Aroma***</th>
<th>Flavour***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference (-18°C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>7.92a**</td>
<td>7.90a</td>
</tr>
<tr>
<td>2</td>
<td>7.86a</td>
<td>7.87a</td>
</tr>
<tr>
<td>3</td>
<td>7.83a</td>
<td>7.80a</td>
</tr>
<tr>
<td>4</td>
<td>7.73a</td>
<td>7.62a</td>
</tr>
<tr>
<td>Experimental (30°C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1)</td>
<td>6.77b</td>
<td>6.73b</td>
</tr>
<tr>
<td>(2)</td>
<td>5.33c</td>
<td>4.75c</td>
</tr>
<tr>
<td>(3)</td>
<td>4.25d</td>
<td>3.64d</td>
</tr>
<tr>
<td>(4)</td>
<td>3.73d</td>
<td>2.92d</td>
</tr>
</tbody>
</table>

* Time without parentheses is for reference sample.

** Means followed by the same letters are not significantly different from each other (p>0.05)

*** 8 (not at all rancid), 6 (slightly rancid), 4 (moderately rancid), 2 (very rancid) and 0 (extremely rancid) for both aroma and flavour.
Table 3-2. Compounds identified by gas chromatography mass spectrometry (GC-MS) and authentic compounds.

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>Octane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentanal</td>
<td>octane</td>
</tr>
<tr>
<td>hexanal</td>
<td>pentanol</td>
</tr>
<tr>
<td>(heptanal)*</td>
<td>hexanol</td>
</tr>
<tr>
<td>(octanal)</td>
<td>octanol</td>
</tr>
<tr>
<td>(nonanal)</td>
<td>(4-methylpentan-2-one)</td>
</tr>
<tr>
<td>heptane</td>
<td>(methylbenzene)</td>
</tr>
</tbody>
</table>

*All compounds without parentheses were confirmed by GC-MS only.
<table>
<thead>
<tr>
<th>Time (month)</th>
<th>Compound</th>
<th>hexanal (ppm)</th>
<th>pv (meq/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>none</td>
<td>0.000*</td>
<td>0.000*</td>
</tr>
<tr>
<td>1</td>
<td>pentanal, heptane, pentanol, hexanal, hexanol and heptane</td>
<td>0.259</td>
<td>0.278</td>
</tr>
<tr>
<td>2</td>
<td>pentanal, pentanol, hexanal, octane and 4-methylpentan-2-one</td>
<td>0.393</td>
<td>0.367</td>
</tr>
<tr>
<td>3</td>
<td>pentanal, heptane 4-methylpentan-2-one, pentanol, hexanal, octane hexanol, heptanal, heptanol and octanal octanol and nonanal</td>
<td>1.546</td>
<td>0.606</td>
</tr>
<tr>
<td>4</td>
<td>4-methylpentan-2-one pentanal, heptane pentanol, hexanal, octane hexanol, heptanal, heptanol and octanal octanol and nonanal</td>
<td>2.197</td>
<td>4.077</td>
</tr>
</tbody>
</table>

*Each data point is average of triplicate.
Table 3-4.
Presentation of the $R_f$ values of tocopherol as determined by researchers and those obtained from Macadamia nuts.

<table>
<thead>
<tr>
<th>T*</th>
<th>M^a</th>
<th>G^b</th>
<th>ST^c</th>
<th>SM^d</th>
<th>Se</th>
<th>SS^f</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-T</td>
<td>0.50</td>
<td>0.50</td>
<td>0.520</td>
<td>0.54</td>
<td>0.54</td>
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</tr>
<tr>
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<td>γ-T</td>
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</tr>
<tr>
<td>γ-T₃</td>
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<td>0.28</td>
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<tr>
<td>δ-T</td>
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<td>0.25</td>
<td>0.26</td>
<td>0.26</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Tocopherol

^aResults by Müller Mulot (1976) as cited by Contreras-Guzmán et al. (1982)

^bAdapted from Guzmán et al. (1982)

^cIndividual standards

^dStandard mixture of α-, γ- and δ-tocopherol

^etocopherols from sample of Macadamia nuts

^fsample spiked with α-tocopherol
FIGURES
Figure 3-1.
Sensory score versus storage time for Macadamia kernels.
8 (not at all rancid), 6 (slightly rancid), 4 (moderately rancid), 2 (very rancid) and 0 (extremely rancid) for both aroma and flavour.
Figure 3-2.

