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Presence of Testa and Shell Maintains Oil Stability in Almond and Canarium Nuts

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Abstract: (1) Background: The oil stability of tree nuts during storage can be influenced by storage conditions such as temperature, humidity, and moisture concentration. However, few studies have assessed how the presence of testa and shell affects the oil stability of tree nuts during storage. We aimed to determine how storage conditions affect oil stability in almond and canarium, in particular, the presence of testa and storage time of nut-in-shell (NIS). (2) Methods: We measured peroxide value (PV), free fatty acid (FFA) and hexanal concentrations of almond and canarium (blanched vs. kernel-in-testa) stored at 45 °C for 24 days. We also measured PV, FFA and fatty acid composition of canarium samples at days 0 and 140 stored as NIS under ambient conditions. (3) Results: The presence of testa in almond and canarium decreased hexanal and PV concentrations at day 24 of incubation. Canarium PV and FFA concentrations increased over 140 days of storage in the shell compared to day 0. However, both PV and FFA concentrations remained within the acceptable threshold during storage. No changes in fatty acid composition were found during NIS storage. (4) Conclusions: Testa and shell could act as a natural coating, slowing down oxidation rates. Hence, long-term storage on nuts in testa or nuts in shell are recommended for tree nuts.

Keywords: *Prunus communis*; *Canarium indicum*; blanched almond; hexanal; peroxide values; free fatty acid; accelerated ageing

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1. Introduction

Global nut industries have grown 65% between 2010 and 2020, as more consumers recognise the health benefits of nuts, and production in 2020 is 5.3 million metric tons [1]. Nuts contain a high unsaturated fatty acid content, making them susceptible to oxidation during storage [2]. The unstable carbon-carbon double bonds in unsaturated fatty acids can be quickly degraded, causing nuts to become rancid [3–6]. Oil rancidity leads to volatile compounds and off-flavours [7,8] which are undesirable for consumption. Poor post-harvest storage practices such as elevated humidity and temperature are two of the main factors initiating oil oxidation [2,9–11]. Post-harvest storage practices that alleviate or slow down oil oxidation under ambient conditions are needed to prolong the shelf life of nuts.

Oil rancidity can be measured using various assessment indicators. For example, the ratio of unsaturated:saturated fatty acid in oils determines the stability of oils [2,6]. Nut rancidity can also be measured using peroxide values (PV) and free fatty acids (FFA) [3].

Peroxides and FFAs are produced through autoxidation and hydrolytic rancidity in the presence of enzymes, respectively [12,13]. Hexanal is a secondary product of oxidation and is produced through oxidation of linoleic acid [14,15]. Hexanal concentration can be used to determine off-flavour development in oil and is directly linked to oil quality [15]. Relying on one oil oxidation indicator can be misleading. For example, both PV and FFA can be degraded to secondary products and having low PV and FFA values does not necessarily indicate low oil oxidation [16,17]. Hence, more than one assessment method is usually needed to determine oil rancidity.

Various food preservation methods such as synthetic food coating are used to prolong the shelf life of fruits, nuts and vegetables [18–20]. However, applying nonthermal and natural preservation methods in nuts is preferred due to their ability to prolong shelf life and preserve naturally occurring compounds [21]. Nuts can be sold with or without the shell, and also with or without the testa. Post-harvest storage and processing of nuts such as removing shell and/or testa, roasting, drying and packaging affect nut oxidation leading to altered hexanal production, PV and FFA [2,10,22,23]. For example, when kernels are exposed to high (120 °C) roasting temperatures for up to 20 min, testa remaining on a kernel can reduce oil oxidation compared with kernels that have the testa removed [24]. The naturally occurring kernel covers such as shell or testa are protective outer layers of kernels in tree nuts especially after proper post-harvest drying methods are practiced [9,25]. However, only few studies have examined how storage in shell or in testa affects oil stability.

