biology and life sciences forum

MDPI

Proceeding Paper

Functional Foods or Over-Hyped? Observations on the Antioxidant and Phenolic Content of Australian Foodstuffs †

Joel B. Johnson [1,2,*], Janice S. Mani [1], Ryan J. Batley [1], Beatriz E. Hoyos [1], Nicola Novello [1], Parbat Raj Thani [1], Charitha Priyadarshani Ekanayake Arachchige [1], Pasmita Neupane [1] and Mani Naiker [1]

[1] School of Health, Medical and Applied Sciences, Central Queensland University, North Rockhampton, QLD 4701, Australia; email1@email.com (J.S.M.); email2@email.com (R.J.B.); email3@email.com (B.E.H.); email4@email.com (N.N.); email5@email.com (P.R.T.); email6@email.com (C.P.E.A.); email7@email.com (P.N.); m.naiker@cqu.edu.au (M.N.)

[2] Centre for Nutrition and Food Sciences, Queensland Alliance for Agriculture and Food Innovation (QAAFI), The University of Queensland, Brisbane, QLD 4067, Australia

* Correspondence: joel.johnson@cqumail.com

† Presented at the 4th International Electronic Conference on Foods, 15–30 October 2023; Available online: https://foods2023.sciforum.net/.

Abstract: Consumers are showing increasing awareness of the concept of 'functional foods': foods which can provide health benefits in addition to their nutritional value. There is particular demand for foods with a high antioxidant and phenolic content, which may improve cardiovascular health, reduce inflammation and slow or prevent the onset of chronic, non-communicable diseases. However, there is a lack of comprehensive databases using consistent analytical protocols to analyse the antioxidant and phenolic content of different food types—particularly in regional areas such as Australia. Over the past four years, our laboratory has analysed over 1000 food-related samples using several antioxidant capacity assays (ferric reducing antioxidant power—FRAP—and cupric reducing antioxidant capacity—CUPRAC), as well as the total phenolic content (TPC) by the Folin-Ciocalteu method. Here, we provide a summary of this data by different food types, to inform researchers, policy planners, nutritionists, and consumers about the typical levels of antioxidants and total phenolics found across a range of Australian foodstuffs, particularly grains. The highest antioxidant and phenolic contents were typically found in native Australian fruits, while grains, nuts and non-native fruits showed lower antioxidant and phenolic contents. Spices, processed foodstuffs, and non-fruit native Australian foods showed an intermediate content. Furthermore, medicinally used plants showed a much higher phenolic content and antioxidant capacity compared to non-medicinal plants. Finally, we present correlations between the various analytes.

Keywords: phytochemicals; total phenolic content; antioxidant capacity; correlation; health benefits; bioactives

Citation: Johnson, J.B.; Mani, J.S.; Batley, R.J.; Hoyos, B.E.; Novello, N.; Thani, P.R.; Arachchige, C.P.E.; Neupane, P.; Naiker, M. Functional Foods or Over-Hyped? Observations on the Antioxidant and Phenolic Content of Australian Foodstuffs. *Biol. Life Sci. Forum* **2023**, *26*, x. https://doi.org/10.3390/xxxxx

Academic Editor(s): Name

Published: date

1. Introduction

There are contradictory opinions in the scientific literature about the true health benefits of antioxidant compounds and polyphenols. Numerous authors have argued that total antioxidant activity is not a good indicator of food quality or health benefits [1,2]. On the other hand, numerous epidemiological studies indicate a strong correlation between antioxidant and/or polyphenol intake and reduced risk of chronic disease, particularly cardiovascular-related conditions [3–7].

Further complicating the issue, other authors suggest that antioxidants may not be beneficial in their isolated forms, but do provide health benefits in their endogenous forms, where there is a mix of phytochemicals present in a natural matrix [8].

A recent study suggested that dietary total antioxidant capacity (DTAC), as measured by the ferric reducing antioxidant potential (FRAP) method, could be considered an indicator of healthy diet quality [3]. Consequently, establishing databases of the typical phenolic and antioxidant contents of common foodstuffs is an important step toward establishing the potential health benefits of different food groups [2].

