

Proceeding Paper

# Factors Influencing Bioactive Constituents in Desi Chickpea: Variety, Location and Season <sup>†</sup>

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**Abstract:** Chickpea (*Cicer arietinum* L.) is a significant pulse crop in Australia, with an industry value of over AU \$1.3 billion. However, there are few studies investigating the levels of health-benefiting constituents in desi chickpea, and the impacts of variety, growing location and season on these constituents. This study aimed to study the levels of health-benefiting constituents in desi chickpea, including 97 samples of Australian desi chickpea, comprising 18 varieties, grown in a range of field trials across four Victorian locations and 3 growing seasons. Various physical characteristics and phytochemical composition were determined in the samples, including 100-seed weight, colour, moisture content, total phenolic content (TPC), ferric reducing antioxidant potential (FRAP), cupric reducing antioxidant potential (CUPRAC) and total monomeric anthocyanin content (TMAC). The screening results showed a significant difference in TPC, TMAC, and FRAP among different desi varieties, suggesting there may be variation in their potential health benefits. Furthermore, the growing location and growing season significantly impacted all analytes. Correlation analysis revealed a number of significant correlations, including a moderate positive correlation between the b\* colour and the antioxidant capacity and total phenolic content. This work provides the first detailed insight into the range of phenolic and antioxidant contents found in Australian desi chickpea, and the impact that genotype, location and season can have.

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

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## 1. Introduction

Chickpea (*Cicer arietinum* L.) is one of the oldest known pulse crops and is widely grown across the world [1,2]. Globally, it is ranked as the second-most produced cool season food legume crop, with 15.9 million tonnes harvested in 2021 [3]. Although this crop was not grown commercially in Australia until the 1970s [4,5], Australia has grown to become the 8th largest producer and the largest exporter of chickpea. A total of 876,468 tonnes were harvested in 2021 [3], with over 95% of this being exported, primarily to the Indian subcontinent [6]. Chickpeas have been divided into two market classes, light coloured and larger-seeded kabuli type, and dark-seeded and smaller-seeded desi type [7]. Desi chickpeas are the dominant variety cultivated in Australia, accounting for approximately 90–95% of the total production [8]. The remaining 5–10% of production consists of kabuli chickpeas. The current value of the Australian chickpea industry is estimated at AU \$1.33 billion [8]. Furthermore, Australian chickpeas are highly regarded on the international market for their quality [8].

There is considerable potential for Australian growers to expand the production of chickpea, particularly in northern Australia [9]. Notably, data from the International Trade Centre estimates the current untapped demand for chickpea in international export markets to be worth over \$400 million USD [10].

One notable nutritional characteristic of chickpea is its high protein content [11], making it an excellent replacement for meat in vegetarian diets. Furthermore, proteins and protein hydrolysates can be readily extracted from chickpea using wet or dry extraction methods [12]. These protein fractions can then be used in the production of artificial meat analogues and other protein-fortified products such as noodles, bread and cookies [12].

In addition to this, chickpea has recently attracted interest due to its potential health-benefitting activity [13–16]. Previous work has shown that chickpeas or compounds isolated from chickpeas display a broad range of advantageous biological activities, including antioxidant activity [17], anti-cancer activity [18–20], hypocholesterolemic activity [21,22], hypoglycaemic activity [23–25], anti-hypertensive activity [26,27], and anti-inflammatory activity [28,29]. The major compound classes believed to be responsible for these beneficial effects include polyphenols, carotenoids, tannins, sterols and peptides [13,14]. International research has shown that the content of these phytochemicals—including phenolics and carotenoids—can vary significantly between different chickpea varieties [30–35], similar to that observed in other pulse species [36–38]. Consequently, the primary objective of this study was to investigate the variability in key phytochemical constituents among various varieties and under different growing conditions in the Australian setting.

This study exclusively focused on the levels of health-benefitting constituents in desi type chickpeas, as they represent the dominant variety cultivated in Australia. Specifically, this study aimed to investigate the impact of variety, location and season on the phytochemical content of the chickpea samples.

## 2. Materials and Methods

### 2.1. Seed Material

The 97 desi chickpea samples included in this study were sourced from archived samples stored at Agriculture Victoria Research (Horsham Victoria). The samples comprised 18 different varieties, grown in a range of field trials across four sites in Victoria and 3 growing seasons (2017, 2019 and 2020). The number of samples from each variety ranging from 1 to 20 (mean = 5 samples/variety). The majority of samples (55) were grown under ambient conditions with no imposed treatments; however, 16 of the samples were from herbicide treatment trials and 25 samples were part of pathology trials.

