

Article

Vitamin D Content of Australian Native Food Plants and Australian-Grown Edible Seaweed

Laura J. Hughes ¹, Lucinda J. Black ¹, Jill L. Sherriff ¹ , Eleanor Dunlop ¹ , Norbert Strobel ², Robyn M. Lucas ^{3,4}  and Janet F. Bornman ^{5,*}

¹ School of Public Health, Curtin University, Bentley WA 6102, Australia;

laura.j.hughes@postgrad.curtin.edu.au (L.J.H.); lucinda.black@curtin.edu.au (L.J.B.);

j.sherriff@curtin.edu.au (J.L.S.); eleanor.dunlop@postgrad.curtin.edu.au (E.D.)

² National Measurement Institute, 1/153 Bertie Street, Port Melbourne VIC 3207, Australia;

norbert.strobel@measurement.gov.au

³ National Centre for Epidemiology and Population Health, Research School of Population Health,

The Australian National University, Canberra ACT 2600, Australia; robyn.lucas@anu.edu.au

⁴ Centre for Ophthalmology and Visual Science, University of Western Australia, Perth WA 6009, Australia

⁵ School of Veterinary and Life Sciences, Murdoch University, Murdoch WA 6150, Australia

* Correspondence: janet.bornman@murdoch.edu.au; Tel.: +61-478-473-439

Received: 24 May 2018; Accepted: 3 July 2018; Published: 6 July 2018



Abstract: Vitamin D has previously been quantified in some plants and algae, particularly in leaves of the Solanaceae family. We measured the vitamin D content of Australian native food plants and Australian-grown edible seaweed. Using liquid chromatography with triple quadrupole mass spectrometry, 13 samples (including leaf, fruit, and seed) were analyzed in duplicate for vitamin D₂, vitamin D₃, 25-hydroxyvitamin D₂, and 25-hydroxyvitamin D₃. Five samples contained vitamin D₂: raw wattleseed (*Acacia victoriae*) (0.03 µg/100 g dry weight (DW)); fresh and dried lemon myrtle (*Backhousia citriodora*) leaves (0.03 and 0.24 µg/100 g DW, respectively); and dried leaves and berries of Tasmanian mountain pepper (*Tasmannia lanceolata*) (0.67 and 0.05 µg/100 g DW, respectively). Fresh kombu (*Lessonia corrugata*) contained vitamin D₃ (0.01 µg/100 g DW). Detected amounts were low; however, it is possible that exposure to ultraviolet radiation may increase the vitamin D content of plants and algae if vitamin D precursors are present.

Keywords: liquid chromatography with triple quadrupole mass spectrometry (LC-QQQ); liquid chromatography; triple quadrupole; vitamin D; serum 25-hydroxyvitamin D (25(OH)D); plants; algae

1. Introduction

Approximately a quarter of Australian adults are deficient in vitamin D (serum 25-hydroxyvitamin D (25(OH)D) < 50 nmol/L) [1]. There is seasonal variation in the prevalence of vitamin D deficiency, with 14% of the adult population deficient in summer and 36% in winter [1]. Certain population groups, such as people with dark skin, those wearing covering for religious or cultural reasons, and people living largely indoors, are at greater risk of deficiency due to inadequate sun exposure, particularly in winter months [2]. Although the major source of vitamin D for humans is cutaneous synthesis of vitamin D₃ following exposure of the skin to solar radiation [3], when sun exposure is inadequate to maintain vitamin D status, dietary sources make a small but useful contribution [4].

In the Australian food supply, fish, meat, eggs, dairy, and fortified margarine are known sources of vitamin D₃, while mushrooms are mainly a source of vitamin D₂ and small amounts of vitamins D₃ and D₄ [5,6]. Vitamin D₃ has been found in shiitake mushrooms, a few algae, and several species of Angiosperms (flowering plants) [7] belonging to the plant families of Cucurbitaceae, Fabaceae, Poaceae,

and Solanaceae (Table 1). However, although D₃ is found in several plants, previous results have come mostly from non-edible leaves rather than the edible portions of the different species, such as fruits or seeds. The hydroxylated metabolite of vitamin D₃, 25(OH)D₃, is found in animal products [8–10] and likely has a greater biological activity than vitamin D₃ itself [11,12]. There is some evidence that vitamin D₃ taken orally is more effective than vitamin D₂ at increasing levels of 25(OH)D [13,14].

Vitamin D regulates and maintains critical levels of calcium and phosphates in the skeleton of vertebrates by promoting absorption of these nutrients from the gastrointestinal tract. Rickets, the softening of bones in children (or osteomalacia in adults) due to vitamin D deficiency, has been increasing globally [15,16]. Vitamin D deficiency has also been linked to a number of other health conditions besides bone health, including reduced muscle function, autoimmune disease, cardiovascular disease, and some cancers [17–19].

The anti-rachitic properties of plants were originally discovered by animal feeding studies [20–22], although the active compound in these early studies was later identified as vitamin D₂ produced from fungal contamination, rather than endogenous to the plants. More recently, high-performance liquid chromatography–ultraviolet (HPLC–UV) with mass spectrometry or liquid chromatography–tandem mass spectrometry (LC–MS/MS) have been used to measure vitamin D metabolites directly in the plant matrix [23–25].