This study aims to contribute to that aim, by providing a retrospective analysis of the phytochemical content of foodstuffs and related samples analysed by our laboratory.

2. Materials and Methods

2.1. Samples

Data from a broad range of samples are included in this study, principally plant-based foods or foodstuffs grown in Australia. These samples were procured from various sources and analysed in our laboratory over a four-year period between 2019 and 2023. Table 1 provides an overview of the sample types and numbers included in the dataset.

Table 1. Summary of the sample types investigated in this study.

Category	Subcategory	No. Samples
Foodstuffs	Edible leaves	2
	Fruit	18
	Grain	519
	Native food (non-fruit)	19
	Native fruit	18
	Nuts	36
	Processed foodstuff	5
	Spice	271
	Vegetable	10
Animal foodstuffs	Animal supplement	5
	Livestock fodder	298
Medicinal plants	Medicinal plant (non-Australian)	14
	Medicinal supplement (plant-based)	2
	Native medicinal plant	60
Other samples (non-edible)	Byproduct (of food)	52
	Native plant	29
	Root	34

2.2. Sample Processing

Fresh plant samples were washed with distilled water. Vitamin C extraction was performed on selected samples, using fresh material. The remainder of the material was freeze-dried using an FTS Flexidry system (−50 °C, 50 mT); a few of the sample types were oven-dried at low temperatures (<60 °C).

For most samples, the moisture content was recorded from the loss in mass upon drying and calculated as a percentage of the original sample (by weight).

The dried material was ground to a fine, homogenous flour, typically using a Breville Coffee & Spice Grinder (Botany, NSW, Australia), and stored in darkness at 4 °C until used for further chemical analysis.

2.3. Measurement of Vitamin C Content

After extraction with 3% *w*/*v* metaphosphoric acid, the vitamin C content of selected samples was measured on an Agilent 1100 HPLC-DAD system, as previously reported [9]. Results were expressed as mg per 100 g of sample.

2.4. Measurement of Phytochemical Composition

Polar phenolic compounds were extracted with 90% methanol, following the protocol described in Johnson, et al. [10], using a sample-solvent ratio of around 1:15 (typically a sample mass of ~1 g and a final volume of 14–15 mL). While the sample masses extraction volumes varied between sample types (depending on the mass of each sample available for analysis), the steps and times in the extraction protocol were kept consistent. Extractions and subsequent assays were performed in duplicate for each sample.

The TPC, FRAP, CUPRAC and TMAC were analysed following the methods described in Johnson, et al. [10]. As a further measure of antioxidant activity, the ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) decolourisation assay was conducted in selected samples using the methods of Re, et al. [11].

Results for TPC were expressed in gallic acid equivalents (GAE), results for FRAP, CUPRAC and ABTS in Trolox equivalents (TE), and results for TMAC in cyanidin-3-glucoside equivalents (C3G); all expressed as mg per 100 g of original sample material (dry weight basis—DW).

2.5. Measurement of Protein Content

The crude protein content was measured on a selection of samples using LECO TruMac Series Carbon and Nitrogen Analyser (LECO, USA); protein content was calculated using an appropriate conversion factor (typically 6.25, but dependent upon the specific foodstuff type) [12].

2.6. Statistical Analysis

Statistical tests were performed on the phytochemical and phenolic data using R Studio running R 4.0.5 [13]. Where applicable, results are presented as mean ± 1 standard deviation. A significance value of $p \leq 0.05$ was taken as statistically significant.

3. Results and Discussion

3.1. Antioxidant Contents of Different Foodstuffs

As shown in Table 2, there was an extensive range of variation in the composition of different foodstuffs and related groups. Although this was not aimed to be a comprehensive or strictly representative study, the categories with larger sample sizes (see Table 1) are likely to be reasonably representative of the category in general.