### 2.2. Seed Processing and Analysis of Physical Characteristics

The 100-seed weight (HKW) of the whole seed was determined using an IC-VA seed counter (AIDEX Co, Japan), with measurements performed in triplicate for each sample. The chickpea samples were then ground to a fine flour using a Breville Coffee & Spice Grinder (Botany, NSW).

The colour of the chickpea flour was quantified using a calibrated Konica Minolta chroma meter (CR-400), reported as CIE values of lightness ( $L^*$ ), yellow/blue ( $b^*$ ) and red/green colouration ( $a^*$ ). Measurements were performed in triplicate for each sample.

The moisture content of the flour was determined according to AOAC Official Method 925.10. Briefly, flour samples (~3 g) were dried in a laboratory oven (Memmert 400; Buechenbach, Germany) at 105 °C and the loss in mass quantified. All subsequent results were expressed on an oven-dry weight basis.

### 2.3. Measurement of Phytochemical Composition

Polar phenolic compounds were extracted from the chickpea flour samples with 90% methanol, following the protocol described in Johnson, et al. [9], using 1 g of flour and a final volume of 14 mL. Extractions and subsequent assays were performed in duplicate for each sample.

The total phenolic content (TPC), ferric reducing antioxidant potential (FRAP), cupric reducing antioxidant potential (CUPRAC) and total monomeric anthocyanin content (TMAC) were analysed using microplate-based methods, as previously described in detail [39]. Results for TPC were expressed in gallic acid equivalents (GAE), results for FRAP and CUPRAC in Trolox equivalents (TE), and results for TMA in cyanidin-3-glucoside equivalents (cyd-3-glu); all per 100 g of original sample material (oven-dry weight basis).

### 2.4. Statistical Analyses

Statistical tests were performed on the phytochemical and phenolic data using R Studio running R 4.0.5 [40]. Where applicable, results are presented as mean  $\pm$  1 standard deviation. When investigating statistical differences between varieties, only the varieties with  $\geq 10$  samples were included ( $n = 5$  varieties in total) to ensure a high level of statistical power. However, all samples were included in statistical analyses by year or location.

## 3. Results and Discussion

### 3.1. Impact of Chickpea Variety

As the samples were not from a balanced genotype  $\times$  environment  $\times$  year trial with equal numbers of samples for each condition (see Appendix D), the impact of these variables was unable to be explored through a three-way ANOVA. However, each of these variables was investigated separately, thus averaging out the impacts of the other two variables (Tables 1–3). Consequently, while the interactions between these terms were unable to be investigated, their broad impacts on phytochemical composition and physical seed parameters could be observed.

Examination of these parameters by variety (Table 1) revealed a significant level of variation in the FRAP, TPC and TMAC between the major chickpea varieties, as well as in the seed size (HKW) and the yellow-blue colouration of their flour (CIE b value). The variety Howzat displayed the highest FRAP and TPC, while PBA Slasher showed the lowest concentrations of these analytes. However, this latter variety did contain the highest TMAC.

**Table 1.** Impact of variety on the size, colour and phytochemical composition of desi chickpea. Note that only varieties with  $\geq 10$  samples were included. Varieties with the same superscript were not statistically different according to a post hoc Tukey test at  $\alpha = 0.05$ .

