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First stage development of an Australian anthocyanin food composition database for dietary studies - A systematic process and its challenges

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First stage development of an Australian anthocyanin food composition database for dietary studies - A systematic process and its challenges

Abstract

In the last decade, there has been an increased interest in anthocyanin-based research with a growing need to accurately measure anthocyanin intake in population studies. Anthocyanin content in foods is known to vary across regions due to climate, soil content and harvesting practices. To accurately measure nutrient intake in population studies, food composition databases tailored to specific regions need to be developed. The aim of this study was to describe the first stage development of an Australian anthocyanin food composition database focusing on fruit and vegetables. A systematic literature search found analytical data on the anthocyanin content of five fruits and two vegetables (purple dragon carrot and red cabbage) out of the total plant-based food category (58 individual fruits and 62 vegetables). In addition, values were found for ten Australian native fruits, of which 9 are not included in the Australian database. Development of an anthocyanin food composition database relies on the availability of analytical food data. In the case of Australian fruits and vegetables, there are limited data available for anthocyanin content and imputations from other polyphenol datasets will be necessary. Regardless, development of an anthocyanin database tailored specifically for Australian research will facilitate better estimation of intake.

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1 **Original Research Article**

2 **First stage development of an Australian anthocyanin food**
3 **composition database for dietary studies – A systematic process**
4 **and its challenges**

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16 **Highlights**

- 17 • Consumption of anthocyanins has been associated with beneficial health effects.
- 18 • These observations have led to an increased interest in anthocyanin based research.
- 19 • Accurate measurement of anthocyanins in food is important in population studies.
- 20 • Similar foods from different regions are known to differ in nutritional content.
- 21 • It is imperative to develop food composition databases for different regions.

22

Abstract

In the last decade, there has been an increased interest in anthocyanin-based research with a growing need to accurately measure anthocyanin intake in population studies. Anthocyanin content in foods is known to vary across regions due to climate, soil content and harvesting practices. To accurately measure nutrient intake in population studies, food composition databases tailored to specific regions need to be developed. The aim of this study was to describe the first stage development of an Australian anthocyanin food composition database focusing on fruit and vegetables. A systematic literature search found analytical data on the anthocyanin content of five fruits and two vegetables (purple dragon carrot and red cabbage) out of the total plant based food category (58 individual fruit and 62 vegetables). In addition, values were found for ten Australian native fruit, of which 9 are not included in the Australian database. Development of an anthocyanin food composition database relies on the availability of analytical food data. In the case of Australian fruits and vegetables, there is limited data available for anthocyanin content and imputations from other polyphenol datasets will be necessary. Regardless, development of an anthocyanin database tailored specifically for Australian research will facilitate better estimation of intake.

KEYWORDS: Anthocyanins, micronutrients, antioxidants, Food analysis, Australia, Food composition database, fruit, vegetables.

Introduction

Anthocyanins are a subclass of flavonoids, a group of polyphenols. They are commonly found in plant based foods in the human diet and are known to be responsible for the deep rich purple, red, and blue colours in many fruits and vegetables. In nature, about 17 different anthocyanins have been discovered but to date only six (cyanidin, delphinidin, petunidin, peonidin, pelargonidin and malvidin) have been shown to be of dietary importance and are ubiquitously distributed in the food supply (Fernandes et al., 2014). Some common fruit and vegetable sources of anthocyanins include berries, cherries, apples, red and purple grapes, plums and pomegranates (Warner, 2015), as well as red wine and certain vegetables such as red cabbage, red onions and radishes (Zhang, 2015). In the last decade there has been an increased interest in anthocyanin based research (Mahdavi et al., 2014) because of their potential health benefits including improved cognition (Kent et al., 2015a) and vision (Kalt et al., 2014), as well as their protective effects against cardiovascular risk factors (Pojer et al., 2013) and inflammation (Cassidy et al., 2015, Mena et al., 2014). Animal and clinical trials continue to show promising results on these observed beneficial health effects (Wallace et al., 2016, Bhaswant et al., 2015).

