Optimising kernel processing for shelf-life

Dr Cameron McConchie
CSIRO Plant Industry

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Final Report

Optimising kernel processing for shelf-life

MC 06010
(June 2010)

Cameron McConchie, Aijun Yang, Robert Forrester, Scott Underdown and Kirsty Macpherson

CSIRO

TABLE OF CONTENTS

CHAPTER 1 – Project Details

Project Title:	Optimising kernel processing for shelf-life
Project Number:	MC 06010

Project Leader:	Dr C. McConchie CSIRO Plant Industry Queensland Bioscience Precinct 306 Carmody Rd St Lucia Qld 4067 Phone :07 3214 7789 Fax: 07 3214 2272 Email: cameron.mcconchie@csiro.au
Other Key Personnel:	Aijun Yang, Robert Forrester, Kirsty Macpherson

Purpose of the report:
The aim of this project is to improve the shelf life of roasted macadamia by:

1) Describing the effects of roasting on kernel colour, moisture content, texture and structure.
2) Subjecting variously stored raw and roasted kernel to accelerated aging conditions.
3) Application of analytical methods to quantify kernel deterioration.
4) Developing models to predict deterioration.

Funding sources:	HAL, AMS, CSIRO and collaborating Growers
Date of report:	May 2010

CHAPTER 2 – Summaries

2.1 Media summary

Freshly roasted macadamia kernels are highly desired for their unique flavour and texture. The capacity to reliably deliver this experience to consumers is proving a challenge. The Australian macadamia industry is striving to find reliable diagnostic measurements linked to processing and consumer perception of kernel quality. This project has examined the effects of kernel moisture content, roasting and storage duration on kernel deterioration using accelerated shelf-life testing (ASLT). This information was used to develop kinetic models to describe the effects of temperature and storage duration on kernel deterioration. This information is a key element for the future development of a system for the prediction of shelf-life of macadamia products. In the interim the ASLT was used to detect kernels with low oxidative stability in commercial consignments as they pose greatest threat of alienating consumers. The ASLT is able to simulate the effects of 6 months storage in 6 weeks, and the outcome from these ALST trials could be predicted within two weeks. In controlled research trials little or no change in kernel composition is observed with this treatment where as less than half of the commercial samples were within acceptable product specifications after this time. This indicates a widespread problem throughout the Australian macadamia industry. It was found that there was no relationship between oxidative stability after 6 weeks of ASLT and the visual assessment of kernel quality showing there is currently limited capacity to manage kernel shelf-life. The variation in oxidative stability within consignments was very large so significant difference between consignments was detected. Development of sampling protocols and quality control procedures will be required to manage oxidative stability commercially. In these

investigations there were significant cultivar and kernel moisture content effects that need to be understood to prevent confounding this issue.

2.2 Technical summary

Macadamia kernel is roasted to improve visual appeal and flavour. Kernel moisture content and roast conditions are known to affect oxidative stability and kernel texture of other tree nuts. Even mild oven roasting conditions have been shown to remove more than 60% of the pre-roast moisture content but the effects of this on kernel keeping properties are unknown. Drying macadamia nuts to current recommended processing moisture contents is energy and time consuming. In this project, the following components were investigated:

- The potential to manipulate raw kernel moisture and oven conditions to achieve acceptable kernel colour.
- The effects of initial raw kernel moisture content, storage temperature and duration on kernel texture and oxidative stability.
- The kinetics of oxidative rancidity development during macadamia storage using hexanal production for varying temperatures and storage duration.
- The capacity of the tools developed within this project to identify commercial consignments with low oxidative stability.

In the first component of this project the potential to manipulate kernel moisture contents and roast conditions to achieve acceptable colours were investigated for use in subsequent storage trials. The experiments were performed using three cultivars (A16, 814, and 849), with three pre-roast kernel moisture contents (1.5, 3, and 5% w/w). The kernels were roasted at 4 temperatures for 5 durations that were repeated 4 times in a completely randomized design. Pre- and post-roast masses were recorded as were pre- and post-roast colour. There were highly significant ($P<0.001$) main effects due to temperature, heat sum, cultivar and moisture content for all colour variates and changes to kernel mass. There were also highly significant ($P<0.001$) interactions between temperature and heat sum, moisture content and heat sum for all colour variates, and between moisture content and temperature for ΔL^* and Δa^*. The

interactions between moisture content and heat sum were not as strong for the mass change variates. There were several kernel moisture and roasting combinations that provided acceptable roast colours with 3% kernel moisture content the highest, safe moisture content tested to roast kernel before the post-roast *L** value became excessive. Changing the moisture content of the nut-in-shell to effectively change kernel moisture had significant effects on whether the kernel remained intact or was cleaved into halves. This effect differed between cultivars though in all cultivars the nuts that failed to crack tended to have a lower mass. In all cultivars cracking nut-in-shell with a kernel moisture content of 5% reduced the proportion of whole kernel. These roasting and cracking results indicate there is potential to process macadamias at higher kernel moisture contents but this should only be contemplated provided sensory attributes, storage characteristics and processing efficiencies are unaffected.

The effects of storing raw and variously roasted kernel of two macadamia cultivars at two initial kernel moisture contents at 22^{o}C and 40^{o}C for periods up to 60 weeks on kernel quality were also assessed. The kernel of A16 and Own Venture were selected for investigation because of their different linoleic acid content that had previously been shown to be associated with oxidative stability. Own Venture kernel had the higher linoleic content and invariably had lower oxidative stability as measured by hexanal levels and peroxide values after all treatments and storage conditions. Kernel were roasted at three different conditions 125^{o}C for 25 min, 135^{o}C 10 min and 156^{o}C for 2min. All roasting treatments reduced kernel peak fracture force compared to raw kernel. The fracture force of all kernel whether raw or roasted also lowered with storage duration. However the amount of moisture loss and initial changes to fracture force were significantly less when roasted at 156^{o}C. There were no differences in hexanal levels or peroxide values between kernels of the same cultivar after similar storage durations due to any of the roasting treatments. After some of these roasting treatments the kernel moisture content was below the recommended level and as low as 1.2% (w/w) but still showed the same pattern of deterioration as higher moisture content kernel indicating that over drying does not accelerate deterioration. While the target moisture contents were between 1.5 and 2.5% these precise levels were not achieved. The mean kernel moisture contents for these classes of kernel in reality differed by less than 0.5% but kernel at a lower moisture content were significantly more stable. Examination of stained kernel examined under florescence microscopy

confirmed that oil was stored in numerous oleosomes within the kernel cells however after roasting these were invariably ruptured resulting in oil coalescence into large droplets. The response of macadamia kernel to roasting is similar to other tree nuts in that fracture force is reduced with roasting. However, unlike other tree nuts, the oxidative stability was not affected.

The kinetics of oxidative rancidity development during macadamia storage was described using hexanal production for a temperature range of 25-55^{o}C for storage up to 140 days. Hexanal was used because it had previous been shown to have the best correlation with taste panels perception of rancidity of any chemical indices. The rate of hexanal production was affected by time and storage duration. The results showed that deterioration was satisfactorily described by an Arrhenius-type temperature dependence model of coefficients. The activation energy for the hexanal level was found to be 55.9 kJ/mol over the temperature range of the study. A generalised model to describe the production of hexanal during storage of raw macadamia kernel as a function of temperature and time was established. This information can be used to develop models to predict shelf life but will need to be supplemented by investigation on different kernel styles, storage conditions and packaging materials.

While many of the elements needed for shelf-life prediction in macadamia exist, including chemical indices to monitor rancidity that are related to consumer perception of deterioration and kinetic models to describe deterioration and putative thresholds for consumer acceptance, these require further validation and development to encompass the diversity of kernel quality, package and storage conditions. In the interim it was proposed to determine the capacity of existing chemical indices and accelerated shelf-life testing protocols to segregate the least stable consignments at delivery for processing. This information will also provide information on the variability of consignments and sampling needs. To do this the oxidative stability of premium and commercial grade kernel retained from the visual assessment testing of commercial nut-in-shell consignments of macadamias was assessed. The samples came from 4 processors at monthly intervals from May to December 2009. These samples contained only kernel that was considered saleable, premium and commercial grade, with all reject kernel removed and recorded. In addition to these 200 g samples a single 1 kg sample was requested for each month. The initial kernel moisture

content was measured and reduced to below 2% (w/w) if required. Initial rancidity tests were made using extracted oil for peroxide value (PV), % free fatty acids (FFA), conjugated dienes (CDHP), and p–anisidine value (pAV). A single 50 g subsample was taken from the 200g sample with 5 samples taken from the 1 kg samples. Repeated hexanal measures were made on the same jars after storage at accelerated shelf-life testing (ASLT) conditions of 0, 2, 4 and 6 weeks when the trial was terminated and kernel again tested with the same suite of rancidity tests. There was no correlation between visual quality assessments and any of the measures of kernel rancidity before or after ASLT. Initial PV and FFA values were also only weakly related to final ASLT results indicating minimal potential to manage kernel quality with existing tests. All other measures, CDHP and pAV, were not or only weakly related to the hexanal level at week 6. By comparison the correlation between hexanal at week 0 and hexanal at week 6 was $R^2 = 0.556$ and by week 2 this increased to a significant $R^2 = 0.921$, ($P < 0.016$). Kinetic models indicated that storing kernel for 6 weeks ASLT conditions was equivalent to 6 months at ambient (20^oC). Our results indicate that the response to 6 weeks ASLT can be predicted after 2 weeks and that this may be further reduced by optimizing the ASLT conditions. However the variability between subsamples within the 1 kg samples was so great that it was not possible to demonstrate any significant differences between consignments. In order to implement the use of a chemical index of oxidative stability, a revision of sampling protocols and perhaps reduction in analytical costs and time will be required.

In conclusion, this project has

- Shown that current commercial dry roast conditions due not necessarily reduce the oxidative stability of raw kernel.
- Confirmed the importance of kernel moisture content and cultivar in determining oxidative stability but challenges the recommended safe moisture contents for storage
- Develop a kinetic models that describes kernel deterioration due to storage temperature and duration that can be used to predict storage properties
- Demonstrated that accelerated shelf life testing using hexanal measurement can identify consignments with low oxidative stability but will require development of sampling protocols for commercial application.

CHAPTER 3

3.1 Roasting and processing

There is little evidence to indicate that there is a safe NIS moisture content above 3.5%. Drying to low kernel moisture contents should be considered as early as possible throughout the supply chain.

In the absence of any sensory data the adoption of higher roast temperatures for shorter durations should be commercially evaluated for potential to increase throughput.

Due to numerous interactions in cracking nuts the effects of size of nut in shell and kernel moisture contents need to be considered in evaluating cultivar kernel processing characteristics and cracker performance.

3.2 Detection of unstable kernel

The sampling strategy used in commercial trials was very limited. The use of hexanal for the detection of kernel low oxidative stability needs to be further evaluated within a commercial environment taking sampling into account to determine whether it can satisfactorily characterise consignments.

Since these trials indicate that nut in shell received by the current process is already compromised, appropriate sampling protocols will need to be developed to efficiently describe the oxidative stability of kernel through the value chain. Based on these results appropriate control points and actions need to be developed.

Potentially much more intensive sampling for oxidative stability will be required for cost effective detection of kernel with low oxidative stability after accelerated shelf life testing. These options should include near infra red spectroscopy and e-nose.

Previous research indicated that a higher storage temperature could be used for accelerated aging of kernel. This needs to be evaluated to determine the potential to

further streamline accelerated aging conditions and reduce the time taken to assess kernel stability.

It would be desirable to have a non-destructive analytical system that could predict oxidative stability. To this end the spectral properties of raw kernel should be investigated to determine whether unstable kernel can be detected without accelerated aging.

The existing SPME-GC technology in combination with accelerated aging is to the stage where it should be used to review the effects of current harvest and post-harvest practices to minimize the effects of kernel oxidative stability, and not just visual defects.

The existing SPME-GC technology in combination with accelerated aging should be used to describe the genetic, developmental and environmental basis of unstable kernel to ensure industry development does not exacerbate this problem.

3.3 Shelf-life prediction

Chemical indices to define thresholds for acceptability of kernel that are related to consumer perceptions of kernel rancidity and staleness need to be developed and adopted.

The distribution of unstable kernel within retail packs and the impact this has on the perception of quality by consumers needs to be understood to support the development of quality control processes.

Kinetic models that relate temperature and storage duration under defined conditions need to be developed for a greater range of kernel styles and packaged product.

The effects of low temperatures on oil crystallization and oxidative stability needs to be described to optimize long term storage.

The knowledge of the physical properties of macadamia oil and the existing accelerated shelf life test need to be combined with kinetic models of kernel deterioration and thresholds of consumer acceptance to provide reliable predictors of kernel shelf life for the different macadamia products.

3.4 General

Payment schemes to reward and penalize growers based on the oxidative stability of related the commercial value have to be developed to drive industry change.

Chapter 4

The effects of roasting conditions on macadamia kernel moisture content and colour

4.1 Abstract

Macadamia kernel is roasted to improve visual appeal and flavour. Kernel moisture content and roast conditions are known to affect oxidative stability and kernel texture of other tree nuts. Even mild oven roasting conditions have been shown to remove more than 60% of the pre-roast moisture content but the effects of this on kernel keeping properties are unknown. Drying macadamia nuts to current recommended processing moisture contents is energy and time consuming. The aim of the current experiments was to investigate the potential to manipulate kernel moisture contents and roast conditions to achieve acceptable colours for use in subsequent storage trials. The experiments were performed using three cultivars (A16, 814, and 849), with three pre-roast kernel moisture contents (1.5, 3, and 5% w/w). The kernels were roasted at 4 temperatures for 5 durations that were repeated 4 times in a completely randomized design. Pre- and post-roast masses were recorded as were pre- and post-roast colour. There were highly significant ($P<0.001$) main effects due to temperature, heat sum, cultivar and moisture content for all colour variates and changes to kernel mass. There were also highly significant ($P<0.001$) interactions between temperature and heat sum, moisture content and heat sum for all colour variates, and between moisture content and temperature for ΔL^* and Δa^*. The interactions between moisture content and heat sum were not as strong for the mass change variates. There were several kernel moisture and roasting combinations that provided acceptable roast colours with 3% kernel moisture content the highest, safe moisture content tested to roast kernel before the post-roast L^* value becomes excessive.

Changing the moisture content of the nut-in-shell to effectively change kernel moisture had significant effects on whether the kernel remained intact or was cleaved into halves. This effect differed between cultivars though in all cultivars the nuts that

failed to crack tended to have a lower mass. In all cultivars cracking nut-in-shell with a kernel moisture content of 5% reduced the proportion of whole kernel.
The roasting and cracking results indicate there is potential to process macadamias at higher kernel moisture contents but this should only be contemplated provided sensory attributes, storage characteristics and processing efficiencies are unaffected.

4.2 Introduction

Macadamia kernel, like other tree nuts, is roasted to improve flavour, texture and nutritional value. Most early investigations of roasting in macadamia relied emersion in coconut oil to achieve a rich golden colour (Leverington, 1972). Due to increasing concerns of dietary intake of saturated fats, oven roasting has become the preferred means of roasting kernel. However roasting now makes a minimal change to the raw kernel colour, a practice that reduces the level excessively dark kernel and minimises the risk of differential roasting responses due to mixing kernel from different cultivars (McConchie and Albertson, 2006). Models to describe the effect of roasting parameters such as oven temperature, residence time and air speed on kernel colour have been developed (McConchie et al., submitted) that enable comparisons between different sources of kernel or processing parameters.

This knowledge has been used address concerns that the macadamia improvement program was destined to produce flavourless kernel. This was achieved by examining the effects of two roasting treatments on consumer perceptions of nine cultivars sourced from six properties in two growing regions (O'Riordan, 2004). The two roasting treatments employed the same oven temperature but different residence times. No cultivar effects were shown as most sensory differences could be traced to roasting treatments. However it was found that consumers preferred the lighter colour of the short roast duration but the flavour of the longer roast time. At the same time it was shown that it was possible to roast kernel at higher pre-roast moisture contents than the recommended 1.5% (w/w) and obtain acceptable roasted kernel colour (McConchie and Albertson, 2006). The varying moisture content may enable other reactions that may affect kernel flavour as has been reported in other roasted products

such as coffee beans (Baggenstoss et al., 2008), peanuts (Moss and Otten, 1989) and barley for use in brewing (Robbins and Fryer, 2003)

Models of kernel colour development have also demonstrated that macadamia kernel can achieve comparable kernel surface colours using a range of roast conditions (McConchie et al, submitted). Many of these roast treatments remove in excess of 50% of the pre-roast moisture content. It other tree nuts roasting has been shown to reduce shelf life (Perren and Escher, 1999) but reports in macadamia are contradictory (Mason et al., 1995, Lemmer and Kruger, 2000 a & b, Kruger et al., 2009). This is thought to be due to an increase in intercellular pore sizes (Perren and Escher, 1999). The porosity of raw macadamia kernel is also thought to increase at low moisture contents (Palipane, 1992) suggesting the oxidative stability of macadamia will be reduced by roasting due to loss of moisture from the kernel. This conclusion is also supported by the observations that high oil content products such as nuts may become more susceptible to oxidative rancidity at low water activities (Labuza, 1979). The effects of roasting conditions on moisture loss and kernel stability have not been investigated in macadamia.

Most studies on the keeping properties of macadamia have used raw kernel (Himstedt, 2002) although the effects of different roast temperatures and durations on the sensory and chemical indices of rancidity have been investigated by Mason et al. (1995). While differences in the sensory properties of kernel were demonstrated immediately following the different roasting treatments and kernels deteriorated after 6 and 12 months storage under vacuum no relationship with roast temperature or duration was found. The authors noted these trials were conducted under controlled conditions that were unlikely used under commercial conditions. Differences between various cultivars in the keeping properties of roasted kernel have also been reported in South Africa (Kruger et al., 2009). Research from Hawaii has supported this conclusion but also found the relationship between cultivars varies between seasons (Wall, 2010). Due the potential differences in kernel dimensions that are known to affect roasting responses it is unclear whether there are also interactions between roast conditions and the resultant kernel composition and kernel stability.

The moisture content of macadamia kernel is determined by the level of drying applied to the nut-in-shell prior to cracking. The recommended cracking moisture content is 1.5% (w/w) kernel moisture content or 3.3% nut-in-shell moisture content. The effects of different cracking techniques on the production of whole kernel from different sources of nut-in-shell have been described by Wallace et al. (2001). However the effects of different moisture contents on the performance of these crackers and potential interactions are unknown. Raising moisture content is known to increase the displacement distance required to break the macadamia shell and therefore may affect the integrity of the enclosed kernel (Liang et al., 1984)

The aim of the current project was to describe the effects of a range of roast temperatures and duration on moisture loss and colour development in kernel form three commercial macadamia cultivars. The pre-roast kernel moisture content will be varied to determine whether this affects the quality of the roasted product. The aim is to determine whether these parameters can be adjusted to increase the moisture content of pre-roast kernel to reduce drying costs while producing a roasted kernel with acceptable appearance and moisture composition. To achieve these pre-roast moisture contents the nut-in-shell drying process will be modified. The impact of cracking nut-in-shell at different moisture contents will be described.

4.3 Material and methods

Three tons of macadamia nut-in-husk were collected from a commercial orchard in Bundaberg, Queensland, in April 2006, which comprised 750 kg of ‘A16’, 1500 kg of ‘849’ and 750 kg of ‘814’. The nut-in-husk were ground harvested and mechanically dehusked. Any obviously discoloured nut-in-shell or nuts with damaged shell was removed. The de-husked nut-in-shell was placed in open mesh bags and transported to Brisbane on the same day as being harvested. The nut-in-shell was then placed into forced air dehydrators (Wessberg & Tulander, Hurricane Forced Air Heating, Sydney, Australia) at ambient air temperature over two days to bring the nut-in-shell field moisture content from around 25% down to 5% ± 1.5%. Once this moisture content was reached the nut-in-shell were separated into the three experimental treatment moisture contents of 1.5% ± 0.5%, 3% ± 1% and 5% ± 1.5%. The nut-in-shell for the 1.5% and 3% treatments were then further dried using a heat-

pump drier (Australian Heat Pump Systems Pty Ltd, Queensland, Australia) set at 33 ºC and 30% relative humidity. The final moisture content of kernel was determined for 10 kernels using an Adam, AMB 50 moisture balance. The kernel for these measures was ground using a barrel-type cheese grater. Results of three replicates for each of the cultivar by moisture content treatments are shown in Table 4.1.

Target Moisture Content (Cultivar-moisture content)	Measured moisture content (% w/w)
A16-1.5%	1.757
A16-3%	2.198
A16-5%	4.574
849-1.5%	1.603
849-3%	2.938
849-5%	5.411
814-1.5%	1.665
814-3%	2.849
814- 5%	5.539

Table 4.1. Target and measured experimental moisture content

The dried nut-in-shell were sealed into aluminium-lined barrier bags and stored at 3.5ºC until cracked. Bags of nuts were removed from the cool room the day before cracking to allow nut-in-shell to come to room temperature before cracking. Shell was removed using an Amanasco mechanical cracker and whole undamaged kernels were selected for the experiment. The whole kernel from each cultivar were separated into moisture content levels and sealed in aluminium-lined barrier bags and stored at 3.5ºC.

In total 20 roasts treatments were performed each week that were repeated weekly over a 4-week period. Four roasts were completed each day and these were completely randomized within a week. The sequence of roasts within weeks is shown in (Appendix 4.1 A - D). The kernel were arranged in 9 rows on a roasting tray. Each row was composed of 6 kernel of a single cultivar with same moisture content, making a total of 54 kernel used per roast.

Before roasting these kernel were individually weighed to determine their mass. The kernel were then set out on the roasting tray and the colour was measured on each side of the suture line, top and bottom of the kernel using CIE *L*, a** and *b**

colour scheme of a Minolta ChromaMeter CR-300 (Minolta Co Ltd Osaka, Japan) (Light source D65 and 10^o standard observer). This instrument is a tristimulus colourimeter, that measures 4 specific wavelengths in the visible range, specified by the Commision Internationle de l'Eschairge (CIE).

Kernels were roasted in a modified pizza oven (Lincoln Impinger, model 1304, USA) at 126, 132, 144, and 156 ºC for durations set out in Table 4.2.

Roast temperature (ºC)	Degrees above 120 ºC	Roast duration (min)				
126	6	12	24	36	48	60
132	12	6	12	18	24	30
144	24	3	6	9	12	15
156	36	2	4	6	8	10

Table 4.2. Roasting conditions.

The roast durations were selected to give the same degree x minutes, or heat sum, using 120ºC as the base temperature

heat sum = (roast temperature – 120) x roast duration (minutes)

This was used because McConchie and Albertson (2006) had previously shown minimal colour change in kernel roasted for 60 min at 120^oC in the same oven.
To ensure roasting temperatures were maintained, 3 Type N thermocouples (Datataker 500, Pacific Data Systems, Brisbane) were placed across the roasting tray at kernel height.

After each roast, the kernel were cooled to room temperature under a pedestal fan for 10 minutes to stop the cooking process and the *L**, *a**, *b** values measured again as previously described. The kernel colour was calculated as the mean of all *L**, *a** and *b** values. The change (Δ) in *L**, *a** and b* was calculated by subtracting the mean pre-roast *L**, *a** and *b** measurement from the mean post-roast value for each kernel.

The kernels were re-weighed to determine the change in mass (Δg) during roasting that was assumed to be due to loss of water. Since kernel differ in size, the relative moisture loss based on the pre-roast kernel mass ($\Delta g \cdot g^{-1}$) and rate of moisture loss $\Delta g \cdot (degree \cdot minute)^{-1}$ were also calculated. To provide a reasonable number of

significant digits in the tables of means, Δg and $\Delta g \cdot g^{-1}$ were multiplied by 100 and $\Delta g \cdot (degree \cdot minute)^{-1}$ was multiplied by 1000.

Data were analysed using ANOVA and means and least aquare of difference (LSD) were then tabulated. The original numbers were used in all covariate analyses. All analyses were carried out using the statistical package GenStat.

The effects of nut-in-shell moisture contents on the state of the kernel after being processed through an Amanasco nut cracker were also assessed. One hundred nuts from the three replicates of the three cultivars (814, 849 and A16) at three kernel moisture contents (1.5, 3 and 5% w/w) were individually weighed and cracked. The result for each nut was recorded as whole when visually 75% of the kernel remained in one piece, half when less than 75% of the kernel was in one piece and uncracked when the shell was not broken. A total of 2700 observations were made.

4.4 Results

Kernel colour change

Kernel of all cultivars had reduced *L** values with higher kernel moisture contents (Fig 4.1). Images of the kernel at different moisture contents is shown in Fig 4.2.The kernel of A16 had the highest *L** value across all moisture contents indicating this cultivar had the lightest kernel colour. The kernel of 814 and 849 had comparable *L** values at kernel moisture contents 1.5 and 3%. The greatest reduction in *L** was evident between 3% and 5% kernel moisture content. A16 kernel still had the highest *L** value at 5% moisture content but 849 *L** value was lower than 814.

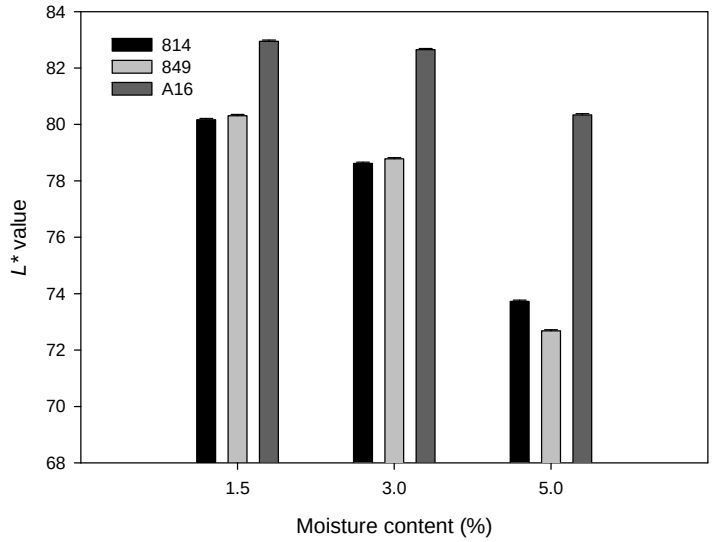

Figure 4.1. Mean pre-roast L* value of 814, 849 and A16 kernel at 1.5%, 3% and 5% kernel moisture content (Error bars = LSD).

Due to the roast durations differing between roast temperatures the anova was performed using heat sum which made the data balanced. Results for the anova of kernel colour data are presented in Table 4.3. There were highly significant (P<0.001) main effects due to temperature, heat sum, cultivar (CV) and moisture content (MC) for all three colour variates. There were also highly significant (P<0.001) interactions between temperature and heat sum, moisture content and heat sum for all variates, and between moisture content and temperature for ΔL^* and Δa^*, and between moisture content and heat sum for Δb^*.