| Parameters                | Howzat (n = 10)               | Kyabra (n = 14)                | PBA Slasher (n = 11)          | PBA Striker (n = 20)           | Sonali (n = 10)                | p Value               |
|---------------------------|-------------------------------|--------------------------------|-------------------------------|--------------------------------|--------------------------------|-----------------------|
| HKW (g/100)               | 18.6 $\pm$ 1.7 <sup>bc</sup>  | 23.0 $\pm$ 1.9 <sup>a</sup>    | 18.8 $\pm$ 1.2 <sup>bc</sup>  | 20.2 $\pm$ 2.6 <sup>b</sup>    | 16.6 $\pm$ 0.8 <sup>c</sup>    | <0.001 <sup>***</sup> |
| Flour colour—L*           | 78.05 $\pm$ 1.41              | 80.66 $\pm$ 0.79               | 78.18 $\pm$ 6.30              | 79.28 $\pm$ 1.37               | 77.72 $\pm$ 1.10               | 0.066 <sup>NS</sup>   |
| Flour colour—a*           | 1.93 $\pm$ 0.86               | 1.74 $\pm$ 0.39                | 1.78 $\pm$ 0.20               | 1.41 $\pm$ 0.60                | 1.51 $\pm$ 0.41                | 0.096 <sup>NS</sup>   |
| Flour colour—b*           | 27.04 $\pm$ 1.65 <sup>a</sup> | 26.09 $\pm$ 1.28 <sup>ab</sup> | 24.96 $\pm$ 1.27 <sup>b</sup> | 26.27 $\pm$ 1.52 <sup>ab</sup> | 26.44 $\pm$ 0.30 <sup>ab</sup> | 0.013 <sup>*</sup>    |
| Moisture (%)              | 9.21 $\pm$ 0.86               | 9.13 $\pm$ 0.83                | 9.24 $\pm$ 0.83               | 8.86 $\pm$ 0.72                | 8.39 $\pm$ 0.75                | 0.087 <sup>NS</sup>   |
| FRAP (mg TE/100 g)        | 40.3 $\pm$ 16.2 <sup>a</sup>  | 29.5 $\pm$ 6.7 <sup>ab</sup>   | 24.9 $\pm$ 8.9 <sup>b</sup>   | 33.1 $\pm$ 10.4 <sup>ab</sup>  | 28.5 $\pm$ 13.3 <sup>ab</sup>  | 0.028 <sup>*</sup>    |
| CUPRAC (mg TE/100 g)      | 124 $\pm$ 20                  | 129 $\pm$ 21                   | 123 $\pm$ 17                  | 132 $\pm$ 42                   | 150 $\pm$ 26                   | 0.232 <sup>NS</sup>   |
| TPC (mg GAE/100 g)        | 93.7 $\pm$ 11.6 <sup>a</sup>  | 80.3 $\pm$ 14.1 <sup>ab</sup>  | 72.6 $\pm$ 8.8 <sup>b</sup>   | 91.1 $\pm$ 9.5 <sup>a</sup>    | 82.2 $\pm$ 13.7 <sup>ab</sup>  | <0.001 <sup>***</sup> |
| TMAC (mg cyd-3-glu/100 g) | 5.8 $\pm$ 1.5 <sup>ab</sup>   | 5.0 $\pm$ 3.2 <sup>ab</sup>    | 7.2 $\pm$ 1.5 <sup>a</sup>    | 4.2 $\pm$ 2.0 <sup>b</sup>     | 4.5 $\pm$ 1.4 <sup>b</sup>     | 0.006 <sup>**</sup>   |

NS—not significant ( $p > 0.05$ ), \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

The FRAP of the chickpea samples was approximately 7–8 times lower than the results previously found for faba bean [41], but higher than the values found for wheat and mungbean [42]. However, the CUPRAC was around three times lower than that found for mungbean. Overall, the FRAP values were lower than the results reported by Johnson, et al. [9] in the kernel flour of five new chickpea genotypes from Australia. No literature values were found for the CUPRAC analysis of chickpea.

The TPC of the chickpea extracts was around three times lower compared to faba bean, but comparable to the results observed in mungbean. The TPC of these chickpea samples were also comparable to those found by Johnson, et al. [9] in several new varieties of Australian desi chickpea. Furthermore, the TPC was comparable to values reported by Heiras-Palazuelos, et al. [30] for desi chickpea cultivars from Mexico, but lower than most values reported by Segev, et al. [43] for Australian chickpeas.

### 3.2. Impact of Growing Location

All parameters differed significantly with the growing location (Table 2). The highest FRAP and TPC were found for the Curyo site, while the highest CUPRAC was at observed Horsham. Conversely, the highest TMAC was at Banyena.

**Table 2.** Impact of growing location on the size, colour and phytochemical composition of desi chickpea. Note that one location (Rupanyup) was excluded as it contained only 5 samples. Locations with the same superscript were not statistically different according to a post hoc Tukey test at  $\alpha = 0.05$ .