Table 1. Content of vitamin D₂, vitamin D₃, and 25-hydroxyvitamin D₃ in plants, microalgae, and macroalgae derived from previously published literature.

Common Name (Botanical Name)	Plant part	Vitamin D ₂ (µg/100 g)	Vitamin D ₃ (µg/100 g)	25(OH)D ₃ (µg/100 g)
Plants				
Tomato (<i>Lycopersicon esculentum</i>)	Leaf	Not tested	78 (DW) ^a	2 (DW) ^a
	Leaf	Not tested	110 (FW) ^b	1.5 (FW) ^b
	Leaf	Not tested	0.17 (DW) ^c	n/d ^c
Waxy leaf nightshade (<i>Solanum glaucophyllum</i>)	Leaf	Not tested	0.32 (DW) ^c	0.08 (DW) ^c
Potato (<i>Solanum tuberosum</i>)	Cell culture derived from leaf material	Not tested	220.00 (FW) ^d	100.00 (FW) ^d
		Not tested	15 (FW) ^b	n/d ^b
Bell pepper (<i>Capsicum annuum</i>)	Leaf	Not tested	n/d ^c	n/d ^c
Day blooming jasmine (<i>Cestrum diurnum</i>)	Leaf	Not tested	10 (DW) ^e	10 (DW) ^e
Zucchini (<i>Cucurbita pepo</i>)	Leaf	Not tested	23 (FW) ^b	Not tested
Alfalfa/Lucerne (<i>Medicago sativa</i>)	Leaf	4.8 (DW) ^f	0.06 (DW) ^f	Not tested
Rimu (<i>Dacrydium cupressinum</i>)	Fruit	70 (DW) ^g	11.5 (DW) ^g	Not tested
Algae				
<i>Microalgae</i>				
Phytoplankton (<i>Pavlova lutheri</i>)	Whole algae	1.9–4.3 (DW) ^h	2.2–14.7 (DW) ^h	Not tested
		5.3 (DW) ⁱ	80.4 (DW) ⁱ	Not tested
		72.4 (DW) ⁱ	271.7 (DW) ⁱ	Not tested
(<i>Tetraselmis suecica</i>)	Whole algae	3900 (DW) ^j	Not tested	Not tested
Marine centric diatom (<i>Skeletonema costatum</i>)	Whole algae	1400 (DW) ^j	Not tested	Not tested
		1100 (DW) ^j	Not tested	Not tested
(<i>Isochrysis galbana</i>)	Whole algae	500 (DW) ^j	Not tested	Not tested
(<i>Chaetoceros calcitrans</i>)	Whole algae	n/d ^j	Not tested	Not tested
<i>Macroalgae</i>				
Japanese Wireweed (<i>Sargassum muticum</i>)	Not specified	90 (DW) ^j	Not tested	Not tested

Decimal places are reported as per the original reference. n/d, not detected; DW: dry weight; FW: fresh weight
^a [26] ^b [24] ^c [25] ^d [23] ^e [27] ^f [28] ^g [29] ^h [30] ⁱ [31] ^j [32].

This paper focuses on some of the Australian native foods in an effort to detect possible new sources of vitamin D in plants and seaweed. The commercial production of native foods across Australia is estimated to have a gross value of more than 15 million Australian dollars [33]. In a recent stocktake of the Australian native plant industry, it was found that production of lemon myrtle and

mountain pepper dominated over other native species, with cultivated supplies of most species [33]. A majority of native food production is used as raw material for value-added products [33].

Given the emerging interest in Australian native food plants for local consumption and export [33], we measured vitamin D₂, vitamin D₃, 25-hydroxyvitamin D₂ (25(OH)D₂), and 25(OH)D₃ in a selection of Australian native food plants and Australian-grown edible seaweed. Because the metabolisms of calcium and vitamin D are closely linked in animals and there is a potential link between calcium and vitamin D metabolism in plants [34], we selected plants and seaweed with known high calcium (Ca) content. These included *Acacia victoriae* (wattleseed; 434 mg Ca/100 g, seeds [35]), *Tasmannia lanceolata* (Tasmanian mountain pepper; 495 and 148 mg Ca/100 g, dried leaves and berries, respectively [35]), *Backhousia citriodora* (lemon myrtle; 1583 mg Ca/100 g, dried leaves [35]), and the seaweeds, *Lessonia corrugata* (kombu; 706 mg Ca/100 g, dried [36]) and *Undaria pinnatifida* (wakame; 1100–3000 and 150 mg Ca/100 g, dried and fresh, respectively [36,37]).

