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Shelf Life of Tropical Canarium Nut Stored under Ambient Conditions

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Abstract: There is a need to develop alternative crops to improve the food security and prosperity of developing countries. The tropical nut *Canarium indicum* (canarium nut) is increasingly used as a shade tree for cocoa and has potential for commercialization as a sustainable crop that will improve food security and livelihoods in Melanesia and East Asia. There is no information on canarium nut shelf life characteristics. Canarium kernels may be prone to rancidity, due to a high content of unsaturated fatty acids. Kernels at 5.4% moisture content were vacuum-packed with a domestic vacuum-packaging system and stored for six months in Papua New Guinea and for nine months in Southeast Queensland, Australia at both ambient temperatures (22 to 31 °C and 22 to 25 °C, respectively) and under refrigeration. Nuts were analysed for changes in peroxide values and free fatty acids (FFAs) over the storage periods that might indicate development of rancidity. Peroxide values indicated very low levels of oxidation in all treatments. Free fatty acids were at low levels but increased significantly during storage at ambient temperatures. The results suggested that vacuum-packed Canarium nuts can be stored safely under ambient tropical conditions for six months with daytime temperatures around 31 °C, and for nine months at 25 °C. Increasing FFA levels at ambient temperatures indicate caution about longer storage time at ambient temperatures. Storage under refrigeration greatly prolonged shelf life.

Keywords: nuts; *Canarium indicum*; canarium nut; shelf life; peroxide values; free fatty acids; food security

1. Introduction

Only 103 domesticated species of the world's 250,000 species of flowering plants provide 90% of the world's food [1]. A diversification away from over-reliance on staple food crops will be important as part of the process of achieving security of food production for an increasing world population in the future [2,3]. There is potential for expansion of species currently used as indigenous food crops. Nuts in particular are increasing in importance as food crops, with total world tree nut production for 2014–2015 at ~3.5 million metric tons (kernels) [4]. Edible nuts in the form of raw or roasted kernels [5,6] are increasingly recommended for inclusion in healthy diets due to evidence of beneficial effects on blood cholesterol, coronary heart disease, diabetes, sudden death, inflammation, thrombosis and vascular reactivity [7–10]. More than 80% of the world trade in tree nuts is comprised of just four species, walnuts, hazelnuts, pistachios, and almonds [4].

Many other species of edible nuts around the world have potential to be developed and produce a commercial return, for example, macadamia and Brazil nuts are case studies. Macadamia originated in the sub-tropical rainforest of eastern Australia, and is a rare example of a nut from the tropics grown commercially in plantations [11–17]. Brazil nuts have also become an important industry for countries along the Amazon River such as Brazil, Peru and Colombia, however, Brazil nuts are harvested from the forest floor rather than plantations [18]. In Papua New Guinea, the Solomon Islands and Vanuatu, potential exists for domesticating a variety of new tree nuts that have been used in traditional cultures [19–21].

The genus *Canarium* contains tree nuts with beneficial properties, including *Canarium odontophyllum*, *C. ovatum* and *C. album* [22–26]. *Canarium ovatum* (Pili) is cultivated commercially in the Philippines, producing 23,000 metric tons of edible nuts in 2011 [19,27]. *Canarium indicum* L. (Burseraceae) is a tropical nut species indigenous to lowland rainforests of eastern Indonesia, Papua New Guinea (PNG), the Solomon Islands and Vanuatu, producing nutritious nuts and high quality timber [20,28]. Canarium nuts have been an important food for the people of Papua New Guinea for thousands of years [29,30]. *Canarium indicum* has great potential for commercialization and is in a unique position to develop as a sustainable industry, adding to food security in Melanesia [20,31,32]. Traditionally, the nut has been mainly harvested from the ground in forests, similar to the Brazil nut, but large numbers of trees are now being planted as shade trees in cocoa plantations in Papua New Guinea [20]. In traditional culture, surplus nuts are typically stored in woven baskets above a fireplace. This practice is not suitable for a commercial product.

