# Horticulture Innovation Australia

**Final Report** 

# Developing a commercial shelf life test for macadamias

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Project Number: MC12002

#### MC12002

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## Summary

This project builds on the results from MC10008 to produce protocols for a standard objective test of macadamia kernel rancidity development.

The aims of the project were:

- Develop protocols for a rapid and accurate commercial test for measuring the potential shelf life of macadamias (an objective test for kernel quality).
- Demonstrate that the rapid aging process correlates with natural long-term ageing.
- Propose a hexanal concentration that correlates with consumer perceptions of stale and rancid kernel.

A commercially viable, cost effective, rapid test has been developed that allows the macadamia industry to assess the shelf life and quality of raw and roasted macadamia kernel.

A prototype of a new electronic nose instrument, OdourScan®, has been developed by Next Instruments Pty Ltd, and assessed during these experiments. The OdourScan has proved to be an efficient machine able to measure hexanal concentrations in the headspace of packaged macadamia nuts.

Protocols have been developed that will enable a cost effective commercial test for shelf life. Using the equipment and methodology developed in these experiments a prediction for the shelf life can be obtained within seven to 14 days.

The results demonstrate that rapid ageing coupled with measuring hexanal concentration in the headspace has potential as a predictive tool for macadamia shelf life.

The protocols developed in this project are simple, fast and economical and show real promise for incorporation into existing industry quality assessment procedures.

Each test can be completed in under 15 minutes. The cost of the e-nose and simplicity of equipment and test procedures mean that processing companies can now use rapid ageing and hexanal concentrations as part of their quality control program.

The benefits from a cost effective and reliable shelf life test span all sectors of the industry:

Test results can be used:

- To educate farmers, and other and handlers, through the supply chain of the impact on kernel quality and shelf life from poor handling practices
- To monitor and measure quality decline through the supply chain
- To quickly and objectively identify product on supermarket shelves that are stale or rancid even though they may not be past their "Best by Date"
- To assess the potential shelf life of new cultivars being developed in the industry breeding project
- To aid re-packers to set realistic and meaningful "Best by Dates"
- To rapidly assess the effect on storage stability of different packaging materials

Using the methodology and equipment developed in this project it is proposed that a preliminary threshold level of 100µg/g be set as the concentration that approximates a peroxide value (PV) of 3meq/kg. Further analyses of hexanal and peroxide values are required as there was considerable variation in results around the limits of PV 3meq/kg and 100µg/g hexanal. Peroxide value not exceeding 3meq/kg is currently the Australian macadamia industry's minimum standard guideline for premium kernel (AMS 2008).

It is recommended that in order to establish a stronger correlation between hexanal and peroxide values further analyses are required.

The methodology described has been shown to have application for determining immediate kernel quality and potential shelf life. Industry confidence will only be achieved through repeated use and continual reporting of results.

A small continuing project involving cooperation and participation of macadamia processors could act as an extension phase to gain industry adoption of this technology.

# Keywords

Macadamia shelf Life; hexanal; Electronic Nose; rancidity

# Introduction

A predictive test for macadamia kernel shelf life has been a long-term goal for the macadamia industry. Research conducted by CSIRO developed a test that could predict the shelf life using rapid aging to speed up the accumulation of hexanal in the headspace of containers of macadamias. The hexanal concentration was measured using solid-phase micro extraction and gas chromatography (SPME-GC) (McChonchie et al 2010). They also developed an Arrhenius equation to describe the kinetics of kernel deterioration at different temperatures for cultivar A16. By knowing the Arrhenius constant it becomes possible to determine the time that a product will remain within defined limits under specified storage conditions, and hence the ability to apply a meaningful "use by" or "best by" date.

Research by Mason et al (2003) showed that hexanal was a more reliable measure of rancidity than peroxide value. Their results showed that peroxide values of 3.0-5.0meq/kg was approximately equal to hexanal concentrations of between 15-20µg/g. Mason et al (2003) utilised a sensory panel in their research and comparing the sensory with hexanal concentration and peroxide values concluded that rancidity becomes detectable at a hexanal concentration between 10-25µg/g and objectionable at levels between 25- 45µg/g.

Measuring the hexanal concentration in the headspace is only one step in developing a meaningful shelf life test. Further trials need to be done on different cultivars to check that the Arrhenius equation developed by McConchie et al (2010) holds across varieties. Also further work needs to be done on shortening the rapid aging phase to make the test a more commercially viable prospect. Mason et al (2003) used accelerated ageing at 37.5°C for up to 28 weeks. Long-term storage trials to simulate product being stored in retail shops or consumer cupboards need to be conducted and compared with rapid aged product to test the validity of the predicted "use by" or "best by" dates.

Previous research has been done using glass jars. Flexible pouches are an attractive

alternative, being more in line with traditional storage and packaging, as well as being more cost effective. It is possible that flexible pouches could be used with the E-nose provided the pouches do not give off any volatiles that interfere with the hexanal measurements.

The HAL funded project, "Developing a commercial test for macadamia shelf life; Proof of Concept" MC10008 clearly demonstrated that the E-nose, OdourScan®, was capable of discriminating between batches of kernel in varying stages of oxidation. Data obtained by SPME-GC and the E-nose showed a good correlation for batches of kernel that had been treated to give varying shelf life durations. The results demonstrate that E nose could be developed as a fast economical test.

Electronic noses require minimal sample preparation and can objectively, safely and quickly characterise the global taste and odour profiles of ingredients or formulations. It is for this reason that E nose is now widely used in major food and drink companies to measure and monitor product quality.

# Methodology

#### Experiment 1: Optimising the rapid aging process.

Temperatures of 45°C, 55°C and 65°C were trialled with the aim of reducing the time it takes to demonstrate an increase in the headspace concentration of hexanal. Four varieties were included in the trial including; A16, Daddow, 849 and 344. Macadamia nut in shell was purchased directly from growers in August 2012. The nuts were dried to 1.5% kernel moisture and stored at 15°C until the OdourScan® was received in late October. As well as the four single cultivars tested, a commercial batch of raw kernel was purchased from a local processor and added to the experimental treatments.

50 grams of raw kernel (5 reps for each treatment) was sealed in 250 ml or 375ml glass jars and placed in incubators at 45°C, 55°C and 65°C.

The concentration of hexanal in the headspace of each jar was measured each week until the hexanal concentration had exceeded 50µg/g.