Headspace gas chromatography profiles for volatiles in fresh and stored Macadamia kernels at 2 and 4 months of storage.
Figure 3-3.

Gas chromatogram obtained from four months of storage with the compounds identified in it. Conditions are explained in the materials and methods section.
F I D detector response

Peak name
1. pentanal
2. heptane
3. 4-methylpentan-2-one
4. pentanol
5. hexanal
6. hexanol
7. heptanal
8. heptanol
9. octanal
10. octanol
11. nonanal
Figure 3-4.

Chromatogram of headspace volatiles identified by GC-MS.
Peak name

1. 2-methylpropanol
2. 3-methylbutanal
3. 2-methylbutanal
4. pentanal
5. heptane
6. 4-methylpentan-2-one
7. pentanol
8. methylbenzene
9. hexanal
10. octane
11. hexanol
12. heptanal
13. heptanol
14. octanal
15. octanol
16. nonanal

Abundance

Time ->  6.00  8.00  10.00  12.00  14.00  16.00  18.00  20.00
Figure 3-5.

Structure of tocopherol adapted from Schuler (1990).
Figure 3-6.

Identification of tocopherols and tocotrienols by TLC.

a = standard γ-tocopherol
b = sample spiked with α-tocopherol
c = sample
d = standard α-tocopherol
e = standard mixture of α-, γ- and δ-tocopherol
f = standard δ-tocopherol
CHAPTER 4

THE EFFECT OF WATER ACTIVITY ON TEXTURE OF MACADAMIA NUT KERNELS
SUMMARY

The objective of the present study was to evaluate the relationship between crunchiness (a desirable texture attribute) and water activity in raw *Macadamia integrifolia* kernels. The two sensory textural attributes were correlated with six instrumental parameters. Discriminative parameters were found to be peak force and % expressible moisture. Significant correlations ($r>0.80$) were observed between sensory (crunchiness and overall texture acceptability) and % expressible fluid, % expressible fat, compressive force at failure and the number of peaks in each curve. The critical water activity where the product became organoleptically unacceptable was above 0.415. The computed monolayer value was 1.93% H$_2$O whose corresponding water activity was 0.188 at 20°C. A dramatic increase in the amount of % expressible fluid and fat was observed at the critical water activity.
INTRODUCTION

Nuts have been outlined by Burdon (1989) as one of the four main product categories of snack foods. He has mentioned two deterioration processes that take place during shelf life. The first one is the loss of texture due to moisture gain and the second is rancidity.

A considerable amount of literature exists to describe the relationship between water activity and microbial growth. The current study was therefore carried out in order to contribute to the understanding of the relationship between texture and water activity.

Texture, as an important food attribute, has been described by Bourne (1982) as "a group of physical characteristics that arise from the structure of a food, which belong to the mechanical or rheological subheading, they are sensed by the feeling of touch and are measured objectively by functions of mass, time and distance".

Water activity has been defined by Hardman (1976) as the ratio of the partial vapour pressure of water in equilibrium with the solution (Pequ) to the vapour pressure of pure water at the same temperature and pressure as the solution (Po).

The following formula is used:

$$ a_w = \frac{Pequ}{Po} $$

Of great concern in the processing of foods is the water activity below 0.95. The water content and water activity relationship is the moisture sorption isotherm (Troller 1989).

Rockland (1969) listed the characteristics of foods in terms of the moisture sorption isotherm as in Figure 4-1.
Labuza (1968) described region 1 as corresponding to the adsorption of a monomolecular film of water; region 2 as adsorption of additional layers over this monolayer; and region 3 as the condensation of water in the pores resulting in dissolution of the soluble matter.

Three types of isotherms (Figure 4-2) and the cause of their shape have been described by Labuza (1984a). The type I isotherm shown in Figure 4-2 is that of pure crystalline sugar in which the moisture gain is significant at an \( a_w \) above 0.7-0.8. The reason for type I is that it illustrates that the only effect of water is the hydrogen bonding to the -OH groups in the crystal. The sugar, ground to smaller particles will increase the surface area hence the capability of adsorption of water will increase at low water activity. Type II isotherm is a sigmoid shaped isotherm in which foods such as cereals follow. Two bends in the curve are noticed (at \( a_w \) 0.2-0.4 and 0.6-0.7) which arise as a result of the changes in magnitude of the separate physical chemical effects (Raoult's law, capillary effects and surface water interactions).