In other crops postharvest handling methods have been developed to reduce waste in the postharvest chain and maintain quality and nutritional attributes, [12–17,33]. Nuts are prone to rancidity because of high content of unsaturated fatty acids. Rancidity is one of the most important defects of this food [34,35]. Canarium nut has very high oil content of around 67 to 75%, with 40% oleic acid (monounsaturated, with one double bond) and 14% linoleic acid (polyunsaturated, with two double bonds) [36]. The high degree of unsaturation of canarium oil, especially linoleic acid, creates potential for oxidative rancidity to develop [37,38]. Hydrolytic rancidity can also occur if moisture content of foods is not reduced and maintained sufficiently [39,40]. It is the first step in the deteriorative rancidity pathway [39]. Fatty acids generated from hydrolysis are more prone to oxidation than intact triglycerides [38,41] and are the preferred substrate for respiration, generating heat and more water [42]. Tropical conditions with high temperatures and humidity may favour development of rancidity [43,44]. There is some knowledge of culture and postharvest processing of canarium nuts [45–49], however more research is needed to identify suitable postharvest treatments.

We conducted trials to investigate postharvest shelf life of *Canarium indicum* nuts stored as whole nuts and as kernels. Canarium kernels were packed using a domestic vacuum packaging system and stored under ambient and refrigerated conditions to investigate the shelf life characteristics of canarium under conditions likely to prevail in the postharvest chain in a tropical climate. For comparison, kernels were also stored in a laboratory under ambient laboratory conditions that may approximate conditions sometimes pertaining within a building in the tropics. Knowledge of shelf life of canarium kernels will provide information on suitable storage conditions to maintain quality of nuts in conditions encountered in developing countries.

2. Materials and Methods

2.1. General Methods

Canarium indicum fruit of up to two weeks post-abscission age was gathered from the ground in plantations in East New Britain, PNG. Fruit was depulped in PNG by a traditional method of soaking fruit in water, then allowing it to ferment in wet hessian sacks for two to three days until the pulp (exocarp) was soft enough to remove from the nuts. Sacks were maintained wet by external application of water as required. Three shelf-life experiments were conducted: (1) shelf-life of kernels stored in-shell as whole nuts at three different initial moisture contents (16.1% ± 0.3%, 10.3% ± 0.2% and 5.4% ± 0.1%) at mean laboratory temperatures of 22.2 ± 0.04 °C minimum and 24.5 ± 0.04 °C maximum; (2) shelf-life of kernels dried to an initial moisture content of 5.4% ± 0.1 stored at both ambient tropical temperatures (mean minimim 22.0 ± 0.01 °C to mean maximum 31.2 ± 0.26 °C) and under refrigeration (4 °C); and (3) shelf-life of kernels dried to an initial moisture content of 5.4% ± 0.1% stored at both laboratory temperature as above and under refrigeration (4 °C). Nuts were dried in a Memmert fan-forced laboratory oven (Memmert Gbh & Co. KG, Schwabach, Germany). The moisture content (wet basis (w.b.)) of nuts for shelf life studies was determined by drying three replicates of five nuts at 105 °C for 24 h to remove all free water [50] Moisture content was determined by the formula: Moisture content (w.b.) = (initial weight − final weight) × 100 / initial weight.

All moisture contents were calculated on a wet basis (w.b.). For shelf-life of kernels, whole nuts were dried for 3 days at 38 °C followed by 3 days at 45 °C to achieve nut moisture content of 5.4% ± 0.1% (w.b.). Nuts were cracked by hand with a modified TJ's™ macadamia nutcracker to release the kernels [45]. Kernels in testa were vacuum-packed for storage using a Foodsaver®Vac 440 domestic vacuum-sealer (Sunbeam Corporation, Botany, Australia) in polyethylene packaging film (Foodsaver VSO520, Sunbeam Corporation, Botany, Australia). At assessment kernels were blanched by pouring hot water at 100 °C over kernels in testa and allowing them to stand for 1.5 min before removing the testa. Kernels were then re-dried for 15 min at 60 °C to remove moisture absorbed during blanching. Shelf-life was assessed by peroxide value (PV) for oxidative rancidity and free fatty acids (FFA) for hydrolytic rancidity.