#### Results:

The OdourScan® has proved very sensitive and able to measure hexanal concentrations over a much broader range than was expected. Initially there was some time spent on understanding the machine and to establish reliable calibration curves. The sensitivity of the machine is set near its maximum resolution which has meant that in these experiments the headspace concentration of hexanal rapidly exceeded the limits of the instrument in the 65°C treatments.

Figures 1.1 to 1.5 below show the correlation of the hexanal concentration with temperature and length of time for accelerated ageing. In all cases, except the commercial batch, the headspace concentration of the 65°C treatment exceeded 70µg/g within 14 days. Also all cultivars at all temperatures demonstrated a measurable and significant increase in hexanal concentrations within seven days. The only exception to this was the commercial batch of raw kernel. This batch of kernel, which obviously has a longer shelf life, showed a slight but non-significant rise at 45°C, however the 55°C and 65°C treatments were both significant (Fig 1.5).

Table 1.1 shows the time taken for each treatment to reach the theoretical threshold  $50\mu g/g$  hexanal in the headspace. The time to reach  $50\mu g/g$  was reduced by approximately half with each ten degree increase in incubation temperature. Based on the Arrhenius rate constant developed by McConchie et al (2010) the deterioration rate of macadamias at  $45^{\circ}$ C,  $55^{\circ}$ C and  $65^{\circ}$ C will respectively occur at a rate 6, 11 and 22 times faster than at 20°C. Using the Arrhenius equation (Figure 1.6) the predicted time it will take the kernel to reach  $50\mu g/g$  hexanal has been calculated for long-term storage for each variety at 20°C in ambient air. The predicted time ranged from 132 days to 220 days for the four cultivars while the commercial batch had a longer predicted time of 341 to 360 days (Table 1.1).

Note, initially it was expected that 50µg/g of hexanal was the end point for consumer acceptance and hence the shelf life cut off. This was based on research by Mason et al where they concluded that hexanal concentrations of 25-45µg/g would be unacceptable for consumers. After further experiments using the OdourScan® and methodology developed in this project it is recommended that this threshold is revised

upwards to about 100µg/g. This will be discussed further in context of the long-term ageing trial.



Figure 1.1 Effect of temperature on accelerated ageing of raw macadamia kernel, cultivar Daddow. Note: Maximum concentration recorded was  $70\mu g/g$ . By day 14 all reps at 65°C had exceeded 70  $\mu g/g$  and 3 of 5 reps at 55 C had exceeded 70  $\mu g/g$ .



Figure 1.2 Effect of temperature on accelerated ageing of raw macadamia kernel, cultivar 344. Note: Maximum concentration recorded was 70µg/g. By day 14 all reps at 65°C had exceeded 70µg/g.



Figure 1.3 Effect of temperature on accelerated ageing of raw macadamia kernel, cultivar 849. Note: Maximum concentration recorded was  $70\mu g/g$ . By day 14 all reps at  $65^{\circ}$ C had exceeded  $70\mu g/g$ .



Figure 1.4 Effect of temperature on accelerated ageing of raw macadamia kernel, cultivar A16. Note: Maximum concentration recorded was 70µg/g. By day 14 all reps at 65°C had exceeded 70µg/g.



Figure 1.5 Effect of temperature on accelerated ageing of a commercial batch of style 4 raw macadamia kernel.

Time to reach 50µg/g hexanal in accelerated aged raw kernel					
		Days	s to reach 50µ	g/g	
Temperature	Daddow	344	849	A16	Commercial
65	7	9	8	8	16
55	14	20	20	20	31
45	22	34	35	35	60
	Predi	cted time to re	each 50µg/g at	20°C	
65	154	198	176	176	352
55	154	220	220	220	341
45	132	204	210	210	360

Table 1.1 Days of accelerated ageing to reach the theoretical threshold of 50  $\mu$ g/g hexanal with the predicted time it would take at 20°C, calculated using the Arrhenius equation (Fig 1.6).

#### **Discussion:**

A useful generalization supported by the Arrhenius equation is that, for many common chemical reactions at room temperature, the reaction rate doubles for every 10°C increase in temperature. The results obtained for all four cultivars and the commercial batch of kernel that was tested in this trial reflect this generalization. Table one shows that for each 10°C increase in the incubation temperature the time for each treatment to reach 50µg/g was approximately halved.

The four cultivars use in this trial were collected directly from farms in late August. The fact that the nuts were collected during the latter part of the season, after an extended wet period, meant that the shelf life was probably reduced. The results bear this out when compared to the commercial batch. This commercial batch was harvested in the early to mid season, was vacuum packed in October 2012 and had a best by date of October 2013.

The predicted time to reach 50µg/g for each cultivar was remarkably constant across all temperatures, indicating that all temperatures would be suitable for accelerated aging. The benefit of the higher temperatures is that the result is obtained faster. Product that has a short shelf life can be determined within seven days, even at 45°C.



Figure 1.1 Arrhenius equation for raw A16 macadamia kernel as developed by McConchie et al (2010)

# Experiment 2. Confirming that roasted kernel ages in the same fashion as raw kernel.

The methodology used in experiment one was repeated using dry roasted kernel in experiment two. Kernel from the same varieties and batches as used in experiment one were oven roasted at 135°C for 20 minutes. The kernel was allowed to cool then 50 grams of kernel was placed in 375ml jars, sealed and placed in incubators at 45°C, 55°C and 65°C (5 reps of each). The concentration of hexanal in the headspace was measured using an OdourScan® on a weekly basis.

#### **Results:**

Figures 2.1 to 2.4 show the correlation of the hexanal concentration in the headspace with temperature and length of time of accelerated ageing for dry roasted macadamia kernel. All cultivars at all temperatures demonstrated a measurable and significant increase in hexanal concentrations within seven days.

The trends and correlations were very similar to the results from the raw kernel used in experiment one, suggesting that roasted kernel ages in a similar fashion to raw kernel.

Table 2.1 shows the time taken for each roasted kernel treatment to reach the theoretical threshold of  $50\mu g/g$  hexanal in the headspace. The time to reach  $50\mu g/g$  was reduced by approximately half with each ten degree increase in incubation temperature, with the exception of 344 in the 65°C and 55°C treatments. In this instance it took only 6 days at 65°C and 21 days at 55°C.