In type III isotherms (anticaking agents) water is at specific sites while the binding energy is large resulting in low water activity (vertical rise at low water activity). The large increase in water activity is due to the fact that all the binding sites and narrow capillaries are filled. Therefore the material is insoluble and thus water added interacts with the water present through weak hydrogen bonding resulting in a solution on the surface.

The moisture content at which each polar and ionic group has a water molecule bound to form the beginning of a liquid like phase is the Brunauer Emmet Teller (BET) isotherm as defined by Labuza, 1984b. Labuza (1968) cited the BET model from Brunauer et al. (1938 and Acker (1963) as follows:
\[
\frac{V}{V_m} = \frac{C\alpha}{(1-\alpha)[1+(C-1)\alpha]}
\]  
(eq. 1)

where

- \( V \) = volume adsorbed either in mL/gram solid or g/g solid
- \( V_m \) = monolayer value (same units as \( V \))
- \( C \) = \( k \exp (Q_s/RT) \)
- \( k \) = accommodation coefficient/frequency factor \( \approx 1 \)
- \( Q_s \) = heat of adsorption on homogeneous sites
- \( R \) = gas constant
- \( T \) = absolute temperature in degrees kelvin
- \( \alpha \) = activity

Equation (1) is arranged into equation 2:

\[
\frac{\alpha}{(1-\alpha)V} = \frac{1}{V_mC} + \left[ \frac{\alpha(C-1)}{V_mC} \right]
\]  
(eq. 2)

A straight line is obtained from plotting \( \alpha/(1-\alpha)V \) versus \( \alpha \). The intercept \( (I) \) and slope \( (S) \) of this line enables one to calculate the monolayer value as follows:

\[
V_m = \frac{1}{S+I}
\]

A limited number of studies have taken place to describe the relationship between textural properties of a food and the BET monolayer value. Bourne (1986) observed major textural changes just near the BET monolayer value (\( a_w 0.14 \)) in apple flesh.

Katz and Labuza (1981) studied the effect of water activity on the texture of four snack foods (potato chips, popcorn, puffed corn curls and saltines). It was hypothesised that the considerable hydrogen bonding and Van der Waals forces that occur between the carbohydrate macromolecular matrix at the BET monolayer could aid in the crispness sensation when eating.
They also found that, as the water activity decreased, the sensory acceptability increased and the critical water activity was observed in the range 0.35-0.50. This water activity was above the BET monolayer values calculated for all four snack foods (aw 0.17-0.22). Studies on rates of chemical reactions have shown that, a moisture content exists at which the rates of quality loss are insignificant (Labuza, 1984b). This moisture content is related to the BET monolayer value determined from the BET isotherm equation which usually is near a water activity of 0.2-0.4. An increase in water activity causes an increase in solubility and mobility and therefore the rate is faster. However, at some point the reaction rate falls as no more species dissolve.

This is contrary to the findings of Veeraju et al. (1978) who observed that the colour and flavour of walnut, Juglan regia kernels were more stable at a moisture content of 3.2% and 3.6% rather than the monolayer value of 2.2%.

Szczeniak (1986) outlined the reasons why researchers sought correlations between sensory and instrumental analyses as: a) to predict consumer response; b) to understand perception in sensory texture assessment; c) and to develop improved/optimised instrumental methods that would in turn duplicate the sensory evaluation. In addition, instruments for quality control are required.

Researchers have found correlations between sensory and instrumental analyses in foods. Brady et al. (1985) found a high correlation between sensory springiness and adjusted downstroke cohesiveness for beef and beef soy loaves. Lee et al. (1987) observed high correlations (r>0.80) between compressive force and sensory elasticity, firmness and chewiness, stress relaxation and elasticity, total expressible fluid and juiciness, and expressible moisture and wateriness.

Vickers (1980) found Young's Modulus to have the highest correlation with sensory firmness of all the instrumental parameters.
Vickers (1987) discovered that combined measurements of acoustical and force-deformation gave a good measure of oral chip crispness. In 1988, Vickers reported on the correlation of acoustical and force deformation measurements with sensory crispness as high as $r = 0.99$. This measurement appears to be useful with such high correlations. Mohammed et al (1982) also found a very high correlation between sensory crispness and both sensory sound intensity ($r = 0.91$) and instrumental level of sound ($r = 0.70$) suggesting that a combination of the two provide great use in the measurement of sensory crispness.

