Hindawi
Journal of Food Quality
Volume 2018, Article ID 7836745, 6 pages
https://doi.org/10.1155/2018/7836745

*Research Article*

# Effect of Processing on Antioxidant Activity, Total Phenols, and Total Flavonoids of Pigmented Heirloom Beans

**Lisa Garretson,[1] Catrin Tyl,[1] and Alessandra Marti[1,2]**

[1]*Department of Food Science and Nutrition, University of Minnesota, St. Paul, MN, USA*
[2]*Department of Food, Environmental and Nutritional Sciences, University of Milan, Milan, Italy*

Correspondence should be addressed to Alessandra Marti; alessandra.marti@unimi.it

Received 29 December 2017; Accepted 31 May 2018; Published 21 June 2018

Academic Editor: Elena González-Fandos



While extensive research has been performed on the composition and cooking quality of commodity beans, relatively little is known about pigmented heirloom varieties and the effects of processing on their antioxidant capacity. The aim of this study was to evaluate the effect of soaking and cooking on antioxidants in four heirloom bean varieties compared to Pinto. Water absorption kinetics, soaking and cooking time, DPPH radical scavenging activity, and total phenolic and total flavonoid content were determined in raw, soaked, and cooked samples. Heirlooms required less time to hydrate compared to Pinto, whereas cooking times were similar. The effect of soaking on antioxidant capacity and flavonoids was minimal compared to cooking, which led to losses of up to 57%. Each pigmented heirloom bean had specific characteristics, and three of them had equal or higher amounts of antioxidants or antioxidant activity than Pinto at every processing step. Among heirlooms, Koronis Purple and Jacob's Cattle had the highest antioxidant activity and Jacob's Cattle and Tiger's Eye the highest amount of flavonoids, even after cooking.

## 1. Introduction

Common dry beans (*Phaseolus vulgaris*) are an important class of legumes based on their accessibility, nutritional content (i.e., high amounts of protein, fiber, and slowly digestible starch), and low cost. Despite a wide range of available varieties, widespread production is limited to a relatively small set of commodity varieties. Recently, consumers are increasingly seeking out heirloom crop varieties as a representation of small-scale, sustainable food production. The emergence of heirloom crop varieties is a direct result of changing socioeconomic trends that have brought new attention to sustainable food production with regards to land usage and fertilizer consumption [1]. Most existing research on heirloom beans has been from an agricultural standpoint, and studies on the postharvest performance of heirlooms are relatively scarce. Recent studies [2–4] have begun to select heirloom varieties based on agronomic performance including productivity and water usage. Miles et al. [5] have investigated cooking time in relation to growing practices, few studies have characterized heirloom beans for their nutritional value and functionality as an ingredient. Garretson and Marti [6] investigated the chemical composition of four varieties of pigmented heirloom beans in comparison with Pinto, mainly focusing on starch properties. The unique seed coat pigmentation differentiates heirloom varieties from their commodity counterparts and makes them an appealing source of bioactive compounds.

However, the introduction of pigmented heirloom beans in the diet of Western countries needs to be supported by studies assessing quality from multiple angles, including processing time and quality characteristics. A major limitation of incorporating beans on a regular basis in our diet is their relatively long preparation time. Therefore, in the effort of selecting bean varieties for product applications, soaking and cooking times should be considered.

Processing may have a detrimental effect on antioxidants, as many are lost to the cooking water or degraded due to extended exposure to high temperatures [7–9]. While pigmented heirloom beans have already been demonstrated to be rich sources of antioxidants [6], their characteristics through processing have not been investigated yet.

The aim of this study was to (1) improve the knowledge on preparation time and cooking quality of pigmented heirloom beans and compare the findings to a commodity bean and (2) determine the effects of processing *in vitro* DPPH scavenging and total phenolics and total flavonoids in pigmented heirloom beans.

## 2. Materials and Methods

*2.1. Materials.* Four heirloom bean (*Phaseolus vulgaris* L.) varieties—Hutterite Soup, Jacob's Cattle, Koronis Purple, and Tiger's Eye—were kindly provided by the Department of Horticulture of the University of Minnesota (St. Paul, MN, US). Pinto (Lariat cv) bean was used as control. All samples were harvested in 2013. Samples were collected from four farmers in Minnesota and Wisconsin (US), and mixed together. For DPPH, Folin-Ciocalteu, and flavonoids assays, beans were ground (particle size ≤0.5 mm) using a Cyclone Sample Mill (UD Corporation, Boulder, CO).