As research continues to elucidate beneficial health effects of anthocyanins, it is important that the amount of anthocyanin intake is accurately measured in both epidemiological and experimental studies in order to allow translation of this evidence into dietary messages for overall health improvement. For this purpose, a number of specific and general polyphenol databases, including the American USDA Database for the Flavonoid Content of Selected Foods and the European Phenol-Explorer, exist for the measurement of polyphenol (anthocyanin) intake (Bhagwat et al., 2011, Neveu et al., 2010). Comparing these two databases for the estimation of dietary polyphenol intake in Polish adults, Witkowska et al. (2015) demonstrated significant discrepancies between the amount of flavonoid intake estimated when using the USDA and Phenol-Explorer databases (525 mg/day vs 403.5 mg/day, respectively

p<0.001). Epidemiological studies commonly utilise either one of these databases for the measurement of polyphenol intake. Such discrepancies between databases may lead to differing conclusions regarding the amount of anthocyanins in the human diet and potential dose responses (Kent et al., 2015b, Yahya et al., 2015, Witkowska et al., 2015).

Within the USDA and Phenol Explorer databases, analytical data are not country or region specific but are reported as mean values for all available data for a particular food. As a result, there is the possibility that this can lead to inaccurate measurement of polyphenol intakes in specific populations. Evidence has shown that differences in climate, soil conditions and methods of plant harvesting among other factors are major determinants of natural variation in the amounts of nutrients contained in foods (Karl, 2009, Hornick, 1992). For example, evidence suggests that the levels of anthocyanin in fruit may be directly proportional to the amount of sunlight exposure. A study by Song et al. (2015) showed that anthocyanin levels in grapes were substantially increased with grape bunch exposure to sunlight in comparison to shading. Average monthly hours of sunshine over the year has been shown to be significantly different in various fruit producing regions of the world (World weather and Climate Information, 2016). Focusing on this difference in climate and harvesting practices, the amount of micronutrients in apples are found to differ substantially between the USDA (USDA, 2015) and the Australian Food, Supplement and Nutrient Database (AUSNUT) (Food Standards Australia and New Zealand, 2014) (Table 1). For these reasons, it is important that databases tailored to specific regions of the world are developed to facilitate better measurement of anthocyanin intakes in population level studies.

In utilising the available databases for epidemiological studies, another drawback is the underrepresentation of native fruit and fruit hybrids specific to particular regions. For example, there is an underrepresentation of some common Australian native fruit including quandong, riberry and fingerlimes which are known to have high levels of anthocyanins (Cherikoff, 2015).

Table 1: Micronutrient comparison between USDA and AUSNUT food composition database values of apple

	AUSNUT* values	USDA ⁺ values
Preformed vitamin A (retinol) (µg)	0	3
Beta-carotene (µg)	10	27
Provitamin A (b-carotene equivalents) (µg)	13	
Vitamin A retinol equivalents (µg)	2	0
Thiamin (mg)	0.022	0.017
Riboflavin (mg)	0.013	0.026
Vitamin C (mg)	4	4.6
Dietary folate equivalents (µg)	13	3

*AUSNUT – Australian Food and Nutrient Database ⁺USDA – U.S Department of Agriculture

Source: USDA (2015) and Food Standards Australia New Zealand (2014)

The increased interest in anthocyanin-based research has also resulted in fruit breeders developing different hybrids of fruit and vegetables high in anthocyanin content and antioxidant capacity. One such fruit is a new variety of the Japanese plum (*Prunus salicina* Lindl), named the Queen Garnet plum (QGP) which was developed within a Queensland Government (Australia) breeding program. (Fanning et al., 2014). It is thus important for the phytochemical content of these novel plant foods to be captured within regionally appropriate food composition databases.