Zabik (1979) examined the effects of humidity on the texture of sugar-snap cookies. The correlation between crispness and relative humidity was $r = -0.94$ for high quality flour cookies and $r = -0.89$ for low quality flour cookies. Vickers (1984) found a difference in pitch between crispness and crunchiness (foods that were more crispy than crunchy produced higher-pitched sounds than those which were more crunchy than crispy). Vickers (1981) observed close correlations between oral crunchiness and oral crispness ($r = 0.97$) as well as auditory crispness and auditory crunchiness ($r = 0.96$) in sixteen foods she studied. In 1985, Vickers observed a higher pitch and loudness in crispy foods than in crunchier ones with a few exceptions.

Crunchiness is an important attribute in snack foods. It has been described by Szczesniak (1990) as a universally accepted textural characteristic which refers to materials that break readily and abruptly giving a particular sound effect once energy is released. Its loss (resulting in sogginess) could lead to loss of consumer acceptance. Seymour and Hamann (1988) studied crispness and crunchiness of five low moisture foods by subjecting each food to three different water activities. They observed that both sensory crispness and crunchiness decreased as the water activity increased and strong correlations ($r > 0.90$) were also observed between sensory crispness and crunchiness and mechanical force and work.
As there has been no known reported work conducted on water activity and texture relationship of macadamia nuts, it was essential to explore this area in order to gain a better understanding.

The purpose of the present study was to:

1. evaluate the effect of water activity on the sensory crunchiness and overall acceptability of macadamia nut kernels;

2. determine the critical water activity where the product was rejected due to lack of crunchiness or poor texture;

3. correlate the sensory textural attributes (crunchiness and overall textural acceptability) with six instrumental parameters (% expressible fluid, % expressible fat, % expressible moisture, compressive force at failure, number of peaks in each curve and peak force); and

4. determine if there is any major textural change at the BET monolayer.
MATERIALS AND METHODS

MATERIALS
First grade whole shelled Macadamia nuts (Macadamia integrifolia) of the cultivar Keauhou (Hawaii Agricultural Experimental Station, cultivar 246) purchased from Macadamia Plantations of Australia were used. Before delivery the nuts where harvested and dehusked at the factory. The nuts were treated at the Plantation before receipt as described in Chapter 2.

STORAGE CONDITIONS
Eight duplicate 400 g lots of samples were placed in canisters over saturated salt solutions (eight salts whose relative humidities ranged from 0% to 75%) based on Labuza (1984c). These samples were stored at 20°C until they were equilibrated with the surrounding atmospheres.

DETERMINATION OF MOISTURE CONTENT
Initial and final moisture (after equilibration) content was determined in triplicate using the Dean and Stark method as described by WHO (1973). Samples were 10-40 g.

DETERMINATION OF WATER ACTIVITY
The nuts were crushed to about 1 mm size using a mortar and pestle for measuring the water activity. The initial water activity (at the start) and end (upon equilibration) was determined at 20°C using the Novasina Water Activity meter type TH2 after calibration.

SENSORY ANALYSES
Sensory evaluation was carried out in a quiet and comfortable area having individual sensory evaluation booths.
Fourteen panellists were selected on the basis of their previous sensory panel experience in macadamia and pecan nuts. The nuts used had different levels of texture due to the varying extreme storage relative humidities. A triangle test was carried out in order to select the panellists on their ability to differentiate between crunchy and soggy macadamia nuts.

After the nuts were equilibrated to water activities 0.090, 0.105, 0.200, 0.270, 0.350, 0.415, 0.550 and 0.63 at 20°C they were cut to 10 mm height. Samples (15 g) from each treatment were placed in plastic containers covered with aluminium foil.

A balanced incomplete block design was used for sample presentation using fourteen panellists with each panellist being presented with four samples as described by Cox (1958). The four samples were coded and presented to the panellists in a random order with ambient temperature water for rinsing. Panellists were instructed to evaluate crunchiness which they indicated on a numberline (10 cm) with 0 cm (not crunchy), 5 cm (moderately crunchy) and 10 cm (extremely crunchy). Texture acceptability was then evaluated and recorded numerically using the hedonic scale which ranged from 1 (dislike extremely) to 9 (like extremely) with 5 indicating "neither like nor dislike".