Relatively little detailed research has been carried out on Australian native foods, although the existing studies have shown many of the traditional foods to have high nutrient content. For example, the edible seed of the wattleseed tree (from the family Leguminosae) has a strong nutty or coffee-like flavor and has been included in sweet dishes or as a coffee substitute [33]. It is also a good source of calcium, magnesium, and zinc [35]. The lemon myrtle tree, family Myrtaceae, produces leaves with an intense lemon/lemongrass flavor. These leaves are used fresh or dried as a culinary herb, as a tea, or for use in cosmetics and food flavoring agents in the form of extracted oil [33]. The lemon myrtle leaves are high in calcium, vitamin E, and antioxidants [35]. Mountain pepper (from the family Winteraceae), a shrub native to Tasmania, is a versatile plant with both the berries and leaves being used as a food source [33]. The fresh and dried berries are used as an alternative to traditional pepper, and fresh and dried leaves are used as a culinary herb [33]. Both the leaves and berries have been used as therapeutic agents and as a preservative [33]. The plant is high in vitamin E, folate, and antioxidants with moderate levels of calcium [35]. Finally, two types of kelp seaweeds were selected: kombu (family Lessoniaceae), a native of Tasmania [38]; and wakame (family Alariaceae), an introduced kelp species [38,39], hand-harvested in the wild, mostly in Tasmania. Both kombu and wakame are high in calcium [36,37].

With this initial study, we highlight the possibility of using the selected plants and algae as a natural and additional source of vitamin D for reducing the incidence of vitamin D deficiency in vulnerable population groups. For the vitamin D analyses of the selected species, we used liquid chromatography with triple quadrupole mass spectrometry (LC-QQQ), which has been validated for the detection of low levels of vitamin D metabolites in biological samples [40], modified to suit the complex matrices of plants and algae.

2. Materials and Methods

2.1. Sample Acquisition

Samples of Australian native food plants (wattleseed (*Acacia victoriae*), lemon myrtle (*Backhousia citriodora*), and Tasmanian mountain pepper (*Tasmannia lanceolata*)) and Australian-grown edible seaweeds (wakame (*Undaria pinnatifida*) and kombu (*Lessonia corrugata*)) were sourced from commercial growers or wild harvesters. The selected plants were identified as commonly consumed and commercially available in the Australian food supply [33,35].

The samples were shipped directly from the growers and harvesters to the National Measurement Institute of Australia (NMI), Port Melbourne, Victoria, for preparation and analysis. To maintain their integrity, fresh samples were shipped in an insulated box containing cooler bricks. The details of quantity and source of samples, along with any processing by the growers and harvesters, are outlined in the Supplementary Materials (Table S1).

2.2. Sample Preparation

Upon arrival at NMI, dried samples were stored at room temperature, and fresh samples were stored at $<5\text{ }^{\circ}\text{C}$. Dried and freeze-dried samples were homogenized. Fresh samples were prepared as follows: the leaves were cut to 1 cm squares, and fresh fruit was blended; the weight was recorded; the samples were frozen overnight at $-70\text{ }^{\circ}\text{C}$ and then freeze dried for 48 h to $-50\text{ }^{\circ}\text{C}$ and $<10\text{ mTorr}$; the weight was recorded again; and the freeze-dried factor was determined. Each dried and fresh sample yielded one analytical sample. The prepared samples were stored between $-16\text{ }^{\circ}\text{C}$ and $-20\text{ }^{\circ}\text{C}$ until extraction and analysis.

2.3. Sample Analysis

The instrumentation used was similar to that of Jäpelt et al. [25]. Extraction procedures were derived from published methodology [25,41]. An equivalent deuterated internal standard was added for each vitamin D analogue under investigation: 100 μL of a mixed internal standard solution was added to each sample. This contained 100 ng/mL each of vitamin D₃ [²H₃], vitamin D₂ [²H₃], 25(OH)D₃ [²H₃], and 25(OH)D₂ [²H₃] (Iso Sciences/PM Separations).

The samples were homogenized with 1 g ascorbic acid, 10 mL deionized water, 30 mL absolute ethanol, 2 g potassium hydroxide pellets, and 100 μL of 100 ng/mL deuterated internal standard mix and made to 50 mL with deionized water. The headspace was flushed with nitrogen gas, capped, and placed in a shaker for saponification overnight. The samples underwent centrifugation and 10 mL of the ethanol layer was extracted onto diatomaceous earth Solid Phase Extraction (SPE) cartridges (ChemElute Agilent). The organic soluble compounds were washed off with two 30 mL aliquots of petroleum spirits. The washes were collected into 80 mL glass EPA vials and then evaporated to dryness under high purity nitrogen gas. The residue was reconstituted into 400 μL heptane and transferred to a LC vial containing a 400 μL glass insert. The prepared extracts were stored at $-20\text{ }^{\circ}\text{C}$.

The reagent, PTAD (4-phenyl-1,2,4-triazoline-3,5-dione) reacts non-specifically with dienes under a reaction mechanism called the Diels–Alder Reaction. While in theory, excess PTAD is added to derivatize the vitamin D analogues, samples with high diene content may limit the amount of PTAD available for derivatization. Therefore, where samples were determined to have high diene content, extract clean-up via normal phase chromatography fraction collection was performed. The extracts were inspected for cold precipitate: if present, the liquid extract was transferred to a fresh 400 μL glass insert. Of the remaining liquid extract, 200 μL were injected onto a normal phase chromatographic system with a silica column, 1 mL/min 2% isopropyl alcohol in heptane mobile phase, and a photodiode array detector set to 265 nm. Vitamin D and 25(OH)D fractions were collected.