While there are some differences between the raw and roasted kernel they are generally within expectation given normal variation of a natural product. The predicted time to reach 50µg/g for roasted Daddow kernel, 132 to 244 days, (Table 2.1), is very similar to that for raw Daddow kernel, 132 to 254 days, (Table 1.1). Similarly for 344 the predicted range is 154 to 246 for roasted kernel and raw 198 to 204 for raw. For 849 roasted kernel the range is 154 to 204 compared to the raw kernel which is 176 to 220 and finally for A16 roasted kernel, the range is 154 to 168 which is a bit lower than the range for the A16 raw kernel of 176 to 220 (Table 2.1).







Figure 2.2 Effect of temperature on accelerated ageing of dry roasted macadamia kernel, cultivar 344. Note: Maximum concentration recorded was  $70\mu g/g$ . By day 14 all reps at  $65^{\circ}$ C and  $55^{\circ}$ C had exceeded 70  $\mu g/g$ .



Figure 2.3 Effect of temperature on accelerated ageing of dry roasted macadamia kernel, cultivar 849.



Figure 2.4 . Effect of temperature on accelerated ageing of dry roasted macadamia kernel, cultivar A16.

Time to reach 50µg/g hexanal in accelerated aged roasted kernel						
	Days to 50µg/g					
Temperature	Daddow	344	849	A16		
65	6	7	7	7		
55	12	21	18	18		
45	24	41	34	28		
Predicted time to reach 50µg/g at 20°C						
65	132	154	154	154		
55	132	231	198	198		
45	144	246	204	168		

Table 2.1 Days of accelerated ageing to reach the theoretical threshold of 50  $\mu$ g/g hexanal with the predicted time it would take at 20°C, calculated using the Arrhenius equation (Fig 1.6)

#### Experiment 3. Comparison of container and headspace volumes.

The purpose of this experiment was to test the variation between different containers including glass jars, flexible foil pouches, and plastic bags with varying amounts of kernel and volumes of headspace.

Test one; 20mls of a standard solution containing 10µg/g hexanal was placed into either 500g foil pouches or 375ml glass jars. The pouches and jars were then sealed and incubated at three different temperatures, 45°C, 55°C and 65°C for seven days. The concentration of hexanal in the headspace of each treatment was measured using an OdourScan®.

Test two; varying amounts of rancid kernel was placed in different containers and sealed with a range of headspace volumes. Treatments included:

- 1. 50g kernel sealed in 375ml glass jar
- 2 150 g kernel sealed in 375ml glass jar
- 3 150g kernel sealed in a plastic bag with small headspace

- 4 150g kernel sealed in a plastic bag with large headspace
- 5 50g kernel sealed in foil pouch with small headspace
- 6 100g kernel sealed in foil pouch with small headspace
- 7 100g kernel sealed in foil pouch with large headspace.

#### **Results:**

There was no significant difference between the headspace hexanal concentration between the foil pouches and glass jars (Fig 3.1). Significantly the variation in headspace volume made no difference to the concentration measured by the OdourScan®. The ease of use was much greater with the glass jar than the foil pouches. The glass jars were faster and easier to fill, were faster to test and faster to reseal after measuring the headspace. This trial demonstrates that it is feasible to use foil pouches in a test to predict the shelf life of macadamia but as a routine test glass jars are much more efficient.



Figure 3.1 Comparison of 500g foil pouch versus 375 ml glass jar as a vessel for rapid ageing macadamia kernel



Figure 3.2 Comparing different volumes of headspace with varying volumes of kernel and different packaging. Results show that headspace and kernel volume do not impact  $\mu$ g/g concentration.

Figure 3.2 shows no significant difference between packaging type, headspace volume or kernel volume. This result means that retail packs can be immediately assessed without needing to be treated in any way. The headspace can simply be sampled by the OdourScan® and an almost instant result achieved. Commercial samples can be rapid aged in their original packaging enabling residual shelf life to be estimated. It also demonstrates the applicability of rapid ageing and headspace analysis as an efficient method to assess different packaging materials.

#### **General Discussion:**

The main purpose of the first stage of this project was to establish protocols for a rapid and accurate commercial test to measure the potential shelf life of macadamias.

The protocol for accelerated ageing trials has now been refined as follows:

- Use 375ml glass jars with a septum in the lid for sampling.
- Place approximately 50g of macadamia kernel in each glass jar and seal.
- Place the glass jar in an incubator at elevated temperature for 7 to 14 days at 45°C or 55°C.
- To assess the hexanal concentration remove the sample jar from the incubator and allow it to cool to room temperature.

- Measure the headspace in the jar using 40ml sample extracted and assessed by the OdourScan®
- Sample each jar twice and average the two readings.
- If the jar needs to be replaced in the incubator the syringe hole can be sealed with cello tape.
- A second reading can be taken after at least 4 hours to allow the headspace to equilibrate.
- Check the calibration with a standard solution at least every two hours

#### Experiment 4. Compare long-term ageing with rapid ageing

Experiment 4 is a long-term ageing treatment at room temperature. The aim is to confirm that rapid ageing correlates with normally aged product. Raw kernel of four cultivars was stored at room temperature in glass jars containing ambient air. There was no active control over the temperature of the room.

The concentration of the hexanal in the headspace was monitored each month.

#### Results

Figures 4.1 to 4.5 show the accumulation of hexanal in the headspace for the four different macadmia cultivars and the commercial kernel during long-term ageing at room temperature. The coefficients of determination ( $R^2$  values) range from a low of 0.76 for 849 to a high of 0.91 for 344 and Daddow. This gives us a high degree of confidence in the prediction time to reach our theoretical threshold level of 50µg/g Hexanal.



Figure 3.1 Hexanal concentration in the headspace of 344 aged at room temperature.



Figure 4.2 Hexanal concentration in the headspace of 849 aged at room temperature.



Figure 4.3 Hexanal concentration in the headspace of A16 aged at room temperature.









Table 4.1 shows the predicted time to reach  $50\mu g/g$  hexanal for each of the four cultivars and the commercial kernel. As a general comment the long-term ageing process seems to be predicting a longer shelf life than the rapid aged product. This is likely to be a result of the samples being aged in a room with no temperature control. With the fluctuating temperature from day to night over the recent cooler months it is likely that the average temperature is less than  $20^{\circ}$ C which is the theoretical temperature used to predict the end point. The important result from this experiment is that the order of the prediction for the five samples has not changed. The commercial sample remains the sample with the longest predicted shelf life. The predicted time to reach  $50\mu g/g$  from the original rapid ageing experiment for the commercial kernel was 351 days. The predicted time to reach  $50\mu g/g$  after 140 days of ageing at room temperature is 490 days based from the first day of ageing.