| Parameters                | Banyena (n = 25)          | Curyo (n = 18)            | Horsham (n = 49)          | p Value    |
|---------------------------|---------------------------|---------------------------|---------------------------|------------|
| HKW (g/100)               | 20.1 ± 2.6 <sup>a</sup>   | 17.4 ± 1.2 <sup>b</sup>   | 19.8 ± 3.1 <sup>a</sup>   | 0.003 **   |
| Flour colour—L*           | 79.80 ± 1.06 <sup>a</sup> | 77.81 ± 1.17 <sup>b</sup> | 79.70 ± 1.46 <sup>a</sup> | <0.001 *** |
| Flour colour—a*           | 1.67 ± 0.25 <sup>a</sup>  | 1.26 ± 0.74 <sup>b</sup>  | 1.98 ± 0.56 <sup>a</sup>  | <0.001 *** |
| Flour colour—b*           | 24.95 ± 0.68 <sup>c</sup> | 26.86 ± 1.04 <sup>b</sup> | 27.77 ± 0.98 <sup>a</sup> | <0.001 *** |
| Moisture (%)              | 9.34 ± 0.51 <sup>a</sup>  | 9.05 ± 0.48 <sup>a</sup>  | 8.20 ± 0.96 <sup>b</sup>  | <0.001 *** |
| FRAP (mg TE/100 g)        | 27.0 ± 6.9 <sup>b</sup>   | 38.3 ± 13.9 <sup>a</sup>  | 34.9 ± 11.6 <sup>a</sup>  | 0.002 **   |
| CUPRAC (mg TE/100 g)      | 114 ± 18 <sup>b</sup>     | 133 ± 23 <sup>b</sup>     | 157 ± 36 <sup>a</sup>     | <0.001 *** |
| TPC (mg GAE/100 g)        | 74.1 ± 6.9 <sup>b</sup>   | 92.8 ± 13.9 <sup>a</sup>  | 87.0 ± 11.6 <sup>a</sup>  | <0.001 *** |
| TMAC (mg cyd-3-glu/100 g) | 6.6 ± 2.5 <sup>a</sup>    | 5.0 ± 1.5 <sup>b</sup>    | 3.5 ± 1.4 <sup>c</sup>    | <0.001 *** |

NS—not significant ( $p > 0.05$ ), \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

### 3.3. Impact of Season

Similarly, the growing season had a significant impact on all parameters measured (Table 3). Both FRAP and CUPRAC were higher in the 2020 samples, while the TPC was significantly lower in the 2017 samples. It is important to caution that as all samples were stored following harvest, there may have been some change in their composition over this period, particularly for the older samples. Although there does not appear to be any work documenting this specifically in chickpea, Nasar-Abbas, et al. [44] noted a minor reduction in the TPC of faba bean samples over the period of one year, with the loss accelerated under higher temperatures or exposure to light. However, Ziegler, et al. [45] found contrasting results in soybean, with the free phenolic content increasing slightly over a storage period of one year.

In addition to possessing the highest TMAC, the oldest samples (2017) also tended to have a larger kernel size and higher moisture content. This latter parameter may be related to the absorption of moisture by the chickpea samples over the longer storage time.

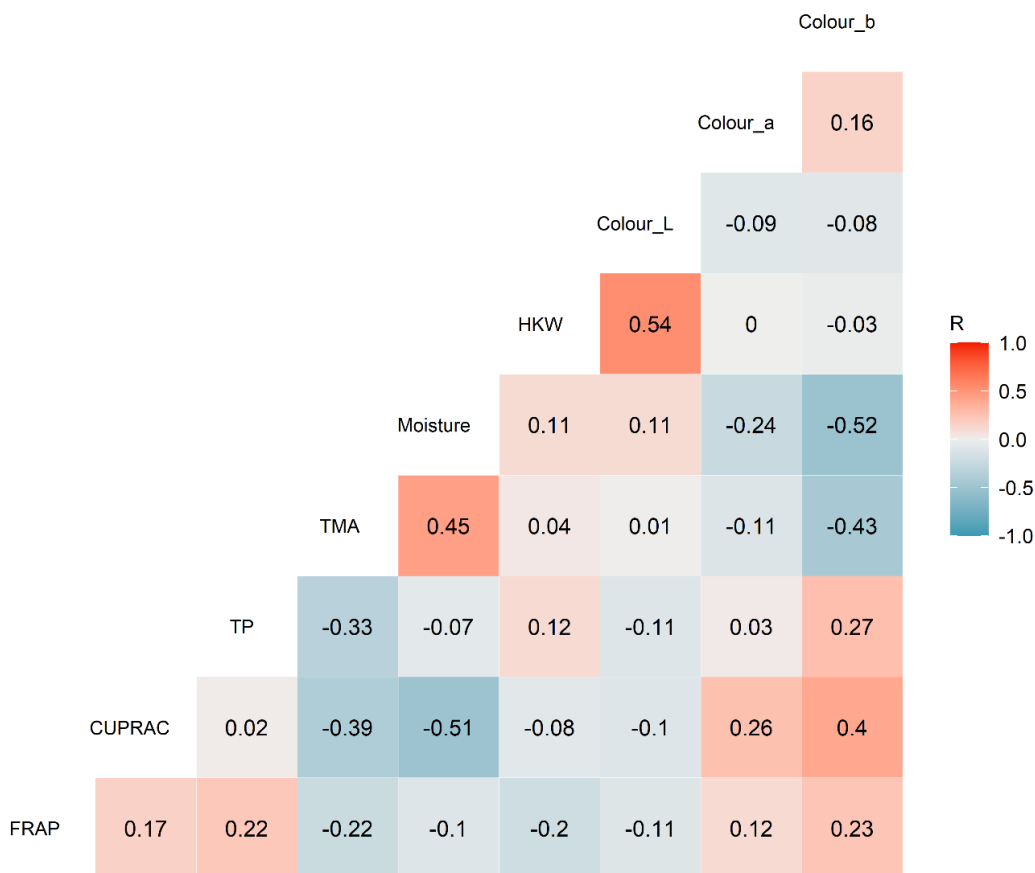
**Table 3.** Impact of growing year on the size, colour and phytochemical composition of desi chick-pea. Years with the same superscript were not statistically different according to a post hoc Tukey test at  $\alpha = 0.05$ .