2.2. Shelf Life of Canarium Kernels Stored as Nuts (Nut-in-Shell)

Nuts at three different initial moisture levels were stored in open-mesh polypropylene bags under laboratory conditions (25 °C) for 11 months. Initial moisture contents were 16.1% ± 0.3%, 10.3% ± 0.2% and 5.4% ± 0.1% (w.b.). For each moisture content, there were five replicates each of 10 nuts. Moisture contents were determined again after six weeks ambient drying in the laboratory. Kernels from nuts oven-dried to 5.4% moisture content (w.b.) as above were used to obtain initial and final values for PV and FFA.

2.3. Shelf Life of Canarium Kernels Stored at Ambient Tropical Temperatures (22 °C to 31 °C)

Canarium nuts at 5.4% moisture content (w.b.) were cracked using a "TJ" macadamia cracker [45]. The kernels obtained were vacuum packed and stored for six months under ambient tropical conditions (mean minimum 22.0 ± 0.01 °C to mean maximum 31.2 ± 0.26 °C and under refrigeration (4 °C). Each temperature treatment consisted of five replicates of 10 kernels. Free fatty acids were determined at the start of the experiment, and PV and FFA analyses were conducted after one month, after three months and after six months of storage.

2.4. Shelf-Life of Canarium Kernels Stored at Laboratory Temperature

Canarium nuts at 5.4% moisture content (w.b.) were cracked as above [45]. The kernels obtained were vacuum packed and stored for nine months under laboratory conditions (22.2 ± 0.04 °C minimum and 24.5 ± 0.04 °C maximum) to examine Canarium shelf life at a lower temperature than tropical, and under refrigeration (4 °C). Each temperature treatment consisted of five replicates each of 10 kernels. FFA were determined at the start of the experiment and PV and FFA analyses were conducted after 1, 3, 6 and 9 months of storage.

Following testa removal, each replicate of ten kernels was crushed twice in a garlic press and mashed in a mortar and pestle with n-pentane. The sample was then stirred in a covered beaker with pentane for half to one hour. The liquid was then transferred to test tubes and centrifuged at 3000 rpm for 3 min to separate the oil and solvent from the nut residue. The supernatant liquid was then decanted and the pentane stripped from the oil in a Buchi Rotavapor (BÜCHI Labortechnik AG, Flawil, Switzerland).

2.5. Peroxide Values and Free Fatty Acids

Peroxide Values were determined according to AOAC Official Method 965.33 modified as follows: smaller samples of oil were used (1 g instead of 5 g); 0.01N $Na_2S_2O_3$ was used instead of 0.1 N $Na_2S_2O_3$; and $Na_2S_2O_3$ titration was accomplished with a 100 µL HPLC syringe instead of a burette. Peroxide value (expressed as milliequivalents of peroxide per kilogram of sample) was calculated according to the formula:

$$PV\ (meq\ perkg) = \frac{(S - B) \times N \times 1000}{sample\ wt\ (g) \times 1000} \quad (1)$$

where S = sample titration (µL); B = Blank titration; and N = normality of $Na_2S_2O_3$.

Free fatty acids were determined using AOAC method 940.28, modified as follows: smaller samples of oil were used (1 g instead of 5 g); 0.1 N NaOH was used instead of 0.25 N; and NaOH titration was accomplished with a 100 µL HPLC syringe instead of a burette. Free fatty acids were calculated as follows:

$$\%\ FFA\ (as\ oleic) = \frac{(\mu L) alkali \times N\ of\ alkali \times 28.2\ mg}{sample\ wt\ (g) \times 1000} \quad (2)$$

2.6. Statistical Analysis

Data for PV and FFA for kernels stored for 11 months as nut-in-shell were analysed for significant difference by a non-parametric Mann-Whitney U test. Data for PV and FFA for the kernel shelf life studies (stored at tropical temperatures (22 °C to 31 °C) and stored under laboratory conditions (constant 25 °C) and under refrigeration (4 °C) were analysed with a two-way ANOVA with time and temperature of storage as factors. Where significant differences were detected, means were compared using Duncan's Multiple Range test.