Overall, the highest TPC values were found for the native Australian fruit (mean of 8500 mg GAE/100 g DW), plant-based medicinal/herbal supplements (6000 mg/100 g), non-Australian medicinal plants (3850 mg GAE/100 g), and native Australian medicinal plants (2500 mg/100 g). Among other common foodstuffs, fruits, grains, nuts, and vegetables tended to show a low TPC (140–300 mg GAE/100 g), while processed foodstuffs, native Australian bushfoods (excluding native fruit) and spices showed a moderate TPC (550–1400 mg GAE/100 g).

Similarly, the highest FRAP values were found in native Australian fruit (mean of 17,700 mg TE/100 g DW), followed by plant-based herbal supplements (6300 mg TE/100 g), native Australian medicinal plants (4800 mg TE/100 g) and non-Australian medicinal plants (4700 mg TE/100 g). Most common foodstuff groups (e.g., nuts, grain, fruit) showed a relatively low FRAP (90–410 mg TE/100 g), while with moderate values seen in spices and Australian bushfoods (700–900 mg TE/100 g). Interestingly, the processed foodstuffs included in this study contained a higher average FRAP (2100 mg TE/100 g), although this may not be the case for all processed foods.

The CUPRAC was also highest for native Australian fruit (76,400 mg TE/100 g DW), followed by Australian medicinal plants (17,500 mg TE/100 g), other native Australian plants (12,500 mg TE/100 g), non-Australian medicinal plants (10,600 mg TE/100 g) and food by-products (10,300 mg TE/100 g).

Table 2. Average content of total phenolics, antioxidants, anthocyanins, moisture, protein, and vitamin C in different groups of Australian foodstuffs and related samples. Results are given on a dry-weight basis (mean ± SD). See Table 1 for sample sizes.

Category	Subcategory	TPC (mg GAE/100 g)	FRAP (mg TE/100 g)	CUPRAC (mg TE/100 g)	TMAC (mg C3G/100 g)	Moisture (%)	Protein (%)	ABTS (mg TE/100 g)	Vitamin C (mg/100 g)
Foodstuffs	Edible leaves	2666 ^	2471 ± 1054	10,470 ± 3706	60 ^	-	19 ^	-	-
	Fruit	268 ± 534	414 ± 914	1659 ± 2379	22 ± 78	80 ± 23	8 ^	617 ± 659	99 ± 63
	Grain	251 ± 299	182 ± 258	720 ± 837	9 ± 7	10 ± 2	24 ± 5	-	-
	Native food (non-fruit)	858 ± 594	711 ± 582	4573 ± 1070	28 ± 33	58 ± 14	-	-	54 ± 24
	Native fruit	8486 ± 6205	17,735 ± 18,745	76,412 ± 42,402	29 ± 55	65 ± 25	-	6008 ± 7993	290 ± 178
	Nuts	139 ± 18	89 ± 16	138 ± 18	-	-	27 ± 2	-	-
	Processed foodstuff	548 ± 552	2093 ± 1446	2914 ± 2231	124 ± 176	-	-	-	7 ± 1
	Spice	1362 ± 620	896 ± 1231	3070 ± 2454	14 ± 9	49 ± 40	-	-	-
	Vegetable	304 ± 102	213 ± 178	3129 ± 1840	2 ± 4	82 ± 11	4 ^	1837 ± 1708	55 ± 49
Animal foodstuffs	Animal supplement	1385 ± 1124	448 ± 194	2980 ± 2015	-	-	-	-	-
	Livestock fodder	1022 ± 562	754 ± 387	2931 ± 1695	19 ± 13	10 ^	20 ± 6	-	-
Medicinal plants	Medicinal plant (non-Australian)	3846 ± 2841	4686 ± 5998	10,553 ± 10,183	5 ± 11	-	-	-	-
	Medicinal supplement (plant-based)	6025 ± 1719	6284 ^	7153 ± 3277	-	-	-	-	-
	Native medicinal plant	2493 ± 1667	4776 ± 4764	17,501 ± 18,279	-	50 ± 16	-	-	-
Other samples (non-edible)	Byproduct (of food)	811 ± 1263	1083 ± 1917	10,281 ± 5801	9 ± 14	-	13 ± 2	1290 ± 541	296 ± 335
	Native plant	1501 ± 1035	2569 ± 4169	12,498 ± 13,529	6 ± 8	31 ± 11	-	-	54 ± 74
	Root	390 ± 119	467 ± 175	390 ± 119	-	-	7 ± 1	-	-

A dash (-) indicates no data (not tested). ^ SD cannot be calculated, as only one sample was measured for this analyte.