| Parameters                | 2017 (n = 30)  | 2019 (n = 53)  | 2020 (n = 14)   | p Value    |
|---------------------------|----------------|----------------|-----------------|------------|
| HKW (g/100)               | 20.2 ± 2.4 a   | 19.3 ± 2.8 a   | 18.5 ± 3.1 a    | 0.003 **   |
| Flour colour—L*           | 80.00 ± 1.14 a | 79.09 ± 1.71 b | 79.57 ± 1.12 ab | <0.001 *** |
| Flour colour—a*           | 1.68 ± 0.27 b  | 1.62 ± 0.65 b  | 2.42 ± 0.40 a   | <0.001 *** |
| Flour colour—b*           | 24.82 ± 0.74 b | 27.54 ± 1.12 a | 27.48 ± 0.88 a  | <0.001 *** |
| Moisture (%)              | 9.46 ± 0.56 a  | 8.45 ± 0.97 b  | 8.35 ± 0.81 b   | <0.001 *** |
| FRAP (mg TE/100 g)        | 26.9 ± 6.4 c   | 33.8 ± 11.9 b  | 43.7 ± 10.1 a   | 0.002 **   |
| CUPRAC (mg TE/100 g)      | 116 ± 18 c     | 142 ± 32 b     | 181 ± 28 a      | <0.001 *** |
| TPC (mg GAE/100 g)        | 76.4 ± 8.8 b   | 88.7 ± 12.2 a  | 88.3 ± 10.6 a   | <0.001 *** |
| TMAC (mg cyd-3-glu/100 g) | 6.4 ± 2.4 a    | 4.0 ± 1.6 b    | 3.6 ± 1.0 b     | <0.001 *** |

NS—not significant ( $p > 0.05$ ), \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

### 3.4. Correlation Analysis

To further investigate the inter-relationships that may exist between the bioactive phytochemical constituents and the physical characteristics of the seed, Pearson R linear correlation analysis was performed. The results are summarised in the correlogram presented in Figure 1. Significant correlations were observed between TPC and FRAP, but not between TPC and CUPRAC or FRAP and CUPRAC. The CUPRAC was positively correlated with the b\* colour, but negatively correlated with moisture content.



**Figure 1.** Correlogram showing the correlations between the phytochemical constituents and physical parameters of the chickpea seed (n = 97 samples). Correlations with R values above 0.21 or below -0.21 were statistically significant at  $\alpha = 0.05$ .

#### 4. Conclusions

This study results demonstrated significant variation in the TPC, TMAC and antioxidant capacity (FRAP but not CUPRAC) of different desi chickpea cultivars grown in Victoria, Australia. Similarly, the growing location and year had a significant impact on the levels of these phytochemical constituents. Finally, correlation analysis showed a significant correlation between TPC and FRAP in these samples, but not between TPC and CUPRAC or FRAP and CUPRAC.

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