**INSTRUMENTAL ANALYSES OF TEXTURE**

After equilibrating samples to the desired water activities, they were then cut in halves (10 mm height each) and analysed using an Instron Universal Testing machine (model 1140).

Samples were analysed at room temperature using a 3.6 cm diameter compression flat plate at a 50 mm/min crosshead speed and 200 mm/min for chart speed. Fifty samples were tested from each treatment.
Half of each lot (twenty five), 10 mm halves were each compressed to 70% of their original height (from which compressive force at failure, number of peaks in each curve and peak force were determined). Expressible fluids were determined using the method employed by Lee et al (1987) in their study on frankfurters. To determine expressible fluids the other twenty five samples were each compressed to 70% and the fluid was collected on previously dried filter paper (Whatman #41 having a 9 cm diameter). Expressible fluid was determined by reweighing the filter paper and taking it as a sample percentage. The filter papers were then dried at 100°C for 12 hours and reweighed to evaluate the expressible fat. Percent expressible moisture was calculated as the difference between the percentage total expressible fluid and percentage fat.

STATISTICAL ANALYSIS

Correlation coefficients were determined using the BMDP, statistical package. Analysis of variance and Duncans multiple range test (Duncan, 1955) at 5% significance level was carried out for each parameter at different water activities. The upper and lower confidence interval was determined at 5% significance level of confidence based on Steel and Torrie (1980).
RESULTS AND DISCUSSION

To ensure that an equilibrium had been reached, three nuts from each treatment were put aside in a petri dish and weighed each week until a constant weight was achieved as shown in Figure 3. This gradual uptake or loss of water could be due to the high fat content of about 75% dry basis (fat is hydrophobic in nature and has less sorption capacity) in the nuts. At relative humidities 69% and 75% the changes in mass of sample were greater (as expected because the environment was more saturated than the others) compared to the lower relative humidities. Around the fourth week a constant weight was achieved. This is similar to the findings of Saravacos (1969) who discovered that defatted soybean sorbed more water and at a faster rate than full fat soy bean.

Figure 4. is a plot of the difference between the expected water activity and the attained equilibrium with the immediate environment provided by the salt solution. The nuts did not show an equilibrium water activity corresponding to the specific relative humidity. It is observed that at very low (0.00%) and high (54%, 69% and 75%) relative humidities, the difference is quite large. At high relative humidity the large difference could be due to the slow sorption of water as this was a much higher water activity to attain. However there has been no explanation found for the large difference at the low relative humidity. Perhaps the size and internal structure of the nut also impedes equilibration. In any case the data suggest that macadamia nuts will be slow to adsorb moisture in humid climates. Veeraju et al (1978) in his study on walnuts attained water activities corresponding to their relative humidities by introducing a vacuum to facilitate equilibrium. Since it was required to have a range in water activity, the nuts were analysed without attaining the corresponding relative humidities.
THE BET MONOLAYER

The moisture sorption isotherm for Macadamia nuts is shown in Figure 5. This is a sigma shaped isotherm which was described by Labuza (1984a) as a type II isotherm in which two inflection points are noted. At water activity 0.100 and 0.35 the equilibrium relative humidity of the kernels changed significantly for a little change in moisture. Veeraju et al (1978) observed similar effects in walnuts at water activity between 0.2 and 0.6.

The BET monolayer value was calculated using linear regression for the plot of \( a/(1-a)m \) (Figure 6) which was as follows:

\[
\text{for } a_w 0.20-0.415, r^2 = 0.980, I = 0.0384, S = 0.4808.
\]

From the above calculations the BET monolayer value was calculated as 1.926% whose corresponding water activity from the moisture sorption isotherm (Figure 5) is 0.188. As studied by Labuza (1984b), below this BET the rate of quality loss is negligible below this moisture content. There was no major textural changes observed at the BET monolayer although Bourne (1986) observed major textural changes near the BET monolayer value \((a_w, 0.14)\) in apple flesh.

SENSORY EVALUATION

Crunchiness is an important attribute whose absence (sogginess) indicates poor quality and therefore reduces consumer acceptance. The values in Table 1 show that as the water activity increased beyond 0.270, the nuts became less crunchy and less liked. These trends were statistically significant. Lack of crunchiness in the nuts appeared to be related to a reduced textural acceptability of the product. Figures 7 and 8 show the critical water activity for crunchiness and the overall textural acceptability for the raw macadamia nuts (0.415) whose crunchiness score was 6.14 which is just above the moderately
crunchy score as in Figure 7. Also for the overall textural acceptability the score was 6.43 which was above the "like slightly" value of the hedonic scale as in Figure 8. This investigation was similar to the one carried out by Katz and Labuza (1981) who found differences in the crispness of snack foods and established critical water activities (0.35-0.50) from the sensory results. Seymour and Hamann (1988) also observed a decrease in sensory crispness and crunchiness as the water activity of five low moisture foods increased.