Canarium nut has recently been commercialized [26,27]. *Canarium indicum* L. is a forest timber tree in the Pacific with edible nuts [24,28,29] and is used as a shade-tree for cash crops including cocoa [30,31]. It is important to understand to what extent the oil stability of canarium is comparable with other commercially available tree nuts. Globally, there are many tree nut species that are traded formally and informally [32] and almond (*Prunus communis* L., Rosaceae) production is greater than any other crop according to INC [1]. Almond is one of the most nutritionally and commercially valued tree nuts consumed as a snack worldwide [1]. Hence, we assessed the oil stability of the canarium kernel compared with that of almond. Additionally, canarium nuts are stored as nut-in-shell in the factory under ambient conditions between 3 and 5 months, on average 4 months, before being cracked and further processed. The oil stability of canarium nut-in-shell samples has been examined over eleven months at a constant temperature of 25 °C [11] in which the results are not applicable to storage under ambient conditions, ranging between 28 °C and 34 °C and high humidity (https://www.accuweather.com/en/pg/kokopo/257846/november-weather/257846?year=2023 accessed on 25 August 2023). This study had two aims (1) to determine how oil stability is affected by the presence of testa using accelerated ageing, and (2) to assess how oil stability is affected by long-term storage of NIS under ambient conditions.

2. Materials and Methods

2.1. Sample Collection and Experimental Design

2.1.1. Experiment 1: Oil Stability of Blanched and Kernel in Testa of Almond and Canarium Using Accelerated Ageing

The almond with testa samples were sourced from a retail outlet. The origin of almond samples was unknown; however, all almond samples were well within the time of 'best to use' when purchased. All samples were then stored in a refrigerator prior to chemical analysis. The purple fruits of canarium were soaked in warm water for 5 min and the pulp was then manually removed from the shells [29]. All nuts of both species were immersed in hot water (100 °C) for 90 s to facilitate blanching (the removal of the testa) [29]. There were two treatments: blanched (testa removed) and kernel-in-testa (control). The testa of blanched samples was manually removed. However, the testa of kernel-in-testa samples were not removed. All samples were placed into a laboratory oven and dried at 40 °C until the moisture of the samples reached 5.9% in almond and 4.5% in canarium.

A sub-sample of almond and canarium samples (n = 5) from each treatment was used to assess the initial PV and FFA concentration. Then, five replicated foil pouches, each containing five nuts, were assigned for each treatment (blanched and kernel-in-testa) and each species (almond and canarium) to be used for accelerated ageing. Accelerated ageing is commonly used to understand oil oxidation stability where samples are exposed to relatively low temperatures (usually between 40 °C and 60 °C) from a few days to a few weeks compared with roasting temperatures which are usually over 100 °C [12,24,33–36]. Nuts for each treatment and species were placed into pouches (14 cm × 8 cm) and sealed with ambient air. The samples were then placed in an incubator at 45 °C and incubated for 24 days. At days 1, 10 and 24 of incubation, the pouches were removed from the incubator and allowed to reach room temperature. The headspace was then sampled for hexanal concentration. After each sampling, the pouches were re-sealed and placed in the incubator until the next sample collection. At each sampling, approximately 40 mL of headspace gas was removed via a hypodermic needle and passed through an e-nose (OdourScan®). The concentration of hexanal was calculated from standard curves generated by measuring macadamia oil with a known concentration of hexanal.

2.1.2. Experiment 2: Oil Stability of Canarium Kernels Stored as Nut-in-Shell (NIS)

The purple fruit of canarium was de-pulped as described above and then the nut-in-shell (NIS) was dried to a moisture concentration of 10% equivalent to kernel moisture concentration of 5% and randomly placed in seven large containers at ambient temperature (22–31 °C) for 140 days. Six NIS samples were randomly sampled at days 0, 7, 84 and 140 from each container following the incubation. Sampled nuts were prepared as described in Section 2.1.1. of this manuscript [29]. Kernels were stored at 4 °C in air-tight plastic bags before further analysis.