344 had the second longest predicted time from rapid ageing at 207 days to reach 50µg/g and after room temperature ageing for 140 days a predicted time of 323 days.

Daddow had the shortest predicted time after the rapid ageing experiment of 147 days and after ageing for 140 days at room temperature a prediction of 167 days. The fact that the order of the four cultivars remains constant between the rapid ageing and the long-term ageing is an indication that the Arrhenius equation remains relavent for the four cultivars used in these trials.

#### Combining rapid ageing with long-term ageing

After 140 days of ageing at room temperature a sample of the kernel was removed and rapid aged at 45°C for seven days (Table 4.1, Long-term + rapid 45°C). The predicted time to reach  $50\mu g/g$  after this rapid ageing phase still holds the same order as with all other experiments. 344 has the longest predicted shelf life and Daddow the shortest (Table 4.1).

Predicted time to reach 50µg/g hexanal						
Cultivar	344	A16	849	Daddow	Commercial	
Long-term ageing at room temperature	323	205	225	167	490	
Average rapid ageing	207	202	202	147	351	
Long-term plus rapid ageing	253	220	180			

Table 4.1 Comparison of the predicted shelf life of long-term aged product with rapid ageing.

While there is some difference in predicted shelf life between each of these trials the critical result is that in every experiment the prediction for Daddow is significantly shorter than the other three cultivars. This is a clear indication that macadamia kernel with a reduced shelf life can be identified through the protocols defined in this project. Determining the precise length of the shelf life requires further development.

Figure 4.6 shows the hexanal concentration of the four macadamia cultivars after 140 days ageing at room temperature followed by seven days rapid ageing at 45°C. Once again it can be seen that the order of hexanal concentration, and hence predicted shelf life corresponds with both the rapid ageing and long-term treatments. 344 has the

lowest concentration of hexanal and therefor longest shelf life while Daddow has the highest concentration of hexanal and hence the shortest shelf life. It is clear that the Daddow kernel that was supplied for this project has a significantly shorter shelf life than the other three cultivars. This does not mean that cultivar Daddow has shorter shelf life than other macadamia cultivars, only that the sample of Daddow kernel supplied for this research had a shorter shelf life than the other samples.





Figures 4.7- 4.10 show the accumulation of hexanal for individual samples of the different cultivars aged at room temperature for 140 days. Cultivar 344 seems to have a lag phase over the first 40 days before hexanal concentration begins to increase. This induction phase occurs because the kernel is protected by anti-oxidants. Once the anti-oxidants are metabolised the development of rancidity will accelerate. We see that this lag phase is longer and more pronounced in 344 than either A16 or Daddow. After 36 days aging at room temperature the hexanal concentration for 344 remains below  $2\mu g/g$  (Fig 4.8) while both A16 and Daddow are at about  $4\mu g/g$  (Figs 4.9 and 4.10 respectively)



Figure 4.7 Accumulation of hexanal in individual samples of raw 344 kernel aged at room temperature



Figure 4.8 Accumulation of hexanal in individual samples of raw A16 kernel aged at room temperature



Figure 4.9 Accumulation of hexanal in individual samples of raw Daddow kernel aged at room temperature

Figure 4.11 shows a comparison between assessing dried NIS (Nut-In-Shell) or dried kernel. NIS was dried to 3.5% moisture and stored at room temperature for three months. After three months storage half the NIS sample was removed and cracked and the kernel sorted. Approximately 50g of kernel was sealed in 375ml glass jars. The remaining dry NIS was also sealed in 375ml jars. Both the NIS samples and the kernel samples were rapid aged at 45° for seven days. The concentration of hexanal in the headspace was then measured using the OdourScan®. A second batch of NIS was stored wet in plastic bags at room temperature for three months. The plastic bags had small holes punched in them to allow air movement but to restrict moisture loss. After three months half the wet NIS was cracked and the kernel removed. Wet NIS samples and wet kernel samples from the same batch of nuts was then placed into glass jars, sealed and rapid aged for seven days at 45°C. In all cases the NIS had a lower concentration of hexanal then the kernel samples. The wet samples, both NIS and kernel had significantly higher hexanal concentrations than the dry samples (Fig 4.11). The results from this trial show that it is not possible to gain an accurate picture

of the state of the kernel, in terms of hexanal production, unless the kernel is removed from the shell.

#### **Commercial Testing**

The eventual outcome for this project will be a test that can be used in commercial laboratories to test kernel quality on delivery or for monitoring kernel quality within the warehouse and through the value chain. With this in mind some preliminary measurements were conducted on commercial laboratory kernel recovery samples from the 2013 season. Two samples of NIS that had been submitted for a standard kernel recovery test were selected for this first look at how a commercial test might be used. The two samples were obviously poor quality with high levels of reject kernel. Sample number 100755 was delivered to the laboratory for analysis on 8/2/2013. It appeared to contain nuts from the previous season and it had a high level (18.3%) of reject kernel, mostly due to mould (10.2%). Sample number 1308H was delivered on 29/5/2013 and had 9.9% reject kernel, mostly due to internal discolouration (8.2%).

Following the standard laboratory kernel assessment test additional NIS from the retention sample was cracked and placed into 375ml jars and sealed. The jars were left on the laboratory bench for two hours to allow the headspace to equilibrate. Two reps of unsorted kernel from sample  $\mu$ 755 were tested. With sample 1308H the kernel was separated into premium and reject and each grade was tested separately. The two reps of sample 100755 had high levels of hexanal 40.3 $\mu$ g/g and 42.2 $\mu$ g/g respectively (Table 4.2). The premium kernel from sample 1308H had lower levels at 7.1 $\mu$ g/g hexanal and the reject kernel, with a high percentage of internal discolouration, had high levels at 39.4 $\mu$ g/g (Table 4.2). The results from this preliminary trial demonstrate that it is possible to incorporate hexanal headspace analysis into the current industry quality assessments and that the results could be reported back to the grower at the same time that the kernel recovery results are provided.



Figure 4.9 This shows that NIS doesn't compare with kernel once it starts to age. The NIS always has a lower hexanal value than the kernel. Also note when NIS is stored wet the hexanal concentration is rapidly increases to very high levels.