Anthocyanins, as measured by TMAC, were most abundant in processed foodstuffs, although there was a very high level of variability. Among non-processed foods, the highest TMAC values were seen for native fruits, native non-fruit foods, and commercial fruits.

The remaining parameters (moisture, protein, ABTS and vitamin C) were only measured in a smaller selection of the samples. However, most sample classes fell into fairly clear groups such as low moisture content (grain and fodder), moderate moisture content (native plants, spices, native foods and native fruit), and high moisture content (fruit and vegetables). Similarly, low-protein content (<10%) classes included vegetables (one sample), roots, fruit (one sample) and food by-products, while a high protein content (>20%) was found in the grain and nut samples.

Similar to the FRAP and CUPRAC assays, much higher ABTS values were found for native Australian fruit compared to introduced, commercial fruits (6000 vs. 600 mg TE/100 g, respectively). Finally, a low average vitamin C content (<10 mg/100 g) was found in processed foodstuffs; a moderate content (~50 mg/100 g) in vegetables, native bushfood, and native (non-food) plants; and a high vitamin C content (~300 mg/100 g) in food by-products and native Australian fruit.

Of particular note are the considerably higher TPC and antioxidant capacity observed among medicinal plants (both international and Australian species), compared to other plants. This supports previous proposals that the medicinal properties of these plants may be mediated in part by their antioxidant-active compounds [4].

Additionally, it was noted that the native Australian medicinal plants showed a lower average TPC compared to their international counterparts, but a higher antioxidant capacity (as measured by FRAP and CUPRAC).

3.2. Correlation between Different Analytes

As seen in Table 3, there was a very strong positive linear correlation between the TPC, FRAP, and CUPRAC across all sample types. The strongest correlation was seen between TPC and CUPRAC ($r_{1094} = 0.900$, $p < 0.001$), while the correlation strength was similar between FRAP and TPC ($r_{1304} = 0.845$, $p < 0.001$), and between FRAP and CUPRAC were similar ($r_{1097} = 0.848$, $p < 0.001$). Numerous previous studies have reported positive correlations between TPC and antioxidant capacity [14–16], albeit to varying extents. However, this study confirms the strong positive correlation between these assays, for a very large number of samples (>1000) across a wide range of matrix types. One benefit of only using data from our laboratory is that all samples were tested using consistent methodology, which is likely to provide a better picture of the true correlation between these assays.

Table 3. Pearson linear correlation analysis between various analytes measured across the sample types. The sample size (number of samples where both analytes were measured) are shown below each correlation.

Analyte	TPC	FRAP	CUPRAC	TMAC	Moisture	Protein	ABTS	Vitamin C
TPC	-	0.845 *** (n = 1304)	0.900 *** (n = 1094)	0.275 *** (n = 528)	0.327 *** (n = 671)	−0.013 NS (n = 706)	0.096 NS (n = 6)	0.783 *** (n = 78)
FRAP	-	-	0.848 *** (n = 1097)	0.309 *** (n = 536)	0.167 *** (n = 620)	−0.046 NS (n = 706)	0.909 *** (n = 16)	0.744 *** (n = 84)
CUPRAC	-	-	-	0.413 *** (n = 325)	0.168 *** (n = 443)	0.123 ** (n = 538)	0.978 *** (n = 22)	0.698 *** (n = 76)
TMAC	-	-	-	-	0.094 NS (n = 365)	−0.083 NS (n = 251)	0.917 *** (n = 20)	0.015 NS (n = 41)
Moisture	-	-	-	-	-	−0.356 *** (n = 215)	−0.083 NS (n = 22)	0.279 ** (n = 91)
Protein	-	-	-	-	-	-	ND	ND
ABTS	-	-	-	-	-	-	-	0.185 NS (n = 21)

Vitamin C	-	-	-	-	-	-	-	-

NS—not significant ($p > 0.05$), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. ND = no data.