In Table 2, the relationship of the critical water activity and the moisture content for sensory evaluation to the monolayer value are shown. The critical water activity values obtained were higher than the monolayer values which were similar to the findings of Katz and Labuza (1981). The critical water activities for both crunchiness and overall acceptability in this study were the same showing that the crunchiness intensity is related to the textural attribute for acceptance.

MECHANICAL AND SENSORY ANALYSES

The panelists were able to establish critical water activities from the degree of crunchiness as well as overall textural acceptability. The critical water activity determined using the sensory evaluation was compared with the mechanical analyses in order to observe any similarities. Compressive force at failure was studied as in Figure 9. Initially at water activity 0.09, the force required is fairly small in the force deformation curve. There are differences as detected but they were not exactly the same as those perceived by the panel. Although the samples after water activity 0.415 were significantly the same as those determined in the overall textural acceptability, this change was very similar to that observed at the critical water activity in sensory evaluation.
The peak force (Figure 10) was also examined but there was no indication of any change at the critical water activity. No significant difference was observed between water activity 0.105 and 0.200 as well as water activity 0.35 to 0.63. The observations for % expressible moisture (Figure 11) were the same as those observed in peak force. Lee et al (1987) used expressible fluid, fat and moisture in the study for frankfurters. It was adapted in this study although no researcher has previously used it to study the evaluation of texture in macadamia nuts. Some very promising results were obtained. For expressible fluid and fat, similar findings were observed as shown in Figure 12 and 13. A linear rise occurs initially and then a leveling between water activity 0.105 and 0.27 and another leveling is between water activity 0.35 and 0.415. After that, a change at the critical water activity was very similar to the one observed in sensory evaluation.

It is observed that the amount of fat and fluid expressed at low water activity is lower than that at high water activity indicating that probably at low water activity the nuts are much more compact thus making it difficult to express the fluid. However at high moisture they are not so compact thus more fluid is expressed.

The number of peaks in each curve was done by direct counting of the number of jags in each curve. This measure, although not presented previously in this manner, is similar to the criterion used for presenting mechanical analyses for puffed corn curls in which a jagged curve (representing a more crispy product) and a smooth (representing a less crispy product) as presented by Katz and Labuza (1981). In Figure 14, there was a difference in which the number of
peaks decreased with the increase in water activity (the number of jags decreased with increase in water activity and vice versa).

Results in Table 3 show a significant relationship between sensory (crunchiness and overall acceptability) and mechanical analyses. Strong correlations were found between compressive force to failure and crunchiness of five low moisture foods by Seymour and Hamann (1988).

A low correlation was observed between peak force, % expressible moisture and sensory analyses. The low correlation between % expressible moisture and sensory analyses could be due to the fact that there was a very small amount of moisture to be expressed. A second reason could be due to the fact that the method itself was not sensitive enough to pick up any differences in the amount of moisture present.
CONCLUSION

A significant relationship was found between instrumental and sensory analyses. It was found that of the instrumental analyses, compressive force at failure, number of peaks in each curve, % expressible fluid and fat were good indicators of crunchiness. The results suggested that these parameters could be useful in measuring texture acceptability in Macadamia nuts without the involvement of costly subjective analyses. A critical water activity based on texture was established as 0.415. Below this value, the product was organoleptically acceptable, therefore suggesting that the product could be kept to this water activity and its texture be accepted. The BET monolayer was established. However, there was no major textural changes observed at the BET monolayer. Further investigation needs to be carried out to find how these instrumental parameters could actually be related to sensory responses.
REFERENCES


TABLES
Table 4-1.
Average scores for crunchiness and overall textural acceptability.