*2.2. Processing*

*2.2.1. Soaking.* Bean samples were soaked in distilled water (sample/water ratio 1 : 3 w/v) at 25°C for up to 28 hours. Soaking time was determined as the time needed for beans to reach 50% of maximum hydration [10, 11]. The soaking test was performed in duplicate. After soaking, beans were treated immediately with liquid nitrogen, freeze-dried, and ground using a mortar and pestle (particle size ≤0.5 mm) for further analysis.

*2.2.2. Cooking.* Soaked beans were cooked in duplicate in boiling water (sample/water ratio 1 : 3 w/v) until the optimal cooking time, which was determined using the tactile method described by Xu and Chang [10, 11]. Cooked beans were treated immediately with liquid nitrogen, freeze-dried, and ground using a mortar and pestle (particle size ≤0.5 mm).

*2.3. Extracts Preparation.* Sample extraction was performed in duplicate following the procedure proposed by Xu and Chang [10, 11]. The sample (0.2 g) was extracted with 10 mL of acetone/water/acetic acid (70 : 29.5 : 0.5) at 25°C for 2 h. After centrifugation (10 min, 13,000 × g), the supernatant was recovered and stored in the dark at −20°C until analysis. Each extract was analyzed in duplicate.

*2.4. DPPH Assay.* DPPH radical scavenging of the extracts was determined using the method reported by Ragaee et al. [12] and slightly modified by Garretson and Marti [6]. Results were expressed as the percentage of DPPH scavenging based on the equation $[1 - (A_1/A_2)] \times 100]$, where $A_1$ is the absorbance of the sample and $A_2$ is one of the controls represented by the extraction solvent.

*2.5. Folin-Ciocalteu Assay (Total Phenols Assay).* The Folin-Ciocalteu assay was carried out as reported by Xu and Chang [10] and slightly modified by Garretson and Marti [6]. The calibration curve for gallic acid in the linearity range of 50–1000 $\mu$g/mL was used to express the results as milligrams of gallic acid equivalent (GAE) per gram of the sample on a dry weight basis (mg of GAE/g of the sample).

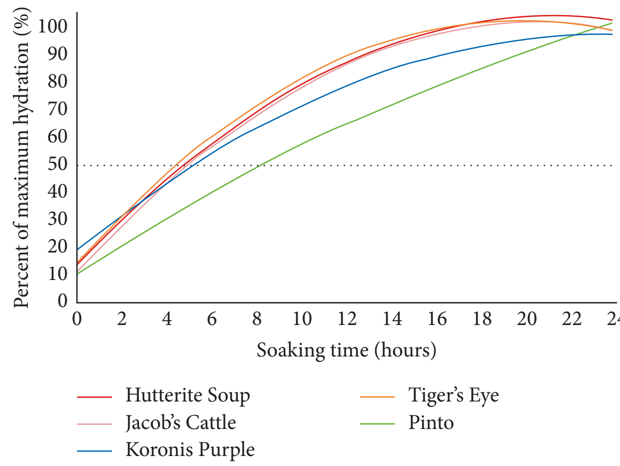


Figure 1: Water absorption of beans during soaking.

*2.6. Total Flavonoids.* Total flavonoids content was determined as reported by Xu and Chang [13] and slightly modified by Garretson and Marti [6] on the extracts obtained as described above. The calibration curve for (+)-catechin in the linearity range of 25–1000 $\mu$g/mL was used to express the results as milligrams of (+)-catechin equivalents (CE) per gram of the sample on a dry weight basis (mg·of CE/g of the sample).

*2.7. Statistical Analysis.* ANOVA was performed utilizing Statgraphics XV version 15.1.02 (StatPoint Inc., Warrenton, VA, USA). Samples were used as factor. When a factor effect was found significant ($P \leq 0.05$), significant differences among the respective means were determined using the LSD test.

## 3. Results

*3.1. Water Absorption, Hydration Rate, and Soaking Time.* The water absorption curves of bean samples are reported in Figure 1. All the heirloom beans exhibited an initial phase of rapid water pickup followed by an equilibrium phase, during which the beans approached their full soaking capacity. Heirlooms reached the saturation after 16 hours and the plateau phase of water absorption after approximately 20 hours. Compared to Pinto, heirloom samples had fast hydration rates, with Tiger's Eye showing the fastest hydration rate, while Koronis Purple the lowest one. The absorption curve for Pinto was linear, requiring longer time to reach saturation and subsequently a plateau. All heirloom varieties had similar soaking times, ranging from 4.5 to 5.5 h (Table 1), which is similar to previous studies for black beans [10].

*3.2. Cooking Time.* Stove top boiling was selected as the cooking method for this study due to relative ease of implementation and widespread use in home kitchens.