Fractions of vitamin D and 25(OH)D were combined and evaporated under high purity nitrogen gas. The dry material was reconstituted in 200 μL of dry acetonitrile containing 1 mg/mL of 4-phenyl-1,2,4-triazole-3,5-dione (PTAD) and transferred to a fresh LC vial. Two hours were allowed to complete derivatization. The sample was evaporated under high purity nitrogen gas. The dry material was reconstituted in 100 μL of methanol and water (70:30), transferred to a fresh 400 μL glass insert, and placed into an LC vial. A limit of quantitation was conservatively set at 0.05 $\mu\text{g}/100\text{g}$, which is half of the ‘spiked’ recovery level of 0.1 $\mu\text{g}/100\text{g}$.

The recoveries were determined for each sample analyzed. The recoveries at the 0.1 $\mu\text{g}/100\text{g}$ ‘spiked’ recovery level were as follows: vitamin D₃, 86–104%; vitamin D₂, 76–105%; 25(OH)D₃, 85–114%; and 25(OH)D₂, 90–114%. The recovery for 25-hydroxyvitamin D₃ in wattleseed (roasted/milled/ground seed) could not be determined due to a matrix interference.

The samples were analyzed for vitamin D₂, vitamin D₃, 25(OH)D₂, and 25(OH)D₃ using LC-QQQ (Agilent, San Jose, CA, USA). The calibration samples of vitamin D₂, vitamin D₃, 25(OH)D₂, and 25(OH)D₃ were prepared. The calibration concentrations (in ng/mL) were 0, 2.5, 5, 7.5, 10, 15, 25, 50, 75, and 100. Each calibration sample also contained 10 ng/mL of deuterated internal standard for each vitamere (vitamin D analogue) tested. The calibrations and samples were analyzed using 1290 Infinity Series LC System/6460 Triple Quad liquid chromatography–tandem mass spectrometry

(LC–MS/MS; Agilent Technologies Mulgrave, Victoria, Australia) fitted with a Jet Stream ESI source in positive ion mode using a Supelco Ascentis Express C18 10 cm × 2.1 mm, 2.7 µm LC chromatographic column (Sigma-Aldrich, Sydney Australia).

For each vitamer analyzed, each sample was tested in duplicate, and duplicate values were averaged to obtain one mean value for each sample. A third sample, spiked with the same vitamer, was analyzed for each sample tested to provide quality control data. The mean percentage recovery and mean relative percentage difference were calculated for each vitamer. At the time of writing, the expected limit of detection, post validation study, is expected to be 0.05 µg/100 g (N. Strobel, email communication, 10 October 2017).

The mean recovery percentage across all samples for vitamin D₂, vitamin D₃, 25(OH)D₂, and 25(OH)D₃ was 96%, 98%, 101%, and 94%, respectively. Across all samples, the mean relative percentage difference between duplicate samples for vitamin D₂, vitamin D₃, 25(OH)D₂, and 25(OH)D₃ was 71%, 15%, 50%, and 56%, respectively.

3. Results

Of the 13 samples tested, three contained quantifiable vitamin D metabolites. Vitamin D₂ was found in dried lemon myrtle leaves (0.24 µg/100 g) and the dried leaves and berries of Tasmanian mountain pepper (0.67 and 0.05 µg/100 g, respectively). In addition, three samples contained detectable vitamin D metabolites at levels below the limit of quantitation. Approximate levels are provided for indicative purposes only. Vitamin D₂ was detected in raw wattle seed (≈0.03 µg/100 g) and fresh lemon myrtle leaves (≈0.03 µg/100 g). Vitamin D₃ was detected in fresh kombu (≈0.01 µg/100 g). There were no detectable vitamin D metabolites in the other samples (Table 2).

Table 2. New data on the content (dry weight) of vitamin D₂, vitamin D₃, 25-hydroxyvitamin D₂, and 25-hydroxyvitamin D₃ in Australian native food plants and edible seaweed.

Common name (Botanical name)	Food Type	Part Tested	Vitamin D ₂ (µg/100 g)	Vitamin D ₃ (µg/100g)	25(OH)D ₂ (µg/100 g)	25(OH)D ₃ (µg/100 g)
Wattleseed	Plant	Leaf	<0.05	<0.05	<0.05	<0.05
(<i>Acacia victoriae</i>)		Raw seed	0.03 *	<0.05	<0.05	<0.05
		Roasted, milled seed	<0.05	<0.05	<0.05	<0.05
Tasmanian mountain pepper	Plant	Fresh leaf	<0.05	<0.05	<0.05	<0.05
(<i>Tasmannia lanceolata</i>)		Dried leaf	0.67	<0.05	<0.05	<0.05
		Fresh berries	<0.05	<0.05	<0.05	<0.05
		Dried berries	0.05	<0.05	<0.05	<0.05
Lemon myrtle	Plant	Fresh leaf	0.03*	<0.05	<0.05	<0.05
(<i>Backhousia citriodora</i>)		Dried Leaf	0.24	<0.05	<0.05	<0.05
Wakame	Algae	Fresh upper leaf and central stem	<0.05	<0.05	<0.05	<0.05
(<i>Undaria pinnatifida</i>)		Dried upper leaf and central stem	<0.05	<0.05	<0.05	<0.05
Kombu	Algae	Fresh leaf	<0.05	0.01*	<0.05	<0.05
(<i>Lessonia corrugata</i>)		Dried leaf	<0.05	<0.05	<0.05	<0.05

* This result is below the limit of quantitation and is provided for indicative purposes only.