2.2. Oil Extraction and Chemical Analysis

Oil was also extracted from all samples used for Experiment 1 (including both blanched and kernel-in-testa for almond and canarium) on day 1 before commencing accelerated ageing and day 24 after collecting headspace gas. Oil was extracted from Experiment 2 samples collected on days 1, 7, 84 and 140. The samples in each pouch were pooled to constitute one sample per pouch. Thus, five replicate oil samples were obtained for both treatments (blanched vs. kernel-in-testa) of each species (almond and canarium). The samples of each replicate were crushed three times using a garlic crusher and added to 80 mL of pentane. After stirring the mixture for 20 min, the pentane was removed from the oil using an air-tight vacuum rotator, (BÜCHI Labortechnik AG, Flawil, Switzerland), for 15 min. The extracted oil was collected and stored at 4 °C before further analyses.

An OxiTester Touch Analyser (Olive OxiTester, Sw version 1.22, CDR FoodLab®, Ginestra, Fiorentina, Florence, Italy) was used to measure PV and FFA. The required reagents were provided by CDR FoodLab®, and placed into single-usage prefilled cuvettes (PV kit catalogue number F33 and FFA kit catalogue number F300128). No other reagents were used, and oil was directly injected into the prefilled cuvettes [33]. To test PV and FFA, 5 µL and 2.5 µL oil, respectively, were placed in the prefilled cuvettes with relevant reagents and the colour intensity was measured. The colour intensity was measured at 505 nm for PV samples and at 630 nm for FFA samples. The PV was expressed as meqO_2/kg oil and FFA and presented as a percentage of oleic acid.

Samples collected at days 1 and 140 following the incubation in Experiment 1 were assessed for their fatty acid composition as described by Bai et al. [28]. In brief, 1 µL of oil from each replicate was mixed with a dry methanol solution (0.7 mL) containing butylated hydroxytoluene and HCl 32% (25 µL). The mixture was incubated at 65 °C overnight followed by mixing with 0.5 mL of hexane and 0.5 mL of MilliQ water. The methylated fatty acids (FAME) were collected and the fatty acid compositions were determined using Gas Chromatography–Mass Spectrometry (GCMS).

2.3. Statistical Analysis

In Experiment 1: a two-way repeated measures analysis was performed to detect differences in hexanal concentrations between treatments (blanched and kernel-in-testa) and sampling days for each nut species. Significant interactions were observed between treatments (blanched and kernel-in-testa) and sampling time. Therefore, a one-way ANOVA was used to test for differences in hexanal concentrations using six treatments (each treatment/time combination) for each nut species. In Experiment 1, a one-way ANOVA was also used to test for differences in PV and FFA were measured on days 1 and 24 following incubation to test for differences between blanched and kernel-in-testa for each nut species throughout the incubation. In Experiment 2: a one-way analysis of variance (ANOVA) was undertaken to detect differences in PV and FFA concentrations among sampling times followed by a Tukey HSD where significant differences were found. A *t*-test was used to examine changes in the fatty acid composition of canarium kernels between days 1 and 140 following incubation. A regression was also performed between hexanal concentration and PV. All statistical analyses were conducted using SPSS 24 software.

3. Results

3.1. Oil Stability of Blanched and Kernel-in-Testa of Almond and Canarium Using Accelerated Ageing

The blanched almond kernel had significantly higher hexanal concentrations at day 24 than at days 1 and 10 following accelerated ageing (Figure 1a). In contrast, almond kernel-in-testa showed no significant differences in the hexanal concentrations between days 10 and 24 following the incubation (Figure 1a). Almond kernel-in-testa had an 87% increase in hexanal production between days 1 and 10. Canarium kernel-in-testa had significantly lower hexanal concentrations than that of blanched kernels at days 10 and 24 following the incubation (Figure 1b). Hexanal concentration was significantly higher on day 24 than on day 1 for canarium kernels both with and without testa (Figure 1b). There was a 46% increase in hexanal production in canarium kernel-in-testa between days 1 and 10.