Testing commercial deliveries				
Sample No.	Hexanal (µg/g)			
10075A (high levels of mould)	40.3 µg/g			
10075B (high levels of mould)	42.2 μg/g			
1308H Premium kernel	7.1 μg/g			
1308H Reject kernel (high % internal discolouration)	39.4 µg/g			

Table 4.2 Using the OdourScan® to test kernel quality of commercial deliveries

#### Discussion

These results clearly demonstrate the potential of using the OdourScan<sup>®</sup> to assess hexanal concentrations in the headspace of jars containing macadamia kernel. Protocols for rapid ageing have been developed that are simple and repeatable.

The preliminary tests conducted on the commercial deliveries demonstrate that this test could be incorporated into current industry quality tests with very little effort.

#### Long-term storage

Figure 4.12 shows the progression of the long-term storage kernels over a 20month period at a constant 25°C. Hexanal increased to an average  $30\mu g/g$  in 250 days and to 55  $\mu g/g$  after 600days. Samples were removed at each sampling point vacuumed packed and cool stored for further analysis.

Four batches of kernel that were used in the marketing consumer testing which had four different PV levels were assessed for hexanal concentration. The kernel had been provided by processors from stored commercial kernel. Each batch had a different PV level, 1.6, 2.2, 4 and 4.6meq/kg. Three reps were taken from each batch and assessed for hexanal. Figure 4.13 shows a strong correlation between PV and hexanal concentrations with  $R^2$  value of 0.85. It is worth noting that there is a range of hexanal concentrations for each PV. For example kernel with a PV of 2.2meq/kg had a hexanal range from 17 to 42µg/g and kernel with a PV of 4.6meq/kg had a hexanal range from 62-100µg/g. Experience would suggest that if a number of peroxide values had been measured on each batch there would also have been a range for PV.

Not all kernel in a single batch ages at the same rate. Figure 4.14 demonstrates the variation that exists within a normal population of aged kernel. Samples A1Comp to A6Comp are all 50gram samples taken from previously aged Daddow kernel (2012 harvest). The concentration of the 50gram samples varies from a high of  $187\mu g/g$  hexanal to a low of  $116\mu g/g$ . Samples A1 to A10 are from 10 individual kernels removed from the same treatment. The variation between kernels ranges from a high of  $231\mu g/g$  to a low of  $38\mu g/g$ .



Figure 4.10 Hexanal concentration in the head space of kernel aged at 25°C



Figure 4.11 Correlation of headspace hexanal concentration with peroxide value in rapid aged macadamia kernel.

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Figure 4.12 Variation of Hexanal concentrations in 50 gram composite samples and of single kernels drawn from the same batch of rapid aged kernel.

#### Discussion

The long-term aging treatment has shown a steady increase in the hexanal concentration up to day 600.

A correlation between the PV and hexanal concentration has been demonstrated. The development of a methodology to assess individual kernels will enable us to manufacture consistent batches of kernel for research purposes. Although it is time consuming, taking about 10-15 minutes for each kernel, it is feasible to use this to produce relatively homogenous samples for use in research projects where flavour and quality is being assessed.

#### Long-term ageing compared to rapid ageing

Macadamia kernel was naturally aged at 25°C for up to 20 months and rapidly aged at 55°C for up to 28 days to generate a range of hexanal concentrations in the headspace. Five levels of hexanal concentrations were produced for each ageing treatment by removing kernel at intervals, sealing into foil pouches and cool storing. The treatments were analysed by a sensory panel to determine if there was a difference between the naturally aged and rapid aged kernel.

The sensory analysis was conducted using duo-trio tests to determine if there was any perceivable sensory difference in aroma and taste between rapidly aged macadamia and fresh macadamia samples. The test also compared aroma and taste of naturally aged macadamia with fresh macadamia. There were 5 samples of rapidly aged macadamia samples (RA1, RA2. RA3. RA4 and RA5) and five samples of naturally aged samples (NA1, NA2, NA3, NA4 and NA5) (Table 4.3). Samples were presented to the panellists in a balanced random order.

Twenty panellists evaluated the samples. Five sessions were conducted, the first session involved familiarising the panel with Duo-trio test. The remaining four sessions comprised of two aroma sessions (fresh vs. naturally aged and fresh vs. rapidly aged macadamia samples) and two taste sessions (fresh vs. naturally aged and fresh vs. rapidly aged macadamia samples). The results suggest there was a significant difference in aroma between fresh and all the naturally aged macadamia samples (p<0.05), however in taste there was no significant difference between fresh and naturally aged kernel for up to four months (NA1 and NA2) but the panel easily differentiated fresh samples from NA3, NA4 and NA5 (aged for 8-20 months). With the rapidly aged samples, there was a significant difference in aroma of fresh and rapidly aged samples (RA2, RA3, RA4 and RA5). But the panel could not differentiate the flavour of fresh samples from all the rapidly aged samples (RA1, RA2, RA3, RA4 and RA5) of macadamia kernel.

There was a difference between the naturally aged and rapidly aged treatments in that the sensory panel was able to discriminate the taste of kernel with hexanal concentrations above  $40\mu$ g/g (NA3, NA4 and NA5) but recorded no significant difference in flavour of any of the rapidly aged kernel, even with hexanal concentrations as high as  $60\mu$ g/g.

#### Long-term storage

Macadamia kernel was incubated at a constant temperature of 25°C for up to 20 months. Kernel was removed at various stages (Table 4.3) sealed into foil pouches and stored at 4°C to maintain quality until the end of the ageing treatment.

As a comparison kernel from the 0 days storage treatment was rapidly aged for up to 28 days (table 4.3). Samples were drawn each week, sealed in a foil pouch and stored at 4°C until the end of the ageing treatment.

Macadamia Kernel Samples for sensory analysis						
Natural Age at	25Deg C	Rapid aged at	55deg C			
Sample No.	Ageing	Sample No.	Ageing			
NA 1	0 days	RA 1	0 days			
NA 2	4 months	RA 2	7 Days			
NA 3	8 months	RA 3	14 Days			
NA 4	16 months	RA 4	21 Days			
NA 5	20 months	RA 5	28 Days			

Table 4.3 Ageing treatments for headspace and sensory analysis

Sensory analysis was conducted at the University of Qld, School of Agriculture & Food Sciences, by Dr Sangeeta Prakash.

The following information has been prepared by Dr Prakash.