4. Discussion

We detected low levels of vitamin D₂ in raw wattleseed, dried leaves and fruit of Tasmanian mountain pepper, and fresh and dried lemon myrtle leaves. Although fungal infection was not tested for in our study, the vitamin D₂ content found in the plants may have been due to fungal contamination [42]. Vitamin D₂ is considered a marker for fungal contamination in some crops, such as ryegrass (*Lolium perenne* L.) and hops (*Humulus lupulus* L.) [43,44]. Vitamin D₃ and, in some cases, 25(OH)D₃ have previously been detected in the leaves of tomato [24–26], waxy leaf nightshade [23,25],

potato [24], day blooming jasmine [27], zucchini [24], and alfafa [28]; however, we did not detect these metabolites in our samples of native Australian plants and detected only very low levels in seaweed.

Recently, the fruit of the rimu tree (*Dacrydium cupressinum*), a podocarp native to New Zealand, was found to contain substantial amounts of both vitamin D₂ and D₃ [29]. Measured by isocratic reversed-phase HPLC, the average vitamin D₂ and D₃ contents of rimu berries were reported as 70 µg/100g and 11.5 µg/100g, respectively, although no quality control data were provided. In another study, the precursors of vitamin D₂ and D₃ (ergosterol and 7-dehydrocholesterol, respectively) were detected in plant oils [45]. Other studies have found that the vitamin D₃ and 25-hydroxyvitamin D₃ content of leaves and cell cultures of certain plants increases after ultraviolet (UV) irradiation [25,27,46]. For example, exposure to UV radiation increased the vitamin D₃ content of tomato (*Solanum lycopersicum* L.) leaves by almost 60 times to 100 ng/g, compared with 1.7 ng/g in non-UV-exposed leaves [25]. Future investigations into other potential plant sources of vitamin D and the effect of exposure to UV radiation appear warranted by the finding that consumption of plant oils, particularly UV B-irradiated wheat germ oil, increased 25(OH)D plasma concentration in mice [45].

Sargassum, an edible macroalgae [38], was first discovered to have anti-rachitic properties when the lipid fractions of the algae were fed to rats with induced rickets [47]. Since then, vitamin D₂ and vitamin D₃ have been found in microalgae and macroalgae using high-performance liquid chromatography (HPLC) in much larger quantities than were found in our study [30–32]. Vitamin D metabolites were largely undetected in macroalgae in our study, with the exception of vitamin D₃ in kombu (*Lessonia corrugata*) measured at 0.01 µg/100 g. In other studies, Japanese wireweed (*Sargassum muticum*) was found to contain 90 µg/100 g, while vitamin D₂ and D₃ contents in microalgae ranged from not detected to 3900 µg/100 g and 2.2–271.7 µg/100 g, respectively [30–32]. Ergosterol and 7-dehydrocholesterol have also been found in microalgae [31]. As with plants, it has been suggested that the significant vitamin D content of algae is dependent on exposure to UV radiation [30,48]. The role of UV radiation has been implicated by the finding that microalgae harvested in summer have a higher vitamin D₂ and D₃ content than those harvested in autumn and winter [30]. Although we detected only low levels of vitamin D₃ in kombu and no vitamin D₂ or vitamin D₃ in wakame, the algae tested in our study were harvested in the winter months and were not sundried or exposed to UV radiation after harvest.

Plant and algal matrices present challenges for the quantification of vitamin D₂, vitamin D₃, 25(OH)D₂, and 25(OH)D₃, due in part to the presence of interfering compounds such as chlorophyll and lipophilic pigments [48]. Therefore, any method used must be highly sensitive and selective [48]. When compared to single mass spectrometry (MS), LC-QQQ has higher sensitivity and selectivity when applied to the detection of pesticides in water and soil samples [49]. To our knowledge, this method has not been used previously to detect vitamin D metabolites in complex plant and algal matrices and is a major strength of our study due to the low detection limits of the instrumentation. The mean recovery from all spiked samples in our study was high, indicating that LC-QQQ is highly accurate in detecting low levels of vitamin D in plant and algal matrices. All samples were measured in duplicate, and where possible, we tested the edible portion in addition to the leaf material. However, we tested only a few species of Australian native food plants and Australian-grown edible seaweed. Although regional and seasonal variation have been shown to influence the vitamin D content of plants [43,48], we analyzed only single samples sourced from single locations and during months of relatively low UV radiation levels.

In conclusion, this study has demonstrated the high sensitivity of LC-QQQ methodology, which will be used in future studies in search of natural dietary sources of vitamin D. Our results show that the selected Australian native plants and algae have very low levels of vitamin D. However, given that the vitamin D precursors, ergosterol and 7-dehydrocholesterol, have previously been found in both plants and algae, testing the effect of exposure to UV radiation on the vitamin D content of Australian native food plants and Australian-grown edible seaweed is warranted. Also, a larger sample size across a greater number of plant species and habitats will increase the likelihood of identifying nutritionally

relevant amounts of vitamin D. An important factor in future studies will be taking the seasonal effect of vitamin D into account in natural ecosystems, because this will relate back to the levels of solar UV radiation for potentially increasing vitamin D levels.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2072-6643/10/7/876/s1>, Table S1: Description of plant and algae samples included in the current study.