The antioxidant capacity also showed a strong positive correlation with vitamin C content, a weak correlation with TMAC, and a very weak positive correlation with moisture content. TPC showed similar correlations with most of these parameters, but not ABTS. The moisture content also showed a weak positive correlation with vitamin C, but a negative correlation with protein content. Finally, the CUPRAC (but not other measures of antioxidant capacity) was very weakly correlated with protein content.

4. Conclusions

This study provided information on the typical phytochemical composition of >1000 samples of principally Australian foodstuffs and related plant products, including their phenolic contents and antioxidant capacities. Typically, the highest contents were found in native Australian fruits, while grains, nuts and non-native fruits showed fairly low antioxidant and phenolic contents. Spices, processed foodstuffs and Australian (non-fruit) bushfoods showed an intermediate content. Notably, medicinally used plants showed a much higher phenolic content and antioxidant capacity compared to other, non-medicinal plants. Additionally, this work also highlighted the significant nutrient potential which can occur in food by-products, including high antioxidant and vitamin C contents. Continued attention should be given to valorising these by-products into higher-value products—either for food or non-food purposes.

Author Contributions: Conceptualization, J.B.J.; methodology, J.B.J. and M.N.; software, J.B.J.; validation, J.B.J.; formal analysis, J.B.J.; investigation, J.B.J., J.S.M., R.J.B., B.E.H., N.N., P.R.T. and P.N.; resources, J.B.J. and M.N.; data curation, J.B.J.; writing—original draft preparation, J.B.J.; writing—review and editing, J.B.J., J.S.M., R.J.B., B.E.H., N.N., P.R.T., C.P.E.A., P.N. and M.N.; visualization, J.B.J.; supervision, M.N.; project administration, M.N.; funding acquisition, J.B.J. and M.N. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by a New Staff Grant (RSH/5343) awarded by CQUniversity to one of the authors (MN). The research was also supported in part by the Australian Government in the form of a Research Training Program Stipend provided to two of the authors (J.B.J. and J.S.M.).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The dataset underlying the results of this study is available upon request from the corresponding author.

Acknowledgments: We would like to thank the colleagues and students who have assisted with these phytochemical analyses over the years, including Tania Collins, Sobia Ikram, Megan Reynolds, Emily Bolton and Federico Caravani.

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

References

1. Pompella, A.; Sies, H.; Wacker, R.; Brouns, F.; Grune, T.; Biesalski, H.K.; Frank, J. The use of total antioxidant capacity as surrogate marker for food quality and its effect on health is to be discouraged. *Nutrition* **2014**, *30*, 791–793. https://doi.org/10.1016/j.nut.2013.12.002.
2. Pellegrini, N.; Vitaglione, P.; Granato, D.; Fogliano, V. Twenty-five years of total antioxidant capacity measurement of foods and biological fluids: Merits and limitations. *J. Sci. Food Agric.* **2020**, *100*, 5064–5078. https://doi.org/10.1002/jsfa.9550.
3. Zujko, M.E.; Waśkiewicz, A.; Witkowska, A.M.; Cicha-Mikołajczyk, A.; Zujko, K.; Drygas, W. Dietary Total Antioxidant Capacity—A New Indicator of Healthy Diet Quality in Cardiovascular Diseases: A Polish Cross-Sectional Study. *Nutrients* **2022**, *14*, 3219. https://doi.org/10.3390/nu14153219.
4. Mani, J.S.; Johnson, J.B.; Hosking, H.; Ashwath, N.; Walsh, K.B.; Neilsen, P.M.; Broszczak, D.A.; Naiker, M. Antioxidative and therapeutic potential of selected Australian plants: A review. *J. Ethnopharmacol.* **2020**, *268*, 113580. https://doi.org/10.1016/j.jep.2020.113580.