		ΔL*		Δa*		Δb*	
Source of variation	**d.f.**	**m.s.**	**v.r.**	**m.s.**	**v.r.**	**m.s.**	**v.r.**
Week stratum	3	926.8	2.75	573.585	4.33	40.2	0.37
Week x Roast stratum							
Temperature (°C)	3	6172.95	18.29***	2863.294	21.62***	827.43	7.54***
Heat sum	4	80228.39	237.7***	44916.89	339.1***	28186.95	257***
Temperature x Heat sum	12	1203.1	3.56***	789.329	5.96***	911.83	8.31***
Residual	56	337.49	1.13	132.455	1.19	109.68	1.09
Week x Roast x CV x MC stratum							
Cultivar (CV)	2	884.12	2.96	201.772	1.82	4892.62	48.58***
Moisture content (MC, %)	2	21054.01	70.52***	27535.47	247.9***	6379.46	63.34***
CV x MC	4	775.34	2.6	156.357	1.41	123.58	1.23
CV x Temperature	6	271.1	0.91	122.824	1.11	117.74	1.17
MC x Temperature	6	1012.2	3.39**	410.25	3.69***	63.25	0.63
CV x Heat sum	8	290.5	0.97	71.391	0.64	550.45	5.47***
MC x Heatsum	8	2517.31	8.43***	1483.74	13.36***	424.7	4.22***
CV x MC x Temperature	12	211.3	0.71	96.134	0.87	52.93	0.53
CV x.MC x Heat sum	16	232.32	0.78	67.154	0.6	93.83	0.93
CV x Temperature x Heat sum	24	316.41	1.06	108.062	0.97	115.98	1.15
MC x Temperature x Heat sum	24	342.28	1.15	110.833	1	183.26	1.82
CV x MC x Temperature x Heat sum	48	249.74	0.84	106.95	0.96	90.51	0.9
Residual	472	298.53	8.92	111.08	11.6	100.71	5.62
Week x Roast x CV x MC x Nut stratum	3555	33.46	4.35	9.574	4.85	17.93	2.75
Wk x Roast x CV x MC x Nut x position stratum	4266	7.69	0.53	1.973	0.53	6.52	0.64
Week x Roast x CV x .MC x Nut x position x dup stratum	8531	14.59		3.697		10.13	

Table 3.3. ANOVA table of the effects of roast temperature, heat sum, moisture content (mc) on colour parameters *L**, *a** and *b**, (**= P<0.01, *** = P<0.001).

The means and LSD for the significant temperature by heat sum interactions for the change in colour variates are given in Table 4.4. The interaction is largely due to the different responses of the colour variates at the 4 temperatures with the increase in heat sum. The pattern of response is essentially similar for in *L**, *a** and *b**. When kernel darkens in response to roasting this is evident as an increase in *L** but a reduction in the values of a* and b*. At low heat sums the colour change is smaller at the high temperatures than at the low temperatures, whereas at high heat sums this change at the high temperatures is the same or greater than that of the low temperature roast(s).

Colour variate	Oven Temperature (^{o}C)	Heat sum (degree above 120^{o}C x minutes)				
		72	**144**	**216**	**288**	**360**
Δ*L**	**126**	1.696	5.533	9.855	11.096	12.456
	132	0.514	3.333	6.452	10.136	11.927
	144	0.002	1.994	4.845	8.811	11.037
	156	0.045	1.648	4.649	9.034	14.433
	LSD	1.7678				
Δ*a**	**126**	-1.908	-5.368	-8.046	-8.766	-9.125
	132	-0.775	-3.154	-6.029	-8.389	-9.266
	144	-0.262	-1.929	-4.756	-7.782	-9.389
	156	-0.499	-1.687	-4.451	-7.624	-10.865
	LSD	1.1074				
Δ*b**	**126**	-2.678	-5.526	-6.858	-6.884	-6.857
	132	-0.996	-4.144	-6.923	-7.351	-7.138
	144	0.181	-2.815	-5.754	-7.41	-8.083
	156	-0.176	-2.501	-5.225	-8.269	-8.517
	LSD	1.0078				

Table 4.4. Mean ΔL*, Δa* and Δb* and standard errors of difference to enable comparisons within the same heat sum for the oven temperature by heat sum interactions

The means and their LSDs for the significant moisture content by heat sum interactions are given in the Table 4.5. This interaction is largely due to the very different response of kernels at the highest moisture level of 5%, where the colour change is most dramatic for kernels subjected to the three highest heat sum levels. The change for all colour variates increases with increasing heat sums but the response for kernels at 1.5% and 3% moisture contents is very similar. The Δ *b** values are not quite so pronounced for the moisture content by heat sum measurements as for Δ*L** and Δ*a**.

Colour Variate	Kernel Moisture content (% w/w)	Heat sum (degree.minutes)				
		72	**144**	**216**	**288**	**360**
Δ*L**	**1.5**	0.949	2.746	5.096	8.308	10.656
	3	0.308	2.532	4.854	7.976	10.332
	5	0.436	4.103	9.402	13.023	16.402
	LSD	1.4708 (1.4398)				
Δ*a**	**1.5**	-0.645	-2.047	-3.909	-6.379	-7.765
	3	-0.518	-2.23	-4.252	-6.551	-8.127
	5	-1.419	-4.827	-9.3	-11.491	-13.092
	LSD	0.906 (0.8782)				
Δ*b**	**1.5**	-0.906	-3.156	-5.13	-7.318	-7.92
	3	-0.246	-2.971	-5.087	-6.475	-6.89
	5	-1.6	-5.111	-8.353	-8.642	-8.137
	LSD	0.8486 (0.8362)				

Table 4.5. Mean ΔL*, Δa* and Δb* and standard errors of difference for the moisture content by heat sum interaction. The first LSD value allows comparison within a heat sum while the LSD in parenthesis is used to make comparison within a moisture content.

The means of Δ *L** and Δ *a** variates for the significant interactions between moisture content and roast temperature are given in the Table 4.6. The interactions detected here for both variates are largely due to the different roast behaviour of kernel roasted at the highest moisture content of 5%. However the changes in both Δ *L** and Δ *a** decrease with increasing roast temperature at 3% and 5% but are similar at a moisture content of 1.5%.

Kernel Moisture content (% w/w)	Colour Variate							
	Δ *L**				**Δa***			
	Oven Temperature (°C)				**Oven Temperature (°C)**			
	126	132	144	156	126	132	144	156
1.5	6.258	5.314	4.808	5.824	-4.674	-4.074	-3.693	-4.154
3	6.602	5.286	4.166	4.747	-5.301	-4.427	-3.679	-3.936
5	11.521	8.818	7.038	7.315	-9.953	-8.066	-7.099	-6.986
LSD	1.3156 (1.2878)				0.8104 (0.7856)			

Table 4.6. Mean ΔL* and Δa* and standard errors of difference for the moisture content by oven temperature interaction. The first LSD value allows comparison within an oven temperature while the LSD in parenthesis is used to make comparison within a moisture content.

The means for cultivar by heat sum interaction for the Δ*b** variate are shown in Table 4.7. This interaction is largely due to the different response of A16 to the increasing levels of heat sum. Cultivars 814 and 849 behave in a similar manner across the range of heat sums with a similar increase in Δ*b** with increasing heat sum.

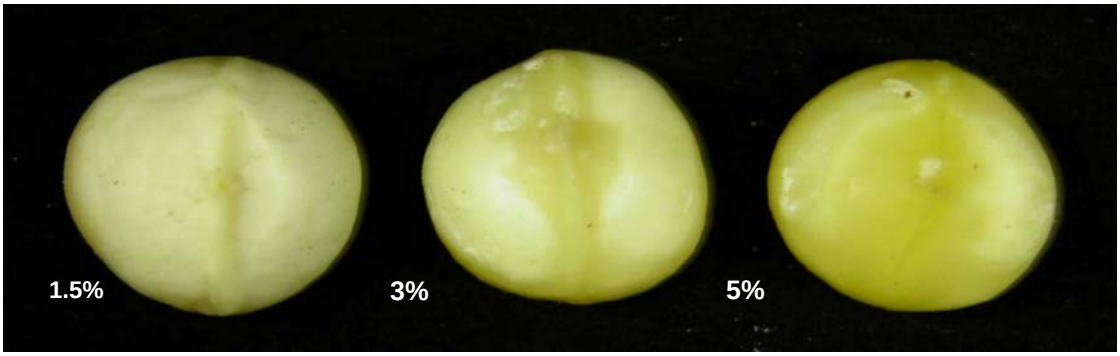

Figure 4.2. kernel of Macadamia cultivar A16 at 1.5, 3 and 5% moisture content (w/w)

Cultivar	Heat sum				
	72	**144**	**216**	**288**	**360**
814	-0.675	-3.467	-5.902	-6.965	-6.864
849	-1.161	-3.248	-5.521	-6.419	-6.454
A16	-0.915	-4.524	-7.147	-9.051	-9.628
LSD	0.8486 (0.8362)				

Table 4.7. Mean Δb^* and standard errors of difference for the cultivar by heat sum interaction. The first LSD value allows comparison within a heat sum while the LSD in parenthesis is used to make comparison within the moisture content.

Moisture loss

Results for the ANOVAs of mass change data are presented in Table 4.8. Again there were highly significant ($P<0.001$) main effects due to temperature, heat sum (Heatsum), cultivar (CV) and kernel moisture content (MC) for weight loss variates. However, the interaction between temperature and heat sum is not as strong.

Variate		Δg		$\Delta g.g^{-1}$		$\Delta g.(degree.Minute)^{-1}$	
Source of variation	d.f.	m.s.	v.r.	m.s.	v.r.	m.s.	v.r.
Week stratum	3	0.067535	9.35	0.003625	4.57	36.1834	2.52
Week x Roast stratum							
Temperature (^oC)	3	0.46189	63.97***	0.059208	74.72***	2130.065	148.4***
Heat sum	4	1.382929	191.5***	0.163464	206.3***	59.8196	4.17**
Temperature x Heat sum	12	0.020785	2.88**	0.002281	2.88**	11.6695	0.81
Residual	57	0.00722	1.37	0.000792	1.5	14.3547	2.39
Week x Roast x CV x MC stratum							
Cultivar (CV)	2	0.079918	15.19***	0.008437	16.01***	110.1194	18.33***
Moisture content (MC, %)	2	8.113302	1542.***	0.97325	1846.***	8255.222	1374.***
CV x MC	4	0.043375	8.24***	0.005725	10.86***	55.4915	9.24***
CV x Temperature	6	0.003363	0.64	0.000251	0.48	10.9182	1.82
MC x Temperature	6	0.036964	7.03***	0.004783	9.07***	634.1055	105.6***
CV x Heat sum	8	0.010717	2.04	0.000201	0.38	13.144	2.19*
MC x Heatsum	8	0.198335	37.7***	0.022387	42.47***	77.9441	12.98***
CV x MC x Temperature	12	0.001685	0.32	0.00018	0.34	6.773	1.13
CV x.MC x Heat sum	16	0.003972	0.75	0.000278	0.53	5.3028	0.88
CV x Temperature x Heat sum	24	0.002135	0.41	0.000259	0.49	2.4973	0.42
MC x Temperature x Heat sum	24	0.00404	0.77	0.000442	0.84	3.4802	0.58
CV x MC x Temperature x Heat sum	48	0.001795	0.34	0.000275	0.52	3.4238	0.57
Residual	480	0.005261	6.26	0.000527	7.79	6.0071	6.22
Week x Roast x CV x MC x Nut stratum	3600	0.00084		6.77E-05		0.9654	
Wk x Roast x CV x MC x Nut x position stratum	4320	0		0		0	
Week x Roast x CV x .MC x Nut x position x dup stratum	8640	0		0		0	

Table 4.8. ANOVA table of the effects of roast temperature, heat sum, moisture content on kernel mass change Δg, $\Delta g.g^{-1}$, $\Delta g.(degree.minute)^{-1}$, (*= P<0.05, **= P<0.01, *** = P<0.001).

The means and LSDs for mass change variates due to moisture content by heat sum interactions are given in the Table 4.9. The change in mass, Δg, increased with increasing heat sum and the initial moisture content of the kernel. The relative mass change, $\Delta g.g^{-1}$, behaved in a similar way but this difference diminished at higher heat sum at the same moisture content. However the rate of moisture loss, $\Delta g.(degree.minute)^{-1}$, increased with heat sum at 1.5% moisture content was steady

and perhaps lower at the highest heat sum at 3 % and reduced with increasing heat sum with a pre-roast moisture content of 5%.

Kernel mass variate	Kernel Moisture Content (%wb)	Heat sum				
		72	**144**	**216**	**288**	**360**
Mass Change (Δg)	**1.5**	0.53	1.28	2.01	2.72	2.98
	3	1.32	2.71	3.87	4.6	5.03
	5	4.28	6.92	9.7	11.45	12.93
	LSD	0.640 (0.604)				
Relative mass change ($\Delta g.g^{-1}$)	**1.5**	0.18	0.45	0.7	0.94	1.05
	3	0.49	1	1.39	1.65	1.78
	5	1.51	2.44	3.39	4	4.43
	LSD	0.206 (0.192)				
Rate of mass loss ($\Delta g.(degree.minute)^{-1}$	**1.5**	0.37	0.4	0.53	0.56	0.54
	3	1.05	1.09	1.05	1	0.91
	5	3.43	2.84	2.75	2.51	2.33
	LSD	0.248 (0.204)				

Table 4.9. The means and LSDs for mass change variates, Δg , $\Delta g.g^{-1}$, $\Delta g.(degree.minute)^{-1}$ due to moisture content by heat sum interactions. The first LSD value allows comparison within a heat sum while the LSD in parenthesis is used to make comparison within the moisture content.

The means and LSDs of kernel mass change variates for the moisture content by oven temperature interactions are given in the Table 4.10. This shows that the change in kernel mass and the relative change in kernel mass reduce with increasing oven temperature probably due to the reduction in oven residence time needed to achieve the same heat sum at increasing temperatures. However the rate of mass change increases with oven temperature and kernel moisture content.

Kernel mass variate	Kernel Moisture Content (% w/w)	Oven Temperature (°C)			
		126	132	144	156
Mass Change (Δg)	1.5	2.73	2.01	1.46	1.42
	3	4.66	3.79	2.89	2.69
	5	11.17	9.36	8.05	7.64
	LSD	0.574 (0.540)			
Relative mass change ($\Delta g.g^{-1}$)	1.5	0.96	0.69	0.5	0.5
	3	1.69	1.36	1.04	0.96
	5	3.9	3.27	2.83	2.62
	LSD	0.184 (0.172)			
Rate of mass loss ($\Delta g.degree^{-1}.minute^{-1}$)	1.5	0.27	0.4	0.51	0.74
	3	0.52	0.81	1.17	1.58
	5	1.26	1.99	3.29	4.54
	LSD	0.220 (0.182)			

Table 4.10. The means and LSDs for mass change variates, Δg, $\Delta g.g^{-1}$, $\Delta g.(degree.minute)^{-1}$ due to the moisture content by oven temperature interactions. The first LSD value allows comparison within an oven temperature while the LSD in parenthesis is used to make comparison within the moisture content.

Effects of nut in shell moisture content of state of kernel after cracking

Since there are quite different numbers of kernel in the different size categories the initial analysis of the NIS mass variate was carried out using REML (residual maximum likelihood). The variance components due to the reps and the combinations of cultivar by moisture content were effectively zero. This is probably due to the large residual variance arising from the mass differences between the NIS within the above combinations. The fixed effects from this analysis are assessed using the table of Wald statistics (Table 4.11). Note that although the terms are added sequentially in this analysis, this is not an issue since the factors are balanced.

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
%mc	108.39	2	54.2	<0.001
cultivar	300.54	2	150.27	<0.001
Kernel status	42.6	2	21.3	<0.001
%mc.cultivar	12.37	4	3.09	0.015
%mc.kernel status	2.1	4	0.52	0.718
cultivar.kernel status	26.44	4	6.61	<0.001
%mc.cultivar.kernel status	6.71	8	0.84	0.568

Table 4. 11. REML analysis of the effects of moisture content, cultivar on kernel status and the associated interactions

There were highly significant effects of moisture content, cultivar, kernel status and interaction between cultivar and kernel status. There was also a significant but reduced interaction between moisture content and cultivar. The mean mass for each of the 81 experimental combinations were then calculated and analysed using an analysis of variance. However there were two outliers in replicate 2 of 849 with 3% and 5% that were removed from the analysis. The residual mean square has been halved in this new analysis and hence the significance level of all effects has been enhanced. The results from the analysis excluding the aberrant values are presented Table 4.12.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replicate stratum	2	0.08227	0.04114	1.16	
replicate.*Units* stratum					
%mc	2	6.85325	3.42662	96.22	<.001
cultivar	2	13.17305	6.58652	184.96	<.001
kernel_status	2	3.4766	1.7383	48.81	<.001
%mc.cultivar	4	0.39479	0.0987	2.77	0.037
%mc.kernel_status	4	0.34064	0.08516	2.39	0.063
cultivar.kernel_status	4	1.33284	0.33321	9.36	<.001
%mc.cultivar.kernel_status	8	0.44697	0.05587	1.57	0.158
Residual	50	1.78053	0.03561		

Table 4.12. ANOVA of the effects of moisture content, cultivar on kernel status and the associated interactions

The means and LSDs for the highly significant interactions between cultivar and kernel status are shown in Table 4.13 The observed interaction is largely due to the different A16 having heavier nuts producing half kernel compared to the other cultivars. The uncracked nuts in all cultivars tend to be lighter.

	Kernel status		
Cultivar	**Half**	**Whole**	**Uncracked**
814	6.431	6.518	6.19
849	6.703	6.768	6.392
A16	7.776	7.328	6.886
LSD	0.3082		

Table 4.13. Means and LSD for the interactions between cultivar and kernel status

The means and LSD for interactions between %mc and cultivar are shown in Table 4.14. The weight of nuts increased with moisture content. The interaction is largely due the comparable mass of cultivar 849 nuts with 3% and 5% moisture content.

	Cultivar		
%mc	814	849	A16
1.5	6.013	6.276	6.904
3	6.421	6.756	7.309
5	6.705	6.831	7.777
LSD	0.198		

Table 4.14. Table of means and LSD for interactions between moisture content (%mc) and the three cultivars

Table 4.15 shows the observed numbers in each kernel state category for each cultivar at the three moisture contents.

In Table 4.16 the mean NIS weight for each of the cultivar, moisture content and kernel state after cracking are shown.

%mc	cultivar	Kernel status		
		Half	Whole	Uncracked
1.5	814	119	150	31
	849	116	164	20
	A16	101	166	33
3	814	119	145	36
	849	138	136	26
	A16	111	160	29
5	814	100	139	61
	849	128	140	32
	A16	115	136	49

Table 4.15 Observed numbers of kernel in each of the states (half, whole and uncracked) for each of the three cultivars at the different moisture contents

%mc	cultivar	Kernel status		
		Half	Whole	Uncracked
1.5	814	6.108	6.138	5.805
	849	6.442	6.508	5.827
	A16	7.384	6.997	6.371
3	814	6.651	6.484	6.127
	849	6.766	6.905	6.163
	A16	7.741	7.226	6.923
5	814	6.558	6.93	6.64
	849	6.912	6.868	6.309
	A16	8.206	7.742	7.385

Table 4.16. Mean nut-in-shell weight for each of the cultivars at the different moisture contents and kernel states after cracking

In all the above analyses NIS masses were correlated with the different moisture contents. Hence the data were adjusted to an estimated zero moisture content using the adjustment values of NIS moisture contents by reducing values by 3.3%, 7.8% and 11.8% for the 1.5%, 3% and 5% based on the published results of Palipane and Driscoll (1992). As expected the results of these new analyses were very similar to those performed earlier. As before there were two outlier observations and these were removed from the data before the final analysis of variance. The results of this analysis are summarized in the Table 4.17. There is no longer a significant interaction

between %MC and cultivar, but a significant interaction between is now evident between %MC and kernel status (P=0.29).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replicate stratum	2	0.0766	0.0383	1.25	
replicate.*Units* stratum					
%mc	2	0.17321	0.0866	2.83	0.068
cultivar	2	11.15424	5.57712	182.31	<.001
kernel_status	2	3.02902	1.51451	49.51	<.001
%mc.cultivar	4	0.25198	0.06299	2.06	0.1
%mc.kernel_status	4	0.36176	0.09044	2.96	0.029
cultivar.kernel_status	4	1.12641	0.2816	9.21	<.001
%mc.cultivar.kernel_status	8	0.36269	0.04534	1.48	0.188
Residual	50	1.52953	0.03059		

Table 4.17. ANOVA of the effects of moisture content, cultivar on kernel status and the associated interactions using NIS masses adjusted for moisture contents.

The means for the significant interactions between Nut-in-shell moisture content and kernel status are given in the Table 4.18. This indicates that recovery of wholes is unaffected by nut-in-shell moisture content, but this is not the case for halves and uncracked NIS kernel.

	Kernel status		
%mc	half	whole	Uncracked
1.5	6.419	6.336	5.804
3	6.507	6.339	6.042
5	6.362	6.339	6.098
LSD	0.1648		

Table 4.18. Means (LSD) for nut-in-shell masses for each of the kernel states after cracking at the different moisture contents using masses that have been adjusted to an estimated zero moisture contents.

The means for the significant interactions between cultivar and kernel status are given in Table 4.19. These results indicate that A16 behave differently to the other cultivars and that uncracked nuts were generally had a lower adjusted mass.

Cultivar	Kernel status		
	half	whole	uncracked
814	5.934	6.009	5.705
849	6.184	6.247	5.893
A16	7.171	6.758	6.346
LSD	0.1648		

Table 4.19. Means (LSD) for nut-in-shell masses for each of the kernel states after cracking for the three cultivars using masses that have been adjusted to an estimated zero moisture contents.

Effect of moisture content on cracking

There were three replications of 100 nuts from the combinations of three cultivars and three moisture contents. The number of wholes, halves and uncracked kernels were recorded for these combinations.

The outcomes for kernel status form three ordered levels of a “quality” factor. The difference between these levels is not measurable. Hence these levels are referred to as ordinal and the data should be modeled accordingly. There are two possible models for such data, the proportional odds model and the proportional hazards model.

Both models can be fitted using GLM (generalized linear models) assuming the multinomial distribution and using the logit link for the proportional odds model or the complementary log log link for the proportional hazards model. Both models have a similar form.

The proportional odds model is

$$\log[\gamma_j(x)/\{1-\gamma_j(x)\}] = \theta_j - \beta^T x$$

And the proportional hazards model is of the form

$$\log[-\log\{1-\gamma_j(x)\}] = \theta_j - \beta^T x$$

The θ_j are known as the cut points. The response is the cumulative set of proportions, that is the probability that the response for unit *i* is in category *j* or lower.

The models fitted included as explanatory factors the % moisture content, the cultivar and the interaction between them. There was a large significant effect due to % moisture content, with a smaller significant effect due to cultivar. There was no evidence to suggest any interaction between them. The proportional hazards model provided a better fit to the data with a residual deviance of 49.31 (48 df), compared to 59.08 (48 df) for the proportional odds model. The inclusion of cultivar in the model was significant with a change of deviance of 6.98 on 2df. The residual mean deviance of 1.037 indicates a very good fit to the data for this model.

The parameter estimates for the fitted model are given in the Table 4.20. It should be noted that the levels of the two factors are compared with the third level of the factor respectively. With respect to moisture content there is a significant difference between 5% and both 1% and 3 %, but no significant difference between 1% and 3%. The result for cultivar is not so obvious from this table. However although there is no significant difference between A16 and either of the other two cultivars, there is a significant difference between 814 and 849. Fitted values for the proportion of wholes are shown in Figure 4.3.

Parameter	estimate	s.e.	t(*)
Cut-point half/whole	-0.5103	0.0522	-9.78
Cut-point whole/uncracked	0.6437	0.0496	12.99
1.5 % mc	-0.2377	0.0529	-4.49
3 %mc	-0.1632	0.053	-3.08
5 %mc	0	*	*
CV 814	0.0816	0.0529	1.54
CV 849	-0.057	0.0526	-1.08
CV A16	0	*	*

Table 4.20. Parameter estimates for the fitted model using moisture contents, cultivar and their interaction as explanatory factors.

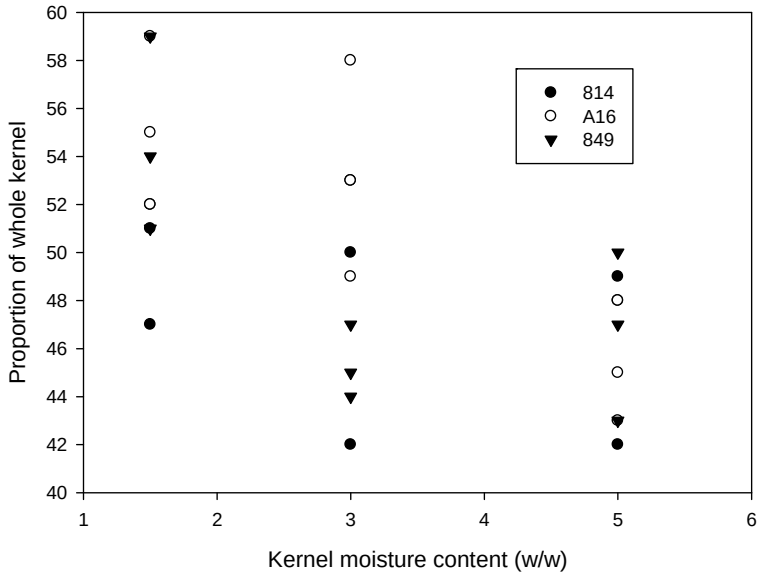

Figure 4.3. Fitted values for the proportion of whole kernel for the three replicates of cultivars 814, 849 and A16 after cracking at different nut-in-shell moisture contents

4.5 Discussion

These results show that there are significant effects of cultivar, moisture content and roast conditions and interactions between these factors that impact the roasted kernel colour in macadamia. In other tree nuts these factor are known to also impact on texture, consumer acceptance and oxidative stability of kernel (Perren and Escher, 1999; Saklar et al. 1999; Saklar et al., 2001; Saklar et al., 2003). These trials

aimed to identify constraints to roasting conditions within which processing parameters could be manipulated. This variation is exploited in other roasted products to optimize roasting conditions to improve consumer acceptance.