#### Test method:

Duo-trio test to see if panellists can detect any significant difference between fresh versus naturally and rapidly aged macadamia samples. Panellists were presented samples in a balanced random order. The data was analysed using statistical software package Minitab® V16.

#### Sample Preparation:

For aroma analysis the samples were served in vials and the panel was instructed to sniff the samples and identify the sample similar in aroma to the reference (REF). For taste, the samples were served in plastic cups and the panel was instructed to identify the samples similar to the reference (REF).

Sample quantity: one or two macadamia nuts served in a vial or plastic cup

# Sample temperature: 22°C Design: A duo-trio difference test with 20 panellists screened for sensory acuity Number and type subject: 20 panellists Serving container: 30 mL plastic cups Method of sample presentation: Samples were presented to the panellists in a balanced random order. Panellist were seated in a sensory booth Lighting conditions: White light

#### Results:

Twenty panellists evaluated the macadamia samples for aroma and taste. The results for aroma are presented in Tables 4.4 and 4.5 and the results for taste are presented in Tables 4.6 and 4.7. For a panel of 20, the minimum number of correct responses for the products to be significantly different at 0.05% level of significance is 15.

#### Aroma Session:

	Aroma - Fresh vs. Naturally aged						
No of	Fresh vs	Fresh vs	Fresh vs	Fresh vs	Fresh vs	Fresh vs	
Panellists	NA 1	NA 1	NA 2	NA 3	NA 4	NA 5	
1	1	1	1	1	1	1	
2	1	1	1	1	0	1	
3	1	1	1	1	1	0	
4	1	1	1	1	1	1	
5	1	1	1	1	1	1	
6	1	1	1	1	1	1	
7	1	1	0	1	1	1	
8	1	1	1	1	1	1	
9	0	1	1	1	1	1	
10	0	1	1	1	1	1	
11	1	0	1	1	0	1	
12	1	1	1	1	1	1	
13	1	1	1	1	1	0	
14	1	1	1	1	1	0	
15	1	0	1	1	1	1	
16	1	0	0	1	1	1	
17	1	1	1	1	1	0	
18	1	0	1	1	1	0	
19	0	1	0	1	1	1	
20	1	1	1	1	1	1	
Total	17	16	17	20	18	15	
correct							
responses							
	S	S	S	S	S	S	
S = Significant Difference NS = No significant difference							

Table 4.4: Comparison of the aroma of fresh macadamia with naturally aged macadamia samples.

Table 4.4 shows the results of the difference test from 20 panellists that compares the aroma of naturally aged samples of macadamia against fresh samples of macadamia. The results suggests that the panellists could significantly (p<0.05) detect differences in aroma between fresh and naturally aged samples of macadamia for all the naturally aged treatments.

		Aroma - F	resh vs. Rap	oidly Aged		
No of	Fresh vs	Fresh vs	Fresh vs	Fresh vs	Fresh vs	Fresh vs
Panellists	RA 1	RA 1	RA 2	RA 3	RA 4	RA 5
1	1	0	1	1	1	1
2	1	1	1	1	1	1
3	1	1	1	1	0	1
4	1	0	1	1	1	1
5	0	1	1	1	1	0
6	0	0	0	0	1	1
7	1	0	1	1	1	0
8	0	0	0	0	1	1
9	1	1	1	1	1	1
10	1	0	1	1	1	1
11	1	1	1	1	1	1
12	0	1	1	1	0	1
13	0	1	1	0	1	0
14	1	0	1	1	0	1
15	1	1	1	0	1	1
16	1	1	1	1	1	1
17	0	1	0	0	1	1
18	0	1	1	1	1	1
19	1	1	0	1	1	1
20	0	1	1	1	0	1
Total	12	13	16	15	16	17
correct						
responses						
	NS	NS	S	S	S	S
S = Significa	S = Significant Difference NS = No significant difference					

Table 4.5: Comparison of the aroma of fresh macadamia with rapidly aged macadamia samples

The results from Table 4.5 suggests that the panellists could significantly (p<0.05) detect differences in aroma between fresh and rapidly aged samples of macadamia RA2, RA3, RA4 and RA5. The panellists however could not differentiate between fresh and rapidly aged sample RA1.

#### **Taste Session:**

The results from the taste session are presented in tables 4.5 and 4.6. Table 4.5 presents the results of the difference test from 20 panellists that compares the taste of naturally aged samples of macadamia against fresh samples of macadamia. The results suggests that the panellists could significantly (p<0.05) detect differences in

taste between fresh and naturally aged samples of macadamia NA3, NA4 and NA5, however there was no significant difference in the taste of fresh and naturally aged samples of NA1 and NA2.

Taste - Fresh vs. Naturally Aged						
No of	Fresh vs	Fresh vs	Fresh vs	Fresh vs	Fresh vs	Fresh vs
Panellists	NA 1	NA 1	NA 2	NA 3	NA 4	NA 5
1	0	0	1	1	1	1
2	1	1	1	0	1	1
3	1	1	1	1	0	1
4	1	1	1	1	1	1
5	1	0	0	0	1	1
6	1	1	0	1	1	1
7	1	0	1	0	1	1
8	1	1	1	1	1	1
9	1	0	1	1	1	1
10	1	1	1	1	0	1
11	0	0	1	1	1	1
12	0	1	0	1	1	1
13	1	1	0	0	1	1
14	0	1	1	1	1	1
15	0	0	0	1	1	1
16	0	0	0	1	1	1
17	1	0	1	0	1	1
18	0	0	1	1	0	0
19	1	1	0	1	1	1
20	0	1	0	1	1	1
Total	12	11	12	15	17	19
correct						
responses						
	NS	NS	NS	S	S	S
S = Signific	ant Differen	ce NS = No	significant di	fference		

Table 4.6: Comparison of the taste of fresh macadamia with naturally aged macadamia samples

Table 4.6 shows the results of the difference test from 20 panellists that compares the flavour of naturally aged samples of macadamia against fresh samples of macadamia. The results suggests that the panellists could significantly (p<0.05) detect differences in flavour between fresh and NA3, NA4 and NA5 naturally aged samples of macadamia.