Table 1: Soaking, cooking, and preparation time for heirloom beans and Pinto.

| | Soaking time (h:min) | Cooking time (h:min) | Preparation time (h:min) |
|---|---|---|---|
| Hutterite Soup | 4:50 | 1:25 | 6:05 |
| Jacob's Cattle | 5:07 | 1:00 | 6:07 |
| Koronis Purple | 5:19 | 1:10 | 6:29 |
| Tiger's Eye | 4:33 | 1:00 | 5:33 |
| Pinto | 8:10 | 1:00 | 9:10 |

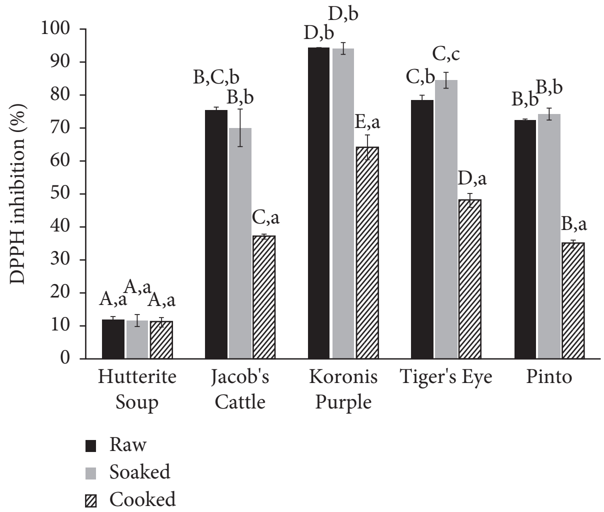


Figure 2: DPPH inhibition for raw, soaked, and cooked beans. For each type of bean, bars with different small letters indicate significant differences (LSD; $P < 0.05$). For each type of treatment (raw/untreated, soaking, and cooking), bars with different capital letters indicate significant differences (LSD; $P < 0.05$).

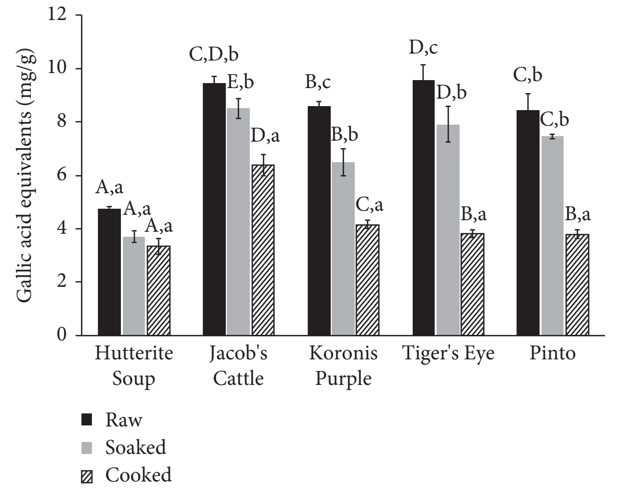


Figure 3: Total phenolics content of raw, soaked, and cooked beans.

Cooking times ranged from 1 hour to 1 hour 25 minutes, with Pinto, Jacob's Cattle, and Tiger's Eye all having the shortest cooking times, while Hutterite Soup had the highest (Table 1).

Total preparation time (Table 1) reflects the total amount of time needed for a raw bean to reach 50% hydration rate and be boiled until thoroughly cooked. The range of total preparation time was over 5 hours, ranging from 5 hours 33 minutes for Tiger's Eye to 9 hours 10 minutes for Pinto. Despite having a similar cooking time than heirlooms, Pinto required over 2 hours more preparation time because of its low rate of hydration during soaking. Hutterite Soup had the longest cooking time; however, total preparation time was the middle value observed. Tiger's Eye had the lowest total preparation time due to both soaking and cooking time being the shortest of those observed.

*3.3. DPPH Assay.* DPPH scavenging ranged from 11.8 to 94.3%, with all raw samples being significantly different from each other (Figure 2; $P \leq 0.05$). The results for raw samples were in the following order: Koronis Purple > Tiger's Eye > Jacob's Cattle~Pinto > Hutterite Soup. This order remained the same after soaking and cooking; however, cooking resulted in statistically significant losses for all samples except for Hutterite Soup. DPPH scavenging of soaked beans' extracts ranged from 11.6 to 94.2%, (Figure 2). The decrease in DPPH scavenging for soaked (compared to raw) Hutterite Soup, Koronis Purple, Jacob's Cattle, and Pinto was not significant. Tiger's Eye was uniquely affected: the soaked sample extract's scavenging activity was significantly higher compared to that of the raw sample.