In summary, the underrepresentation of fruit specific to particular regions in existing databases, as well as the differences in climate and harvesting practices between regions warrants the development of an anthocyanin food composition database specific to different regions.

The aim of this study was, therefore, to describe the first stages of the development of an Australian anthocyanin food composition database using fruit and vegetables to pilot the systematic development process.

This paper will also describe the challenges encountered in developing this tailored Australian database.

Methods

The development of an Australian anthocyanin database involved an expansion of the existing Australian AUSNUT 2011-13 database to include anthocyanin content. The AUSNUT 2011-13 database contains over 5,700 foods and beverages reported by Australians and was developed for use in the 2011-13 Australian Health Survey (Australian Bureau of Statistics, 2013).

As a first stage development, the main focus was fruit and vegetables as these are the major food groups with high levels of anthocyanins. The most reliable method to measure anthocyanin content in foods has been identified as High-Performance Liquid Chromatography (HPLC). When evaluating spectrophotometric methods for antioxidant compound measurement, Tabart et al. (2010) found colorimetric methods were unreliable as reactions could be different for individual compounds within a family (anthocyanins, flavonols or flavan-3-ols) and were not specific to one family. For this reason, only data generated using HPLC methods of analysis were included.

The proposed methodology for estimating the anthocyanin content of Australian foods was adapted from a systematic approach used by Louie et al. (2015) for estimation of added sugar content in Australian foods via an expansion of the AUSNUT database, in which analyses

showed a strong correlation between two researchers in an inter-researcher repeatability analysis.

Step by step systematic approach to estimating anthocyanins in foods

A five staged process was developed for estimating the anthocyanin content of Australian fruit and vegetables. The first four steps were objective while step 5 was considered subjective.

Step 1: Classification of food groups: Food groups were coded into three different categories; plant-based foods, non-plant-based foods and composite foods (made up of plant-based and non-plant based foods). The difference between the non-plant-based food and the composite food group is that the majority of the former were assigned zero values while in the latter, fewer foods were assigned a zero value (Table 2).

The grouping system developed for the AUSNUT 2011-13 database classifies foods according to a major (2-digit) based on key ingredients, followed by sub-major (3-digit) and minor food (5-digit) groups. Using this classification system, there are 24 major food groups covering all the reported foods eaten by Australians, as shown in Table 2.

Step 2: Assignment of zero values: Zero values were assigned to non-plant based food and composite food groups with careful attention being paid not to assign 0 values to foods that contained ingredients from the plant-based food groups.

Step 3: Conduct a systematic literature search for analytical data: A systematic literature search of electronic databases was conducted (Scopus, Web of Science, Medline, PubMed central, ProQuest, and Science Direct) using a combination of search terms; “anthocyanin*”, “Australia*”, “Fruit*”, “vege*”, and “analy*”. Studies were included if they had analysed the anthocyanin content in Australian fruit and vegetables.

Table 2: Major food groups in the Australian Food and Nutrient Database (AUSNUT)

Plant based food groups	Non-Plant based food groups	Composite food groups
16. Fruit products and dishes	14. Fats and oils	11. Non-alcoholic beverages
22. Seed and nut product and dishes	15. Fish and seafood products	12. Cereals and cereal products
24. Vegetable products and dishes	17. Egg products and dishes	13. Cereal based products and dishes
25. Legume and pulse products and dishes	18. Meat, poultry and game products and dishes	20. Dairy & meat substitutes
	19. Milk products and dishes	21. Soup
	30. Special dietary foods	23. Savoury sauces and condiments
	34. Reptiles, amphibia and insects	26. Snack foods
		27. Sugar products and dishes
		28. Confectionery and cereal/nut/fruit/seed bars
		29. Alcoholic beverages
		31. Miscellaneous
		32. Infant formulae and foods
		33. Dietary supplements

152

153 For the vegetable group, additional literature searches using USDA and Phenol-Explorer
 154 databases were conducted to identify vegetables with available anthocyanin analytical values.
 155 Searches used scientific and common names to identify all relevant foods in the USDA and
 156 Phenol-Explorer databases.