3. Results

3.1. Shelf Life of Canarium Kernels Stored as Nut-in-Shell at Constant Temperature (25 °C)

Nut-in-shell stored at three different initial moisture contents (w.b.) (Moist: 16.1% ± 0.3%; Partly dry: 10.3% ± 0.2%; Dry: 5.4% ± 0.1%) equilibrated to ~7.4% ± 0.44% moisture content after six weeks in an air conditioned laboratory at 25 °C. After 11 months the PV and FFA values of these kernels stored as whole nuts were low (Table 1). However, there was a significant increase (p = 0.008) in FFA from 0.11 to 0.31 during storage.

Table 1. Mean peroxide [1] and free fatty acid values (SE) for canarium kernels stored in nut-in-shell for 11 months at laboratory temperatures (~25 °C). Means in the same column with different superscripts are significantly different (p = 0.008).

Treatment	Peroxide Value	Free Fatty Acids
Initial value	NA [1]	0.11 (0.003) ^{a}
After 11 months storage at 25 °C	0.31 (0.032)	0.31 (0.29) ^{b}

[1] Peroxide Values not available. Different superscript letters in the same column indicate significant difference.

3.2. Shelf Life of Canarium Kernels Stored at Ambient Tropical Temperatures (22 °C to 31 °C)

Peroxide values for vacuum-packed kernels stored at tropical temperatures (22 °C to 31 °C) for six months were very low (≤0.61) and there were no significant differences in PV at three months and six months compared with the one-month values (Table 2). All FFAs were also low (0.14 to 0.29), but increased significantly compared with the initial (control, 0.16) after storage at ambient temperature for three months, and were further significantly increased after six months to c. 0.29 (p <0.05) (Figure 1). In contrast, kernels stored under refrigeration showed no significant increase in FFA levels (Figure 1). There were no significant interactions between the time and temperature of kernel storage.

Table 2. Peroxide value [1] means (SE) for vacuum-packed canarium kernels stored at ambient tropical temperatures (mean max. 31.2°C, mean min. 22 °C) and under refrigeration for six months, or at ambient conditions (25°C) and under refrigeration in a laboratory for nine months.

Temperature	Location	1 Month	3 Months	6 Months	9 Months
Tropical	Ambient	0.41 (0.07)	0.40 (0.04)	0.48 (0.07)	
	Refrigerated	0.61 (0.03)	0.42 (0.04)	0.38 (0.03)	
Laboratory	Ambient	NA [1]	0.38 (0.06)	0.25 (0.03)	0.23 (0.01)
	Refrigerated	NA [1]	0.29 (0.03)	0.26 (0.02)	0.19 (0.03)

[1] NA: Peroxide Values not available.

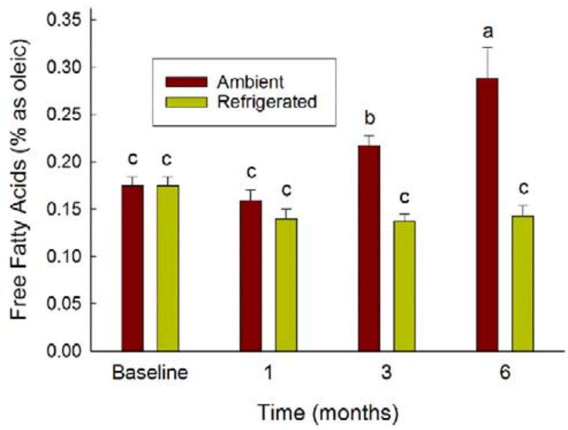

Figure 1. Free fatty acids (% as oleic) for *Canarium indicum* kernels stored at tropical temperatures (22 °C to 31 °C) and refrigerated temperatures (4.0 °C) for six months. Values are means (SE), different superscript letters on columns indicate significant difference between means ($p < 0.05$).