The kernel of all cultivars with 5% kernel moisture contents (kmc) darkened significantly more than kernel at 3% and 1.5% after roasting. This is consistent with the observations of McConchie and Albertson (2006) who found that kernel at pre-roast moisture contents between 3 and 10% darkened more than kernel at higher and lower moisture contents. Again this was evident in all the cultivars tested. This may be explained by the roasting process causing membrane damage and sufficient moisture being present to allow sucrose that in the cellular cytoplasm to come in contact with the cell wall bound invertase and form reducing sugars that react with amino acids as part of a maillard type reaction as has previously been described in macadamia (Albertson et al. 2005; 2006). However, when kernel stored at 5% was re-dried to 1.5% without roasting it became darker, evident as a reduced L^* value, than kernel that had been stored for the same time at 1.5% moisture content (data not shown). Similar effect was also reported by Dominguez et al. (2007) who stored kernel at a_w of 0.215, 0.436, and 0.628 and found kernel at the higher water activity became progressively darker over 8 weeks which they attributed to as evidence of oxidative rancidity. However, they also found that kernel had peroxide value over 20 meq/kg after 2 weeks storage that would indicate the kernel was already compromised and they suggested this change in colour was evidence of oxidative rancidity. Early work by Cavaletto et al. (1996) also found that kernel stored at 4.3% kmc for 16 months at 37^{o}C showed excessive darkening when roasted and developed elevated levels of free fatty acids and reducing sugars. A possible explanation other than kernel becoming rancid is that membranes become permeable at intermediate moisture contents that allows leakage of reactive components or auto-oxidation to occur providing substrates needed for the browning reactions. It is evident that 5% kmc is unsuitable for storage and roasting of macadamia kernel.

A16 kernel underwent greater colour change than kernel of the other cultivars. Research in South Africa has suggested that cultivars such as A16 that are considered to have been derived from both species that have given rise to the commercial macadamia, M. integrifolia and M. tetraphylla, need to be segregated because of their

different roasting responses (Lemmer et al., 2000 a & b; Kruger et al. 2009) . This difference in response was not observed by McConchie and Albertson (2006) who also investigated the roasting behaviour of A16 kernel but concluded that, provided roasting conditions used produced kernel comparable to retail packaged product, a uniform product was best achieved by sorting kernel with comparable pre-roast colour.

The amount of moisture lost at low oven temperatures was always greater than at higher oven temperatures. It would appear that moisture loss at low oven temperatures exceeded that of higher because the residence times has to be extended to have equivalent heat sums. This presumably has provided greater time for heat transfer to the kernel and time for moisture to migrate to the surface. Mason et al. (1995) using oil roasting concluded that it was preferable to use lower oil temperatures and longer roast times as the time range that produces acceptable results is reduced at higher temperatures that may reflect the precision of the process. No differences in kernel stability were observed between kernel roasted from 4 to 25 minutes at temperatures from 115-135^{o}C which they attributed to processing occurring under controlled conditions. In other tree nuts roasting has been shown to increase the intercellular pores by weakening the middle lamella that binds cells together (Saklar et al., 2003). This is in turn allows oxygen access to a modification of the kernel texture and reduced oxidative stability.

Our results would suggest that kernel roasted for extended period would have greatly reduced moisture which should reduce oxidative stability (Labuza, 1979). The effects of reduced moisture content on the storage properties of kernel needs to be assessed under more challenging conditions to determine if there are differential responses.

Larger colour changes were observed at lowest roast temperature (125^{o}C) compared to higher temperatures at comparable heat sums (72 - 216 degree x minutes). A possible explanation for this response is that the longer roast duration at lower roast temperature provides greater heat transfer and time for the roast response to occur. However in roasted peanuts Moss and Otten (1989) found that colour change was minimal during the drying stage where moisture was readily removed from the kernel. However as the amount of kernel moisture loss slowed, the colour change increased. There is some evidence for this in macadamia as the moisture loss increased with kernel moisture content within the same heat sum treatment indicating

greater heat transfer was occurring but the colour change within the 1.5 and 3% moisture content kernel was comparable. Therefore the greater colour change at the lowest roast temperature is due to the increase in time that kernel spends at low moisture contents.

Previous investigations on the cracking efficiency have demonstrated significant differences between cultivars and the performance of crackers at producing whole kernel using nuts that were all dried to 1.5% kernel moisture content (Wallace et al., 2001). These differences can be in part due to separation of the cotyledons during the drying process that was visualized by X-rays. Our results show that the performance of a cracker may be affected by the size distribution of the nuts being processed as well as the moisture content. It is unclear whether the responses are also affected by adjustments to the cracker or an inherent property of the cracking mechanism. The cracker performed badly with the largest and smallest nuts however there were differences in the mean mass of kernel that produced whole kernel between cultivars. The pattern of response differed between A16 and the other cultivars tested which maybe due to the nuts of this cultivar being asymmetric as opposed to being essentially spherical. This could mean that if the dimension of a nut is important in successful cracking, then the short axis in a larger asymmetric nut is comparable to that of a smaller spherical nut. Cracking nuts at 5% kernel moisture content reduced whole kernel in all cultivars that may be explained by the displacement required to crack the shell at these elevated moisture contents causing either the shell failing to break or damage to the kernel as proposed by Liang et al.(1984).

At low moisture levels such as 1.5 and 3 % kernel moisture content water is bound in a monolayer state and requires greater amounts of energy to remove than the free water at high kernel moisture contents (Palipane and Driscoll, 1994). Being able to process nut-in-shell at 3% kmc, equivalent to 7.7% nut-in-shell moisture content (NISmc), compared to 1.5% kmc or 3.3% NISmc could represent a substantial saving. However further research is needed to determine the effects this may have on the efficiency of extracting kernel from the shell and storage properties of kernel. Cavaletto et al. (1968) found that storing nut-in-shell at 3.8% Kmc resulted in loss in quality after 6 months. There was no effect on raw kernel appearance after storage at 7.5% NISmc at 25^{o}C (Mason et al., 1998). However kernel did deteriorate after roasting and storing for 4 months. These combined results suggest that provided

extended storage at high temperatures is avoided, processing at higher moisture contents such as 3% kmc could be justified.

4.6 Acknowledgements

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Appendix 4.1 Randomised sequence of daily roast treatments indicating the position on a tray of each cultivar and moisture content of the kernel. The oven temperature and oven residence time is also shown. Four roasts were performed per day with a total of 20 roasts performed in a week. This was repeated weekly for 4 weeks

	DAY	Replicate 1	Position 1	Position 2	Position 3	Position 4	Position 5	Position 6	Position 7	Position 8	Position 9	Temp (°C)/ Time (min)
WEEK 1	1	roast 1	A16, 3%	849, 5%	814, 3%	849, 3%	814, 5%	849, 1.5%	A16, 1.5%	A16, 5%	814, 1.5%	156/ 8
	1	roast 2	A16, 1.5%	A16, 5%	814, 1.5%	849, 3%	A16, 3%	849, 5%	849, 1.5%	814, 5%	814, 3%	126/ 60
	1	roast 3	A16, 5%	849, 5%	A16, 3%	814, 5%	814, 1.5%	849, 1.5%	814, 3%	A16, 1.5%	849, 3%	132/ 12
	1	roast 4	A16, 5%	A16, 3%	849, 3%	814, 1.5%	814, 5%	814, 3%	849, 5%	849, 1.5%	A16, 1.5%	144/ 3
	2	roast 5	814, 1.5%	814, 3%	849, 1.5%	849, 5%	A16, 3%	849, 3%	A16, 5%	814, 5%	A16, 1.5%	144/ 15
	2	roast 6	A16, 3%	A16, 1.5%	814, 1.5%	849, 1.5%	849, 3%	A16, 5%	814, 5%	814, 3%	849, 5%	156/ 2
	2	roast 7	814, 3%	A16, 1.5%	814, 5%	849, 3%	849, 1.5%	849, 5%	A16, 5%	814, 1.5%	A16, 3%	132/ 24
	2	roast 8	849, 5%	814, 3%	814, 5%	A16, 1.5%	A16, 3%	814, 1.5%	849, 3%	849, 1.5%	A16, 5%	126/ 24
	3	roast 9	A16, 1.5%	A16, 5%	814, 1.5%	849, 3%	814, 3%	814, 5%	A16, 3%	849, 5%	849, 1.5%	126/ 36
	3	roast 10	A16, 3%	814, 1.5%	849, 5%	849, 1.5%	849, 3%	814, 5%	814, 3%	A16, 1.5%	A16, 5%	144/ 6
	3	roast 11	A16, 3%	814, 5%	814, 3%	849, 3%	849, 5%	A16, 1.5%	814, 1.5%	849, 1.5%	A16, 5%	156/ 10
	3	roast 12	849, 3%	849, 1.5%	849, 5%	814, 3%	814, 5%	A16, 3%	A16, 5%	814, 1.5%	A16, 1.5%	132/ 30
	4	roast 13	814, 3%	814, 1.5%	849, 1.5%	A16, 3%	A16, 5%	849, 3%	A16, 1.5%	849, 5%	814, 5%	132/ 6
	4	roast 14	849, 3%	A16, 3%	849, 5%	A16, 1.5%	849, 1.5%	814, 5%	814, 1.5%	814, 3%	A16, 5%	144/ 12
	4	roast 15	849, 5%	A16, 3%	814, 1.5%	849, 3%	849, 1.5%	A16, 5%	A16, 1.5%	814, 5%	814, 3%	126/ 12
	4	roast 16	A16, 3%	849, 3%	814, 3%	814, 5%	A16, 1.5%	A16, 5%	814, 1.5%	849, 1.5%	849, 5%	156/ 6
	5	roast 17	814, 1.5%	849, 5%	849, 3%	A16, 1.5%	814, 5%	A16, 3%	849, 1.5%	814, 3%	A16, 5%	156/ 4
	5	roast 18	849, 5%	814, 3%	814, 5%	849, 3%	849, 1.5%	A16, 1.5%	A16, 5%	A16, 3%	814, 1.5%	132/ 18
	5	roast 19	849, 5%	849, 3%	849, 1.5%	814, 3%	A16, 5%	A16, 1.5%	A16, 3%	814, 5%	814, 1.5%	144/ 9
	5	roast 20	A16, 5%	849, 3%	A16, 3%	A16, 1.5%	814, 5%	814, 1.5%	849, 5%	849, 1.5%	814, 3%	126/ 48

	DAY	Replicate 2	Position 1	Position 2	Position 3	Position 4	Position 5	Position 6	Position 7	Position 8	Position 9	Temp (°C)/ Time (min)
WEEK 2	1	roast 1	814, 5%	849, 5%	849, 1.5%	849, 3%	814, 1.5%	A16, 5%	A16, 3%	814, 3%	A16, 1.5%	126/ 48
	1	roast 2	849, 1.5%	A16, 1.5%	814, 5%	814, 3%	A16, 3%	814, 1.5%	A16, 5%	849, 5%	849, 3%	144/ 3
	1	roast 3	849, 1.5%	849, 3%	849, 5%	814, 1.5%	814, 5%	A16, 3%	814, 3%	A16, 1.5%	A16, 5%	132/ 18
	1	roast 4	814, 1.5%	A16, 5%	849, 5%	814, 5%	A16, 3%	814, 3%	849, 1.5%	A16, 1.5%	849, 3%	156/ 10
	2	roast 5	A16, 1.5%	849, 3%	814, 1.5%	814, 3%	814, 5%	A16, 3%	A16, 5%	849, 1.5%	849, 5%	144/ 9
	2	roast 6	814, 3%	A16, 3%	A16, 1.5%	849, 3%	849, 1.5%	814, 5%	849, 5%	814, 1.5%	A16, 5%	132/ 24
	2	roast 7	A16, 5%	A16, 1.5%	814, 1.5%	849, 5%	814, 5%	814, 3%	849, 3%	849, 1.5%	A16, 3%	156/ 8
	2	roast 8	849, 3%	849, 5%	A16, 3%	849, 1.5%	814, 5%	A16, 1.5%	814, 1.5%	814, 3%	A16, 5%	126/ 24
	3	roast 9	814, 3%	849, 3%	849, 5%	A16, 3%	814, 5%	A16, 5%	814, 1.5%	A16, 1.5%	849, 1.5%	156/ 2
	3	roast 10	849, 3%	A16, 3%	A16, 1.5%	849, 1.5%	814, 3%	814, 5%	814, 1.5%	849, 5%	A16, 5%	126/ 36
	3	roast 11	814, 3%	849, 5%	814, 5%	A16, 1.5%	A16, 3%	849, 1.5%	849, 3%	A16, 5%	814, 1.5%	132/ 30
	3	roast 12	814, 1.5%	A16, 1.5%	814, 5%	849, 1.5%	A16, 5%	814, 3%	849, 3%	A16, 3%	849, 5%	144/ 12
	4	roast 13	814, 3%	849, 5%	849, 1.5%	814, 1.5%	A16, 5%	814, 5%	A16, 1.5%	A16, 3%	849, 3%	144/ 15
	4	roast 14	849, 1.5%	849, 5%	814, 5%	A16, 1.5%	A16, 5%	814, 1.5%	849, 3%	814, 3%	A16, 3%	156/ 4
	4	roast 15	A16, 5%	A16, 1.5%	849, 3%	814, 5%	814, 1.5%	A16, 3%	849, 5%	814, 3%	849, 1.5%	126/ 12
	4	roast 16	A16, 1.5%	814, 3%	849, 5%	814, 5%	849, 3%	A16, 5%	A16, 3%	849, 1.5%	814, 1.5%	132/ 6
	5	roast 17	814, 1.5%	814, 5%	849, 1.5%	814, 3%	A16, 5%	A16, 3%	849, 5%	A16, 1.5%	849, 3%	132/ 12
	5	roast 18	A16, 1.5%	A16, 5%	814, 5%	814, 3%	814, 1.5%	849, 5%	849, 3%	849, 1.5%	A16, 3%	126/ 60
	5	roast 19	849, 5%	814, 3%	A16, 5%	849, 3%	849, 1.5%	A16, 1.5%	814, 5%	A16, 3%	814, 1.5%	144/ 6
	5	roast 20	849, 3%	814, 1.5%	814, 3%	849, 1.5%	A16, 5%	849, 5%	A16, 1.5%	A16, 3%	814, 5%	156/ 6

	DAY	Replicate 3	Position 1	Position 2	Position 3	Position 4	Position 5	Position 6	Position 7	Position 8	Position 9	Temp (°C)/ Time (min)
WEEK 3	1	roast 1	A16, 5%	849, 5%	A16, 3%	814, 3%	814, 5%	814, 1.5%	849, 3%	A16, 1.5%	849, 1.5%	156/ 2
	1	roast 2	849, 5%	814, 1.5%	849, 3%	814, 3%	A16, 5%	A16, 1.5%	814, 5%	A16, 3%	849, 1.5%	126/ 60
	1	roast 3	849, 1.5%	A16, 3%	A16, 5%	814, 5%	A16, 1.5%	849, 3%	814, 3%	814, 1.5%	849, 5%	144/ 9
	1	roast 4	849, 1.5%	814, 1.5%	814, 3%	A16, 5%	A16, 3%	849, 3%	A16, 1.5%	814, 5%	849, 5%	132/ 6
	2	roast 5	849, 3%	A16, 3%	814, 1.5%	849, 5%	814, 5%	849, 1.5%	A16, 1.5%	A16, 5%	814, 3%	144/ 15
	2	roast 6	A16, 3%	814, 5%	A16, 1.5%	814, 1.5%	849, 1.5%	849, 3%	849, 5%	A16, 5%	814, 3%	156/ 6
	2	roast 7	A16, 3%	814, 3%	814, 1.5%	A16, 1.5%	A16, 5%	849, 5%	814, 5%	849, 1.5%	849, 3%	132/ 30
	2	roast 8	A16, 5%	814, 1.5%	849, 5%	849, 1.5%	849, 3%	A16, 3%	814, 3%	814, 5%	A16, 1.5%	126/ 48
	3	roast 9	814, 5%	A16, 3%	849, 1.5%	849, 3%	A16, 5%	A16, 1.5%	849, 5%	814, 3%	814, 1.5%	126/ 24
	3	roast 10	849, 5%	849, 1.5%	A16, 1.5%	814, 3%	A16, 3%	814, 1.5%	814, 5%	849, 3%	A16, 5%	132/ 12
	3	roast 11	849, 3%	849, 5%	849, 1.5%	814, 3%	A16, 5%	814, 5%	814, 1.5%	A16, 1.5%	A16, 3%	144/ 12
	3	roast 12	849, 3%	A16, 5%	A16, 1.5%	849, 1.5%	814, 3%	849, 5%	814, 1.5%	814, 5%	A16, 3%	156/ 10
	4	roast 13	A16, 5%	814, 1.5%	849, 3%	849, 5%	849, 1.5%	A16, 3%	A16, 1.5%	814, 3%	814, 5%	132/ 24
	4	roast 14	814, 1.5%	A16, 3%	A16, 5%	849, 5%	849, 3%	849, 1.5%	814, 3%	814, 5%	A16, 1.5%	144/ 3
	4	roast 15	849, 5%	A16, 1.5%	814, 3%	849, 1.5%	849, 3%	A16, 3%	814, 5%	814, 1.5%	A16, 5%	156/ 4
	4	roast 16	814, 3%	A16, 5%	A16, 3%	814, 1.5%	A16, 1.5%	849, 5%	849, 1.5%	814, 5%	849, 3%	126/ 36
	5	roast 17	849, 1.5%	849, 5%	A16, 1.5%	A16, 5%	A16, 3%	814, 1.5%	814, 3%	849, 3%	814, 5%	132/ 18
	5	roast 18	849, 5%	814, 3%	A16, 1.5%	849, 3%	A16, 5%	A16, 3%	849, 1.5%	814, 5%	814, 1.5%	156/ 8
	5	roast 19	814, 1.5%	A16, 5%	849, 1.5%	A16, 3%	814, 3%	814, 5%	849, 5%	849, 3%	A16, 1.5%	126/ 12
	5	roast 20	849, 5%	A16, 1.5%	849, 1.5%	849, 3%	A16, 5%	814, 5%	A16, 3%	814, 1.5%	814, 3%	144/ 6

	DAY	Replicate 4	Position 1	Position 2	Position 3	Position 4	Position 5	Position 6	Position 7	Position 8	Position 9	Temp (°C)/ Time (min)
WEEK 4	1	roast 1	A16, 3%	849, 1.5%	A16, 5%	814, 5%	A16, 1.5%	849, 5%	849, 3%	814, 3%	814, 1.5%	144/ 9
	1	roast 2	849, 5%	814, 3%	814, 5%	814, 1.5%	A16, 1.5%	A16, 3%	849, 3%	A16, 5%	849, 1.5%	132/ 12
	1	roast 3	A16, 3%	A16, 1.5%	814, 1.5%	814, 5%	814, 3%	849, 5%	849, 1.5%	849, 3%	A16, 5%	156/ 10
	1	roast 4	A16, 5%	A16, 3%	849, 5%	A16, 1.5%	814, 3%	814, 5%	849, 3%	814, 1.5%	849, 1.5%	126/ 12
	2	roast 5	A16, 1.5%	849, 3%	A16, 5%	849, 5%	849, 1.5%	A16, 3%	814, 3%	814, 5%	814, 1.5%	126/ 60
	2	roast 6	814, 3%	849, 3%	814, 1.5%	814, 5%	A16, 3%	849, 1.5%	A16, 1.5%	A16, 5%	849, 5%	144/ 12
	2	roast 7	849, 3%	A16, 5%	814, 1.5%	A16, 3%	A16, 1.5%	814, 5%	849, 1.5%	849, 5%	814, 3%	156/ 4
	2	roast 8	849, 3%	849, 1.5%	849, 5%	A16, 5%	A16, 3%	A16, 1.5%	814, 1.5%	814, 5%	814, 3%	132/ 24
	3	roast 9	849, 5%	849, 3%	A16, 1.5%	849, 1.5%	814, 5%	A16, 5%	814, 1.5%	814, 3%	A16, 3%	156/ 8
	3	roast 10	814, 1.5%	849, 1.5%	A16, 1.5%	849, 5%	849, 3%	A16, 5%	814, 5%	814, 3%	A16, 3%	126/ 36
	3	roast 11	849, 5%	A16, 5%	849, 1.5%	814, 1.5%	849, 3%	A16, 3%	814, 5%	814, 3%	A16, 1.5%	132/ 18
	3	roast 12	A16, 3%	814, 5%	814, 3%	849, 1.5%	814, 1.5%	A16, 1.5%	849, 3%	849, 5%	A16, 5%	144/ 15
	4	roast 13	849, 5%	814, 1.5%	814, 5%	A16, 3%	849, 3%	814, 3%	849, 1.5%	A16, 5%	A16, 1.5%	126/ 24
	4	roast 14	849, 1.5%	A16, 1.5%	849, 3%	A16, 5%	814, 5%	A16, 3%	814, 3%	814, 1.5%	849, 5%	132/ 30
	4	roast 15	814, 3%	A16, 5%	849, 5%	814, 1.5%	814, 5%	849, 1.5%	849, 3%	A16, 1.5%	A16, 3%	144/ 3
	4	roast 16	814, 1.5%	849, 5%	A16, 3%	814, 3%	814, 5%	849, 3%	849, 1.5%	A16, 1.5%	A16, 5%	156/ 6
	5	roast 17	814, 5%	849, 5%	A16, 3%	A16, 5%	A16, 1.5%	814, 3%	849, 1.5%	849, 3%	814, 1.5%	132/ 6
	5	roast 18	849, 3%	814, 5%	A16, 1.5%	814, 1.5%	A16, 3%	849, 5%	A16, 5%	814, 3%	849, 1.5%	156/ 2
	5	roast 19	A16, 3%	814, 1.5%	849, 1.5%	849, 3%	814, 5%	A16, 5%	849, 5%	814, 3%	A16, 1.5%	126/ 48
	5	roast 20	A16, 5%	814, 5%	814, 3%	A16, 1.5%	849, 3%	814, 1.5%	849, 5%	A16, 3%	849, 1.5%	144/ 6

Chapter 5

Effects of roasting on macadamia kernel texture and oxidative stability

5.1 Abstract

The effects of storing raw and variously roasted kernel of two macadamia cultivars at two initial kernel moisture contents at 22^{o}C and 40^{o}C for periods up to 60 weeks on kernel quality has be assessed. The kernel of A16 and Own Venture were selected for investigation because of their different linoleic acid content that had previously been shown to be associated with oxidative stability. Own Venture kernel had the higher linoleic content and invariably had lower oxidative stability as measured by hexanal levels and peroxide values after all treatments and storage conditions. Kernel were roasted at three different conditions 125^{o}C for 25 min, 135^{o}C 10 min and 156^{o}C for 2min. All roasting treatments reduced kernel peak fracture force compared to raw kernel. The facture force of all kernel whether raw or roasted also lowered with storage duration. However the amount of moisture loss and initial changes to fracture force were significantly less when roasted at 156^{o}C. There were no differences in hexanal levels or peroxide values between kernels of the same cultivar after similar storage durations due to any of the roasting treatments. After some of these roasting treatments the kernel moisture content was below the recommended as low as 1.2% (w/w) but still showed the same pattern of deterioration as higher moisture content kernel indicating that over drying does not accelerate deterioration. While the target moisture contents were 1.5 and 2.5% these precise levels were not achieved. The mean kernel moisture contents for this classes of kernel in reality differed by less than 0.5% but kernel a lower moisture content was significantly more stable. Examination of stained kernel examined under florescence microscopy confirmed that oil was stored in numerous oleosomes within the kernel cells however after roasting these were invariably ruptured resulting in oil coalescence into large droplets. The response of macadamia kernel to roasting is similar to other tree nuts in that fracture force is reduced with roasting. However unlike other tree nuts the oxidative stability is not affected.

5.2 Introduction

Roasting is thought to reduce kernel stability in other tree nuts by increasing kernel porosity thereby allowing greater access of air to oil and accelerating oxidative rancidity (Perren and Escher, 1999). Associated an increase in kernel porosity are changes in kernel texture due to heat effects on the pectic middle lamella that holds adjacent cells and their associated walls together (Saklar et al., 2003). Other effects of roasting on kernel microstructure include disruption of oil vesicles and swelling of protein bodies. While a single cell macadamia kernel cell is known to contain numerous vesicles (Walton and Wallace 2009) the effects of roasting cellular and microstructure are unknown.

In the previous chapter it was found that cultivar, kernel moisture content and roast conditions and interactions between these factors affected the roasting response of macadamia kernel. Kernel at moisture contents high as 3% w/w produced acceptable colour kernel if roasted under appropriate conditions. This is substantially higher than the currently recommended 1.5% (w/w) indicating there could be savings to processing by removing shell at higher moisture contents provided there were no effects on processing efficiency and kernel quality. Reducing oil rich products to water activities below 0.3 are thought to increase oxidative rancidity (Labusa, 1979). During roasting macadamia kernel are known to loose over 60% of their initial moisture content. (McConchie and Albertson 2006). Raising the pre-roast moisture content may therefore have impacts on the post-roast kernel stability.

Macadamia when harvested, dried and stored under ideal conditions show minimal deterioration and can be acceptable to consumers after 12 months even after roasting (Mason et al., 1995). To determine whether variables such as oil content or water activity effect the oxidative stability of kernel researchers have routinely stored kernel at elevated temperatures to accelerate this process (Himstedt, 2002, Salter et al., (In prep a). To validate this approach Salter et al (in prep a) showed there similar volatile produced at progressively higher storage temperatures. Despite these and other macadamia kernel storage trials showing more rapid onset of rancidity at elevated

temperatures (Cavaletto et al., 1966, Cavaletto et al., 1968, Chitundu, 1994, Mason et al., 1998, O'Riordan et al., 2005) there is continuing doubt about the relevance of using elevated storage temperatures to reduce the time taken to investigate macadamia kernel deterioration. A comparison of the pattern of deterioration of kernel at ambient temperatures and under accelerated aging conditions would provide further evidence that similar chemical processes are occurring under these different conditions.

While many of the investigations have used trained panels to monitor macadamia deterioration these are impractical and expensive for commercial evaluation purposes. To supplement and replace sensory analysis various chemical indices related to have been employed. Some of these indices such as peroxide value and percentage free fatty acids are used in the product specification for macadamia kernel (Evans and Hoffman, 2005). However there are a range of other measures of oxidative stability used in other high oil content products including TBA, pAV and conjugated dienes. These were not previously investigated because macadamia was thought to have very very low levels of polyunsaturated fatty acids (Himstedt, 2002)

Macadamia oil is known to be mainly composed of monounsaturated and saturated fatty acids with traces of polyunsaturated fats (Himstedt 2002) However the oxidative stability of the polyunsaturated lineoleic acid is known to be acid is known to deteriorate 10 times faster than its monosaturated fatty acid oleic acid with the same length carbon chain (Barnes and Galliard 1982). Previous investigations of raw macadamia have shown that kernel of the same cultivar with higher levels of lineoleic acid have lower oxidative stability (Himstedt 2002, Salter et al, In prep b). Little in known about the variation in linoleic acid content in the different macadamia cultivars or the impact the level of linoleic acid has after roasting on kernel oxidative stability.