157 *Step 4: Contact local researchers and organisations:* Experts in the field of anthocyanin
 158 research were identified from the systematic literature search as well as web searches of
 159 organisations, groups, and individuals to identify where further sources of analytical

anthocyanin data may be located. These sources were contacted to enquire about the existence of any unpublished data for the anthocyanin content of Australian fruits and vegetables.

Step 5: Using borrowed values: Having exhausted steps 1-4, where Australian data was not identified for fruit and vegetables, data will be obtained for similar foods from international databases, including the USDA Database for the Flavonoid Content of Selected Foods and Phenol-Explorer. To assist with the choice of the most appropriate foods from those databases, estimations will be made by calculating a conversion factor to apply to the borrowed values in the Australian food anthocyanin database, considering deviation from average moisture content as shown below: (FAO, 1968)

The conversion factor F is calculated using the following formula:

$$F = \frac{100 - \text{actual moisture content (Australian food composition table)}}{100 - \text{moisture content as shown in the USDA or Phenol - Explorer food comp. table}}$$

Moisture content of the foods in the USDA flavonoid database will be obtained from the USDA National Nutrient Database for Standard Reference (USDA, 2015) and those of the Phenol-Explorer will be obtained from the Danish Food Composition Databank the data source indicated in the Phenol-Explorer database (Saxholt et al., 2008). Having calculated the conversion factor, the corresponding Australian nutrient value will be computed by multiplying specific nutrient value by the conversion factor (F). Following this computation, data from the USDA flavonoid database and Phenol-Explorer will be mapped by the authors (YP and EI) to determine whether to make decisions based on the calculated values from both databases or if further analysis is required. The choice of the international database from which to borrow analytical values will be dependent on the similarity of the foods in their macronutrient content and the similarity of the food supply between Australia and the source country including production and processing techniques.

183

184 **Results**

185 Following the four objective steps outlined above, the existing AUSNUT 2011-13 database
186 was expanded to include anthocyanin content. The foods contained in the database are
187 classified under 24 food groups (Table 2). Of these, four were grouped as plant-based foods,
188 seven under the non-plant based food group and 13 under the composite food group. There was
189 a total of 58 individual fruits and 62 vegetables in the database following this method of
190 categorization.

Figure 1 PRISMA flow diagram for analytical article selection process

191

192 The systematic literature search (Figure 1) for analytical values of Australian fruits and
 193 vegetables yielded analytical values for only five Australian fruit (Table 3), including berries
 194 (Fredericks et al., 2013, Netzel et al., 2006) plums (Fanning et al., 2014, Bobrich et al., 2014),

Table 3: Anthocyanin content of raw Australian fruit

Reference	Food name	Food group	Method of analysis ^a	Total anthocyanin (SD) mg/100g fresh weight	Individual anthocyanins reported
Fredericks et al., (2013)	Strawberry	Fruit products and dishes	HPLC	2.02 (0.04)	yesYes
Netzel et al., (2006)	Blueberry	Fruit products and dishes	HPLC/ESI-MS-MS	381.75 (6.23)	Yes
Bobrich, A., et al. (2014)	Black diamond plum	Fruit products and dishes	HPLC	89.8mg	Yes
Fanning et al., (2014)	Queen Garnet plum	Fruit products and dishes	HPLC	195	No
Takos et al., (2006)	Apple skin (red)	Fruit products and dishes	HPLC	9mg/100g fruit skin	No
Bidon et al., (2013)	Shiraz grape	Fruit products and dishes	HPLC	159 (5)	No
Zabaras et al., (2013)	Red Cabbage	Vegetables	HPLC/ESI-LC/MS	57.4	No
Singh et al., (2012)	Purple dragon Carrot	Vegetables	HPLC	5 (0.2)	No
Netzel et al., (2006)	Muntries	(Native) fruit products and dishes	HPLC/ESI-MS-MS	25.26 (1.24)	Yes
Netzel et al., (2006)	Tasmanian pepper	(Native) fruit products and dishes	HPLC/ESI-MS-MS	607 (52)	Yes
Netzel et al., (2006)	Molucca raspberry	(Native) fruit products and dishes	HPLC/ESI-MS-MS	73.64 (0.41)	Yes