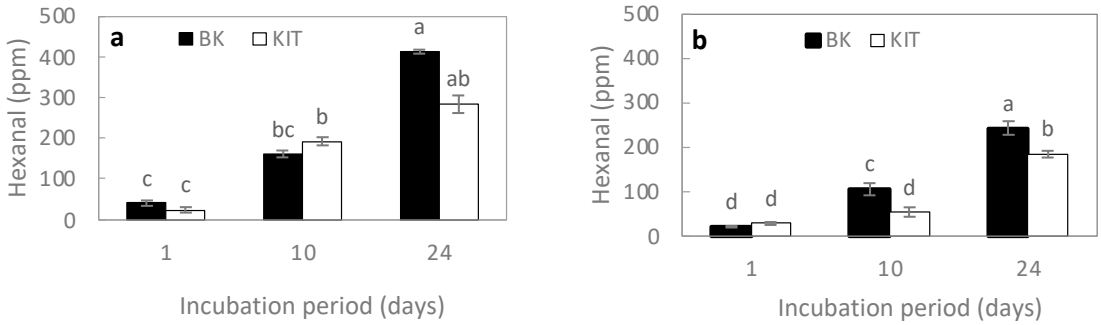


Figure 1. Hexanal concentrations of almond (**a**) and canarium (**b**) for blanched kernels (BK; black columns) and kernel-in-testa (KIT; white columns) at days 1, 10 and 24 following incubation. Lowercase letters indicate significant differences among treatments over the period of study at each nut species (one-way ANOVA; $p < 0.05$).

PV did not differ significantly between blanched and kernel-in-testa on day 1 following incubation (Figure 2). Both almond and canarium kernel-in-testa had significantly lower PV than that of blanched kernels at day 24 following incubation (Figure 2). FFA did not vary significantly between blanched and kernel-in-testa on day 1 following incubation (Figure 3). Blanched kernels in almond had higher FFA than that of kernels-in-testa at day 24 following incubation (Figure 3a). However, FFA of kernels-in-testa in almond did not significantly change between day 1 and 24 following the incubation (Figure 3a). FFA did not vary between blanched kernels and kernels-in-testa of canarium at day 24 following incubation (Figure 3b). However, the FFA of both blanched and kernels-in-testa in canarium were significantly higher on day 24 compared with those on day 1 following the incubation

(Figure 3b). The PV concentrations explained 57% of the variation in hexanal concentrations regardless of nut type (Figure 4).

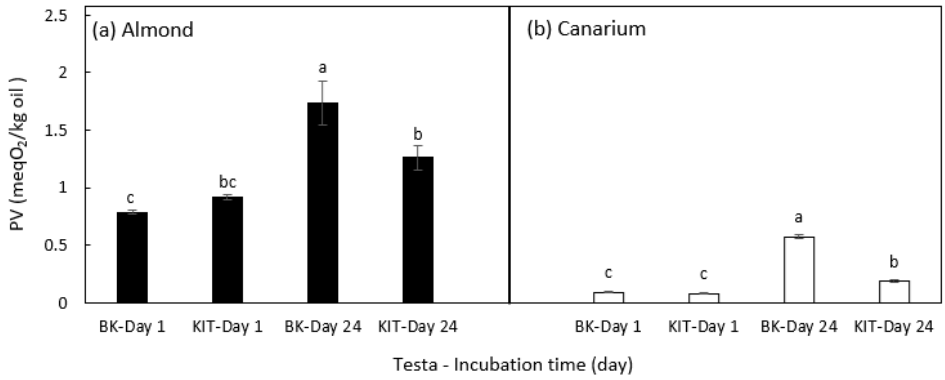


Figure 2. Peroxide value (PV) of (**a**) almond and (**b**) canarium for blanched kernels (BK) and kernel-in-testa (KIT) at days 1 and 24 following incubation. Different lower-case letters indicate significant differences among treatments for each nut species at $p < 0.05$.