		Taste - F	resh vs. Rap	idly Aged		
No of	Fresh vs	Fresh vs	Fresh vs	Fresh vs	Fresh vs	Fresh vs
Panellists	RA 1	RA 1	RA 2	RA 3	RA 4	RA 5
1	0	1	0	1	1	0
2	1	1	0	1	1	1
3	1	0	1	0	1	0
4	1	1	1	0	1	0
5	0	0	0	1	1	1
6	0	0	0	0	1	0
7	0	0	1	0	0	1
8	0	0	1	1	0	1
9	1	1	1	1	1	1
10	0	0	1	1	0	0
11	1	1	0	1	0	1
12	0	0	1	1	1	0
13	1	1	1	1	1	1
14	0	1	0	1	1	1
15	0	0	1	0	0	1
16	0	1	0	0	0	1
17	0	0	1	1	1	1
18	1	1	1	1	0	1
19	0	0	1	1	1	1
20	0	0	1	1	1	1
Total	7	9	13	14	13	14
correct						
responses						
	NS	NS	NS	NS	NS	NS
S – Significant Difference NS – No significant difference						

Table 4.7: Comparison of the taste of fresh macadamia with rapidly aged macadamia samples

The results from Table 4.7 suggests that the panellists could not significantly (p<0.05) detect any difference in taste between fresh macadamia kernel and any of the rapidly aged treatments of macadamia RA1, RA2, RA3, RA4 and RA5.

#### Hexanal concentrations:

The different storage treatments produced kernel with a range of hexanal concentrations as described in Table 4.8 and Figure 4.13. Hexanal concentration ranged from  $1.8\mu$ g/g to  $54\mu$ g/g for the natural aged kernel and from  $1.8\mu$ g/g to  $61\mu$ g/g for the rapid aged kernel. Freshly harvested kernel from the 2015 season had a hexanal concentration of  $1.2\mu$ g/g in the headspace.

Figure 4.14 shows the average peroxide value for each ageing treatment. Average peroxide values increased with time for both the rapid and natural age treatments. There was a large variation within treatments for peroxide value. The maximum average peroxide value reached was 2.7meq/kg for kernel aged at 55°C for 28 days and 2.4meq/kg for kernel aged at 25 °C for 20 months (Fig 4.14).

The sensory panellists found a significant difference between the aroma of the fresh kernel and all the natural aged treatments (Table 4.4) and a significant difference in all but the RA1 treatment in the rapid aged kernel (Table 4.5).

1	Natural aged (2	5°C)	Rapid Aged (55°C)		
Sample ID	Ageing	µg/g Hexanal	Sample ID	Ageing at 55°C	µg/g Hexanal
Fresh			1.2 P	PM	
NA 1	0 days	1.8	RA 1	0 days	1.8
NA 2	4 months	9.8	RA 2	7 Days	17.5
NA 3	8 months	40.3	RA 3	14 Days	23
NA 4	16 months	41.8	RA 4	21 days	38
Na 5	20 months	54.3	RA 5	28 Days	61

Table 4.8: Hexanal concentrations in the headspace of kernel from different ageing treatments.



Figure 4.13 Hexanal concentration for the different ageing treatment. Error bars = one standard deviation (N=4).



Figure 4.14 Average peroxide values for ageing treatments. Error bars = one standard deviation (N=4)



Figure 4.15 Correlation of all hexanal and peroxide values collected. While there is a strong correlation (R2=0.87) there is significant variation in the area of greatest interest around PV 3meq/kg.

Figure 4.15 shows a correlation between hexanal and peroxide values for all the corresponding data collected during the project. While there is a strong correlation with an  $R^2$  value of 0.87 there is significant variation in the area of greatest interest around peroxide values of 3meq/kg. Hexanal concentrations as high as 95µg/g have been recorded for PV values less than 3meq/kg.

#### Discussion:

A significant sensory difference was found between the aroma of fresh kernel and all the natural aged samples of macadamia. However when the samples were rapidly aged the panellists could perceive a difference in aroma only from 14 days on. It was interesting to observe that the panellists did not differentiate the taste of any of the rapid aged samples however with the natural aged samples the panel could detect a perceivable difference in taste for samples stored for eight months and longer (NA3, NA4 and NA5) when compared with fresh samples.

The sensory panel did determine that the flavour of the naturally aged kernel changed over time with storage, however they did not detect any difference in the taste of the rapidly aged kernel, despite the hexanal concentration significantly increasing from 1.8µg/g to 61µg/g and peroxide values increasing from 0.2 to 2.6meq/kg during the ageing treatment. The data for the taste of rapidly aged kernel (table 4.7) shows that an increasing number of panellist indicated that there had been a change in the flavour (13 and 14 panellists for treatment RA 2- RA 5 compared to 7 and 9 panellists for treatment 1). This could indicate that not all kernels that are rapidly aged will change at the same rate. The large variation of the PV values confirms that there was significant variation between kernels within each batch.

While there was significant variation on the peroxide values within treatments the trends between both the rapid ageing and natural ageing treatments are similar and consistent with expected results. The hexanal concentrations also show a consistent trend between both ageing treatments.

The similarity in results of the PV analysis and hexanal concentrations between the long-term ageing and the rapid ageing treatments demonstrate that rapid ageing is a reliable indicator of shelf life for long-term natural aged product. The rapid aged treatment had a PV of 2.7meq/kg and hexanal concentration of  $62\mu g/g$  after 28 days at 55°C, while the natural aged treatment had PV 2.4meq/kg and hexanal concentration of  $54\mu g/g$  after 20 months storage at 25°C.

The results from the sensory panel show that with the rapid ageing treatment there was a significant difference in aroma from fresh kernel after 7 days rapid ageing, however there was no significant difference in flavour for any of the rapid ageing treatments. This could indicate that it takes longer for "off" flavours to develop, even though the chemical signals of PV and Hexanal indicate that there has been oxidation.

The method used in this project appears to give higher readings for PV than the previous study by Mason et al (2003). Mason concluded that peroxide values of 3-5meq/kg were approximately equal to a hexanal concentration of 25-45µg/g. The different methodologies used in the two studies are not directly comparable as Mason measured hexanal concentration in expressed oil using a GC/MS. The absolute values set will be method dependant and therefore it is recommended using the methods described in this project with the OdourScan® that the threshold level of hexanal be set at 100µg/g. The sensory panel found no significant difference in the flavour of fresh kernel and rapidly aged kernel with a hexanal concentration of 60µg/g and PV 2.6meq/kg. Compared to the traditional PV standard of 3meq/kg it would appear that hexanal concentrations will range between about 50 and 90µg/kg (Fig 4.15). Further analyses of PV and headspace hexanal concentrations are required to refine the hexanal threshold.