The aims of the current project were to monitor the deterioration of raw and variously roasted macadamia kernel under ambient and accelerated aging conditions to determine whether the roasting has any effect on oxidative stability. This will also assist in verifying the use of accelerated aging as a process to oxidative stability of kernel. A range of chemical indices will be evaluated to determine their capacity to describe macadamia deterioration. The effects of roast conditions and storage on kernel texture will also be assessed as it is key sensory element of macadamia kernel.

5.3 Material and methods

Collection of nut-in-shell for aging

In April 2008, 20 mature nut-in-shell were harvested from 3 trees of 11 macadamia cultivars. These were dried to ~1.5% kernel moisture content the nuts cracked individually and cold pressed to obtain the oil. The oil was analysed for fatty acid profile. The differences among cultivars were found to be small with Own Venture having the highest percentage of linolenic acid and A16 the lowest. These two cultivars were then selected based on their contrast in linolenic acid content for use in the accelerated aging trial. Bulk harvests were then made of each of these cultivars that were dried to NIS moisture content of ~3 and 5% before cracking.

Whole kernels were selected and dried to two initial raw kernel moisture contents corresponding to the industry standard of 1.5% and the theoretical optimum of 2.5%. Three replicates of each cultivar and moisture combination were stored raw or dry roasted under ambient (22^{o}C) and accelerated aging (40^{o}C) conditions. At 40^{o}C, the kernels were stored for 0, 4, 8, 12, 16, 20 or 24 weeks. At 22^{o}C, the kernels were stored for 0, 12, 24, 36, 48 and 60 weeks. Development of rancidity was monitored at these time points using static SPME-GC measuring head-space hexanal concentrations. Kernel colour and texture were then determined before oil was mechanically pressed on which other indicators for lipid oxidation including PV, CDHP, pAV, FFA and TBA.

Three roasting treatments were used in the accelerated aging trials: long duration low temperature (125^{o}C 36 min), industry standard (133^{o}C 12 min) or short duration high temperature (156^{o}C 2min). For kernels stored at 22^{o}C, only the industry standard roasting condition was used. Approximately 40-50 g samples of kernel for each of the treatments were stored in 250g bottles with septa in the lid. Three replicates of single jars were randomly selected and assessed for their soundness representing “real” replicates.

The measurements made, in the following order, on each of the sample dates were:

1. Headspace hexanal content,
2. Kernel colour,
3. Texture
5. Moisture content (a_w)
6. CDHP, Peroxide value, pAnisidine value, free fatty acid and tiobarbituric Acid (TBA) value

Measurement of rancidity development

Head-space hexanal determination using SPME-GC. Hexanal is produced during the termination phase of lipid oxidation and detected in the headspace above the kernel stored in a sealed jar. In previous studies by Himstedt (2002) hexanal levels gave the best correlation with sensory scores for rancidity compared to PV and FFA. The procedure developed by Salter et al (In prep a) was used with slight modification. The 250 mL TraceClean Tall Wide-Mouth Jar (VWR Scientific Products, PA, USA) containing the kernels was placed into a 30^{o}C water bath for 5 minutes prior to sampling. The fibre, polydimethylsiloxane/divinylbenzene (PDMS/DVB, 65 µm) (Supelco Ltd. Bellefonte, PA, USA), was then placed in the jar and exposed for 25 minutes at 30^{o}C. Following removal from the septum jar, the fibre was withdrawn and desorbed at 220^{o}C for 3 minutes (splitless) in the injection port of the GC (Perkin Elmer, Autosystem Gas Chromatograph) fitted with a SGE BP20 column (30 m x 0.25 mm ID x 0.25 µm Film). The injection port was fitted with a 0.75 mm inlet liner (Supelco Ltd., Bellefonte, PA, USA). The oven temperature was initially held at 40^{o}C for 5 minutes then ramped at 12^{o}C per/min to 240^{o}C and held for 5 mins. The flame ionisation detector (FID) temperature was 280^{o}C.

Hexanal concentration was determined using external standards run under the same conditions over the range of concentrations expected in the sample. The expected concentration range for hexanal in macadamia kernel was thought to be in the range of 0 – 8 µg/g kernel, based on the results of Grosso and Resurrection (2002), who reported that a concentration of 7.4 µg/g hexanal in roasted peanut was unacceptable to consumers. Canola oil solutions containing 0, 0.25, 0.5, 1, 2, 4 and 8 µg hexanal/g oil were made by serial dilution and 20 g of the solution was placed in a 250 mL jar.

Peroxide value (PV). PV is probably the oldest and most commonly used method for measuring rancidity, particularly in the oil industry. Peroxides are the first products of the initiation step of lipid oxidation. Such radicals also react with ferrous ions (Fe^{2+}) in an oxidative process to produce ferric ions (Fe^{3+}) that bind to xylenol orange or ammonium thiocyanate, yielding a colour reaction. The intensity of reaction or concentration of substrate can thus be measured spectrometerically (Shantha and Decker 1994). This colorimetric method requires less material and is more sensitive compared with the iodometric method (AOCS Official Method Cd 8-53) where at least 1 g of sample is needed and the end titration point is somewhat subjective. The current limit used by the macadamia industry is 3.

The procedure reported by Shantha and Decker (1994) was used with slight modification. Approximately 0.2 g of oil was dissolved in 2 mL chloroform:methanol (7:3) and 50 µL 30% ammonium thiocyanate solution was added and vortexed. Five minutes after adding 50 µL iron (II) chloride solution, the absorbance of the mixture was read at 500 nm. The blank contained the solvent and all the above solutions without any oil. PV was expressed as milliequivalents of peroxide per kg.

Conjugated Diene Hydroperoxides (CDHP). Peroxidation of unsaturated fatty acids is accompanied by changes in the position of double bonds to conjugated hydroperoxides and the conjugated structure adsorbs strongly at 232-234 nm. The procedure of measuring CDHP is faster than other methods for rancidity measurements and less sensitive to light. It also requires very little sample although the value is dependent on the fatty acid composition. This method has been used on other oils with high levels of polyunsaturated fatty acids. This method has not previously been used on macadamias which is principally monounsaturated fatty acids. Approximately 0.01 g oil was dissolved in 5 mL hexane and the absorbance was read at 234 nm using air or hexane as background.

p-Anisidine Value (pAV). This test assesses the secondary oxidation products of unsaturated fatty acids, principally conjugated dienes and 2-alkenals. When hydroperoxides breakdown they produce volatile aldehydes like hexanal leaving a non-volatile component of the fatty acid that remains as part of the glyceride molecule. The p-anisidine test measures the non-volatile component. The procedure

of AOCS Official Method (Cd 18-90) was used with slight modification. Approximately 0.3 g of oil was dissolved in 2.5 mL isooctane before 0.5 mL p-anisidine solution was added and vortexed. The absorbance was measured at 350 nm 10 minutes after the addition of p-anisidine solution using 2.5 mL isooctane + 0.5 mL p-anisidine solution as the blank. pAV = 2.5 x (Sample absorbance – Blank absorbance) / sample weight.

Free Fatty Acid (FFA). FFA is a measure of the fatty acids that have been unattached from the triglyceride molecule and they are supposed to be more easily oxidised than when they are esterified as triglycerides. Some processors within the Australian macadamia industry currently use this measurement to evaluate quality of macadamia kernels with a limit of 0.5%. Approximately 0.5 g of oil was dissolved in 5 mL hexane:isopropanol (4:1) before adding a magnetic stirrer and a few drops of 0.1% Bromothymol blue indicator. The mixture was then titrated with standardised 0.002 *N* or 0.02 *N* methanolic KOH until the solution turned green. A triplicate of blanks containing 5 mL solvent with bromothymol blue indicator were also titrated on day of analysis. FFA was expressed as milli equivalent hydroperxides per 100g of oil.

2-Thiobarbituric acid (TBA) value. This method measures secondary-oxidation products and is based on the colour absorbance at 535nm. Because this reaction is not specific and produced by a large number of secondary oxidation products, it is also referred as thiobarbituric acid reactive substances (TBARS). The AOCS Official Method (Cd 19-90) was used with slight modification. Approximately 0.02 g oil was dissolved in 2 mL 1-butanol and 2 mL TBA reagent was added and vortexed. The blank was 2 mL 1-butanol + 2 mL TBA reagent. The mixture was then incubated at 95^{o}C for 2 hours. After the sample cooled down, the absorbance at 532 nm was measured. If the mixture was not clear, the content was filtered through a 0.22 µM membrane filter (Millex GP) before reading.

Colour measurements

The colour of 10 whole kernels from each jar at various storage time points was measured after head-space hexanal determination using the Minolta chromameter using the CIE L*, a* and b* units. Each kernel was measured for the crested and the basal region before the kernel was split and the internal colour was measured. Based

on previous investigations by McConchie et al (submitted) the first principal component (PC1) that had been found to account for 85% of the variation in colour was calculated using the formula, PC1= (0.577*L** -0.615*a** -0.536*b**).

Texture measurements

Texture measurements were made using a Stable Microsystems TA-TX2 Texture analyser (Godalming UK). A modified box cutter blade with a 0.5mm x 50 mm flat cutting edge was used as previously described in McConchie and Albertson (2006). The instrument was set up to run using the library of methods provided with the instrument for almonds with the cutting distance increased from 4mm to 8mm. The program was modified to stop the blade and data recording after travelling 8mm after contacting the kernel surface. Readings of force (kg) were recorded at intervals of 0.005 s. Fracture force was determine using half kernels with kernel resting on the flat surface and blade cutting vertically to the nut axis. The blade contacted the approximate mid point of the kernel.

Microscopy

Transverse hand cut sections of raw kernel central to the cotyledon were made using a single edged razor blade. The region was selected for further processing was proximate to inner cotyledon These sections were fixed in 0.1 m Ph 7.2 Phosphate buffer containing 1.5% gluteraldehyde at 4^{o}C for 24 h. Rinsed in 3 changes of demineralised waters before being foxed in 1% osmium tetroxide for 24 h. Kernel was then rinsed 3 times over 6 h in demineralised water before being dehydrated through an ethanol series and critical point dried using CO_2 as the transition fluid. The dried kernel was then mounted on stubs, sputter coated with gold and examined on a Scanning electron microscope.

Fresh hand sections were also cut of raw and roasted kernel and stained with 1% aqueous Nile Blue, differentiated and mounted in 1% acetic acid. The sections were examined with a Zeiss Axioskop epi-fluorescence microscope fitted with a blue fluorescence excitation filter (maximum 485 nm), and images recorded with an Olympus DP-70 digital camera.

5.4 Results

Colour

The colour analysis were performed on the measure of the top, base and inner surface of 10 kernel. To summarise these results the *L** value and first principal component value that integrates *L**, *a** and *b** value into a single value that accounts for 80% of the total variation were used. The colour data are obtained for the combinations of the factors used in the hexanal analyses.

Since separate kernel samples were used for each time point there was a genuine split plot in time experiment. The kernel stored at 22^{o}C and 40^{o}C were analysed separately.

		L*			**PC1**		
Source of variation	**d.f.**	**m.s.**	**v.r.**	**F pr.**	**m.s.**	**v.r.**	**F pr.**
Rep stratum	2	1.821	0.59		0.25127	1.54	
Rep.combo stratum							
CV	1	83.735	27.12	<.001	5.79893	35.58	<.001
Moisture	1	175.765	56.93	<.001	3.6252	22.25	<.001
Roast_Temp_C	2	77.743	25.18	<.001	7.35467	45.13	<.001
CV.Moisture	1	0.717	0.23	0.635	0.01114	0.07	0.796
CV.Roast_Temp_C	2	4.757	1.54	0.236	0.3036	1.86	0.179
Moisture.Roast_Temp_C	2	11.284	3.65	0.043	0.27368	1.68	0.21
CV.Moisture.Roast_Temp_C	2	4.358	1.41	0.265	0.25521	1.57	0.231
Residual	22	3.087	2.98		0.16296	5.13	
Rep.combo.Post_Roast_Storage stratum							
Post_Roast_Storage	6	5.375	5.18	<.001	0.31393	9.88	<.001
Post_Roast_Storage.CV	6	1.799	1.74	0.116	0.05286	1.66	0.133
Post_Roast_Storage.Moisture	6	0.877	0.85	0.536	0.0184	0.58	0.746
Post_Roast_Storage.Roast_Temp_C	12	1.13	1.09	0.373	0.04175	1.31	0.215
Post_Roast_Storage.CV.Moisture	6	0.931	0.9	0.498	0.02837	0.89	0.501
Post_Roast_Storage.CV.Roast_Temp_C	12	1.631	1.57	0.105	0.03577	1.13	0.343
Post_Roast_Storage.Moisture.Roast_Temp_C	12	1.29	1.24	0.258	0.0403	1.27	0.242
Residual	156	1.037			0.03176		

Table 5. 1. ANOVA for *L** and PC1 for kernel of Own Venture and A16 roasted at 125, 135 and 155^{o}C with moisture content of 1.5 or 2.5% then stored for up to 24 weeks at 40^{o}C.

There were highly significant main effects for both *L** and PC1 for the kernel stored at 40^{o}C (Table 5.1). The means for these are given below, together with their least significant difference (LSD). There seems to be no obvious trend in the L* value but there appears to be a particular trend in the post roast storage colours, but there is a progressive reduction in PC1 over the 24 weeks at 40^{o}C. The Own Venture (OV) kernel was darker than A16 evident by the lower PC1 and *L** values. Similarly kernel at 2.5% (w/w) moisture content was darker that

kernel at 1.5%. The kernel roasted at 125°C and 135°C had a similar colour while kernel roasted at 156°C was lighter.

Post_Roast_Storage (weeks)	0	4	8	12	16	20	24	LSD
PC1	1.179	1.1	0.989	1.089	1.027	0.931	0.929	0.084
L	77.895	77.821	77.274	78.325	78.434	77.951	77.722	0.48

CV	A16	OV	LSD
PC1	1.186	0.883	0.1018
L	78.494	77.341	0.4428

Moisture (% w/w)	1.5	2.5	LSD
PC1	1.155	0.915	0.1018
L	78.753	77.082	0.4428

Roast_Temp_(°C)	125	135	156	SED
PC1	0.881	0.847	1.376	0.1246
L	77.456	77.273	79.023	0.5422

Table 5.2 Table of means and Least significant differences (LSD) for PC1 and L* for kernel of Own Venture and A16 roasted at 125, 135 and 155°C with moisture content of 1.5 or 2.5% then stored for up to 24 weeks at 40°C

The data for the 22°C stored kernel were analysed in two parts – a part for the pre roasted kernel storage and a part for the post roasted kernel. Note that there was only one roast temperature used 135 °C. Both sets of data were analysed by ANOVA. The results are summarised in the table that follows, with L on the left and PC1 on the right. The first table is for the post roast stored data and the second for the pre roast stored data.

		L*			PC1		
Source of variation	**d.f.**	**m.s.**	**v.r.**	**F pr.**	**m.s.**	**v.r.**	**F pr.**
Rep stratum	2	1.954	3.31		0.04084	6.3	
Rep.CV.Moisture_% stratum							
CV	1	10.512	17.81	0.006	0.410828	63.32	<.001
Moisture_%	1	22.11	37.46	<.001	0.082695	12.75	0.012
CV.Moisture_%	1	0.102	0.17	0.691	0.001623	0.25	0.635
Residual	6	0.59	0.53		0.006488	0.68	
Rep.CV.Moisture_%.Raw_storage_time_wks stratum							
Raw_storage_time_wks	5	89.819	80.02	<.001	0.157296	16.39	<.001
CV.Raw_storage_time_wks	5	1.106	0.99	0.439	0.007183	0.75	0.592
Moisture_%.Raw_storage_time	5	2.216	1.97	0.104	0.007265	0.76	0.586
CV.Moisture_%.Raw_storage_time	5	2.184	1.95	0.108	0.018755	1.95	0.107
Residual	40	1.123			0.0096		

Table 5.3 ANOVA looking at the pre-roasting effects of cultivar, moisture content and storage duration at 22^{o}C and any interactions on *L** and PC1.

		*L**			PC1		
Source of variation	**d.f.**	**m.s.**	**v.r.**	**F pr.**	**m.s.**	**v.r.**	**F pr.**
Rep stratum	2	1.609	0.75		0.07823	0.53	
Rep.CV.Moisture_% stratum							
CV	1	65.893	30.69	0.001	2.31429	15.61	0.008
Moisture_%	1	66.145	30.81	0.001	0.45384	3.06	0.131
CV.Moisture_%	1	7.374	3.43	0.113	0.35178	2.37	0.174
Residual	6	2.147	1.76		0.14823	4.92	
Rep.CV.Moisture_%.Post_Roast_Storage_wks stratum							
Post_Roast_Storage_wks	5	7.843	6.43	<.001	0.13134	4.36	0.003
CV.Post_Roast_Storage_wks	5	1.968	1.61	0.179	0.01749	0.58	0.714
Moisture_%.Post_Roast_Storage	5	0.191	0.16	0.977	0.00776	0.26	0.933
CV.Moisture_%.Post_Roast_Storage	5	1.638	1.34	0.266	0.02631	0.87	0.507
Residual	40	1.22			0.03012		

Table 5.4 ANOVA looking at the post-roast effects of cultivar, moisture content and storage duration at 22oC and any interactions on *L** and PC1.

For the roasted kernel stored at 22^{o}C there were highly significant (P<0.001) effects for cultivar, moisture and post roast storage for the *L** and cultivar (P=0.008) and post roast storage (P=0.003) for the PC1 variate. The means for the significant effects (and their LSD's) are given in Table 5.5. The mean L* and PC1 values showed that the kernel of Own Venture

was darker than A16 and the kernel with a target moisture content of 2.5% was darker than the 1.5% kernel. There was no obvious pattern to the kernel colour with storage though kernel on day zero was darker than latter storage dates. This difference is probably not real and was due to a reduced number of kernel being measured on this day.

L*

Cultivars	**A16**	**OV**	**LSD**
	78.66	76.75	0.69

Moisture % (w/w)	**1.5**	**2.5**	**LSD**
	78.66	76.75	0.69

Post_Roast_Storage_wks (weeks)	**0**	**12**	**24**	**36**	**48**	**60**	**LSD**
	76.91	78.21	78.69	78.3	76.72	77.4	0.902

PC1

Cultivars	**A16**	**OV**	**LSD**
	1.327	0.968	0.1814

Post_Roast_Storage_wks (weeks)	**0**	**12**	**24**	**36**	**48**	**60**	**LSD**
	0.956	1.117	1.156	1.249	1.221	1.185	0.1418

Table 5.5. Table of means and their least significant differences (LSD's) for PC1 and L* values of roasted kernel of Own Venture (OV) and A16 at different target kernel moisture contents stored for periods up to 60 weeks.

For the raw kernel we have highly significant effects for cultivar (P=0.006), moisture (P<0.001) and raw storage (P<0.001) for the *L** variate and highly significant effects for cultivar (P<0.001), moisture (P=0.012) and post roast storage (P<0.001) for the PC1 variate. The means for the significant effects (and their LSDs) are given below. The basic pattern that was found in the roasted kernel.

L*

Cultivars	A16	OV	LSD
	77.79	77.02	0.362

Moisture % (w/w)	1.5	2.5	LSD
	77.96	76.85	0.362

Raw_storage_time_wks (weeks)	0	12	24	36	48	60	LSD
	73.8	78.39	79.88	78.85	74.08	79.44	0.866

PC1

Cultivars	A16	OV	LSD
	1.679	1.528	0.038

Moisture % (w/w)	1.5	2.5	LSD
	1.637	1.569	0.038

Raw_storage_time_wks (weeks)	0	12	24	36	48	60	LSD
	1.673	1.504	1.636	1.592	1.449	1.765	0.08

Table 5.6 Table of means and their least significant differences (LSD's) for PC1 and *L** values of raw kernel of Own Venture (OV) and A16 at different target kernel moisture contents stored for periods up to 60 weeks.

Hexanal

The kernel stored at 22^{o}C and 40^{o}C have been analysed separately. In order to stabilize the variance the hexanal values were log transformed prior to analysis. Besides hexanal several other variables (CDHP, PV, FFA, pAV and TBA) were measured but only for the kernel roasted at 135^{o}C. The analysis of variance table for the hexanal analysis is given in the Table 5.7.

Source of variation	m.s.	v.r.	F pr.
Rep stratum	0.5907	0.29	
Rep.cv.Moisture_%.Roast_temp stratum			
cv	472.8416	231.87	<.001
Moisture_%	0.3655	0.18	0.676
Roast_temp	0.2558	0.13	0.883
cv.Moisture_%	2.2482	1.1	0.305
cv.Roast_temp	0.4093	0.2	0.82
Moisture_%.Roast_temp	0.995	0.49	0.62
cv.Moisture_%.Roast_temp	4.1618	2.04	0.154
Residual	2.0393	3.04	
Rep.cv.Moisture_%.Roast_temp.Post_Roast_Storage_wks stratum			
Post_Roast_Storage_wks	20.1034	29.93	<.001
cv.Post_Roast_Storage_wks	2.6574	3.96	0.001
Moisture_%.Post_Roast_Storage_wks	0.5433	0.81	0.565
Roast_temp.Post_Roast_Storage_wks	0.7083	1.05	0.403
cv.Moisture_%.Post_Roast_Storage_wks	1.0139	1.51	0.179
cv.Roast_temp.Post_Roast_Storage_wks	1.1233	1.67	0.078
Moisture_%.Roast_temp.Post_Roast_Storage_wks	1.4349	2.14	0.018
Residual	0.6717		

Table 5.7. ANOVA for the effects of cultivar, moisture content, roast temperature, storage time and their interactions on hexanal level.

There are highly significant (P<0.001) main effects due to cultivar and post roast storage, and a highly significant (P=0.001) interaction between cultivar and post roast storage. There appears to be a significant three factor interaction but since it contains moisture and roast temperature, neither of which are significant anywhere else, and therefore were considered to be of little consequence. The means for the cultivar by post roast storage interaction together with the LSD are given in the Table 5.8.

Cultivar	Weeks Post Roast storage						
	0	**4**	**8**	**12**	**16**	**20**	**24**
A16	-3.259	-3.581	-2.791	-2.367	-1.817	-2.447	-2.413
OV	-1.547	-0.862	-0.091	0.511	0.728	0.826	0.937
LSD	0.6208						

Table 5.8. Mean log hexanal levels for the cultivar by roast interactions for cultivars A16 and Own Venture (OV) stored for periods up to 24 weeks

The data for the 22 ^{o}C stored kernel were analysed in two parts – the part for the raw kernel storage and the part for the post roasted kernel storage. Note that there was only one roast temperature here (135 ^{o}C). Both sets of data can be analysed by ANOVA. The results are summarised in the Tables 5.9 for the raw and 5.10 for the post roast data.

Source of variation	d.f.	m.s.	v.r.	F pr.
Rep stratum	2	5.1261	1.71	
Rep.cv.Moisture_% stratum				
cv	1	102.602	34.26	0.001
Moisture_%	1	7.3455	2.45	0.168
cv.Moisture_%	1	7.3455	1.71	0.239
Residual	6	7.3455	3.61	
Rep.cv.Moisture_%.Raw_storage_time_wks stratum				
Raw_storage_time_wks	4	6.8847	8.31	<.001
cv.Raw_storage_time_wks	4	0.098	0.12	0.975
Moisture.Raw_storage_time	4	3.1852	3.84	0.012
cv.Moisture.Raw_storage_time	4	1.1245	1.36	0.271
Residual	32			

Table 5.9 ANOVA of hexanal product by raw kernel stored at 22^{o}C examining the main effects of cultivar, moisture content, storage time and their interactions.

Source of variation	d.f.	m.s.	v.r.	F pr.
Rep stratum	2	1.2043	0.5	
Rep.cv.Moisture_% stratum				
cv	1	128.2812	53.51	<.001
Moisture_%	1	13.4356	5.6	0.056
cv.Moisture_%	1	2.0333	0.85	0.393
Residual	6	2.3974	5.61	
Rep.cv.Moisture_%.Post_Roast_Storage_wks stratum				
Post_Roast_Storage_wks	4	5.7717	13.51	<.001
cv.Post_Roast_Storage_wks	4	0.7848	1.84	0.146
Moisture.Post_Roast_Storage	4	1.4259	3.34	0.022
cv.Moisture.Post_Roast_Storage	4	1.1202	2.62	0.053
Residual	32	0.4272		

Table 5.10. ANOVA of post roast hexanal production of kernel stored at 22^{o}C of examining the main effects of cultivar, moisture content, storage time and their interactions.

For both post roast stored and raw stored data there were highly significant (P<0.001) effects due to cultivar and storage time. There was also a significant (P=0.022 for post and P=0.012 for raw data) interaction for both data sets between moisture content and storage time, although there was no significant main effect due to moisture in either case.

The cultivar means for the post roast data are -2.574 for A16 and 0.350 for Own Venture with an LSD 0.8. The cultivar means for the raw stored data are -2.34 for A16 and 0.28 for Own Venture with an LSD of 0.894. These are means of log transformed data.

The means for the interaction between moisture content and storage time are given in the following Table 5.11 together with their LSDs. In both the raw and roasted kernel the kernel at the target moisture content of 1.5% (w/w) moisture content deteriorated less than the kernel at 2.5%. In all cases the hexanal tends to increase with the highest level after 60 weeks storage.

Moisture % (w/w)	Weeks Post roast storage				
	12	**24**	**36**	**48**	**60**
1.5	-1.872	-1.848	-1.951	-1.155	-1.101
2.5	-1.694	-0.972	-1.108	-0.389	0.969
LSD	1.0464				

Moisture % (w/w)	Weeks Raw storage				
	12	**24**	**36**	**48**	**60**
1.5	-1.64	-1.6	-1.56	-1	-1.1
2.5	-2.16	-1.69	-0.39	-0.12	0.97
LSD	0.1298				

Table 5.11. Mean and LSD log hexanal value for the interaction between moisture content and storage duration for raw kernel and kernel roasted at 135^{o}C then stored.

Peroxide value

The peroxide value (PV) data has also been analysed, for raw kernel and roasted at 135^{o}C stored at both 22^{o}C and 40^{o}C. The PV variable also needed a log transform to stabilize the variance.

For the roasted kernel stored at 40^{o}C the results of the ANOVA are shown in Table 5.12. There is a highly significant (P=0.002) cultivar effect. The means are 0.177 for A16 and 1.026 for Own Venture with an LSD of 0.34 indicating that PV like hexanal levels showed that A16 kernel was more stable than Own Venture.