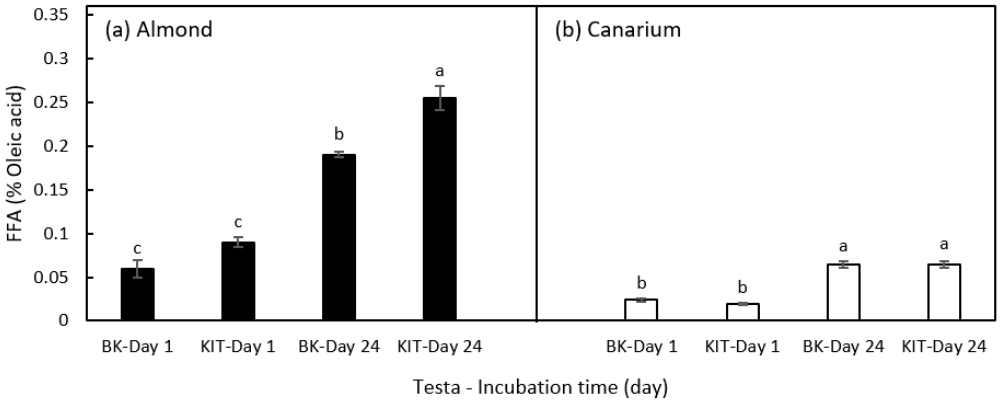


Figure 3. Free fatty acid (FFA) of (**a**) almond and (**b**) canarium for blanched kernels (BK) and kernel-in-testa (KIT) at days 1 and 24 following incubation. Different lower-case letters indicate significant differences among treatments for each nut species at $p < 0.05$.

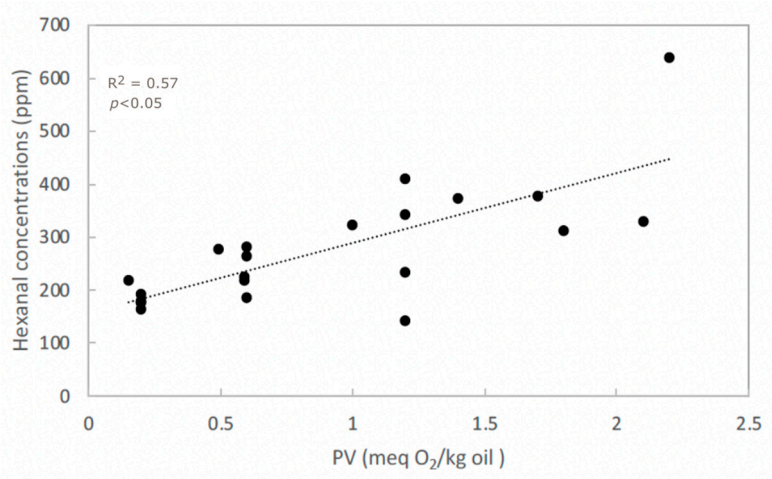
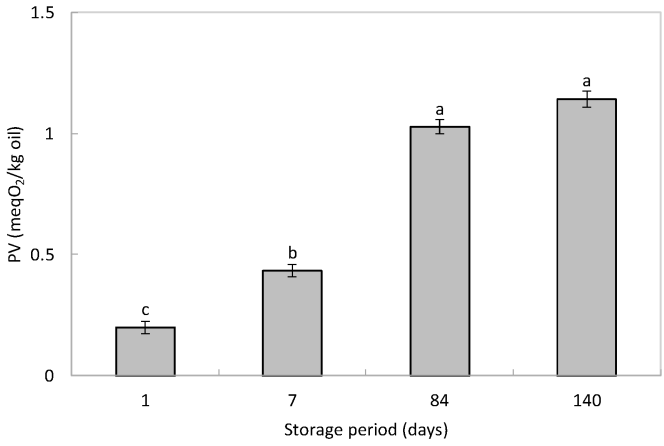


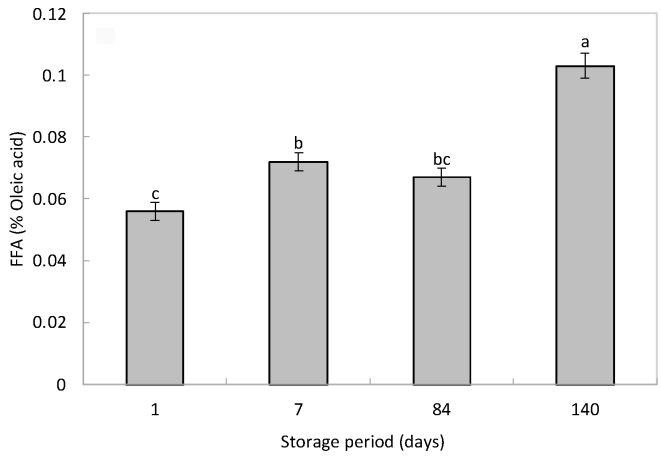
Figure 4. Relationship between peroxide values (PV) and hexanal concentrations.