Author Contributions: J.F.B., R.M.L., J.L.S., and L.J.B. conceived of and designed the study; L.J.H. sourced the samples and wrote the paper; N.S. conducted laboratory analyses; L.J.B., J.L.S., J.F.B., R.M.L., E.D., and N.S. provided critical revision of the manuscript for important intellectual content. All authors are in agreement, and this material has not been published elsewhere.

Funding: This work was supported by the School of Public Health, Curtin University. RML is supported by a National Health and Medical Research Council of Australia Senior Research Fellowship.

Acknowledgments: The authors would like to thank the following growers, producers, and wild harvesters for supplying samples for testing: Australian Rainforest Products, Diemen Pepper Supplies, Kai Ho/Ocean Treasure, and Mootooroo.

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

References

1. Australian Bureau of Statistics. *Australian Health Survey: Biomedical Results for Nutrients, 2011–2012*; ABS: Canberra, Australia, 2014.
2. Nowson, C.A.; Mason, R.S. Vitamin D and health in adults in Australia and New Zealand. *Med. J. Aust.* **2013**, *199*, 394. [[CrossRef](#)] [[PubMed](#)]
3. Mann, J.; Truswell, A.S. *Essentials of Human Nutrition*, 4th ed.; Oxford University Press: Oxford, UK, 2012.
4. Liu, J. Vitamin D content of food and its contribution to vitamin D status: A brief overview and Australian focus. *Photochem. Photobiol. Sci.* **2012**, *11*, 1802–1807. [[CrossRef](#)] [[PubMed](#)]
5. Urbain, P.; Valverde, J.; Jakobsen, J. Impact on vitamin D₂, vitamin D₄ and agaritine in *Agaricus bisporus* mushrooms after artificial and natural solar UV light exposure. *Plant Foods Hum. Nutr.* **2016**, *71*, 314–321. [[CrossRef](#)] [[PubMed](#)]
6. Keegan, R.J.H.; Lu, Z.; Bogusz, J.M.; Williams, J.E.; Holick, M.F. Photobiology of vitamin D in mushrooms and its bioavailability in humans. *Dermato-Endocrinol.* **2013**, *5*, 165–176. [[CrossRef](#)] [[PubMed](#)]
7. Boland, R.; Skliar, M.; Curino, A.; Milanesi, L. Vitamin D compounds in plants. *Plant Sci.* **2003**, *164*, 357–369. [[CrossRef](#)]
8. Dunlop, E.; Cunningham, J.; Sherriff, J.L.; Lucas, R.M.; Greenfield, H.; Arcot, J.; Strobel, N.; Black, L.J. Vitamin D(3) and 25-hydroxyvitamin D(3) content of retail white fish and eggs in Australia. *Nutrients* **2017**, *9*, 647. [[CrossRef](#)] [[PubMed](#)]
9. Liu, J.; Greenfield, H.; Strobel, N.; Fraser, D.R. The influence of latitude on the concentration of vitamin D₃ and 25-hydroxy-vitamin D₃ in Australian red meat. *Food Chem.* **2013**, *140*, 432–435. [[CrossRef](#)] [[PubMed](#)]
10. Liu, J.; Greenfield, H.; Fraser, D.R. An exploratory study of the content of vitamin D compounds in selected samples of Australian eggs. *Nutr. Diet* **2014**, *71*, 46–50. [[CrossRef](#)]
11. Cashman, K.; Seamans, K.; Lucey, A.; Stöcklin, E.; Weber, P.; Kiely, M.; Hill, T. Relative effectiveness of oral 25-hydroxyvitamin D₃ and vitamin D₃ in raising wintertime serum 25-hydroxyvitamin D in older adults. *Am. J. Clin. Nutr.* **2012**, *95*, 1350–1356. [[CrossRef](#)] [[PubMed](#)]
12. Ovesen, L.; Brot, C.; Jakobsen, J. Food contents and biological activity of 25-hydroxyvitamin D: A vitamin D metabolite to be reckoned with? *Ann. Nutr. Metab.* **2003**, *47*, 107–113. [[CrossRef](#)] [[PubMed](#)]
13. Lehmann, U.; Hirche, F.; Stangl, G.I.; Hinz, K.; Westphal, S.; Dierkes, J. Bioavailability of vitamin D(2) and D(3) in healthy volunteers, a randomized placebo-controlled trial. *J. Clin. Endocrinol. Metab.* **2013**, *98*, 4339–4345. [[CrossRef](#)] [[PubMed](#)]
14. Itkonen, S.T.; Skaffari, E.; Saaristo, P.; Saarnio, E.M.; Erkkola, M.; Jakobsen, J.; Cashman, K.D.; Lamberg-Allardt, C. Effects of vitamin D₂-fortified bread v. supplementation with vitamin D₂ or D₃ on serum 25-hydroxyvitamin D metabolites: An 8-week randomised-controlled trial in young adult Finnish women. *Br. J. Nutr.* **2016**, *115*, 1232–1239. [[CrossRef](#)] [[PubMed](#)]
15. Prentice, A. Nutritional rickets around the world. *J. Steroid Biochem. Mol. Biol.* **2013**, *136*, 201–206. [[CrossRef](#)] [[PubMed](#)]