<table>
<thead>
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<th>water activity</th>
<th>crunchiness**</th>
<th>overall textural acceptability***</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.090</td>
<td>6.93ab*</td>
<td>6.86ab</td>
</tr>
<tr>
<td>0.105</td>
<td>7.76a</td>
<td>7.86c</td>
</tr>
<tr>
<td>0.200</td>
<td>7.43a</td>
<td>7.57ac</td>
</tr>
<tr>
<td>0.270</td>
<td>7.50a</td>
<td>7.71ac</td>
</tr>
<tr>
<td>0.350</td>
<td>6.49b</td>
<td>6.43b</td>
</tr>
<tr>
<td>0.415</td>
<td>6.14b</td>
<td>6.57b</td>
</tr>
<tr>
<td>0.550</td>
<td>3.14c</td>
<td>2.86d</td>
</tr>
<tr>
<td>0.630</td>
<td>1.64d</td>
<td>2.57d</td>
</tr>
</tbody>
</table>

* Means followed by the same letters are not significantly different from each other (p <0.05).

** 0 (not crunchy), 5 (moderately crunchy) and 10 (extremely crunchy).

*** 1 (dislike extremely), 5 (neither like nor dislike) and 9 (like extremely).
Table 4-2. Relationship of critical water activity and moisture content for sensory evaluation of crunchiness to the monolayer value.

<table>
<thead>
<tr>
<th></th>
<th>water activity</th>
<th>moisture content</th>
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</thead>
<tbody>
<tr>
<td>Monolayer value</td>
<td>0.188*</td>
<td>1.93**</td>
</tr>
<tr>
<td>crunchiness</td>
<td>0.415</td>
<td>3.00</td>
</tr>
<tr>
<td>acceptability</td>
<td>0.415</td>
<td>3.00</td>
</tr>
</tbody>
</table>

* corresponding water activity to the monolayer value.

** BET monolayer value.
Table 4-3.  
Correlation coefficients for instrumental and sensory analyses*  

<table>
<thead>
<tr>
<th></th>
<th>crunchiness</th>
<th>acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak force (kg)</td>
<td>0.3656</td>
<td>0.3363</td>
</tr>
<tr>
<td>number of peaks in each curve</td>
<td>0.8919</td>
<td>0.8132</td>
</tr>
<tr>
<td>compressive force at failure (Kg)</td>
<td>-0.9298</td>
<td>-0.8737</td>
</tr>
<tr>
<td>Total expressible fluid (%)</td>
<td>-0.8405</td>
<td>-0.8141</td>
</tr>
<tr>
<td>Expressible fat (%)</td>
<td>-0.8488</td>
<td>-0.8096</td>
</tr>
<tr>
<td>expressible moisture (%)</td>
<td>-0.2334</td>
<td>-0.3072</td>
</tr>
</tbody>
</table>

*p<0.01
FIGURES
Figure 4-1.

Food texture characteristics as a function of localised water sorption isotherms (Rockland, 1969).
Region 1
(low moisture)
dry
hard
crisp
shrunken

Region 2
(intermediate moisture)
dry
firm
flexible

Region 3
(high moisture)
moist
soft
flaccid
swollen
sticky

Water activity
Figure 4-2.

Three standard moisture isotherms. Crystalline substances (type I), dehydrated foods (type II) and anticaking agents (type III) as outlined by Labuza (1984a).
Figure 4-3.
The increase and decrease (%) of sample due to water uptake and loss during equilibration at eight different relative humidities.
Figure 4.

Comparison of the water activity attained by the nuts at the relative humidity set by each saturated salt solution.
Figure 4-5.

Moisture content versus water activity at 20°C for Macadamia nut kernel.
Figure 4-6.

BET monolayer plot for sorption data of macadamia nuts.
\[ \frac{a}{1-a}m \]

Water activity

- \( m = \frac{1}{s+1} \)
- \( \text{aw} \ 0.20-0.415 \)
- \( r = 0.980 \)
- \( I = 0.0384 \)
- \( S = 0.4808 \)
Figure 4-7.

Sensory crunchiness intensity as a function of water activity.
Figure 4-8.

Hedonic rating (sensory acceptability) versus water activity.
Figure 4-9.

Compressive force (kg) at failure from the force deformation curve versus water activity.
Compressive force at failure (Kg)

Water activity

Trial 2 n=25
Figure 4-10.

Peak force (kg) versus water activity.
Figure 4-11.

Expressible moisture (%) versus water activity.
Figure 4-12.

Expressible fat (%) versus water activity.
Figure 4-13.