3.2. Oil Stability of Canarium Kernels Stored as Nut-in-Shell (NIS)

Storage time at ambient temperature significantly increased the PV of kernels (Figure 5a). The initial PV was 0.2 meq O_2/kg oil but increased to 1.14 meq O_2/kg oil after 140 days of storage (Figure 5a). The FFA increased significantly from 0.06% to 0.1% oleic acid after 140 days of storage at ambient temperature (Figure 5b). The concentration of total saturated and total unsaturated fatty acids did not change significantly over the storage period of 140 days (Table 1). The ratio of unsaturated:saturated fatty acid did not significantly vary between day 1 and 140 following the NIS storage (1.15 vs. 1.10, respectively) (Table 1). The C18:1 cis was the dominant fatty acid on both days 1 and 140 following the NIS storage (Table 1).



(**a**)



(**b**)

Figure 5. Peroxide values ((**a**) PV) and free fatty acid ((**b**) FFA) concentrations of canarium kernels stored as nut-in-shell (NIS) over 140 days under ambient conditions. Lower-case letters indicate significant differences among sampling days over the period of study (one-way ANOVA; $p < 0.05$).

Table 1. Fatty acid composition (%), total saturated fatty acid (SFA), mono-unsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA) and total unsaturated fatty acid (USFA) and the USFA:SFA of canarium kernels at days 1 and 140 stored as nut-in-shells. * represents a significant difference between day 1 and 140 of the species of fatty acid concentration.

Fatty Acid Composition	Storage Period (Days)	
	1	140
C16:0 *	27.84 ± 1.28	32.26 ± 0.90
C18:0	18.60 ± 0.64	15.36 ± 0.12
C18:1 *cis*	45.27 ± 1.74	43.26 ± 0.87
C18:1 *trans*	1.23 ± 0.22	1.67 ± 0.24
C18:2	7.07 ± 3.21	7.25 ± 0.47
Total SFA	46.44 ± 0.68	47.62 ± 0.90
Total MUFA	46.50 ± 0.23	45.11 ± 1.04
Total PUFA	7.07 ± 3.21	7.25 ± 0.47
USFA:SFA	1.15 ± 0.03	1.10 ± 0.04

4. Discussion

The presence of the testa slows down oil oxidation in both almond and canarium nuts. In our study, the presence of the testa decreased the oxidative activity of both almond and canarium (as determined by hexanal and PV data) which supported our hypothesis. Our results were consistent with other studies which have also suggested that the testa protects against oxidation subject to different post-harvest processing [23–25]. The presence of the testa is expected to slow down oxidation processes through different mechanisms. For example, the testa is a natural coating on kernels that prevents a direct connection between kernels and air [23,25]. Testa also contains polyphenolic compounds which act as an antioxidant leading to decreased oil oxidation activities [23,25]. It should be noted that nuts usually are stored in a protected environment which then helps to slow down the oil oxidation [2]. In our study, the PV did not exceed the acceptable PV for almond ($\leq$2 meq O_2/kg oil) and canarium ($\leq$3 meq O_2/kg oil) [2] even though the samples were kept under 45 °C for 24 days which suggested both tree nuts had strong oil stability.

Canarium showed generally a slower oil oxidation compared with that of almond. For example, there was a 46% increase in hexanal production in canarium kernel-in-testa compared with an 87% increase in almond kernel-in-testa between days 1 and 10. The ratio of unsaturated:saturated fat in canarium is smaller than that of almond [2,37]. Smaller unsaturated:saturated fat ratio indicates decreased carbon–carbon double bonds leading to increased stability in oils [2]. However, other factors such as antioxidants and environmental factors also play important roles in oil oxidation stability [34]. We observed that almonds had higher moisture concentrations than canarium (5.9% vs. 4.5%, respectively) which may have contributed to faster oil oxidation in almonds compared with that of canarium.