16. Uday, S.; Hogler, W. Nutritional rickets and osteomalacia in the twenty-first century: Revised concepts, public health, and prevention strategies. *Curr. Osteoporos. Rep.* **2017**, *15*, 293–302. [[CrossRef](#)] [[PubMed](#)]
17. Wintermeyer, E.; Ihle, C.; Ehnert, S.; Stockle, U.; Ochs, G.; de Zwart, P.; Flesch, I.; Bahrs, C.; Nussler, A.K. Crucial role of vitamin D in the musculoskeletal system. *Nutrients* **2016**, *8*, 319. [[CrossRef](#)] [[PubMed](#)]
18. Lucas, R.M.; Norval, M.; Neale, R.E.; Young, A.R.; de Gruijl, F.R.; Takizawa, Y.; van der Leun, J.C. The consequences for human health of stratospheric ozone depletion in association with other environmental factors. *Photochem. Photobiol. Sci.* **2015**, *14*, 53–87. [[CrossRef](#)] [[PubMed](#)]
19. Bais, A.F.; Lucas, R.M.; Bornman, J.F.; Williamson, C.E.; Sulzberger, B.; Austin, A.T.; Wilson, S.R.; Andradý, A.L.; Bernhard, G.; McKenzie, R.L.; et al. Environmental effects of ozone depletion, UV radiation and interactions with climate change: UNEP Environmental Effects Assessment Panel, Update 2017. *Photochem. Photobiol. Sci.* **2018**, *17*, 127–179. [[CrossRef](#)] [[PubMed](#)]
20. Hess, A.F.; Weinstock, M. Antirachitic properties imparted to inert fluids and to green vegetables by ultra-violet irradiation. *J. Biol. Chem.* **1924**, *62*, 301–313.
21. Bechtel, H.E.; Huffman, C.F.; Ducan, C.W.; Hoppert, C.A. Vitamin D studies in cattle: IV. Corn silage as a source of vitamin D for dairy cattle. *J. Dairy Sci.* **1936**, *19*, 359–372. [[CrossRef](#)]
22. Wasserman, R.H.; Corradino, R.A.; Krook, L.; Hughes, M.R.; Haussler, M.R. Studies on the 1 α , 25-dihydroxycholecalciferol-like activity in a calcinogenic plant, *Cestrum diurnum*, in the chick. *J. Nutr.* **1976**, *106*, 457–465. [[CrossRef](#)] [[PubMed](#)]
23. Aburjai, T.; Bernasconi, S.; Manzcocchi, L.; Pelizzoni, F. Isolation of 7-dehydrocholesterol from cell cultures of *Solanum malacoxylon*. *Phytochemistry* **1996**, *43*, 773–776. [[CrossRef](#)]
24. Aburjai, T.; Al-Khalil, S.; Abuirjeie, M. Vitamin D₃ and its metabolites in tomato, potato, egg plant and zucchini leaves. *Phytochemistry* **1998**, *49*, 2497–2499. [[CrossRef](#)]
25. Jäpelt, R.B.; Silvestro, D.; Smedsgaard, J.; Jensen, P.E.; Jakobsen, J. Quantification of vitamin D₃ and its hydroxylated metabolites in waxy leaf nightshade (*Solanum glaucophyllum* desf.), tomato (*Solanum lycopersicum* L.) and bell pepper (*Capsicum annuum* L.). *Food Chem.* **2013**, *138*, 1206–1211. [[CrossRef](#)] [[PubMed](#)]
26. Prema, T.P.; Raghuramulu, N. Vitamin D₃ and its metabolites in the tomato plant. *Phytochemistry* **1996**, *42*, 617–620. [[CrossRef](#)]
27. Prema, T.P.; Raghuramulu, N. Free vitamin D₃ metabolites in *Cestrum diurnum* leaves. *Phytochemistry* **1994**, *37*, 677–681. [[CrossRef](#)]
28. Horst, R.L.; Reinhardt, T.A.; Russell, J.R.; Napoli, J.L. The isolation and identification of vitamin D₂ and vitamin D₃ from *Medicago sativa* (alfalfa plant). *Arch. Biochem. Biophys.* **1984**, *231*, 67–71. [[CrossRef](#)]
29. von Hurst, P.R.; Moorhouse, R.J.; Raubenheimer, D. Preferred natural food of breeding kakapo is a high value source of calcium and vitamin D. *J. Steroid Biochem. Mol. Biol.* **2016**, *164*, 177–179. [[CrossRef](#)] [[PubMed](#)]
30. Takeuchi, A.; Toshio, O.; Makoto, T.; Tadashi, K. Possible origin of extremely high contents of vitamin D₃ in some kinds of fish liver. *Comp. Biochem. Physiol. A* **1991**, *100*, 483–487.
31. Sunita Rao, D.; Raghuramulu, N. Food chain as origin of vitamin D in fish. *Comp. Biochem. Physiol. A* **1996**, *114*, 15–19. [[CrossRef](#)]
32. de Roeck-Holtzhauer, Y.; Quere, I.; Claire, C. Vitamin analysis of five planktonic microalgae and one macroalga. *J. App. Phycol.* **1991**, *3*, 259–264. [[CrossRef](#)]
33. Clarke, M. *Australian Native Food Industry Stocktake*; Rural Industries Research Development Corporation in Association with ANFIL: Canberra, Australia, 2012.
34. Black, L.J.; Lucas, R.M.; Sherriff, J.L.; Björn, L.O.; Bornman, J.F. In pursuit of vitamin D in plants. *Nutrients* **2017**, *9*, 136. [[CrossRef](#)] [[PubMed](#)]
35. Konczak, I.; Zabarás, D.; Dunstan, M.; Aguas, P.; Roulfe, P.; Pavan, A. *Health Benefits of Australian Native Foods—An Evaluation of Health-Enhancing Compounds*; Rural Industries Research and Development Corporation: Canberra, Australia, 2009.
36. US Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory. USDA National Nutrient Database for Standard Reference, Legacy. 2018. Available online: <https://www.ars.usda.gov/northeast-area/beltsville-md-bhnrc/beltsville-human-nutrition-research-center/nutrient-data-laboratory/docs/usda-national-nutrient-database-for-standard-reference/> (accessed on 1 June 2018).