Source of variation	d.f.	m.s.	v.r.	F pr.
Rep stratum	2	0.5789	0.95	
Rep.cv.Moisture_% stratum				
cv	1	15.151	24.96	0.002
Moisture_%	1	0.8294	1.37	0.287
cv.Moisture_%	1	0.0113	0.02	0.896
Residual	6	0.6071	2.4	
Rep.cv.Moisture_%.Post_Roast_Storage_wks stratum				
Post_Roast_Storage_wks	6	0.3007	1.19	0.327
cv.Post_Roast_Storage_wks	6	0.1482	0.59	0.739
Moisture_%.Post_Roast_Storage_wks	6	0.2476	0.98	0.449
cv.Moisture_%.Post_Roast_Storage_wks	6	0.2532	1	0.435
Residual	48	0.2526		

Table 5.12. ANOVA of raw kernel peroxide values stored at 40^{o}C for the main effects of cultivar, moisture content, storage time and their interactions.

Data for the kernel stored at 22 ^{o}C were analysed separately for the raw kernel and for storage after roasting at 135^{o}C kernel. The PV variable also needed a log transform to stabilize the variance. Both sets of data can be analysed by ANOVA. The results are raw data are summarised in Table 5.13.

Source of variation	d.f.	m.s.	v.r.	F pr.
Rep stratum	2	0.1374	0.38	
Rep.cv.Moisture_% stratum				
cv	1	35.403	98.39	<.001
Moisture_%	1	2.1435	5.96	0.05
cv.Moisture_%	1	0.2114	0.59	0.472
Residual	6	0.3598	0.93	
Rep.cv.Moisture_%.Raw_Storage_wks stratum				
Raw_Storage_wks	4	0.6334	1.64	0.188
cv.Raw_Storage_wks	4	1.4541	3.77	0.013
Moisture_%.Raw_Storage_wks	4	0.8113	2.1	0.104
cv.Moisture_%.Raw_Storage_wks	4	0.0348	0.09	0.985
Residual	32	0.3858		

Table 5.13. ANOVA of the peroxide values of raw kernel stored at 22^{o}C for the main effects of cultivar, moisture contents and storage duration and their interactions.

There was a highly significant (P<0.001) effect due to cultivar, a borderline significant moisture effect (P=0.5) and a significant (P=0.013) interaction between cultivar and storage time. The means for cultivar are 0.007 for A16 and 1.543 for Own Venture with an LSD of 0.31. The means (and LSD) for the significant interaction are given in the Table 5.14. A16 under went minimal change over the 60 weeks while there was significant degradation in Own Venture kernel

Cultivar	Weeks storage Raw				
	12	**24**	**36**	**48**	**60**
A16	0.1	0.175	-0.188	-0.49	0.437
OV	0.968	1.28	1.467	2.173	1.828
LSD	0.7124				

Table 5.14. Means and LSD peroxide values for the significant interaction between cultivar and moisture content for raw kernel stored at 22^{o}C.

The ANOVA of the data for peroxide value of roasted kernel stored at 22^{o}C are shown in Table 5.15.

Source of variation	d.f.	m.s.	v.r.	F pr.
Rep stratum	2	0.1652	0.3	
Rep.cv.Moisture_% stratum				
cv	1	23.6561	42.99	<.001
Moisture_%	1	1.8542	3.37	0.116
cv.Moisture_%	1	0.0008	0	0.97
Residual	6	0.5503	3.96	
Rep.cv.Moisture_%.Post_Roast_Storage_wks stratum				
Post_Roast_Storage_wks	4	0.6506	4.68	0.004
cv.Post_Roast_Storage_wks	4	0.3806	2.74	0.046
Moisture_%.Post_Roast_Storage_wks	4	0.0856	0.62	0.654
cv.Moisture_%.Post_Roast_Storage_wks	4	0.2407	1.73	0.167
Residual	32	0.1389		

Table 5.15. ANOVA of the peroxide values of roasted kernel stored at 22^{o}C for the main effects of cultivar, moisture contents and storage duration and their interactions.

There is a highly significant (P<0.001) cultivar effect and a highly significant (P=0.004) post roast storage effect. There is a just significant interaction between the se two main effects (p=0.046). In Table 5.16 the means of peroxide values for the post roast storage effect and for the interaction between the storage and the cultivar are presented. There would appear to be an irregularity in the data collect for A16 in week 24.

Weeks Post Roast Storage	12	24	36	48	60
	0.676	0.815	0.626	0.735	1.209
LSD	0.383				

Cultivar	Post roast storage				
	12	24	36	48	60
A16	0.164	0.346	-0.122	-0.146	0.679
OV	1.188	1.284	1.373	1.617	1.739
LSD	0.543				

Table 5.16. Means of peroxide values for the post roast storage effect and for the interaction between the storage and the cultivar.

Kernel Moisture content and Water Activity

Again there were two parts to this experiment with kernel stored at 22°C and 40°C. The two sets of data have been analysed separately. There were two variables measured, water activity and final moisture content. The results of the analyses of variance for the kernel stored at 40°C presented in Table 5.17.

		Water activity			Moisture Content		
Source of variation	**d.f.**	**m.s.**	**v.r.**	**F pr.**	**m.s.**	**v.r.**	**F pr.**
Rep stratum	2	0.001025	0.58		0.017	0.28	
Rep.CV.Roast_Temp_C.Initial_Moisture_% stratum							
CV	1	0.025348	14.26	0.001	0.141	2.37	0.138
Roast_Temp_C	2	0.010418	5.86	0.009	0.971	16.3	<.001
Initial_Moisture_%	1	0.183373	103.13	<.001	3.464	58.18	<.001
CV.Roast_Temp_C	2	0.000949	0.53	0.594	0.092	1.54	0.237
CV.Initial_Moisture_%	1	0.00675	3.8	0.064	0.422	7.09	0.014
Roast_Temp_C.Initial_Moisture_%	2	0.000163	0.09	0.913	0.018	0.3	0.742
CV.Roast_Temp_C.Initial_Moisture_%	2	0.003529	1.98	0.161	0.057	0.96	0.398
Residual	22	0.001778	1.6		0.06	1.6	
Rep.CV.Roast_Temp_C.Initial_Moisture_%.Post_Roast_Storage_wks stratum							
Post_Roast_Storage_wks	6	0.005379	4.85	<.001	0.553	14.91	<.001
CV.Post_Roast_Storage_wks	6	0.000363	0.33	0.922	0.055	1.47	0.192
Roast_Temp_C.Post_Roast_Storage_wks	12	0.001276	1.15	0.325	0.032	0.87	0.576
Initial_Moisture_%.Post_Roast_Storage_wks	6	0.007466	6.73	<.001	0.159	4.28	<.001
CV.Roast_Temp_C.Post_Roast_Storage_wks	12	0.001611	1.45	0.148	0.091	2.46	0.006
CV.Initial_Moisture_%.Post_Roast_Storage	6	0.001716	1.55	0.167	0.059	1.59	0.154
Roast_Temp.Initial_Moisture.Post_Roast_Storage	12	0.002125	1.91	0.036	0.075	2.01	0.026
Residual	156	0.00111			0.037		

Table 5.17. ANOVA of the water activity and kernel moisture content for kernel stored at 40°C for the main effects of cultivar, roast temperature, moisture contents and storage duration and their interactions.

For water activity we have a highly significant (P<0.001) interaction between initial moisture and post roast storage time. There are also a highly significant cultivar (P<0.001) and roast temperature (P=0.009) effects. The cultivar means water activity were 0.3923 for A16 and 0.3723 for Own venture (LSD=0.01062), and the mean water activity for the roast temperature were 0.3785 for 125°C, 0.3736 for 135°C and 0.3948 for 156°C (LSD=0.01302). The mean water activity and LSD for the interaction between initial target moisture and the post roast storage are given in Table 5.18. This indicates that there was little change in the water activity of kernel dried to the 1.5% target moisture content over the 24 weeks of storage

at 40°C while the there was a reduction in the water activity of kernel stored at 2.5% moisture content over the same period.

Water Activity	Weeks storage post roast						
Initial Moisture % (w/w)	**0**	**4**	**8**	**12**	**16**	**20**	**24**
1.5	0.3551	0.3436	0.3464	0.3547	0.3585	0.364	0.365
2.5	0.4629	0.4201	0.3987	0.3942	0.4013	0.3957	0.3919
LSD	0.02314						

Table 5.18. Mean water activity and LSD for the interaction between initial target moisture content and post roast storage duration at 40°C.

For final moisture we also have a highly significant interaction (P<0.001) between initial moisture and post roast storage time (Table 5.19). While the moisture contents varied significantly with storage duration there was a significant loss of moisture content over the 24 weeks in both 1.5% and 2.5% kernel moisture contents.

Moisture contents	Weeks storage post roast						
Initial Moisture % (w/w)	0	4	8	12	16	20	24
1.5	1.456	1.294	1.211	1.399	1.259	1.314	1.221
2.5	1.9	1.612	1.524	1.482	1.5	1.42	1.358
LSD	0.1338						

Table 5.19. Mean kernel moisture content and LSD for the interaction between initial target moisture content and post roast storage duration at 40°C.

There was a highly significant (P=0.006) interaction between cultivar, roast temperature and post roast storage time. The means for these two significant interactions are given in Table 5.20 with their LSD. This shows that kernel roasted at 156°C had significantly greater kernel moisture content in both cultivars but all kernel lost moisture during the 24 weeks of storage at 40°C.

		Weeks storage post roast						
CV	Roast Temp (oC)	0	4	8	12	16	20	24
A16	125	1.548	1.339	1.332	1.329	1.255	1.281	1.365
	135	1.555	1.482	1.216	1.308	1.254	1.266	1.218
	156	1.952	1.472	1.54	1.385	1.641	1.403	1.29
OV	125	1.614	1.392	1.258	1.304	1.361	1.297	1.117
	135	1.616	1.352	1.379	1.723	1.382	1.38	1.283
	156	1.785	1.681	1.482	1.595	1.386	1.574	1.466
	LSD	0.2318						

Table 5.20. Mean kernel moisture content (w/w) and LSD for the interaction between cultivar, roast temperature and post roast storage time for kernel of A16 and Own Venture (OV) roasted at 125^{o}C, 135^{o}C and 156^{o}C then stored for periods up to 24 weeks at 40^{o}C.

The data for the 22^{o}C stored kernel were analysed in two parts – a part for the pre roasted kernel storage and a part for the post roasted kernel. Note that there was only one roast temperature here (135 ^{o}C). Since the post roast data was unbalanced it was analysed by REML whilst the raw data was analysed by ANOVA. The results of the REML analysis for post roast data for kernel stored at 22^{o}C are shown in Table 5.21.

			Water Activity			Final Moisture Content		
Fixed term	n.d.f.	d.d.f.	Wald statistic	F statistic	F pr	Wald statistic	F statistic	F pr
CV	1	6.1	0.06	0.06	0.815	14.65	14.65	0.009
Initial_Moisture_%	1	6.1	56.3	56.3	<0.001	45.69	45.69	<0.001
Post_Roast_Storage_wks	5	60	41.5	8.3	<0.001	141.62	28.32	<0.001
CV.Initial_Moisture_%	1	6.2	0.43	0.43	0.536	0.32	0.32	0.594
CV.Post_Roast_Storage_wks	5	59.9	1.32	0.26	0.931	8.72	1.74	0.138
Initial_Moisture.Post_Roast_Stor	5	60	3.4	0.68	0.641	4.25	0.85	0.519
CV.Initial_Moisture.Post_Roast_Stor	5	59.9	1.89	0.38	0.862	1.8	0.36	0.874

Table 5.21. REML analysis of the water activity and final kernel moisture content for kernel stored at 22^{o}C for the variates cultivar, initial moisture content and storage duration and their interactions after roasting at 135^{o}C.

There were highly significant main (P<0.001) effects due to initial moisture and post roast storage time for both variables and a highly significant (P=0.009) main effect due to cultivar for the final moisture content. For water activity the initial moisture means are 0.4332 (for 1.5%) and 0.5081 (for 2.5%) with an average LSD of 0.02054. For final moisture content the means for initial moisture content are 1.64% for 1.5% target moisture contents and 2.11% for 2.5% target moisture contents a LSD of 0.137.

The cultivar we have means were 1.728 for A16 and 2.11 for Own Venture with an LSD of 0.137. The means for the significant interaction for both variables are given in the Table 5.22. There was a strong linear trend over time showing an increase in water activity and kernel moisture content with storage duration.

Water activity (a_w)

Weeks Storage Post Roast	0	12	24	36	48	60
	0.4302	0.4406	0.4762	0.4832	0.493	0.5007
LSD	0.030					

Final moisture content

Weeks Storage Post Roast	0	12	24	36	48	60
	1.533	1.696	1.883	2.087	1.935	2.119
LSD	0.127					

Table 5.22. Means and LSDs for final water activity and moisture content after roasting at 135^{o}C and storage for periods up to 60 weeks at 22^{o}C.

		Water activity			Final Kernel Moisture content		
Source of variation	**d.f.**	**m.s.**	**v.r.**	**F pr.**	**m.s.**	**v.r.**	**F pr.**
Rep stratum	2	0.001805	3.08		0.11222	2.69	
Rep.CV.Initial_Moisture_% stratum							
CV	1	0.002351	4.01	0.092	1.43487	34.34	0.001
Initial_Moisture_%	1	0.099607	169.95	<.001	1.64561	39.39	<.001
CV.Initial_Moisture_%	1	0.004092	6.98	0.038	0.10468	2.51	0.165
Residual	6	0.000586	0.5		0.04178	0.55	
Rep.CV.Initial_Moisture_%.Raw_storage_time_wks stratum							
Raw_storage_time_wks	5	0.020304	17.33	<.001	0.54191	7.11	<.001
CV.Raw_storage_time_wks	5	0.000655	0.56	0.73	0.13478	1.77	0.142
Initial_Moisture.Raw_storage	5	0.002388	2.04	0.094	0.0731	0.96	0.454
CV.Initial_Moisture.Raw_storage	5	0.000888	0.76	0.586	0.03562	0.47	0.798
Residual	40	0.001172			0.07625		

Table 5.23. ANOVA of the water activity and final water activity and final kernel moisture content for the main effects of cultivar, moisture contents and storage duration and their interactions after storage at 22^{o}C.

There were highly significant (P<0.001) main effects due to initial moisture and raw storage time for both variables. There is also a highly significant main effect due to cultivar for the final moisture content. For water activity the means for initial moisture were 0.4359 for target moisture content of 1.5% w/w and 0.5103 for 2.5% with an LSD of 0.01142. For final moisture content the means for initial moisture are 2.22% for the target moisture content of 1.5% w/w and 2.52 for 2.5% with an LSD of 0.0804 and for cultivar the means are 2.23 % w/w for A16 and 2.51% for Own Venture with an LSD of 0.084. The means for the main effect of raw storage time are given in Table 5.24 for both variables. Note once again that there is a strong linear trend over time for both these variables with both water activity and final kernel moisture contents increasing storage duration.

Water activity

Weeks Storage	**0**	**12**	**24**	**36**	**48**	**60**
	0.3928	0.4744	0.4925	0.4782	0.4923	0.5083
LSD	0.02794					

Final moisture content

Weeks Storage	**0**	**12**	**24**	**36**	**48**	**60**
	2.128	2.372	2.409	2.124	2.65	2.533
LSD	0.2254					

Table 5.24. Means and LSDs for the main effect of storage time on water activity and final moisture content after storage at 22oC for periods up to 60 weeks.

Texture

The data used in these analyses is based on the means of 12 kernels measured for both peak fracture forces (kg) and the distance to fracture (mm). The results for kernel stored at 22^{o}C and 40^{o}C have been analysed separately.

For the 40^{o}C the data was incomplete since kernel roasted at 125^{o}C and 156^{o}C were only measured at the time of roasting and after 24 weeks storage, whereas the kernels roasted at 135^{o}C and unroasted kernel were measured at 0, 4, 8, 12, 16, 20 and 24 weeks. Hence we have used REML to analyse the two variables here. The kernel weight has been used as a covariate in the analyses. There were two apparent outlier observations (detected using diagnostic plots) in the distance to fracture data and these were deleted prior to analysis. The

results for both variables Force and Distance are summarised in the Table 5.25. Note that the numerator df (n.d.f) are the same for both variates.

		Force				Distance			
Fixed term	**n.d.f.**	**Wald stat**	**F stat**	**d.d.f.**	**F pr**	**Wald stat**	**F stat**	**d.d.f.**	**F pr**
Post_Roast_Storage	6	76.53	12.76	123.7	<0.001	131.1	21.85	123.3	<0.001
CV	1	29.55	29.55	95.9	<0.001	0.48	0.48	97.7	0.489
Moisture	1	4.61	4.61	24.9	0.042	1.43	1.43	29.3	0.241
Roast_Temp	3	251.63	83.19	47.4	<0.001	28.28	9.37	49.4	<0.001
Post_Roast_Storage.CV	6	19.43	3.24	123.8	0.005	2.23	0.37	123.4	0.896
Post_Roast_Storage.Moisture	6	8.08	1.35	124.2	0.242	22.94	3.82	123.8	0.002
CV.Moisture	1	2.06	2.06	25	0.164	1.09	1.09	29.4	0.305
Post_Roast_Storage.Roast_T	8	24.47	3.06	124.7	0.004	35.11	4.39	124.1	<0.001
CV.Roast_Temp	3	3.81	1.26	47	0.299	2.06	0.68	49	0.567
Moisture.Roast_Temp	3	17.39	5.75	46.5	0.002	2.85	0.94	48.7	0.427
Post_Roast_Storage.CV.Moist	6	5.71	0.95	123.8	0.461	4.37	0.73	123.5	0.628
Post_Roast_Stor.CV.Roast_T	8	19.6	2.45	124.1	0.017	12.62	1.58	123.6	0.138
Post_Roast_Stor.Moist.Roast_T	8	16.9	2.11	124.1	0.039	7.66	0.96	123.7	0.472
CV.Moisture.Roast_Temp	3	0.51	0.17	46.9	0.916	4.61	1.53	48.9	0.219
Kernel_Weight_g	1	13.04	13.04	149	<0.001	11.02	11.02	146.3	0.001

Table 5.25 REML analysis of peak fracture force (Force) and fracture distance (Distance) for kernel of A16 and Own venture either raw or roasted at 125, 135 or 156^{o}C and stored for periods up to 24 weeks at 40^{o}C.

There were highly significant main effects of post roast storage duration and roast temperature. The covariate kernel weight is highly significant (P<0.001) in both analyses with larger kernel requiring more force and distance to fracture. The coefficient for source is 0.5777 (SE = 0.16004) and distance is 1.218 (SE = 0.3631). The two factor interactions between post roast storage and moisture and between post roast storage and roast temperature for the distance variable (Tables 5.26a and b) and between post roast storage and cultivar, between post roast storage and roast temperature, and between moisture and roast temperature are significant for the force variable (Tables 5.26c-e and 5.26b) .

Distance (a)

Weeks Post roast storage	Moisture % (w/w)	
	1.5	2.5
0	3.424	3.898
4	3.182	3.386
8	2.915	2.919
12	3.024	2.957
16	3.055	2.926
20	3.063	3.036
24	2.956	2.818
LSD	0.2266	

(b)

Weeks post Roast Storage	Roast Temperature ^{o}C			
	0	125	135	156
0	4.193	3.201	3.546	3.705
4	3.27	*	3.321	*
8	2.875	*	2.96	*
12	3.047	*	2.934	*
16	3.179	*	2.802	*
20	3.394	*	2.705	*
24	3.041	2.862	2.727	2.918
LSD	0.3218			

Force (c)

Moisture % (w/w)	Roast Temperature ^{o}C			
	0	125	135	156
1.5	3.169	2.611	2.515	2.951
2.5	2.96	2.689	2.565	2.83
LSD	0.0988			

(d)

Weeks post Roast Storage	Cultivar	
	A16	OV
0	3.122	2.851
4	3.01	2.727
8	2.868	2.58
12	2.728	2.823
16	2.838	2.704
20	2.872	2.683
24	2.668	2.6
LSD	1.460	

(e)

Weeks post Roast Storage	Roast Temperature ^{o}C			
	0	125	135	156
0	3.438	2.759	2.719	3.031
4	3.028	*	2.709	*
8	2.945	*	2.503	*
12	3.021	*	2.53	*
16	3.066	*	2.477	*
20	3.111	*	2.444	*
24	2.845	2.541	2.399	2.75
LSD	0.1748			

Table 5.26 a-e. Table of means and LSDs for the two factor interactions for variable distance (mm) between a) Moisture content and storage duration b) Roast Temperature and storage duration , and force (kg) between c) Moisture and roast temperature d) Cultivar and storage duration and e) roast temperature and storage duration (weeks). The stars indicate that there is no data for these combinations.

The data for kernel stored at 22^{o}C for stored raw kernel, and kernel roasted at 135^{o}C then stored were analysed separately using ANOVA. Kernel weight was a used as a covariate. The results are summarised in the Table 5.27 for raw kernel and roasted kernel in table 5.28.

Source of variation		Force			Distance		
	d.f. (mv)	m.s.	v.r.	F pr.	m.s.	v.r.	F pr.
rep stratum							
Covariate	1	0.00332	11.6	0.182	0.0002	0	0.987
Residual	1	0.00029	0.01		0.4549	9.1	
rep.CV.Moisture_% stratum							
CV	1	0.42509	11.98	0.018	0.0136	0.27	0.624
Moisture_%	1	0.22605	6.37	0.053	2.9428	58.86	<.001
CV.Moisture_%	1	0.10243	2.89	0.15	0.2442	4.88	0.078
Covariate	1	0.26643	7.51	0.041	0.249	4.98	0.076
Residual	5	0.03549	1.07		0.05	0.14	
rep.CV.Moisture_%.Raw_storage_time_wks stratum							
Raw_storage_time_wks	5	0.39543	11.87	<.001	0.8159	2.32	0.062
CV.Raw_storage_time_wks	5	0.13479	4.05	0.005	1.1072	3.15	0.018
Moisture_%.Raw_storage_time	5	0.13899	4.17	0.004	0.1932	0.55	0.737
CV.Moisture_%.Raw_storage_time	5	0.06985	2.1	0.087	0.2529	0.72	0.612
Covariate	1	3.01632	90.52	<.001	0.083	0.24	0.63
Residual	38 (-1)	0.03332			0.3511		

Table 5.27. ANOVA of the fracture force (Force) and fracture distance (Distance) for raw kernel stored at 22^{o}C for the main effects of cultivar, moisture contents and storage duration and their interactions. Kernel weight was used as a covariate.

Source of variation		Force			Distance		
	d.f. (mv)	m.s.	v.r.	F pr.	m.s.	v.r.	F pr.
rep stratum							
Covariate	1	0.00906	0.13	0.776	0.188	1.8	0.408
Residual	1	0.06735	2.8		0.1047	0.28	
rep.CV.Moisture_% stratum							
CV	1	0.03637	1.51	0.274	3.117	8.29	0.035
Moisture_%	1	0.56489	23.49	0.005	8.2993	22.07	0.005
CV.Moisture_%	1	0.01948	0.81	0.409	0.4987	1.33	0.302
Covariate	1	0.14026	5.83	0.061	0.3372	0.9	0.387
Residual	5	0.02405	0.47		0.376	1.2	
rep.CV.Moisture_%.Post_Roast_Storage_wks stratum							
Post_Roast_Storage_wks	5	0.05443	1.07	0.39	0.3588	1.14	0.354
CV.Post_Roast_Storage_wks	5	0.11931	2.35	0.059	0.0455	0.15	0.98
Moisture_%.Post_Roast_Storage	5	0.06273	1.24	0.311	0.1618	0.52	0.763
CV.Moisture_%.Post_Roast_Storage	5	0.01336	0.26	0.93	0.1057	0.34	0.887
Covariate	1	0.02674	0.53	0.472	0.4936	1.57	0.217
Residual	38 (-1)	0.05068			0.3136		

Table 5.28. ANOVA of the fracture force (Force) and fracture distance (Distance) for kernel roasted at 135^{o}C then stored at 22^{o}C for the main effects of cultivar, moisture contents and storage duration and their interactions. Kernel weight was used as a covariate.

In both these analyses the covariate was of no importance. For the analysis of the post roast stored kernel there was only have a highly significant (P=.005) effect of moisture content for both variables. The means for force are 2.732 kg for 1.5% kernel moisture content and 2.916 kg for 2.5% moisture content with a LSD of 0.0792. The means for distance were 3.380mm for 1.5% kernel moisture content and 4.079mm for 2.5% kernel moisture content with an LSD of 0.3128.

For the analysis of the raw kernel there were significant interactions between cultivar and storage time and between moisture content and raw storage time for the force variable and for the distance variable there is a significant interaction between cultivar and raw storage time (Table 5.29). This would indicate that Own Venture kernel had reduced fracture force in some weeks, and there was no consistent difference between kernel with an initial target moisture content of 1.5% compared to 2.5% kernel moisture contents. There was also a difference after 24 weeks for the distance needed

to fracture kernel of A16 compared to Own Venture but this difference was not evident at other storage durations.

Force						
	Weeks storage raw					
Cultivar	**0**	**12**	**24**	**36**	**48**	**60**
A16	3.706	3.212	3.384	3.106	3.049	3.484
OV	3.407	3.096	2.855	3.123	3.049	3.128
LSD	0.2386					

Moisture % (w/w)	Weeks storage raw					
	0	**12**	**24**	**36**	**48**	**60**
1.5	3.462	3.296	3.218	3.341	3.067	3.263
2.5	3.65	3.012	3.021	2.888	3.031	3.349
LSD	0.2148					

Distance						
Cultivar	Weeks Storage raw					
	0	**12**	**24**	**36**	**48**	**60**
A16	4.429	3.933	4.714	3.554	4.416	4.6
OV	4.062	4.161	3.904	4.17	4.892	3.924
LSD	0.7132					

Table 5.29. Means and LSDs for significant interactions for peak fracture force (force) between cultivar and storage time and between moisture content and raw storage time and the distance variable between cultivar and storage time for raw kernel stored at 22^{o}C.