## Outputs

OdourScan®; A prototype electronic nose (OdourScan®) was developed specifically for this project by Next Instruments. The OdourScan® has proved to be an efficient machine able to measure hexanal concentrations in the headspace of packaged

macadamia nuts.

Protocols for rapid ageing and hexanal assessment using the OdourScan® have been developed and are described in detail in this report.

Rapid ageing has been demonstrated to be a reliable indicator for the prediction of lipid oxidation in natural aged macadamia kernel.

# Outcomes

A commercially viable, cost effective, rapid test has been developed that allows the macadamia industry to assess the shelf life and quality of raw and roasted macadamia kernel. While rapid ageing and hexanal concentrations have been previously demonstrated as methods to assess shelf life of macadamias (Mason et al 2003, McConchie et al 2010) the procedures and equipment were impractical for a commercial test. Earlier methods used solid phase micro extraction and gas chromatography (SPME-GC) to measure the hexanal concentrations. This method was reliable but slow and required expensive equipment making the cost of each test beyond what was practical on a commercial basis.

The methodology developed in this project makes a test for macadamia shelf life a commercial reality. The OdourScan® costs approximately \$35,000 and requires minimum maintenance and training. Each test can be completed in under 15 minutes. The cost of the e-nose and simplicity of equipment and test procedures mean that processing companies can now use rapid ageing and hexanal concentrations as part of their quality control program.

The benefits from a cost effective and reliable shelf life test span all sectors of the industry. Test results can be used:

- To educate farmers and handlers through the supply chain on the impact of poor handling practices
- By processors to reward farmers for good handling practices that maintain long shelf life. Or alternatively penalise farmers for poor handling practices.

- To monitor and measure quality decline through the supply chain
- To quickly and objectively identify product on supermarket shelves that are stale or rancid even though they may not be past their "Best by Date"
- To assess the potential shelf life of new cultivars being developed in the industry breeding project
- To aid re-packers to set realistic and meaningful "Best by Dates"
- To rapidly assess the effect of different packaging materials

# **Evaluation and Discussion**

The procedures developed in this project have the potential of wide ranging benefits for the macadamia industry and for other nut industries. The equipment used is affordable for larger processors and requires only minimal maintenance and minimal training. One drawback with the OdourScan® is that it is very sensitive to changes in temperature. It requires an air-conditioned room with constant temperature where there is little traffic. Even just opening the door can affect a reading and a shift of 1°C or 2°C requires a new calibration. For this reason it is important to do spot checks with standard solutions of known hexanal concentration regularly during testing.

Hexanal is a good indicator of the degree of lipid oxidation and has been used to monitor oxidation in a wide range of products including infant formula (Garcia-Llates et al 2006), canola oil (Gromadzka J, Wardencki W (2010), meat products (Shahidi 1987) nut products (Pastorelli et al 2007), pistachio nuts (Leufven et al 2007) and Almonds (Cal Almond Board 2014).

Although hexanal is commonly used as an indicator of oxidative rancidity, no common industry standards currently exist for any nut products. Nor is there any standard methodology for measuring hexanal in nuts.

The current accepted quality measure for macadamia kernel is peroxide value with a maximum level of 3meq/kg oil and free fatty acids with a maximum of 0.5% calculated

as oleic acid (Macadamia Industry Quality Handbook 2008).

Although hexanal is commonly used in research as an indicator and for process control in food processing factories it is still not recognised as a standard quality parameter in product specifications. For this reason hexanal is not likely to be accepted in the market as an alternative to peroxide value and free fatty acids. The tests described here will initially only be used by industry for quality management, education and research and will take time and common usage before it can be included as a quality specification for trading nuts.

The hexanal test developed in this project has many advantages over existing quality assessments. It is non-destructive so product can be assessed using the OdourScan® as well as by additional means either chemical or sensory.

The cost effective nature of this test make it suitable for monitoring quality throughout the supply chain; from farmer to consumer. It's possible for processors to conduct a seven day quality test on nuts received from farmers. The cost of the tests make it such that every delivery can be tested. The results of these tests can be used to reward farmers for supplying quality product with long shelf life. In a time of under supply processors are nervous about introducing new quality tests that may scare off suppliers so it is unlikely that this test will become part of the payment schedule for some time. In the current climate of undersupply data collected can be used to educate growers on the effect of good or bad post-harvest handling practices with real objective data. It also provides the processor with better information about suppliers and gives them the ability to segregate incoming product that may have a short shelf life.

Processors can use the test for stock rotation within their warehouse and to direct product with short shelf life to appropriate markets where it will be consumed rapidly, or further processed into products that may stabilize the kernel e.g. ice cream.

Through this project protocols have been developed for an objective test for macadamia quality and predicting the potential shelf life. A methodology for rapid ageing has been developed and measurement of hexanal in the headspace using the

OdourScan® electronic nose has been refined. The comparison of rapid ageing and long-term natural ageing demonstrates that rapid ageing gives a good indication of the expected natural ageing process. Through rapid ageing, kernel with short shelf life can be identified with confidence. When rapid ageing is used in conjunction with the Arrhenius equation a good prediction of the potential shelf life can be made. The prediction is an estimate of the maximum potential shelf life of kernel stored in defined conditions. The actual shelf life is dependent on how the product is handled, what temperature it is stored under, what type of packaging it is stored, whether it is vacuum packed or gas flushed, and most importantly the kernel moisture content. Shelf life will be shortened if any of these conditions are sub optimal.

There is still some uncertainty surrounding the hexanal threshold limit and this needs further analyses to fine tune. The variability in PV result as well as the variability with hexanal measurements means that a range of 50µg/g to 100µg/g is estimated to approximate a PV of 3meq/kg. Refining the hexanal threshold is important in being able to set an accurate "Best by Date" when predicting the shelf life, but is less important for determining the kernel quality, as any kernel with elevated hexanal levels will have a reduced shelf life.

### Recommendations

In order to establish a stronger correlation between hexanal and peroxide values further analyses are required.

The methodology has been established and proven to have application to determining immediate kernel quality and potential shelf life. Industry confidence will only be achieved through repeated use and continual reporting of results.

A small continuing project involving cooperation and participation of macadamia processors could act as an extension phase to gain industry adoption of this technology.

# Intellectual Property/Commercialisation

No commercial IP generated

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