5. Abshirini, M.; Siassi, F.; Koohdani, F.; Qorbani, M.; Mozaffari, H.; Aslani, Z.; Soleymani, M.; Entezarian, M.; Sotoudeh, G. Dietary total antioxidant capacity is inversely associated with depression, anxiety and some oxidative stress biomarkers in postmenopausal women: A cross-sectional study. *Ann. Gen. Psychiatry* **2019**, *18*, 3. https://doi.org/10.1186/s12991-019-0225-7.
6. Witkowska, A.M.; Waśkiewicz, A.; Zujko, M.E.; Szcześniewska, D.; Pająk, A.; Stepaniak, U.; Drygas, W. Dietary Polyphenol Intake, but Not the Dietary Total Antioxidant Capacity, Is Inversely Related to Cardiovascular Disease in Postmenopausal Polish Women: Results of WOBASZ and WOBASZ II Studies. *Oxidative Med. Cell. Longev.* **2017**, *2017*, 5982809. https://doi.org/10.1155/2017/5982809.
7. da Silva, A.; Caldas, A.P.S.; Pinto, S.L.; Hermsdorff, H.H.M.; Marcadenti, A.; Bersch-Ferreira, Â.C.; Torreglosa, C.R.; Weber, B.; Bressan, J. Dietary total antioxidant capacity is inversely associated with cardiovascular events and cardiometabolic risk factors: A cross-sectional study. *Nutrition* **2021**, *89*, 111140. https://doi.org/10.1016/j.nut.2021.111140.
8. Wahlqvist, M.L. Antioxidant relevance to human health. *Asia Pac. J. Clin. Nutr.* **2013**, *22*, 171–176.
9. Johnson, J.B.; Budd, C.; Mani, J.S.; Brown, P.; Walsh, K.B.; Naiker, M. Carotenoids, ascorbic acid and total phenolic content in the root tissue from five Australian-grown sweet potato cultivars. *New Zealand J. Crop Hortic. Sci.* **2021**, *50*, 32–47. https://doi.org/10.1080/01140671.2021.1895230.
10. Johnson, J.; Collins, T.; Power, A.; Chandra, S.; Portman, D.; Blanchard, C.; Naiker, M. Antioxidative properties and macrochemical composition of five commercial mungbean varieties in Australia. *Legume Sci.* **2020**, *2*, e27. https://doi.org/10.1002/leg3.27.
11. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free. Radic. Biol. Med.* **1999**, *26*, 1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3.
12. Mariotti, F.; Tomé, D.; Mirand, P.P. Converting Nitrogen into Protein—Beyond 6.25 and Jones' Factors. *Crit. Rev. Food Sci. Nutr.* **2008**, *48*, 177–184. https://doi.org/10.1080/10408390701279749.
13. R Core Team *R: A Language and Environment for Statistical Computing*, version 4.2.3; R Foundation for Statistical Computing: Vienna, Austria, 2023.
14. Johnson, J.; Collins, T.; Skylas, D.; Quail, K.; Blanchard, C.; Naiker, M. Profiling the varietal antioxidative content and macrochemical composition in Australian faba beans (*Vicia faba* L.). *Legume Sci.* **2020**, *2*, e28. https://doi.org/10.1002/leg3.28.
15. George, J.; Edwards, D.; Pun, S.; Williams, D. Evaluation of Antioxidant Capacity (ABTS and CUPRAC) and Total Phenolic Content (Folin-Ciocalteu) Assays of Selected Fruit, Vegetables, and Spices. *Int. J. Food Sci.* **2022**, *2022*, 2581470. https://doi.org/10.1155/2022/2581470.
16. Apak, R.; Güçlü, K.; Demirata, B.; Özyürek, M.; Çelik, S.E.; Bektaşoğlu, B.; Berker, K.I.; Özyurt, D. Comparative Evaluation of Various Total Antioxidant Capacity Assays Applied to Phenolic Compounds with the CUPRAC Assay. *Molecules* **2007**, *12*, 1496–1547. https://doi.org/10.3390/12071496.