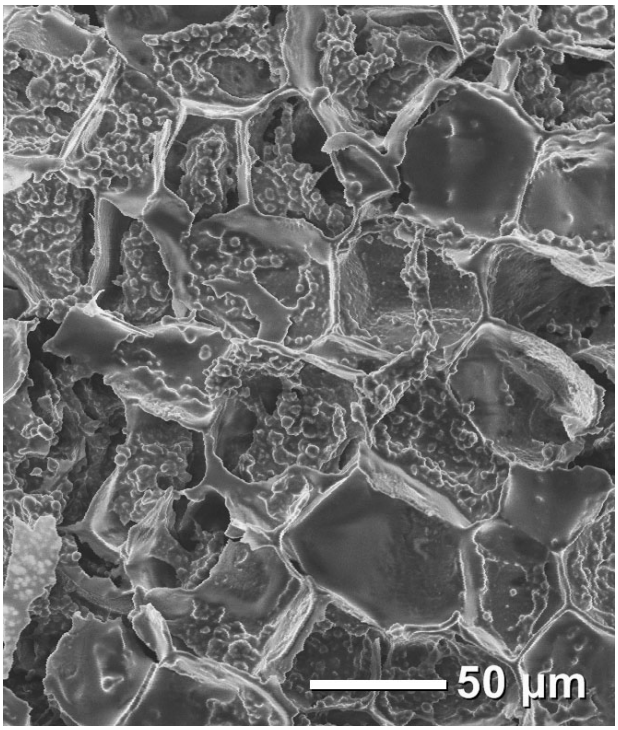

Figure 5.1 Scanning electronmicrograph of a raw macadamia kernel showing the numerous oleosomes within the kernel cells. Scale bar 50 µm

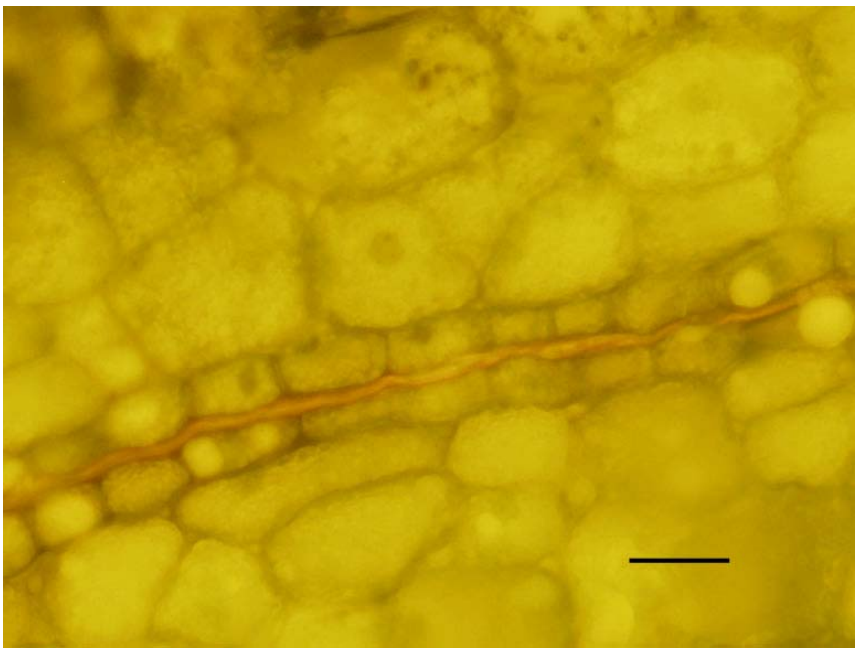

Figure 5.2 Fluorescence micrograph of raw macadamia kernel stained with Nile Blue showing the numerous oleosomes with the kernel cells. Section traverses the junction between the cotyledons. Scale bar = 50 µm.

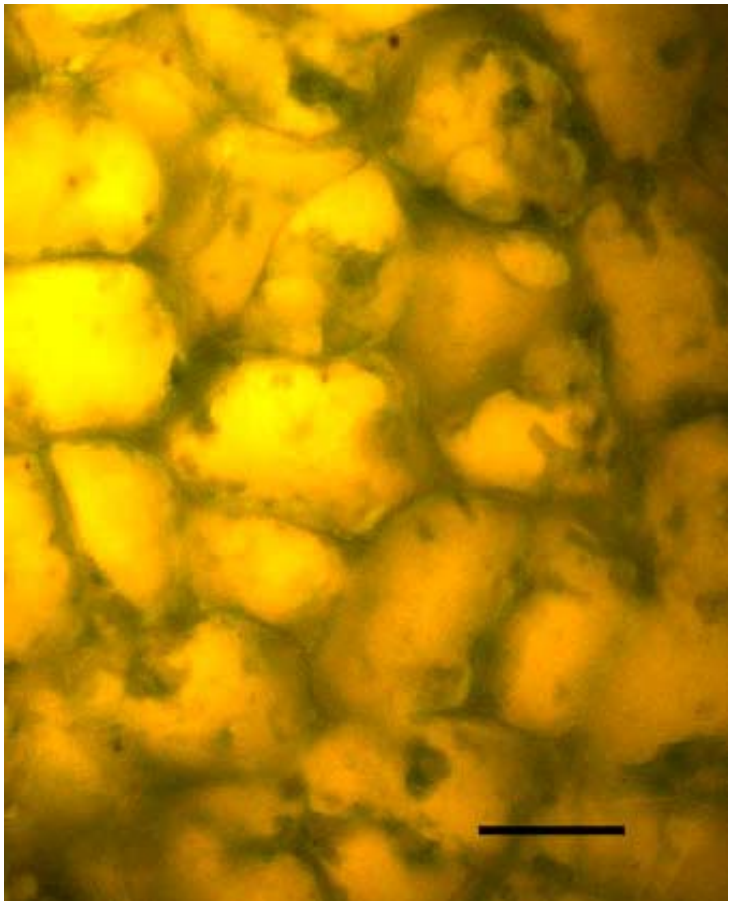

Figure 5.3. Fluorescence micrograph of roasted macadamia kernel stained with Nile Blue showing the numerous oleosomes with the kernel cells. Scale bar = 50 μm.

Morphology

It was very difficult to embed macadamia kernel due to the high oil content preventing infiltration of resin. To obtain images kernel was prepared for scanning electron microscopy using a range solvents including ethanol and acetone to extract the oil. Results are shown for raw kernel in (Fig 5.1) where the numerous oleosomes are generally located with the cells. To avoid the drying process fresh hand sections were stained with Nile blue and examined by fluorescence microscopy revealing the numerous oleosomes within the kernel cells (Fig 5.2). This was repeated with all the various forms of roasted kernel of the different cultivars and invariably the oleosomes were absent and replaced one or two large oil bodies that presumably represent the coalesced oleosomes (Fig 5.3).

5.5 Discussion

These investigations have shown that the selection of cultivar was most important variable in determining the oxidative stability of the kernel assessed. There was no difference in the stability of kernel roasted under a range of conditions whether assessed by hexanal production or peroxide value. However, roasting invariably reduced the peak fracture force indicating the cell to cell adhesion was affected. Roasting also caused the break down of the numerous oleosomes within the kernel cells of macadamia. In other tree nuts reduction of fracture force and ultrastructural breakdown are associated with reduced oxidative stability. This was not the case in macadamia. Extended storage of both raw and roasted kernel also reduce peak fracture force that may have implications for consumer acceptance. While it was intended to have kernel dried to target moisture contents of 1.5% and 2.5% in reality there was only a 0.5% difference between these classes of kernel. Despite this the lower moisture content kernel had higher oxidative stability. Roasting also reduced moisture content below the recommended 1.5% kernel moisture content but this did not affect oxidative stability. In most of the experiments moisture content had a significant affect yet this has not been closely monitored in previous roasting and storage studies.

The effects of roasting on the oxidative stability on kernel stability are contradictory. The investigations by Lemmer and Kruger (2000a &b) concluded that roasting reduces oxidative stability. Three of the cultivars examined A16, A4 and N1 roasted to a much darker colour than other cultivars which they attributed to their hybrid origin. When similar roasting trials were repeated in Australia using A16 this response could not be replicated (McDonagh, 2003). McConchie and Albertson (2006) found A16 kernel to be amongst the lightest coloured kernel after roasting of the range of cultivars examined. When these three aberrant cultivars are separated from the data the more rapid deterioration due to oil roasting is not evident (Lemmer and Kruger (2000a &b). The South African results indicate more rapid deterioration results for dry roasted kernel in roasted kernel compared to raw kernel but again these kernel were roasted for at least twice the duration at 135^{o}C compared to the roasting treatments used our current trials. The roasting conditions we have used are based on

a survey of factory roasting conditions and measurement of commercial packaged kernel (McConchie et al., submitted).

Similar storage and roasting trials were conducted by Mason et al, (1998) in which test NIS was stored for periods up to 12 months at 3.5, 7.5 12.5 and 15% NIS moisture content at 5, 25 and 40^{o}C. The nuts were then shelled and kernel roasted and stored for 0, 4 and 8 months at 25^{o}C. The kernels were then subjected to a range of chemical and sensory analyses provided the kernel was not excessively rancid. The performance of each type of kernel was then compared with control kernel that had been dried to 3.5% then roasted and stored for a comparable 0, 4 and 8 months. Statistical analysis was performed to identify when test kernel differed significantly from control kernel that had been stored for a comparable time after roasting. It was concluded that NIS could be safely stored at NIS moisture contents of 7.5% for 1 month and at 10% for a week despite neither of these conditions being tested. However all kernel that had been stored at 7.5% kernel moisture content then roasted had an overall acceptability of near 50 that is considered unacceptable in many sensor analyses (Cardelli and Labuza, 2001). This suggests that was no safe storage time for NIS at 7.5% moisture content at 25^{o}C. It is interesting to note that the raw kernel peroxide value for NIS stored at 7.5% were invariably higher than the freshly roasted kernel. This may be explained by the roasting temperatures exceeding the boiling point of volatiles of the hydroperoxides detected by this test. However at 25^{o}C the raw kernel invariably had a higher overall acceptability and lower peroxide value compared with roasted stored for comparable combined storage time (Mason et al., 1998) indicating roasting oil roasting had accelerated deterioration. In previous investigation by Mason et al. (1995) no differences in kernel stability were observed between kernel roasted from 4 to 25 minutes at temperatures from 115-135^{o}C which they attributed to processing occurring under controlled conditions.

While there was only slight difference in kernel moisture content between groups of kernel dried too the target kernel moisture contents of 1.5% and 2.5% (w/w) this still resulted in an improved shelf life in the kernel at the lower moisture contents. After roasting these moisture contents were for reduced to as low as 1.2% especially in the lower roast temperatures and longer roast durations. The water activity of this kernel was around 0.3 that is consistent with zone of minimum entropy where lowest

oxidation was observed in raw kernel by Domìnguez et al., (2007). It has been suggested that nut-in-shell can be over dried on farm (Bungay, 2003). Our results would suggest this is unlikely and indicate that higher moisture contents will promote rancidity.

Major differences were observed between the two cultivar used here that were selected for their different linoleic fatty acid content. Linoleic acid content had been previously shown to better correlated with kerne4l oxidative stability in 246 and A16 (Himstedt, 2002) and even between different sites with the same cultivar (Salter et al., *Prep b*). Recently Wall (2010) using a Rancimat with macadamia oil and water mixes has shown significant differences in the stability between years and cultivars. This suggests segregation of nuts from different cultivars may assist in managing through the supply chain provided there are tools to characterize consignments.

It has been suggested the mechanical characteristic of food can be used to replace taste panels and cheaper and easier to perform and are free of the linguistic and cultural limitations (Vincent et al., 2003). Good correlations were reported between incisor penetration and mechanical properties of seven snack foods including macadamia (Agrawal and Lucas, 2003). The mechanical devices used measure the distance travelled after the machined surface contact the nut and the force adplied when the kernel ultimately breaks. McConchie and Albertson (2006) found at reduction in peak fracture force require to break a macadamia kernel after roasting. In hazelnut Saklar et al., (1999) found the first peak in fracture force was related to sensory perception of crispness which they defined as hard but easily breakable. Slope between this peak and the maximum peak fracture force for a kernel was related to crunchiness. These changes in texture are associated with modification of the kernel microstructure including the disruption of cytoplasmic networks, aggregation and swelling of protein bodies and some degree of cell wall separating and increases in intercellular spaces (Saklar et al., 2003). These changes are associated with a reduction in kernel oxidative stability in roasted hazelnuts and almonds (Perren and Escher 1997, 2007). While we observed the break down of subcellular structure in macadamia we did not find a reduction in oxidative stability reported in other tree nuts. As mentioned previously this may have been due to the mild roast conditions

and elevated kernel moisture contents that resulted kernel having comparatively high water activities (0.3) after roasting.

5.6 Acknowledgements

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Chapter 6

Kinetics of macadamia kernel deterioration

6.1 Abstract

The kinetics of oxidative rancidity development during macadamia storage was described using hexanal production for a temperature range of 25-55^{o}C for storage up to 140 days. The rate of hexanal production was affected by time and storage duration. The results showed that deterioration was satisfactorily described by an Arrhenius-type temperature dependence model of coefficients. The activation energy for the hexanal level was found to be 55.9 kJ/mol over the temperature range of the study. A generalised model to describe the production of hexanal during storage of raw macadamia kernel as a function of temperature and time was established.

6.2 Introduction

Lipid oxidation is the main deteriorative affecting packaged macadamia. There are other potential causes such as microbial contamination, enzymic (e.g. lipase) and hydrolytic degradation but these are unlikely at kernel water activities around 0.3 that is associated with recommended kernel moisture contents of 1.5% w/w at ambient temperatures. Since oxidative processes of nuts proceeds slowly at room temperatures the evaluation of shelf life is time consuming and incompatible with industry needs. A frequently used technique to save time is accelerated shelf-life testing (ASLT). This can be achieved in several ways including having light, and raising temperature and moisture contents. Since raising temperature is the most critical of these factors it is usually chosen to accelerate the oxidation processes. To predict the rate of deterioration at a chosen temperature the reaction rate is determined over a range of temperatures that enable the response to be extrapolated by the application of the widely used Arrhenius equation.

The conditions that need to be met to enable successful prediction of shelf life using ASLT are:

1) Minimal deviation from the Arrhenius equation over the temperature range of interest

2) A relationship between the chemical index used and consumer acceptability.

In foods containing lipids both these criteria are dependent on the temperature range chosen as deviations of the oxidation rate can be expected if temperatures cover conditions where crystalisation (solidification) of the lipid are expected. Where this occurs oxidation at rates higher than predicted by the Arrhenius equation are observed.

The general form the Arrhenius equation to relate the temperature dependence to predict oxidation is as follows:

$k = k_0 * \exp(-E_a/RT)$

where

k is the reaction rate constant
R is the molar gas constant (8.314472 J K^{-1} mol^{-1})
T is the temperature in absolute units (^oK)
E_a is the activation energy J mol^{-1}
k_0 is the pre-exponential factor of the frequency factor

Lipid oxidation is known to produce a rage of off-flavoured volatile compounds that explains the consumer rejection of the product. The most appropriate tool to determine when a product has reached the end of its life would be a consumer panel. This is expensive and difficult to perform routinely because of the associated training, calibration and potentially multiple measurements during a product's shelf life. The alternative is to use a quality index that is related to sensory acceptability that can be measured using a quick and simple analytical method. The most frequently used method is peroxide value that quantifies hydroperoxides. However these are flavourless and the relevance of the measurement relies on the relationship of hydroperoxides acting as precursors for volatile secondary oxidation products such as hexanal. Both peroxide values and hexanal levels have been shown to be related to consumer perception of rancidity in macadamia (Chitundu, 1994; Himstedt, 2002). We have used hexanal because

1) The analysis is non-destructive and enables the tested product to be used for other tests
2) It avoids the oil extraction process that is time consuming.
3) Analysis using SPME-GC is objective, fast, repeatable, sensitive and accurate (Purcaro, 2008)
4) It is better correlated with consumer perception of rancidity than peroxide value in a range of food products including macadamia (Himstedt, 2002).

The aim of the present study was to model the temperature dependence of the oxidation rate of macadamia kernel to validate the use of accelerated shelf life testing. It will also contribute to the development of predictive shelf life tests for macadamia kernel products.

6.3 Material and methods

Fruit from cultivar A16 were harvested from the tree in Bundaberg (24^{o} 52' 19" S, 152^{o} 20' 43" E). Trees were7 years old and planted east-west at 7 x 4 m between and within row spacing. A bulk sample of fruit was collected from three blocks of trees and treated separately. Fruit were mechanically dehusked and stored at 4 ^{o}C. The fruit were placed into a heat pump drier (Australian Heat Pump Systems Pty Ltd, Queensland, Australia) at 30 ^{o}C and dried to three water activities (0.27. 0.37, 0.55) as determined on a water activity meter (AquaLab, Decagon Devices Inc. Washington, USA). The fruit shell was then hand-cracked and the kernel collected. A sample of 100 g kernel was placed into a 250 mL septum jar replicated three times. Six incubators were used with one at each of the following temperatures: 25 ^{o}C; 31 ^{o}C; 36 ^{o}C; 43 ^{o}C; 49 ^{o}C and 55 ^{o}C. The 3 water activities (a_w), 6 temperatures and 3 replicates meant that there were 72 jars in total. After 28, 56, 91 and 140 days of storage in the incubators, a 20 g sub-sample was removed from the septum jar and placed into a clean 250 mL septum jar and the hexanal content determined using SPME-GC.

6.4 Results

Hexanal production increased with increasing storage temperature and storage duration. Little or no change was detected in kernel stored at 25^{o}C while at the highest temperatures significant levels were detected after 56 days (Fig. 6.1)

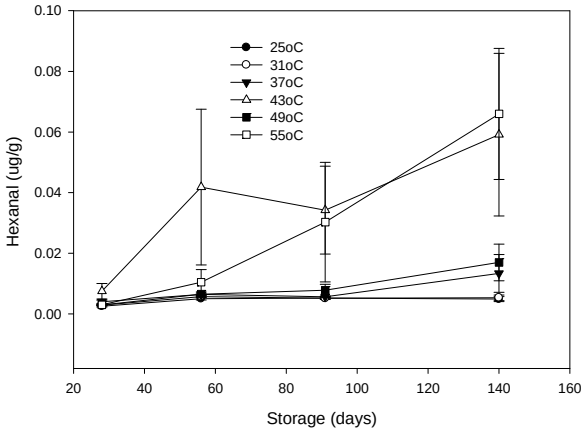

Figure 6.1 Mean hexanal level of A16 kernel stored for up to 140 days at constant temperatures from 25-55^{o}C

The amount (μg/g) of hexanal detected at each time point was of major interest. The natural log transform of hexanal concentration was approximately linear when plotted against time (days) although for temperatures 25 and 31^{o}C at the a_w 0.37 the first observation at 28 days did not observe the linear relationship on the log scale. The effect of this was to make estimated slopes for these two curves are too large. We therefore omitted the initial point for both these situations and refitted the lines to the remaining 3 points where we have now obtained slopes of 0.00457 and 0.00230 instead of 0.00879 and 0.00683 respectively.

Following the usually accepted method k_0 by taking logs of the original equation above, the parameter estimates from the plot of ln(k) versus 1/T were used to estimate the Arrhenius parameters $-E_a/R$ and k_0. This was done using the original estimated values as well as with the modified slope estimates for the 25 and 31 degree data. In both situations there was no significant difference between the three lines for the

different water activity values, so a single pooled line was fitted using data from the three water activities.

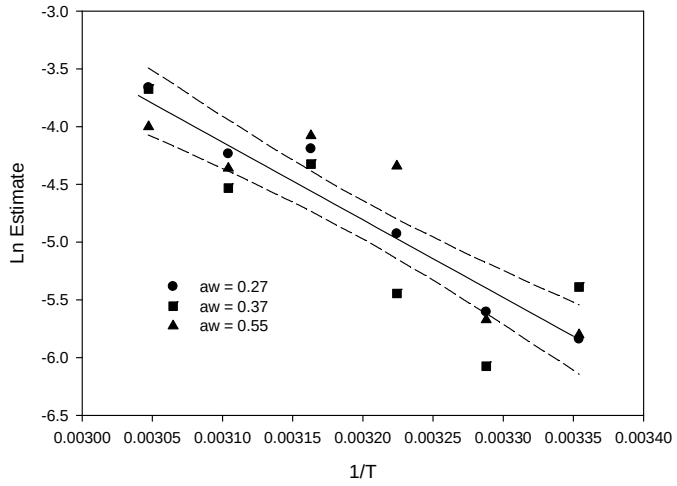

Figure 6.2. Fitted line based on the regression analysis of the ln(k) versus 1/T where T is the storage temperature (^{o}K)

For the original data the intercept was 13.53 (SE = 2.81) and the slope estimate was -5701 (SE = 879) with an adjusted R^2 of 0.707. When we adjusted the two values, as outlined above, we obtained an intercept of 16.71 (2.60) and a slope of -6724 (812) with an adjusted R^2 of 0.799 (Table 6.1). The parameter estimates, error terms and statistics are shown in Table 6.2 and the accumulated analysis of variance in Table 6.3.

Source	d.f.	s.s.	m.s.	v.r	F pr.
Regression	1	8.925	8.9250	68.62	<.001
Residual	16	2.081	0.1301		
Total	17	11.006	0.6474		

Table 6.1. Regression analysis of Log estimate using the fitted terms of the Arrhenius equation. Percentage variance accounted for 79.9 with the standard error of observations is estimated to be 0.361.

Parameter	estimate	s.e.	t(16)	t pr
Ln k_0	16.71	2.60	6.44	<0.001
-E_a/R	-6724.	812.	-8.28	<0.001

Table 6.2. Parameter estimates, error terms and statistical significance derived from the regression of Ln(k) versus 1/T

Change	d.f.	s.s.	m.s.	v.r.	F pr.
-Ea/R	1	8.9250	8.9250	68.62	<.001
Residual	16	2.0810	0.1301		
Total	17	11.0059	0.6474		

Table 6.3. Accumulated analysis of variance.

Since Lnk_0=16.71, then k_0=18074271, and $-E_a/R$ = -6724 where R (molar gas constant) then E_a = 55906.51

By substituting known terms with these values we can show that the Arrhenius equation for the rate constant can be expressed as:

k = 18074271 *exp (-55906.51/ 8.314472 (Temperature (^{o}C) + 273.15)

This is plotted in Fig 6.3 and representing the comparative rate of kernel deterioration at these temperatures.

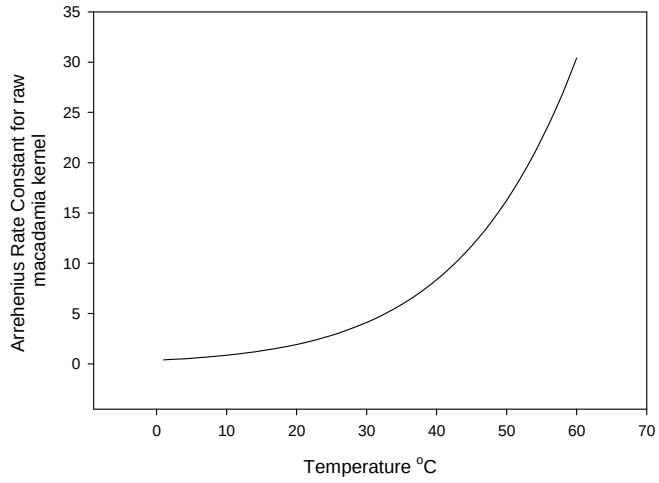

Figure 6.3. Plot of the Arrhenius rate constant developed for raw A16 kernel against storage temperature (^{o}C)

6.5 Discussion

These results provide the basis for estimating the rate of kernel deterioration using hexanal production for raw A16 kernel at 1.5% kernel moisture at a range of temperatures under atmospheric conditions. This could be used to predict shelf-life when combined with knowledge of the threshold for consumer acceptance of rancid kernel when stored in a similar manner. Our results in Chapter 5 would suggest that there would be little modification required for roasted kernel. However this relationship will change when kernel is packaged and stored in modified atmospheres

or the kernel used represent different styles such that the surface area to mass relationship is modified. The process followed here could be expanded to accommodate these requirements if required. The more immediate application for these results is to assist in the interpretation of results for kernel stored under accelerated aging conditions such as elevated temperature which is critical to the oxidative process. Storing macadamia kernel at temperatures of around 37-40^{o}C has been used to accelerate aging by Cavaletto et al., (1966), Himstedt (2002), Salter et al., (*Prep a*), Salter et al., (*Prep b*) and in Chapters 6 & 7. Assuming that the cultivars used behave in a similar manner to A16 the deterioration at 40^{o}C would be occurring at around 4.3 faster than at 20^{o}C.

Similar rate constants have been developed for macadamia kernel based on the peroxide values and free fatty acids generated after storage at temperatures of 30-45^{o}C for periods up to 56 days (Chitundu, 1994). She found that the variation observed in peroxide value was better explained by two regression lines. She described a linear relationship between 20 and 30^{o}C and exponential relationship for 40 to 45^{o}C. In comparison, there was a simple linear relationship between free fatty content across this temperature range. Her analyses were limited by the analytical tools but she concluded that her results, combined with sensory analysis, could be used to predict shelf-life of a range of products being developed by the macadamia industry. We could find no evidence that the macadamia industry has used rate constants to estimate the time that products stay within specification.

To use accelerated shelf-life testing in combination with a rate constant based on the Arrhenius equation to determine the time that a product will be within defined limits for use under the conditions in which the product is stored along with threshold for acceptance needs to be defined. While it would be preferable to use human based sensory panels for this purpose, this is not practical for commercial situations. Therefore chemical indices that are related to consumer acceptance have been developed. In macadamia good correlations have been found between peroxide value and levels of free fatty acids (Chitundu, 1994) but hexanal has been found to be better correlated with the perception of rancidity than either of these methods (Himstedt, 2002). However, as shown in Chapter 7 there was a 10 fold difference in the threshold

of acceptability for hexanal and peroxide values in macadamia. A parsimonious solution will be needed to adopt these findings commercially.

In developing shelf-life predictors of a lipid containing bakery product Calligaris et al. (2008) suggested a four step process. The first of these was to evaluate the physical properties of the fats involved. This investigation is based on the effects of low temperatures on the crystralisation of specific oil components. This crystalisation (solidification) increases the concentration of compounds such as unsaturated fatty triacylglcerols, metals and oxygen, involved in the oxidative process. Since reports of differential scanning calorimetry (Dyszel, 1990) indicate that macadamia oil is generally a liquid at temperatures above 10^{o}C these effects do not appear to be relevant to the results presented here. However, the effects of crystalisation are likely to cause curvature of the Arrehenius equation at low temperatures thereby raising the rate constant. This would result in over estimation of shelf-life of a product that is stored at temperatures at which crystalisation occurs, when using accelerated shelf-life testing at elevated temperatures. In simple terms this rate of deterioration at low temperatures maybe faster than we have predicted and further investigations will be required to determine whether a correction factor is required for kernel stored between zero and 25^{o}C. This effect also implies storing reference samples frozen as adopted in macadamia by Wall (2010) may modify the deteriorative behaviour of kernel. Despite these potential limitations there seems little option but to use kinetic models such as the Arrhenius equation based on chemical indices such as hexanal levels to predict shelf-life in macadamia.