Expressible fluid (%) versus water activity.
Figure 4-14.
Number of peaks versus water activity.
GENERAL CONCLUSION

Significant correlations in studies between objective and subjective measurements were obtained. Correlations observed between sensory (crunchiness and overall texture acceptability) and % expressible fluid, % expressible fat, compressive force at failure and the number of peaks in each curve were significant ($r > 0.80$). From a textural viewpoint the product became organoleptically unacceptable at the critical water activity (above 0.415). The BET monolayer value was 1.93 % H$_2$O with a corresponding water activity of 0.188 at 20°C.

While hydrolytic rancidity measured by free fatty acids followed apparent first order kinetics and was little influenced by variations in water activity between 0.3 and 0.5, oxidative rancidity measured by peroxide value was markedly affected by variations in water activity and further work will be required to definitively establish the kinetics of oxidation in stored macadamia. Volatiles formed by the oxidative deterioration of the nuts were identified as, 2-methylpropanol, 3-methylbutanal, 2-methylbutanal, pentanal, heptane, 4-methylpentan-2-one, pentanol, methylbenzene, hexanal, octane, hexanol, heptanal, heptanol, octanal and nonanal. At two months of storage at 30°C and relative humidity of 50% panellists detected rancidity corresponding to a hexanal concentration of 0.39 ppm and a peroxide value of 0.37 meq/kg. Naturally present antioxidants were $\alpha$-tocopherol and $\alpha$-tocotrienol.

These findings contributed to the understanding of the deteriorative processes (loss of texture and development of rancidity) that take place in *Macadamia integrifolia* nuts during storage.
STUDIES ON THE SHELF
LIFE OF MACADAMIA NUTS

Thesis submitted in partial fulfillment of the
requirements for the Degree of Master of Science (Honours)
in Food Science and Nutrition

by

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1994
PLEASE NOTE

The greatest amount of care has been taken while scanning this thesis,

and the best possible result has been obtained.
DECLARATION

This is to certify that the work in this thesis has not been given to any other University or Institution for a higher degree.

ELIZABETH MALAMA CHITUNDU
ABSTRACT OF THESIS

Shelf life studies of raw *Macadamia integrifolia* nuts were carried out at different storage temperatures, relative humidities and time. An accelerated shelf life test was conducted. The influence of water activity on the texture of the kernels was studied. A comprehensive literature search has revealed no reported use of headspace gas chromatography in the determination of oxidative volatile compounds produced due to deterioration of these nuts. The antioxidants naturally present were identified by the use of thin layer chromatography (TLC).

Accelerated shelf life tests based on the determination of free fatty acids showed that hydrolytic rancidity followed apparent zero order kinetics within the water activity range of 0.3 to 0.5. Oxidative rancidity measured by peroxide value appeared to follow different orders of reaction at different temperatures. However, water activity was not held at a constant value. This work should be repeated to clearly establish the impact of water activity and temperature on the rate of oxidative rancidity. This study could help in predicting the shelf life of the many different products being developed in the macadamia industry.

Headspace gas chromatography and gas chromatography-mass spectrometry (GC-MS) enabled the identification of volatiles such as 3-methylbutanal, 2-methylbutanal, pentanal, nonanal, octanal, heptanal, hexanal, pentanal, 2-methylpropanol, heptane, octane, methylbenzene, pentanol, hexanol, heptanol, octanol and 4-methylpentan-2-one. Hexanal was used as an index for measurement of oxidative rancidity. At two months of storage panellists detected rancidity at which the headspace concentration of hexanal was 0.39 ppm and a peroxide value of 0.37 meq/kg. The antioxidants present were identified as α-tocopherol and α-tocotrienol.
Headspace gas chromatography was done to find a quick and effective method for measuring oxidative rancidity compared to a non-specific method such as peroxide value. The presence of natural antioxidants was verified to explain the shelf life of macadamia nuts.

In textural study, correlations (r>0.80) were found between subjective (crunchiness and overall texture acceptability) and objective measurements (% expressible fluid, compressive force at failure, number of peaks in each curve and % expressible fat). The product became unacceptable organoleptically above water activity 0.415 when stored at 20°C. This level was established as the critical water activity. A dramatic increase in the % expressible fluid and fat was observed at this water activity. The monolayer value was 1.93% H₂O with a corresponding water activity of 0.188 at 20°C. Besides rancidity nuts undergo texture loss as a deterioration process. There is a need to establish a water activity at which the consumer is likely to reject the product.
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