3.3. Shelf Life of Canarium Kernels Stored at Constant Temperature (22–25 °C)

Vacuum-packed canarium kernels did not deteriorate markedly during the nine months of storage and all PVs were very low (≤0.38 meq per kg, Table 2). There were no significant differences between the three, six and nine month treatments or between refrigerated and ambient treatments (Table 2). There were no significant interactions between the time and temperature of kernel storage.

Free fatty acid values were generally low (≤0.2) during laboratory storage for nine months; however, there was a significant upward trend in FFA at ambient temperatures ($p < 0.001$, Figure 2).

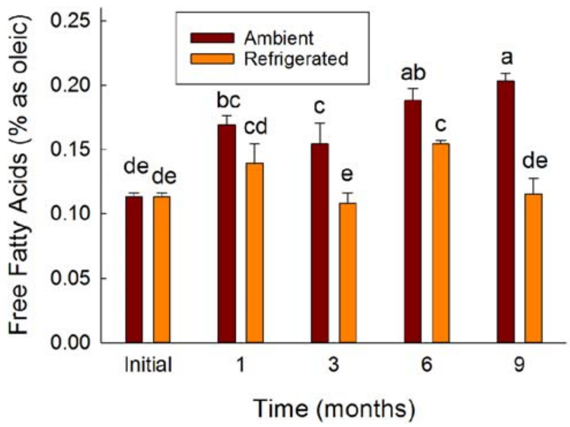

Figure 2. Free fatty acids (% as oleic) for canarium kernels stored at ambient laboratory temperatures (25 °C) and under refrigeration (4.0 °C) for nine months. Values are means (SE), different superscript letters on columns indicate significant difference between means ($p < 0.05$).

4. Discussion

Nuts require appropriate storage because of their high oil content and seasonal cropping nature to maintain eating quality and to make them available out of season [10,51,52]. This study showed that freshly harvested, vacuum packed canarium kernels can be stored for six months at ambient tropical temperatures of 22 to 31 °C, or for nine months at ambient temperature ~25 °C without development of rancidity as indicated by PV and FFA values. The development of unacceptable taste or rancidity is characteristic of nuts that have been stored in inappropriate conditions or stored for too long [53]. Lipid oxidation and hydrolysis during storage are the most common causes of deterioration in the sensory and nutritional quality of nuts [42,54,55]. Rancidity is the most serious defect of edible nuts and success for a new nut crop such as canarium will be greatly affected by the ability to prevent rancidity developing.

We found the PVs for kernels stored for three months, six months and nine months at constant temperature of 25 °C were very low (≤0.38 meq per kg). Similarly, when kernels were stored at ambient tropical temperatures (22 °C to 31 °C) for six months, PVs were low (max. 0.61 meq per kg). We consider that the rancidity indications for macadamias are appropriate for canarium because the two nuts have very similar oil content, of approximately 75% [56]. The Australian Macadamia Industry Quality Handbook has a standard maximum PV of 5.0 [57,58], although other industry sources prefer a maximum of 3.0 [59]. Recognized PV standards for walnuts are less than 3.0, and for almonds less than 2.0 [41]. All PVs were well below all these standards, indicating high quality in terms of oxidative rancidity.

Factors limiting oxidation in this study could be (1) vacuum packing limited oxygen availability; (2) the packaging film limited oxygen availability [52,60,61]; and (3) storing in testa probably helped enhance shelf life, as for almonds [42]. The simple packaging system used enabled safe storage of canarium nuts for six months without refrigeration under ambient, lowland tropical conditions in PNG. Further shelf life studies are needed to determine the storage time limits under tropical conditions using similar and alternative packaging materials.