6.6 Acknowledgements

We thank Hinkler Park Plantations, Bundaberg, for supplying nuts used in these investigations

6.7 References

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Chapter 7

Evaluation of accelerated shelf-life testing using hexanal to detect macadamia kernel with reduced oxidative stability from consignment samples

7.1 Abstract

There are well established processes for developing product shelf-life estimates based on consumer acceptance for oil rich foods but these have not been applied to macadamia kernel. Consequently there have been repeated reports of retail macadamia kernel failing to comply with specifications for rancidity. While many of the elements needed for shelf-life prediction in macadamia exist including chemical indices to monitor rancidity that are related to consumer perception of deterioration, kinetic models to describe deterioration and putative thresholds for consumer acceptance these require further validation and development to encompass the diversity of kernel quality, package and storage conditions is needed. In the interim it was proposed to determine the capacity of existing chemical indices and accelerated shelf-life testing protocols to segregate the least stable consignments at delivery for processing. This information will also provide information the variability of consignments and sampling needs. To do this the oxidative stability of premium and commercial grade kernel retained from the visual assessment testing of commercial nut-in-shell consignments of macadamias has been assessed. The samples came from 4 processors at monthly intervals from May to December 2009. These samples contained only kernel that was considered saleable, premium and commercial grade, and all reject kernel removed and recorded. In addition to these 200 g samples a

single 1 kg was requested for each month. The initial kernel moisture content was measured and reduced to below 2% (w/w) if required. Initial rancidity test were made extracted oil for peroxide value (PV), % free fatty acids (FFA), conjugated dienes (CDHP), and p–anisidine value (pAV). A single 50 g subsample was taken from the 200g sample and while 5 samples from the 1 kg samples. Repeated hexanal measures were made on the same jars after storage at accelerated shelf-life testing (ASLT) conditions of 0, 2, 4 and 6 weeks when the trial was terminated and kernel again tested with the same suite of rancidity tests. There was no correlation between visual quality assessments and any of the measures kernel rancidity before or after ASLT. Initial PV and FFA values were also only weekly related to final ASLT results indicating minimal potential to manage kernel quality. All other measures, CDHP and pAV were not or only weakly related the hexanal level at week 6. By comparison the correlation between hexanal at week 0 and hexanal at week 6 was $R^2 = 0.556$ and by week 2 this increased to significant to $R^2 = 0.921$, ($P < 0.016$). Kinetic models indicate that storing kernel for 6 weeks ASLT conditions was equivalent to 6 months at ambient (20^oC). Our results indicate that the response to 6 weeks ASLT can be predicted after 2 weeks and this may be reduced by optimizing the ASLT conditions. However the variability between subsamples within the 1 kg samples was so great that it was not possible to demonstrate any significant differences between consignments. Indicating that to implement the use of a chemical index of oxidative stability will require revision of sampling protocols and perhaps reduction in analytical costs and time.

7.2 Introduction

The concern that kernel rancidity has contributed to unsatisfactory market growth and reduced prices for macadamia was highlighted at the recent 4^{th} International macadamia conference (Kruger et al. 2009). The need to find cost-effective solutions

has become urgent with the Australian Macadamia Industry's 2009 strategic plan (http://www.horticulture.com.au/industries/Macadamia/default.asp April 2010) noting the need to set quality standards, improve definitions of product specifications and advise how these can be met through handling protocols. This all fitted within an objective to meet market and consumer demand through the value chain.

The requirement to meet consumer needs was anticipated as part of the Australian macadamia improvement program to ensure any new cultivar would be acceptable to the consumer (O'Riordan et al. 2005). Deliberately aged kernel, within current product specifications, were included in this investigation and they were identified by the Australian consumers as rancid. The disturbing consensus from this panel, selected because they were regular purchases of macadamias, was that these aged kernel were preferred to the available retail product.

The Australian macadamia industry has funded research into the problem of kernel quality for over twenty years. The early investigations focused on minimising kernel defects through scheduling harvesting (Mason and Wells, 1984) and improved on-farm drying (Kowitz and Mason, 2001). Moving through the value chain from orchard to processor the safe storage conditions were then investigated (Mason et al., 1998). However, instead of developing models incorporating the key drivers of kernel stability such as water activity, temperature and storage duration, factorial experiments were conducted to test industry nominated temperatures (Kowitz, pers comm.). Highly significant differences were found between the keeping properties of kernel under conditions such as 7.5% nut-in-shell moisture content at 5^{o}C and 25^{o}C but the fragmented conditions used did not enable the kinetic processes involved to be modelled. A similarly restricted approach was taken to examine the effects of kernel composition on keeping properties where it was assumed that total oil content would be associated with kernel stability (Himstedt, 2002). This study showed that the fatty acid profile was more important than the absolute oil content suggesting there would be no benefit from sorting kernel based on specific gravity. All these investigations were performed on raw kernel although separate oil roasting studies showed that this form of processing did not affect kernel keeping properties (Mason et al., 1995). These roasting studies cannot be directly related to current industry practice because most kernel is now dry roasted to a much lighter shade. A more general approach was

taken to develop standardised roast conditions that modelled kernel colour changes in response to oven temperature, air speed, kernel size and oven residence time (McConchie and Albertson, 2006).

Conducting storage trials has been time consuming as nuts were invariably harvested under ideal conditions and deterioration takes several months at ambient temperatures (Mason et al., 1998; Himstedt, 2002). Temperature is generally the most critical factor affecting the rate of oxidative rancidity and this parameter is frequently used to accelerate the reaction rate (Ragnarsson and Labuza, 1977). Storage of macadamia kernel at 30^{o}C was used by Fourie and Basson (1989) to show kernel became rancid within 2 months. The first description of the kinetics of kernel deterioration in macadamia were performed by Chitundu (1994) using storage temperatures from 30-45^{o}C and monitoring deterioration using peroxide values and percentage free fatty acids. She found that the rate of oxidative rancidity as measured by peroxide value increased in linear manner at temperatures from 30 to 35^{o}C but exponentially from 40 to 45^{o}C. In comparison the hydrolytic rancidity as measured by percentage free fatty acids showed a linear increase over this temperature range. Chitundu (1994) was also the first use head-space analysis to monitor rancidity in macadamia kernel but she did not extend her studies to investigate the kinetics of this process. Himstedt (2002) monitored kernel deterioration using peroxide values, free fatty acids and head-space analysis 37^{o}C. Use of elevated temperatures to accelerate deterioration combined with head-space analysis was validated by Salter et al. (*Prep a*) using temperatures from 25 to 55^{o}C and these results enabled the kinetics of these reactions to be described. Using 40^{o}C as the accelerating storage temperature it was shown that delaying harvest reduced kernel stability with therefore justified the use of ethephon to promote nut abscission (Salter et al. *Prep b*). Similar trials were also performed in the current project to determine the effects of pre-roast storage, moisture content and roasting conditions on kernel stability.

The commercial application of this work is to characterise the stability of the kernel to enable it to be directed to suitable products and provide information on the duration that the kernel will comply with the products specifications. This is a two step process

1) To describe the extent that kernel has progressed along the path to being rancid, shelf-life testing.

2) To predict the time the kernel will be within prescribed limits under defined conditions or shelf-life prediction.

The deterioration of high oil content tree nuts is thought to be largely due to oxidative rancidity. This is described as having three successive stages: initiation, propagation and termination (Shahidi, 2001). The first two stages are of greatest relevance to consumers since kernel in the termination stage would be inedible. The major difficulty in describing progress in the initiation stage is there are few chemical signals to indicate the rancidity process. Accelerated aging was therefore employed where raised storage temperature is used to speed up the rancidity development to the propagation stage when detectable changes can be measured. Knowledge of the kinetics of these processes enables the progression along the path to rancid to be described.

Shelf-life prediction uses similar information about the kinetics of deterioration under specific storage conditions to define the time that kernel will fit within specified limits. These limits in this context will be consumer acceptance. The predictions require knowledge of the quality of the kernel when incorporated into a product or when packed. This information is provided by a shelf-life test.

Studies of macadamia rancidity described above have used controlled conditions and precisely defined experimental material to reduce the confounding effects masking responses. There is a gap between this approach and applying the results to manage commercial kernel where knowledge of kernel properties at delivery is minimal.

The aim of the current investigation was determine whether the accelerated shelf-life testing protocol could be used to detect kernel with low oxidative stability in commercially sourced samples. By using commercially material it was intended to provide some insight into the feasibility of incorporating accelerated aging into the current kernel assessment procedures as a shelf-life test and quantify sources of variation. By comparing the visual assessments with measures of oxidative stability it

will be possible to determine the capacity of current grading systems to predict the stability of consignments of nuts.

7.3 Material and methods

Processors were requested to retain samples of 200 g kernel from 6 consignments and a single 1 kg sample of kernel every month from May till September 2009. The September samples were composed of consignments from August and September. Individual laboratory assessments of kernel quality for each consignment were made by the processors. The processors were requested to bias consignments toward poor quality as previous investigations with tree harvested nuts had indicated that no change in kernel properties would be expected within 6 weeks of accelerated shelf-life testing (ASLT) under recommended processing protocols. The kernel samples only contained sound premium and commercial kernel and no reject kernel. The categories for reject kernel included insect damage, mould, surface discolouration, discoloured crest (germination) and shrivelled (immature) as described by Evans and Hoffman (2005). Under this system reject kernel is only placed in a single category and the weight of each category of reject kernel is recorded as a proportion of the total reject kernel. All collaborating processor laboratories were accredited through the Australian Macadamia Society Ltd co-ordinated scheme. After laboratory assessment the kernel was sealed in foil laminated pouches and stored at 4^{o}C-10^{o}C until transfer to CSIRO Plant Industry's Brisbane laboratories where they were stored at 3.5^{o}C until processed.

Moisture contents of the laboratory samples were very variable and deviated considerably from recommended 1.5% (ww). When these exceeded 2% (ww) they were dried in a heat pump drier at 30^{o}C and 30% relative humidity. The dried moisture contents and those already below 2% were recorded.

The samples were allowed to come to ambient temperature (approximately 23^{o}C) before use. Kernel moisture content was measured gravimetrically in a fan forced oven at 105^{o}C for 24 hours.

Approximately 40 g of kernel was then placed in a 250 mL TraceClean Tall Wide-Mouth Jar (VWR Scientific Products, PA, USA) and the hexanal concentration in the head space of the jar was determined as previously described (chapter 6). These jars containing kernel were then placed in the dark in an incubator with a set temperature of 40^{o}C and hexanal was determined again at 2 weeks, 4 weeks and 6 weeks. Following the completion of hexanal measurements after 56 days of ASLT, oil was pressed from the kernel and was measured for peroxide value, free fatty acid content, conjugated diene content and p-anisidine value using the methods previously described. These rancidity measurements for day 0 were performed on an oil sample pressed from a subsample which had been stored at 3.5^{o}C.

The bulk 1 kg samples were treated as for the 200g samples except five subsamples were used from each 1 kg sample.

Statistical analysis

All analyses have been carried out using GenStat. An exponential regression model relating hexanal at week 6 to earlier hexanal values and other measures of kernel quality were fitted to the 1 kg data. These models also included processor as a factor to assess whether there were any processor differences in this relationship.

Repeated measures analyses were carried out using residual maximum likelihood (REML), to assess how the hexanal readings over time interacted with processor and the collection month for the kernel. Although the numbers were small processors and sampling month were also regarded as random effects and REML was used to estimate these components of variance, using the data from each of the 4 hexanal readings in turn.

7.4 Results

Combined 200g and 1kg samples

There was significant correlation between hexanal levels from the commencement of the accelerated shelf-life tests Week 0 through to Week 6. This relationship improved with the time in ASLT conditions as would be expected (Table 7.1). This is diagrammatically shown in Figures 7.1. The fitted exponential equations for the

relationships for hexanal concentration/level and PV between week 6 (Hex-6) and week 0 (Hex- 0), week 4 (Hex-4), initial PV (PV-0) and PV at week 6 (PV-6) are shown in Table 7.2. There were significant differences between processors but these differences were not consistent across time points or analyses. There was a linear relationship between Hex-6 and FFA-6 and again significant differences were observed between the processes that supplied the kernel (Table 7.3). The diagrammatic representation of the relationship of Hex-6 with PV-6 and FFA-6 is shown in Figures 7.2 a & b.

Week	6	4	2
4	0.961	-	
2	0.877	0.965	-
0	0.708	0.738	0.708

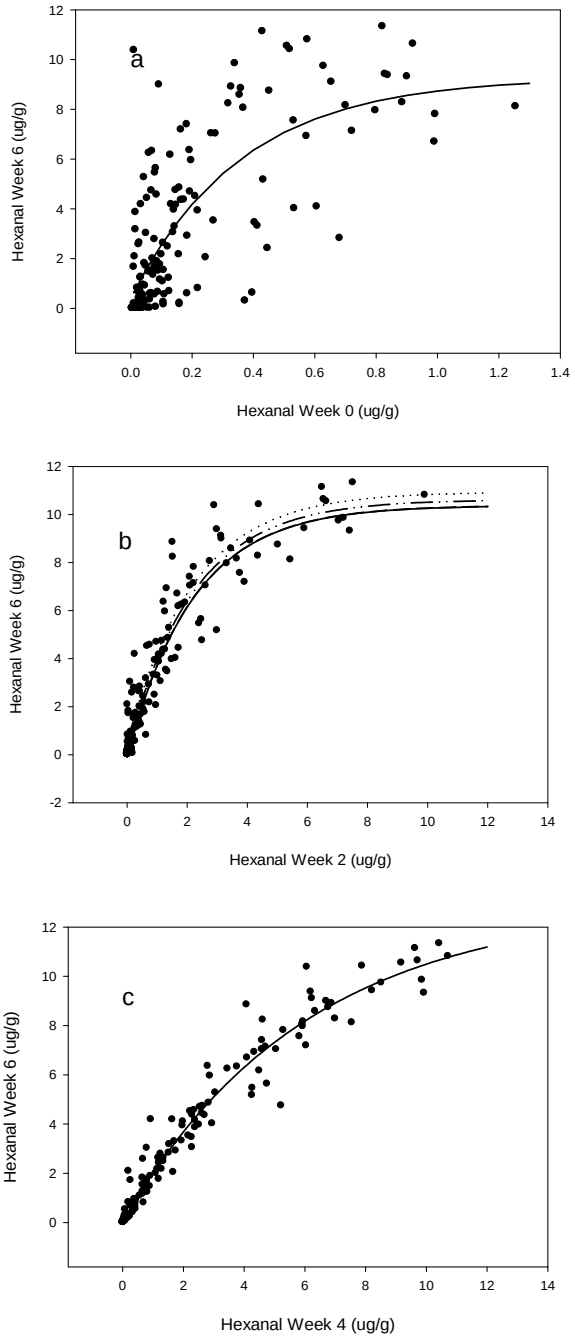
Table 7.1. Correlation matrix of all hexanal level measure in weeks 0, 2, 4, 6

While there was a weak but significant relationship between Hex-0 and PV-0 and FFA-0 (Table 7.2), there was a far better correlation between Hex-0 with PV-6 and FFA-6 than either PV-0 with PV-6 or FFA-0 with FFA-6 (Table 7.5). This indicates that neither PV-0 nor FFA-0 had strong predictive power to identify consignments that would traditionally be considered rancid based on PV or FFA measures. Consistent with this conclusion neither PV-0 nor FFA-0 were strongly correlated Hex-6. The correlation of hexanal level with PV-6 and FFA-6 improved with time during the accelerated aging. It was reassuring that after 6 weeks of ASLT there was a strong reciprocal relationship between Hex-6, PV-6 and FFA-6 confirming that deterioration monitored by hexanal is related to traditional measures of rancidity but hexanal was much more sensitive at predicting the end point.

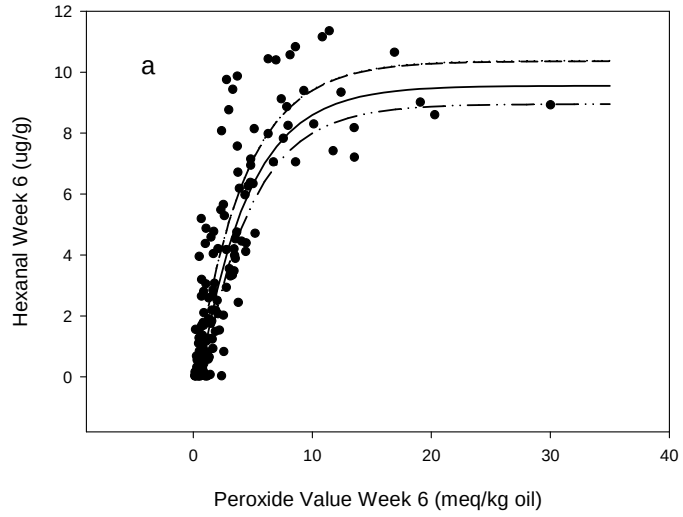
There no relationship or only a very weak relationship between Hex-6 and the measures of CDHP or pAV (Table 7.4). The correlations did improve marginally with measures taken in week 6.

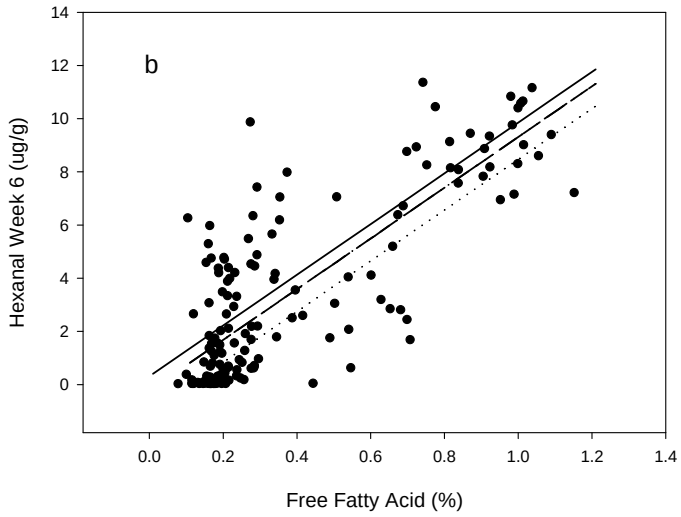
There was no strong relationship with any of the chemical analyses for rancidity and the visual assessment of kernel quality (Table 7.5). The visual assessment characters included reject kernel as a proportion of total kernel, commercial kernel as a proportion of total kernel or reject plus commercial as a proportion of total kernel. Visual representations of these results are shown in Figures 7.3. Since there was no

relationship between reject kernel and chemical assessment of rancidity none of the individual defects such as internal discolouration, insect damage showed any relationship. .



Figures 7.1 a-c. Scatter plots of hexanal levels at a) week 0, b) week 2 and c) week 4 versus week 6. Fitted lines are described in Table 7.2.





Figures 7.2 a & b. Scatter plots of hexanal levels at Week 6 against a) peroxide value at week 6 and b) free fatty acids at week 6. Fitted lines are described in Table 7.2 and 7.3.

Rancidity Measure	Week	Processor	Probability of Processor differences	A (SE)	B (SE)	C (SE)	R^2
Hexanal	0	All equivalent	NS	9.29 (1.08)	-8.895 (0.973)	0.0621 (0.0481)	0.556
Hexanal	2	A	P < 0.016	10.37 (0.199)	-10.48 (0.359)	0.6355 (0.0236)	0.921
		B		10.94 (0.392)			
		C		10.38 (0.202)			
		D		10.62 (0.259)			
Hexanal	4	All equivalent	NS	12.945 (0.615)	-12.912 (0.587)	0.847 (0.0118)	0.965
Peroxide Value	0	All equivalent	NS	8.04 (1.72)	-8.37 (0.587)	0.391 (0.164)	0.282
Peroxide Value	6	A	P < 0.001`	9.556 (0.315)	-10.93 (0.499)	0.7841 (0.0234)	0.804
		B		10.38 (0.565)			
		C		10.36 (0.316)			
		D		8.953 (0.409)			

Table 7.2. Summary of relationships between Hex-6 (hexanal level at 6 weeks) and earlier hexanal measures and peroxide values (PV). The exponential model of the form Hex6 = A + B * (C^x), where x is the explanatory variable Hex-0, Hex-2, Hex-4, PV-0, PV-6.

Rancidity Measure	Week	Processor	Probability of Processor differences	Slope (SE)	Constant (SE)	R^2
Free Fatty Acid	6	A	P=0.014	0.317 (0.360)	9.533 (0.574)	0.627
		B		-1.068 (0.404)		
		C		-0.227 (0.350)		
		D		-0.226 (0.488)		

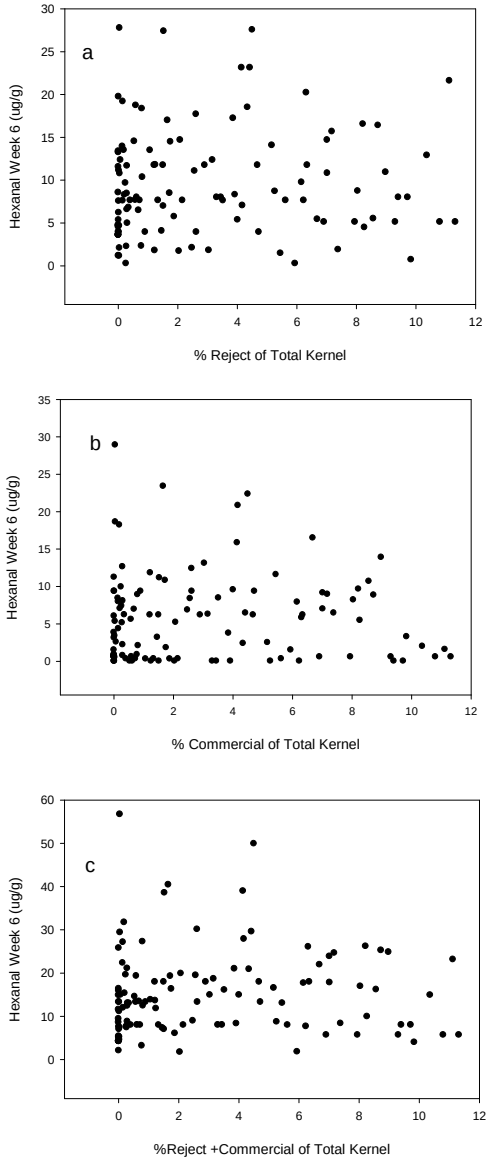
Table 7.3. Summary of relationship between Hex6 (hexanal level at 6 weeks) and free fatty acid at week 6. The linear model of the form Hex6 = A + B x, where x is the FFA-6

Analysis	Hex-6	FFA - 0	FFA-6	CDHP-0	CDHP-6	pAV-0	pAV-6	PV-0
FFA- 0	0.276	-						
FFA-6	0.793	0.417	-					
CDHP-0	-0.036	-0.139	-0.088	-				
CDHP-6	0.239	0.019	0.201	0.594	-			
pAV-0	-0.049	-0.029	-0.157	0.296	0.065	-		
pAV-6	0.169	-0.104	0.061	0.371	0.280	0.439	-	
PV - 0	0.483	0.086	0.353	0.179	0.394	0.109	0.203	-
PV - 6	0.739	0.166	0.670	-0.030	0.374	0.559	0.225	0.559

Table 7.4. Correlation matrix between all chemical analyses for weeks 0 and 6.

Analysis	R
Hexanal Week 6	0.066
Free Fatty Acid Week 6	0.097
Peroxide Value Week 6	0.023
Conjugated Dienes Week 6	0.083
p-Anisidine Value Week 6	0.381

Table 7.5. Correlations between the percentages of total kernel rejected by visual assessment with all chemical analyses made at week 6.



Figures 7.3 a-c. Scatter plots of hexanal level at Week 6 versus a) % Reject, b) % Commercial and c) % Reject + Commercial as a percentage of Total Kernel Recovery

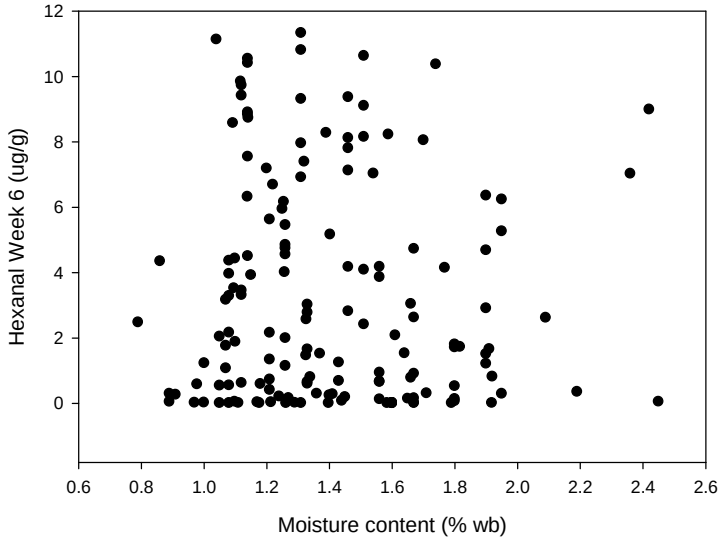


Figure 7.4. Moisture content week 0 plotted against hexanal measured at week 6 after accelerated shelf-life testing

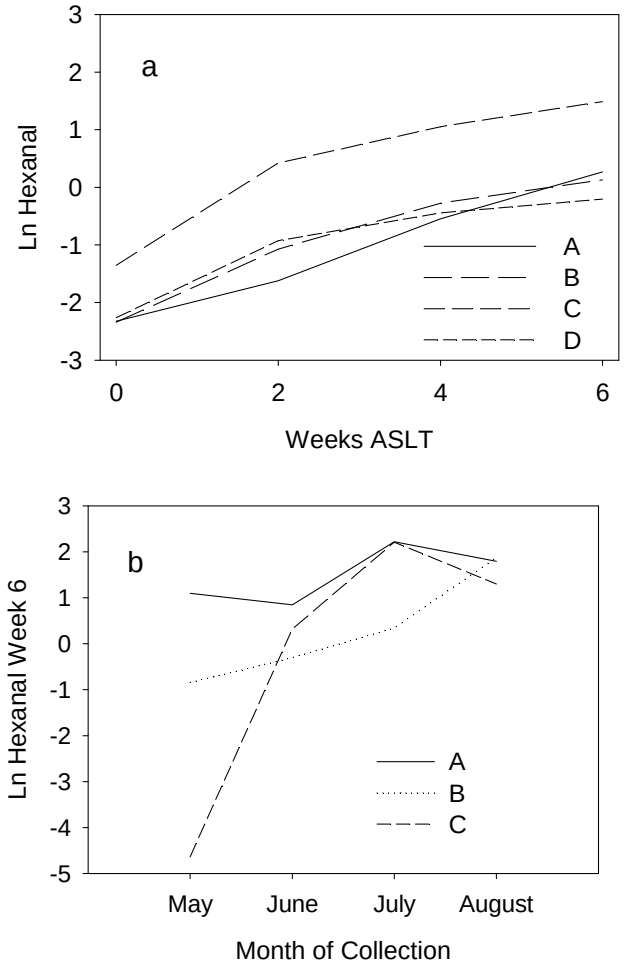
1kg samples only

Results for the 5 subsamples taken from the monthly 1kg samples were analysed separately. The response of individual 40g samples to accelerated aging varied considerably within the 1 kg samples so that no significant differences were detected between the mean performances of the 1kg samples. The variance components were estimated using the untransformed and transformed data for PV at week 0 and week 6 and hexanal measurements at weeks 0, 2, 4 and 6. The data for the two sample sizes were analysed and the results for the log transformed data are summarised in the following (Table 7.6). The results for the transformed and untransformed data were essentially the same. The sample within processor within month component dominates in all situations. There does seem to be a significant component due to month for the 1kg samples but not for the 200g samples. The estimated variance component for processor is effectively 0 for all variables and sample sizes.