Unexpectedly, FFA formation in almond kernel-in-testa was higher than in the blanched kernels. FFA formation is a result of enzymatic hydrolysis of triacylglycerols [13,38]. Increased kernel moisture concentrations stimulate enzymatic activities leading to increased FFA formation [39,40]. Moisture concentration was not a driving factor because the moisture concentrations of both blanched and kernel-in-testa samples in almond did not differ (5.9% and 6%, respectively) after drying. A higher FFA in kernel-in-testa of almond has also been observed compared with kernel with the testa removed (blanched kernel) after 150 days of storage in another study [39]. FFA can be further degraded to secondary products faster in blanched kernels compared with kernel-in-testa samples which explains why kernel-in-testa samples have higher FFA than blanched samples [39]. FFA concentration of less than 1.0% is acceptable for almond [41] and FFA concentrations are linked to off-flavours in nuts [42]. However, FFA should not be used as a sole factor to study rancidity because low FFA in blanched kernels does not necessarily indicate low oil rancidity and our study also confirmed this.

Our results show that the PV concentrations of canarium increased significantly when kernels were stored long-term as NIS. However, PV remained under threshold values after 140 days of storage. The PV threshold for almond and macadamia is 2 meq O_2/kg oil and 3 meq O_2/kg oil, respectively [2]. There is no established threshold for canarium; however, the threshold accepted for macadamia is currently applied for canarium [43]. In a previous study, PV was reported to be 0.31 meq O_2/kg oil when NIS canarium samples were stored for 11 months under a control temperature of 25 °C [11]. The temperature of the storage site fluctuated between 25 °C and 34 °C throughout the storage (Climate-Data.org: http://en.climatedata.org/region/1958/ accessed on 25 August 2023). PV in our study was over three times higher than those reported by Walton et al. [11] which suggests the importance of low storage temperatures to prolong the shelf life of NIS samples. Although PV increased significantly during the storage period, the maximum PV value of 1.14 meq O_2/kg oil is considered generally low in the food industry. The values of PV $\leq$ 3 meq O_2/kg oil are considered acceptable in the macadamia industry [2]. The kernels were intact in shells, which tended to limit oxygen availability in the nuts, hence, limiting peroxidation. The low moisture concentrations of kernels prior to storage also minimised PV production. Macadamia kernels with a higher moisture concentration prior to drying have shown higher PV than kernels with a lower moisture concentration prior to drying [44]. In our study, initial kernel moisture concentration was consistently low in all samples which could explain low PV production over the 140 days of incubation. Therefore, initial low PV and moisture concentration were critical in kernels to prolong the shelf life of NIS stored over 140 days.

The USFA:SFA ratio remained unchanged between day 1 and day 140 of NIS incubation. Unsaturated fatty acid concentrations usually decrease throughout storage and with heat exposure due to the breakdown of the double bonds that exist in unsaturated fatty acids [2,45]. Generally, linoleic acid, C18:2 is more prone to oxidation than oleic acid [37,45]. In our study, oleic acid, C18:1 *cis*, was the dominant fatty acid in canarium oil. A decreased oleic acid concentration after roasting was reported when the oleic acid concentration is greater than that of linoleic acid [45]. Therefore, in our study, it was possible that a larger amount of oleic acid was affected throughout storage leading to a reduction in its relative concentrations, which in turn could explain relative increases in C16:0 at day 140 following storage. The lack of USFA:SFA ratio changes between day 1 and 140 following the NIS storage suggests strong oil stability throughout the storage.

5. Conclusions

Our results show that both testa and shell can slow oil degradation. Long-term storage of kernel-in-testa could be considered before further processing and packaging to maintain nut quality and prevent oil oxidation. Additionally, storing nuts as nuts-in-shell under ambient conditions prior to further processing and packaging could help to delay oil oxidation and prolong shelf life.

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Data Availability Statement: The data will be made available if requested.

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Conflicts of Interest: The authors declare no conflict of interest. The funding body did not influence on the research either.

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