Free fatty acid values showed only limited hydrolysis of vacuum-packed kernels during nine months of storage at 25 °C. The highest value recorded, ~0.3%, was lower than the general standard of 0.4% recommended for hazelnuts and the nut industry in general [55] and for macadamias of <0.5% [62], although it is the maximum desirable level proposed by McConachie [63]. Although FFA values were low, the highest values indicate caution about the potential for development of hydrolytic rancidity during longer term storage. If macadamia kernels develop FFA over 0.15% in 12 weeks of storage they are considered to have a dramatically reduced shelf life [64]. Free fatty acids for the whole nut (nut-in-shell) storage trial, all ambient temperature treatments, and the six month tropical refrigerated treatment increased to over 0.15%, possibly indicating a limited shelf life after 11 months storage at 25 °C in-shell, nine months storage of kernels at constant 25 °C or six months storage at 22 to 31 °C. However, the current results compare very favourably with the accepted FFA standard for walnuts of <1.5% [41]. All refrigerated kernels at both sites maintained excellent FFA levels, demonstrating the value of refrigerated storage. There was some variability in FFA values in the refrigerated treatment stored for nine months, which could be due to inherent variability of samples, e.g., time on the ground before sampling, variable storage time before processing and possible effects of the traditional rotting method (retting) of depulping the fruit in the source country, PNG. Further canarium shelf life studies will be conducted including shelf life of in-shell kernels, an accelerated shelf life study and shelf life of roasted kernels.

The moisture content of the stored kernels was probably a factor in the free fatty acids increases as hydrolytic cleavage of triglycerides can occur when the moisture content is above the critical monolayer level at which enzymes are activated [42]. Blanching to remove the testa of canarium may deactivate or reduce enzymes [65]. Kernel moisture content at the beginning of storage was approximately three percent, sufficient to provide some free water for hydrolysis and suggesting the desirability of a drying regime to achieve lower kernel moisture content for ambient storage, e.g., the recommended moisture content for storage of macadamia kernels is 1.5%. Hydrolysis typically can increase by a factor of eight as water activity increases from 0.3 to 0.5 [40]. Temperature was also a factor, as under all ambient conditions hydrolysis of oils increased as time elapsed, but under refrigeration, hydrolysis was not sufficient to significantly increase FFA. Hydrolysis of nuts appears to be related to storage at excessively high ambient temperature [42,57,66]. Free fatty acids are very important for nuts, as they contribute off-flavours [67–69]. Free fatty acids from hydrolysis are also important for the oxidation pathway as they are more prone to oxidation than intact triglycerides [38,41,42].

Quality of canarium kernels was maintained at satisfactory levels as determined by PV and FFA when kernels were stored in-shell at 25 °C for 11 months (PV, 0.31 meq per kg; FFA, 0.31 % as oleic). This is similar to hazelnuts, which were stored for eight months in-shell at ambient conditions ranging from 10 °C to 26 °C without substantial deterioration as determined by PV and FFA, but after 12 months storage under these conditions, FFA of 0.47% was above the acceptable limit for hazelnuts [55]. Our research shows that canarium kernels can be safely stored in-shell for 11 months at mean laboratory temperatures ranging from 22.2 °C min. to 24.5 °C max. while awaiting further processing, provided the initial nut-in-shell moisture content is no higher than five percent. The inexpensive vacuum seal used is probably affordable technology for a modest operation in a developing country; however, electric ovens as in the current study may not be practical. A further problem is that the electricity network may be unreliable. There may be potential to use solar drying technology.

5. Conclusions

Canarium nut kernels vacuum-packed using simple domestic equipment were stored for nine months at 25 °C or for six months at 31 °C without PV and FFA values indicating rancidity developing. Free fatty acids that developed during these storage periods indicate caution for further storage under these conditions. Storage under refrigeration greatly prolonged shelf life.

Author Contributions: David Walton contributed perimental design and data analysis, and conducted laboratory experiments. Bruce Randall contributed data analysis and manuscript editing. Matthew Poienou conducted tropical experiment. Tio Nevenimo supervised tropical experimentation. John Moxon contributed experimental design and tropical collaboration in project. Helen Wallace contributed experimental design, manuscript editing, and was the project leader.

Conflicts of Interest: The authors declare no conflict of interest.

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