Over the 4 months that these samples were collected there was a reduction in the stability of the kernel as the season progressed based on final level of Hex-6 (Fig 7.5 a). There was also a significant difference between the performance of the individual processors with one of the processors differing significantly from the other three (Fig 7.5b).

Kernel source						
200g				1kg		
Analysis	**Random term**	**component**	**s.e.**	**Random term**	**component**	**s.e.**
PV-0	Processor	0.0591	0.0824	Processor	0.0515	0.177
	Processor.Month	0.0573	0.0599	Processor.Month	0.3862	0.2321
	Processor.Month.Sample	0.454*	0.0765	Processor.Month.Sample	0.669*	0.1252
PV-6	Processor	0.164	0.174	Processor	0.0838	0.3177
	Processor.Month	-0.038	0.066	Processor.Month	0.8812*	0.4261
	Processor.Month.Sample	1.073*	0.18	Processor.Month.Sample	0.383*	0.0718
Hex-0	Processor	-0.106	0.063	Processor	-0.3257	0.3609
	Processor.Month	0.357	0.235	Processor.Month	2.2664*	1.0149
	Processor.Month.Sample	1.048*	0.177	Processor.Month.Sample	0.228*	0.0426
Hex-2	Processor	0.06	0.176	Processor	0.058	0.868
	Processor.Month	-0.154	0.237	Processor.Month	2.904*	1.392
	Processor.Month.Sample	3.941*	0.66	Processor.Month.Sample	1.151*	0.216
Hex-4	Processor	0.127	0.276	Processor	-0.19	0.724
	Processor.Month	-0.109	0.312	Processor.Month	3.103*	1.471
	Processor.Month.Sample	4.673*	0.784	Processor.Month.Sample	1.113*	0.208
Hex-6	Processor	0.254	0.409	Processor	-0.211	0.704
	Processor.Month	-0.077	0.354	Processor.Month	3.046*	1.464
	Processor.Month.Sample	5.041*	0.846	Processor.Month.Sample	1.329*	0.249

Table 7.6. Variance components log transformed data for peroxide and hexanal analyses of the 200g and 1kg. * indicates significant T statistics



Figures 7.5 a & b. **a)** Mean hexanal 6 level for monthly samples from processors A, B, C, and D. ($p<0.005$) **b)** Log of the mean hexanal level from the 5 subsamples taken from the 1kg for the four processors A, B, and C ($p<0.005$)

It was also possible to segregate the analysed samples into levels of oxidative stability based on their hexanal level or peroxide value after 6 weeks ASLT. Assuming that the threshold for consumer acceptance for hexanal is 2ug/g and peroxide value is 3 meq/kg then 50% of sample exceeded this hexanal level and 30% exceed the peroxide level.

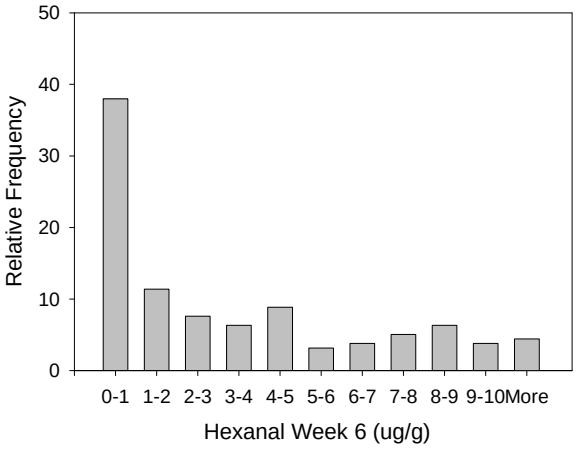


Fig 7.6. Relative frequency of hexanal levels within the 157 samples after ASLT

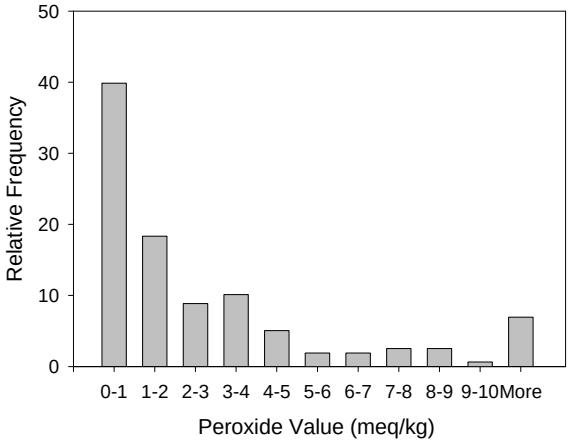


Fig 7.7 Relative frequency of peroxide values within the 157 samples after ASLT

7.5 Discussion

These results demonstrate that commercially sourced kernel could be segregated into different levels of oxidative stability using an accelerated shelf-life testing (ASLT). The major difference from previous investigations using kernel sourced directly from orchards and processed under controlled conditions was that 30-50% of commercial samples, if using peroxide value or hexanal level respectively, were outside product specifications. Where as is little or no change has been observed in orchard sourced material. By setting hexanal levels at 6 week of ASLT as the measure of kernel stability it was found that this level could be accurately predicted after 2 weeks of accelerated ageing. It was also found that visual kernel quality assessment had no capacity to predict any of the chemical measures of rancidity after accelerated aging.

The response of subsamples of kernel from the same consignment to ASLT showed large variation indicating that revised sampling protocols would be required to fully characterise the stability of commercial laboratory samples. However these results did indicate a reduction in shelf-life as the harvest season progressed and significant differences between the processors who supplied the kernel.

These investigations utilised analytical methods for hexanal analysis previously developed by Salter et al. (*Prep a*) and fitted the Arrehenius equation to predict deterioration of kernel stored at temperatures from 25 - 55^{o}C. This equation is frequently used to describe the exponential deterioration in food products and takes the form:

$k = Ae^{-(EA/RT)}$

Where,
k = rate constant
E_A = activation energy = kJmol^{-1}
A= pre-exponential factor
R = gas constant = 8.31 JK^{-1}mol $^{-1}$
T = degrees kelvin

The results from Salter et al. (*Prep a)* predicted that kernel stored at 40^{o}C would deteriorate approximately 4.3 times faster than kernel stored at 20^{o}C. Therefore storing kernel for 6 weeks (42 days) at 40^{o}C was equivalent to just under 6 months storage at 20^{o}C. Hexanal measurements taken on day 0 were far better correlated with hexanal measurements made after 6 weeks of ASLT than any other chemical or visual assessment of quality made at this time. This correlation improved dramatically with 2 weeks of storage at 40^{o}C indicating that deterioration of kernel over 6 months at 20^{o}C can be accurately predicted in as little as 2 weeks.

In designing the ASLT conditions a conservative incubation temperature of 40^{o}C was chosen as macadamia kernel with elevated water activity darkened at higher temperatures suggesting that some form of non-enzymic browning was occurring without kernel being roasted (Salter et al. *Prep a*). It also matched the highest

temperatures used for accelerated shelf-life testing in other tree nuts such hazelnuts (Pastorelli et al. 2007) and Pistachio (Raei et al. 2010). The pattern of volatile production indicative of oxidative rancidity was consistent from 25^{o}C through to temperatures exceeding 50^{o}C in kernel tested at processing water activities comparable to 1.5% kernel moisture content (Salter et al. *Prep a*). This suggests that ASLT could be conducted at higher temperatures so that the kernel would deteriorate at many times the rate observed at 20^{o}C which would further shorten the duration needed to quantify the stability of the kernel. The technical limitation to the aging temperature is when the reactions that occur at higher temperatures are not observed at lower temperatures. For example it has been reported that high temperatures favour the production of 2,4-decadienal over hexanal from fatty acids such as linoleic acid (Frankel 1982). In almond it is recommended accelerating temperature should be below 43^{o}C (Harris et al., 1972).

Results for CDHP and pAV analyses provided little additional information but correlations with PV-6, FAA-6 and Hex-6 did improve after 6 weeks of ASLT indicating there was a relationship to a reduction in kernel quality. These analyses were conducted on crude oil that had been pressed, filtered and centrifuged in a process that took 10-15 minutes alone. It is possible that a more refined extraction process such as soxhlet or accelerated solvent extraction (Albertson et al. 2005; Albertson et al. 2006) would improve the purity of the oil and eliminate confounding compounds. Without these refinements these analyses have no role in monitoring kernel quality.

There was no relationship between the moisture content and kernel stability. Many of the low moisture content kernels used in our current trials were below the industry recommended 1.5% wb. It has been suggested that oxidative stability of products with high oil content is reduced at low moisture contents (water activities) (Labuza 1979) that may occur through over drying. Growers are warned against over drying macadamia nut-in-shell by Bungay (2003) however there is no quantitative data provided to support this conclusion. Work by Cavaletto et al., (1966) has shown kernel stored at 37^{o}C with a moisture content of 4.3% had reduced shelf life compared to kernel with a moisture content of 2.3 and 1.4% stored at the same temperature. The kernel at 4.3% had a free fatty acid value (FFA) of 4% after 16 months storage yet

was rejected by a sensory panel after 2 months suggesting FFA may not have been the appropriate chemical indice of kernel quality. More recently, Dominguez et al (2007) suggested that the zone on minimum entropy and therefore greatest stability for storage of raw kernel was between water activities of 0.36 (1.1 % ww) and 0.44 (1.57% ww). However the kernel used in their investigations had peroxide values in excess of 20 meq O_2/kg nut after 2 weeks storage indicating the kernel was possibly compromised before their experiments commenced and, therefore, should not be the basis for developing industry storage conditions.

The relevance of this investigation is dependent on the relationship between the measured hexanal levels and the level of rancidity of macadamia kernel. Hexanal measurement is not part of any macadamia quality assessment and there would need to be a major change in the analytical assessment methods used in the Australian industry for these results to be adopted. Currently the Australian macadamia industry relies on peroxide values (<3meq/kg oil) and levels of free fatty acids (<0.5%) and kernel not having any off-odours to detect rancidity. These values were set by consensus amongst producers and match the SAMAC export standards (Crous, 2010) used for packaged macadamia. These chemical analyses require the extraction of oil and the hydroporoxides measured by the peroxide value is inherently unstable since they are rapidly converted to aldehydes, alcohols, ketone that are detected by analyses such as para-anisidine, conjugated dienes and head space analysis. Hydroporoxides are actually flavourless and it is the association with these secondary reactions on which peroxide analysis relies upon to act as an indicator of rancidity (Shahidi, 2001). The palette can detect hexanal concentrations as low as 0.06 ug/g (Shahidi, 2001; Himstedt, 2002).

Hexanal production is widely used as an indicator of oxidative rancidity in a range of products including nuts. It has also been found to be well correlated with consumer perceptions of rancidity in a range of products (Purcaro et al., 2008) and invariably out performs peroxide value. The reported threshold for hexanal content for consumer rejection of kernel in tree nuts varies considerably from 1-2 ug/g through to 34 ug/g (Table 7.7). The process of using sensory assessment to nominate when kernel is unacceptable varies between these studies but commonly when a product achieves 5 on scale of 10, or 50 on a scale 100 it is considered as unacceptable. The definitive

work by Mason et al., (1998) for setting safe storage temperatures and moisture contents for macadamia NIS found that overall acceptability was around 50 or lower for all NIS stored at 25^{o}C and then roasted except for NIS at 3.5% for pre-roast storage up to 4 months or 7.5% for pre-roast storage up 2 months. However this only applied to roasted kernel had 0 months post roast storage. Only NIS with 3.5% with no pre roast storage scored was well above 50 after 4 months storage. These changes were only statistically significant when sensory scores approached 40. By fitting a linear regression to the overall acceptability and peroxide value data of Mason et al., (1998) the threshold value for an acceptable peroxide value in macadamia kernel would appear to be is less than 3meq/kg oil (Fig 7.8). Based on this interpretation the only 3.5% NIS moisture content produced roasted kernel acceptable kernel after storage

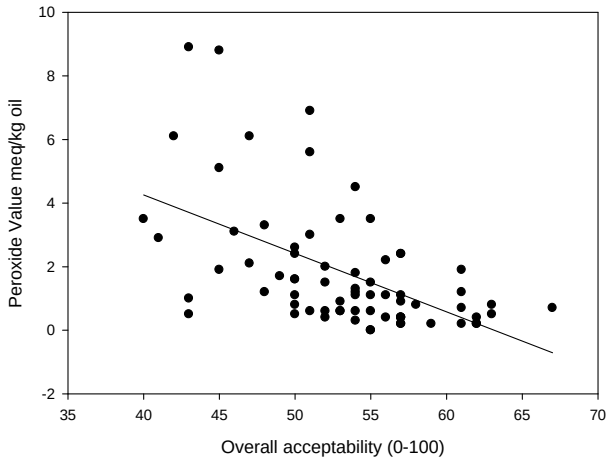


Figure 7.8. Peroxide value and overall acceptability of NIS stored at 3.5, 7.5 and 10% NIS moisture stored at 5 and 25^{o}C content then roasted and stored at 25^{o}C for 0-8 months from Mason et al., (1998).

Our results from previous the sensory analyses of O'Riordan et al. (2005) and comparison with peroxide values and hexanal levels of our current investigations suggested a rancidity threshold of 1-2 ug/g for hexanal and 2-3 meq/kg for PV. This indicates that many of these estimates in literature are an order of magnitude too high. It is interesting to note that a study on hexanal in almond (Mexis et al., 2009a) reported a hexanal level of 14.4 ug/g for kernel rejection while a year later this level had fallen to 1-2 ug/g. Thresholds for hexanal for rejection in the order of 1-2 ug/g are consistent with levels for bakery ingredients however it should be noted the

precise level does vary with the product being investigated (Purcaro et al. 2008). To date, the Australian macadamia industry has not been able to obtain reliable sensory measurements (hedonic and diagnostic) that could be linked to processing and consumer perception of kernel quality.

Tree Nut	**Hexanal mg/kg**	**Peroxide Value meqO_2/kg oil**	**Authors**
Almond	14.4	2.46	Mexis et al., 2009a
	1-2	10	Mexis et al., 2010
Cashew	10.4	1.2	Mexis and Kontominas 2009a
Hazelnut	3.45	1.38	Mexis and Kontominas 2009b
Macadamia	0.393	0.367	Chitundu 1994
	Slightly 11-30 Moderate 30-50 Very >50	Slightly, 2.5-7.5 Moderate, 7.5-12.5 Very >12.5	Himstedt 2002
	n/a	<5	O'Riordan et al., 2004
	n/a	10	Kruger et al., 2009
Walnut	34.5	2.6	Mexis and Kontominas 2009c

Table 7.7. Published threshold values for hexanal levels (ug/g) and peroxide value meq/kg oil for kernel rejection in different tree nuts

At the time when CSIRO co-supervisors suggested Himstedt (2002) use hexanal levels to monitor macadamia rancidity over a decade ago this method was not widespread but head-space analysis had been used to detect rancidity in hazelnuts (Kinderlerer and Johnson, 1992), macadamia (Chitundu, 1992) and walnuts (Forbus et al. 1980, Mate et al. 1996). This early macadamia research was only semi-quantitative as it relied on a purge and capture method analysed by gas chromatography and mass spectroscopy (Chitundu ,1994; Himstedt, 2002). Salter et al. (*Prep a*) developed protocols to use solid phase micro-extraction with gas-chromatography (GC-SPME) to make quantitative measures of hexanal for macadamia. This protocol was then used

to demonstrate a reduction in macadamia kernel stability with retention of the crop on the tree or on the ground (Salter et al. *Prep b*). All these applications were based on raw kernel and in the current project this method has been used to investigate the effects of roasting on kernel stability (McConchie et al., *Prep b*). In parallel with these investigations there has been widespread adoption of hexanal assessment of rancidity in meats (Goodridge et al., 2003; Iglesias and Medina, 2008), bakery products (Purcaro et al., 2008), snack foods such a crisps (Goode and Soutar 1995; Lennersten and Lingnert 1998; Grosso and Resurreccion, 2002; Sanches-Silva et al., 2004; Kaykhaii and Rahmani, 2007; Grosso et al., 2008) and most tree nuts (Table 7.7). This led Purcaro et al,. (2008) to conclude "hexanal can be a realistic index of oxidation process as perceived by consumers. Furthermore, the SPME method set-up is fast, sensitive, repeatable and easy to use for quality control in any bakery food company." Examples of the use of hexanal to measure kernel stability in tree nuts include: almond (Mexis et al., 2009a, Mexis et al., 2009b), Brazil nut (Goode and Soutar, 1995), cashew (Sharma et al., 2000; Mexis and Kontominas, 2009b, hazelnut (Kinderlerer and Johnson 1992; Perren and Escher 1999; Pastorelli et al., 2006; Pastorelli et al., 2007; Mexis and Kontominas 2009a), macadamia (Chitundu 1994, Himstedt 2002, Salter et al., *Prep a;* Salter et al., *Prep b;* McConchie et al., *Prep b*), pecan (Erickson et al., 1994; Baldwin and Wood, 2006), walnut (Forbus et al., 1980, Mate et al., 1996, Jensen et al., 2001, Mexis and Kontominas 2009c).

The better correlation between hexanal levels at week 0 and hexanal at week 6 compared with any other chemical measures at week 0 could be in part due to the repeated measures used for hexanal whereas the other chemical analyses were made on separate subsamples. This was necessary as the other chemical analyses require oil extraction destroying the kernel for subsequent use. It would have been possible to measure hexanal levels on these samples prior to oil extraction to avoid this problem but still would have not have been directly related to Week 6 hexanal measurements.

The performance of the five subsamples from a single monthly 1kg varied considerably. This could be partly explained by the sample variability due to some processors combining multiple samples from several consignments to making up the 1kg. Any commercial assessment method needs to be able to handle high levels of variability as consignment may have nuts from different cultivars, harvests or regions.

Designing a sampling system to handle this variability requires knowledge of the sources of variation and is a major research undertaking. These issues are related to current visual assessment system for grower payment where it was concluded sampling needed to be increased 4-20 fold depending on the level of consistency required (Mengerson, 2000). The difference is rancidity has no visual cues so cannot be rectified on the sorting tables during processing.

The lack of correlation between visual kernel assessments and week 6 hexanal levels or any other chemical analysis after ASLT indicates that it would not be possible to determine the keeping properties of these consignments using the industry's current inspection methods. In hindsight this may have been anticipated as the Australian macadamia industry has had difficulty in providing kernel with reliable keeping characteristics. It also follows that while the co-operating processors were requested to bias their samples towards poor quality they had no capacity to do so. This suggests that the kernel samples used in this study were representative of a random selection of the 2009 Australian macadamia crop.

The poor correlation of the chemical measures of rancidity with the level of kernel defects may reflect the inadequacy of the current industry system of visual kernel assessment (Evans and Hoffman, 2005). The visual assessment process is confounded as kernels are only considered to have a single defect when multiple defects are present so that potentially no category of defect is accurately measured. While this system may serve the purpose of valuing consignments at delivery it would appear inadequate to link any kernel defect to kernel stability. This should not have affected the correlation with the absolute level of reject kernel and measures of rancidity at week 6 after ASLT. However, most forms of discolouration such as discoloured crest, internal discolouration and some forms of insect damage will be affected by the temperature at which the kernel has been dried. More recently McConchie and Macpherson (2008) and McConchie and Forrester (*Prep*) have shown that slight kernel colour changes will occur in damaged kernel under these conditions but to get full expression of damage requires the kernel to be exposed to temperatures between 80^{o}C and 110^{o}C. This is below the temperature required to get colour change in sound kernel for commercial dry roast durations of up to 1 hour for sound kernel (McConchie, 2005). Since the kernel supplied by the processors for our trial was dried

at temperatures below 60^{o}C discolouration defects may have remained concealed that could have affected the relationship between visual assessment and response to ASLT.

It is possible that these laboratory samples are not representative of the stability of an entire consignment due to damage that occurs during preparation for assessment. The current industry recommendation to dry nut-in-shell step-wise for 2 days at 35, 45, 55^{o}C is based on the early work of Prichuvardi and Yamamoto (1965). Since no test for shelf-life for macadamias existed then, it is not certain that this drying regime does not affect kernel stability. While there are industry recommended temperatures for drying nut-in-shell there is no recommended moisture content that the kernel must achieve before an incremental temperature increase. Should kernel have elevated moisture due to lack of air flow or over stocking in the oven then damage may occur (McConchie, 2006). The significant differences between the performance of the kernels from the processors may be due to differences laboratory protocols. Greater understanding on the effects of kernel preparation on its subsequent stability may be required to prevent artefacts in the assessments. Non-invasive techniques such as near infra-red spectroscopy as described by McConchie (2006) that do not require high temperatures to reveal defects could be considered.

Under ASLT condition the majority of the processor supplied samples were rancid within 2 weeks. This indicates that under ambient conditions they would been rancid within 2 months. This is much faster than any macadamia kernel previously reported by Mason et al. (1998) , Wooton (2001), Himstedt (2002), Salter et al., (*Prep a*), Salter et al., (*Prep b*) and even less stable than those reported by Lemmer and Kruger (2000a & b) and Kruger et al., (2009) for South African kernel. Assuming that it is not the preparation of the kernel samples in the processors' laboratory that had caused this deterioration, then there is an obvious need to work back through the production systems on-farm to detect the cause. It would seem unlikely that controlling the causes of visual defects alone will solve this problem.

ASLT only quantifies the progression along the path to rancidity and it needs to be combined with knowledge on storage conditions and levels of consumer acceptance to determine shelf-life. This process will also be altered by the conditions that the kernel

is held. Early work on packaging materials for macadamia focused on preventing physical damage to kernel and quantifying the effects moisture transmission (Cavaletto and Yamamoto 1968). They concluded that provided water vapour transmission rates were low, acceptable quality could be maintained for at least 6-7 months. More recent research on other tree nuts with high level of unsaturated fatty acids similar to macadamia has focused on oxidative rancidity (Table 7.8). There have been numerous studies examining the effects different packaging materials, oxygen scavengers, modified atmosphere and light and temperature on the keeping properties tree nut kernel in various forms from flour to intact nuts (Table 7.8)

Tree nut	**Packaging material**	**Modified atmosphere**		**Storage condition**		**Authors**
		Gases	**Oxygen scavenger**	**Light**	**Temperature**	
Almond	*					Severini et al., (2003)
		*			*	Garcia-Pascual et al., (2003)
	*	*		*	*	Mexis et al., (2009a)
	*	*	*	*	*	Mexis and Kontominas (2010)
Brazil nut	*	*	*			Ribero et al., (1993)
Cashew	*					Lima et al., (1998)
	*					Lima and Borges (2004)
Hazelnut			*			Pastorelli et al., (2006; 2007)
Macadamia	*					Cavaletto and Yamamoto 1968
Pecan	*	*				Dull and Kays (1988)
	*	*				Oro et al. (2008)
Pistachio		*			*	Maskan and Karatas (1998)
		*			*	Maskan and Karatas (1999)
	*	*			*	Raei et al., (2010)
Walnut	*	*	*		*	Jensen et al., (2003)
		*	*			Mate et al., (1996)
	*				*	Vanhanen and Savage (2006)
	*	*		*	*	Mexis et al., (2009c)

Table 7.8. Published investigations examining the stability of packaged tree nuts summarising the variables examined

Almost invariably shelf-life is improved by reducing oxygen levels either by use of oxygen scavengers or gas flushing in combination with a packaging film that has low oxygen and moisture transmission and prevents light access to the kernel. Among the few exceptions are for walnut where is it reported that excessive oxygen reduction can lead to fermentation of the kernel (Dull and Kays 1988). It should be noted that some oxygen absorbers have been reported to have a secondary benefit in reducing hexanal levels during storage (Pastorelli et al. 2006; Pastorelli et al., 2007). The benefit of the barrier films and modified atmospheres to the keeping properties of each of the tree nuts differs because of the kernel fatty acid composition and level of antioxidants (Himstedt, 2002). The most notable is almond where nuts have been stored as nut-in-shell in ambient conditions with no measurable deterioration for 12 months (Senesi et al., 1996; Garcia-Pascual et al., 2003). Kernel deterioration occurs more rapidly once shelled and blanched but almonds have high levels of the anti-oxidant α-tocopherol that is metabolised as the kernel ages (Garcia-Pascual et al., 2003). By comparison levels of α-tocopherol in macadamia are minuscule (0.03-0.07mg $100g^{-1}$ oil) compared to other tree nuts (almond 33.8-40.5 mg $100g^{-1}$ oil) but do diminish with storage duration (Lin et al., 2001). Macadamias are also known to contain tocotrienols (Kaijser et al., 2000) and the phytosterol, β-siterol (Maguire et al., 2004). Other phytochemical with known anti-oxidant activity such as squalene, a phytosterol precursor, are also reported in macadamia kernel but no relationship between the level of these anti-oxidants and oxidative stability of kernel has been demonstrated (Wall, 2010). Even using a film with the appropriate barrier properties does not necessarily ensure a fresh product to the consumer as the sealant, the machinery doing the sealing and blends or style of nuts can affect packaging integrity with failure leading to elevated oxygen levels (Goode and Soutar, 1995).

In this investigation the level of variation in kernel stability within samples prevented the precise description of the stability of a consignment. These measures included the current chemical analyses for rancidity. There was also no relationship between the visual assessment and kernel stability indicting there is currently no capacity to manage kernel storage characteristics or shelf-life. Since the storage characteristics of macadamia kernel are effectively concealed, a chemical or spectral analysis is required to measure kernel stability. Our results, combined with previous studies of

Salter et al., (*Prep a*) and Salter et al., (*Prep b*), indicate that ASLT with head-space analysis can effectively predict deterioration that would expected over nearly 6 months in as little 2 weeks. Additional investigation may enable this period to be further reduced as no measures prior to 14 days were made. There is also potential to increase the storage temperature to accelerate deterioration.

While use of GC-SPME is very accurate it is also time consuming and costly to setup and maintain. This could be prohibitive if it was found that sampling to predict the quality of a product has to occur at several stages during processing as is the case for the removal of visual defects. The cost of analyses could be reduced and the measurement could be performed more rapidly using other technologies that could be built on the GC-SPME technologies in combination with accelerated shelf-life testing. This may include near infra red spectroscopy as reported in almonds (Jensen et al., 2001) or an electronic nose for hazelnut (Pastorelli et al., 2007).

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