Reference	Food name	Food group	Method of analysis ^a	Total anthocyanin (SD) mg/100g fresh weight	Individual anthocyanins reported
Netzel et al., (2006)	Davidson's plum	(Native) fruit products and dishes	HPLC/ESI-MS-MS	38.42 (0.83)	Yes
Netzel et al., (2006)	Illawarra plum	(Native) fruit products and dishes	HPLC/ESI-MS-MS	556.82 (20.73)	Yes
Netzel et al., (2006)	Cedar bay cherry	(Native) fruit products and dishes	HPLC/ESI-MS-MS	27.77 (0.54)	Yes
Netzel et al., (2006)	Burdekin plum	(Native) fruit products and dishes	HPLC/ESI-MS-MS	174.55(10.63)	Yes
Netzel et al., (2007)	Riberry	(Native) fruit products and dishes	HPLC-DAD/ESI/MS-MS	72.39 (1.45)	Yes
Netzel et al., (2007)	Bush Cherry	(Native) fruit products and dishes	HPLC-DAD/ESI/MS-MS	105.79(1.24)	Yes
Netzel et al., (2007)	Finger lime	(Native) fruit products and dishes	HPLC-DAD/ESI/MS-MS	11.09 (0.83)	Yes
Unpublished data from source	pomegranate juice	fruit products and dishes	HPLC	13 ^b	No
Unpublished data from source	pomegranate cranberry juice	fruit products and dishes	HPLC	12 ^b	No
Unpublished data from source	pomegranate blueberry juice	fruit products and dishes	HPLC	12 ^b	No
Unpublished data from source	Dr Red (Dr Purple Shiraz and Purple Carrot Blend)	fruit products and dishes	HPLC	9 ^b	No

Reference	Food name	Food group	Method of analysis ^a	Total anthocyanin (SD) mg/100g fresh weight	Individual anthocyanins reported
Unpublished data from source	Dr Red (Purple carrot blueberry punch)	fruit products and dishes	HPLC	40 ^b	No
Unpublished data from source	Dr Red (Blueberry punch)	fruit products and dishes	HPLC	610 ^b	No
Unpublished data from source	Nature's Goodness (Joint Formula: Cherry juice concentrate with anthocyanin complex)	fruit products and dishes	HPLC	14 ^b	No
Unpublished data from source	Sunraysia (100% Prune juice (from concentrate))	fruit products and dishes	HPLC	<<1 ^b	No

196 ^aHPLC- High Performance Liquid Chromatography, HPLC/ESI-MS-MS High-Performance Liquid Chromatography/Electrospray Ionization Tandem Mass Spectrometry,
197 HPLC/ESI-LC/MS - High-Performance Liquid Chromatography/Electrospray Ionization Liquid Chromatography/Mass Spectrometry, HPLC-DAD/ESI/MS-MS - High-
198 Performance Liquid Chromatography- Diode Array Detection /Electrospray Ionization Tandem Mass Spectrometry

199 ^b Single item analysis

200

apple (Takos et al., 2006), grape (Bindon et al., 2014) as well as ten native Australian fruit (Netzel et al., 2007, Netzel et al., 2006) and two Australian vegetables, namely purple dragon carrots (Singh et al., 2012) and red cabbage (Zabaras et al., 2013).

Unpublished analytical data from local researchers at the Queensland Alliance for Agriculture and Food Innovation, University of Queensland, Australia were obtained for commercial pomegranate juice, pomegranate cranberry juice, pomegranate blueberry juice, Dr Red (Dr Purple Shiraz and Purple Carrot Blend), Dr Red (Purple carrot blueberry punch), Dr Red (Blueberry punch), Nature's Goodness (Joint Formula: Cherry juice concentrate with anthocyanin complex) and Sunraysia (100% Prune juice (from concentrate)).