37. Mabeau, S.; Fleurence, J. Seaweed in food-products - biochemical and nutritional aspects. *Trends Food Sci. Technol.* **1993**, *4*, 103–107. [[CrossRef](#)]
38. Sanderson, J.C.; Di Benedetto, R. *Tasmanian Seaweeds for the Edible Market*; Marine Laboratory: Taroona, Tasmania, Australia, 1988.
39. Sanderson, J. A preliminary survey of the distribution of the introduced macroalga, *Undaria pinnatifida* (Harvey) Suringer on the east coast of Tasmania, Australia. *Bot. Mar.* **1990**, *33*, 153–158. [[CrossRef](#)]
40. Adamec, J.; Jannasch, A.; Huang, J.; Hohman, E.; Fleet, J.C.; Peacock, M.; Ferruzzi, M.G.; Martin, B.; Weaver, C.M. Development and optimization of an LC-MS/MS-based method for simultaneous quantification of vitamin D2, vitamin D3, 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3. *J. Sep. Sci.* **2011**, *34*, 11–20. [[CrossRef](#)] [[PubMed](#)]
41. AOAC International. Cholecalciferol (vitamin D3) in selected foods. In *Official Methods of Analysis of AOAC International*, 20th ed.; AOAC International: Atlanta, GA, USA, 2016.
42. Jäpelt, R.B.; Jakobsen, J. Vitamin D in plants: A review of occurrence, analysis, and biosynthesis. *Front. Plant Sci.* **2013**, *4*, 136. [[CrossRef](#)] [[PubMed](#)]
43. Jäpelt, R.B.; Didion, T.; Smedsgaard, J.; Jakobsen, J. Seasonal variation of provitamin D2 and vitamin D2 in perennial ryegrass (*Lolium perenne* L.). *J. Agric. Food Chem.* **2011**, *59*, 10907–10912. [[CrossRef](#)] [[PubMed](#)]
44. Magalhães, P.; Carvalho, D.; Guido, L.; Barros, A. Detection and quantification of provitamin D2 and vitamin D2 in hop (*Humulus lupulus* L.) by liquid chromatography-diode array detection-electrospray ionization tandem mass spectrometry. *J. Agric. Food Chem.* **2007**, *55*, 7995–8002. [[CrossRef](#)] [[PubMed](#)]
45. Baur, A.C.; Brandsch, C.; König, B.; Hirche, F.; Stangl, G.I. Plant oils as potential sources of vitamin D. *Front. Nutr.* **2016**, *3*, 29. [[CrossRef](#)] [[PubMed](#)]
46. Curino, A.; Milanesi, L.; Benassati, S.; Skliar, M.; Boland, R. Effect of culture conditions on the synthesis of vitamin D(3) metabolites in *Solanum glaucophyllum* grown in vitro. *Phytochemistry* **2001**, *58*, 81. [[CrossRef](#)]
47. Darby, H.H.; Clarke, H.T. The plant origin of a vitamin D. *Science* **1937**, *85*, 318–319. [[CrossRef](#)] [[PubMed](#)]
48. Jäpelt, R.B.; Silvestro, D.; Smedsgaard, J.; Jensen, P.E.; Jakobsen, J. LC-MS/MS with atmospheric pressure chemical ionisation to study the effect of UV treatment on the formation of vitamin D3 and sterols in plants. *Food Chem.* **2011**, *129*, 217–225. [[CrossRef](#)]
49. Schreiber, A. Advantages of using triple quadrupole over single quadrupole mass spectrometry to quantify and identify the presence of pesticides in water and soil samples. *Sciex Concord Ontarion* **2010**, *1*, 1–6.



© 2018 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).