Discussion

Using a systematic approach, for the first time, attempts have been made to bring together in a database the analytical data for the anthocyanin content of Australian foods (fruit and vegetables). The first four objective steps were unambiguous as the food groupings based on major ingredients were readily available; hence classification as plant-based or non-plant-based foods was a straightforward process.

Even though there is currently very limited analytical data on the anthocyanin content of Australian foods which was evident in the few studies identified during the systematic literature search, preliminary comparisons of the available data on fruit with the existing polyphenol databases shows a substantial difference in the reported anthocyanin content (table 4). It is expected that more data will become available as this area of research advances, allowing for continuous sourcing of unpublished data. Expansion of the dataset will enable more precise and consistent estimation of anthocyanin intakes in future studies.

225 **Table 4: Differences in anthocyanin content of similar fruit vegetables from different databases and sources**

226

Fruit/vegetable	USDA database (mg/100g)	Phenol-Explorer database (mg/100g)	Australian data (mg/100g)
Strawberry	2.42	73.01	2.02
Blueberry	163.3	133.99	381.75
Plum	56.05	47.79	89.8
Red cabbage	209.95	- *	57.4

227 *- not reported

To date, the major focus has been on raw fruit and vegetables but as this project progresses, a number of considerations will be made in gathering analytical values. Food processing, for example, will be a major consideration as it has been shown to affect the nutritional content of foods with more effect on micronutrients. Blanching for example was observed to decrease the tannins and antiradical efficiency of plums but increased the total polyphenol content, while osmotic dehydration had no effect on the total polyphenol and ferric reducing power (Valero et al., 2012). On drying, Piga et al. (2003) observed that some anthocyanin and flavonol content of plums had significantly decreased. In line with this observation, Najafabad and Jamei (2014) studied the free radical scavenging capacity and antioxidant activity of both fresh and dried fruit samples (plum) and observed that the fresh samples had more oxygen free radical (superoxide and peroxy radicals) scavenging capacity than the dried samples.

Some of the strengths of the proposed methodology are its systematic approach and the fact that local researchers were contacted for unpublished data in order to gather additional analytical data.

Furthermore, upon completion of the proposed database, there is the possibility of improved study results based on better measurement of anthocyanin consumption. In estimating anthocyanin intake in population studies, a major limitation has been the significant differences in the estimated amount of anthocyanin intake depending on the database used to measure anthocyanin (Witkowska et al., 2015). Witkowska et al. (2015) proposed that the combination of available databases can have a significant effect on accurate measurement of anthocyanin intake. Considering this, a major strength of this proposed methodology will be the combination of analytical data from Australian foods and borrowed data from international databases based on micro and macro nutrient similarity which will provide more accurate estimation.

A major limitation of this project was the limited availability of analytical data for Australian foods and thus the need to borrow international data. Some of the published analytical values only reported total anthocyanin content, rather than individual anthocyanins (Singh et al., 2012, Bindon et al., 2014, Takos et al., 2006, Zabaras et al., 2013, Fanning et al., 2014). This could be seen as a limitation as evidence shows that specific anthocyanins vary substantially in their bioavailability (McGhie and Walton, 2007). .

Conclusion

In conclusion, this proposed methodology provides insight for the compilation of analytical data for an Australian anthocyanin database for fruit and vegetables. Whilst the literature search produced very limited results, this was to be expected as the database is in the early stages of development and anthocyanins are an emerging area of research. As more analytical data becomes available from organisations and independent researchers, updates will be made to produce a robust database that will facilitate accurate measurement of anthocyanin consumption in Australian population studies and clinical trials.

Conflict of interest

The authors of this manuscript have no conflict of interest to declare.

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