After cooking, the values for DPPH inhibition ranged from 11.1 to 64.1% (Figure 2). The range of loss after cooking was 5.7–51.9% and was higher in Pinto than for all heirlooms. Among the heirloom varieties, Hutterite Soup had the lowest amount of loss, while Jacob's Cattle had the highest. Xu and Chang [9] reported similar behavior among various pulses after a boiling process, citing a range of loss between 52.9 and 88.4%. The higher range of loss could have been due to a slightly longer boiling time (90 min) than what was used in this study (60–85 min). DPPH inhibition of raw pulses was significantly correlated with DPPH inhibition of soaked ($r = 0.992$; $P < 0.001$) and cooked ($r = 0.929$; $P = 0.023$) samples.

*3.4. Folin-Ciocalteu (Total Phenols) Assay.* The range for total phenolic content according to the Folin-Ciocalteu method was 4.8–9.6 mg GAE/g (Figure 3) in raw beans. These values are within the range of GAE reported for Pinto cultivars by Mojica et al. [14]. The samples were in the following order: Tiger's Eye and Jacob's Cattle had the highest, followed by Pinto and Koronis Purple, and finally Hutterite Soup. After soaking, all samples' GAEs decreased, but the ranking among varieties remained in the same order. Values ranged from 3.7 to 8.5 mg GAE/g, (Figure 3). Jacob's Cattle experienced the lowest amount of loss (about 10%) and Koronis Purple the highest (about 25%).

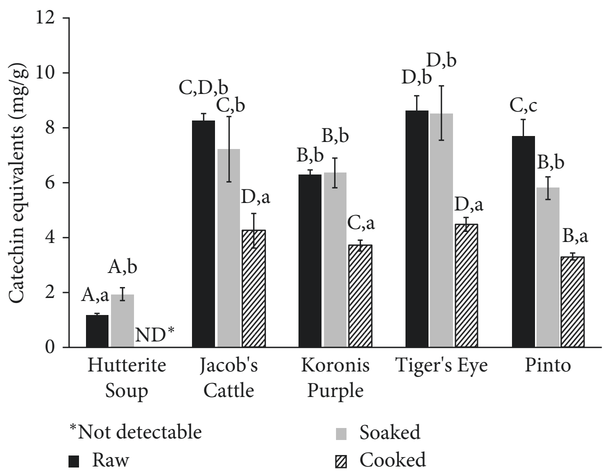


Figure 4: Total flavonoids content of raw, soaked, and cooked beans.

Cooking resulted in significant further decreases of GAE in all samples, to values from 3.34 to 6.37 mg GAE/g (Figure 3). As with DPPH inhibition, Hutterite Soup showed the lowest total phenolics value of all beans while Jacob's Cattle had the highest, being nearly twice that observed for Hutterite Soup. The values for Pinto and Tiger's Eye were not different from each other ($P \leq 0.05$) and significantly higher than that for Hutterite Soup. Loss due to cooking ranged from 30 to 60%, with Hutterite Soup showing the least amount of loss, while Tiger's Eye had the highest one. In addition, Tiger's Eye was the only heirloom variety with a greater percentage of loss after cooking versus Pinto. Xu and Chang [9] reported similar behavior, with loss due to cooking ranging from 33 to 50%. The amount of GAE in raw pulses was significantly correlated with that measured after soaking ($r = 0.966$; $P = 0.007$).

*3.5. Total Flavonoid Content.* Total flavonoid content in raw beans ranged from 1.2 to 8.7 mg·CE/g (Figure 4) and samples followed the order of Tiger's Eye ≈ Jacob's Cattle > Pinto > Koronis Purple >> Hutterite Soup. This order remained after soaking, which however resulted in a decrease for all samples except Hutterite Soup. After soaking, total flavonoids content ranged from 1.8 to 8.5 mg·CE/g, with Hutterite Soup having the lowest of all samples' values. The change after soaking for most varieties was similar to trends previously observed by Boateng et al. [7].

The range observed for total flavonoids after cooking was 3.3–4.6 mg·CE/g; however, the value for Hutterite Soup was below the detectable limit. For the most part, the sample order was the same as for raw and soaked beans. Both Jacob's Cattle and Tiger's Eye had the highest values, with their values not being significantly different from each other ($P \leq 0.05$). All heirlooms besides Hutterite Soup had higher total flavonoids than Pinto. The range of total flavonoids lost due to cooking was 41–57%. All heirlooms showed less loss versus Pinto; among heirlooms, Jacob's Cattle had the highest loss (48.5%). The amount of flavonoids in raw pulses was significantly correlated with that measured after soaking ($r = 0.951$; $P = 0.013$) and cooking ($r = 0.971$; $P = 0.006$).

## 4. Discussion

In recent years, consumer awareness about the nutritional benefits of beans has made them a popular choice for increasing protein and fiber in the diet. Additionally, desirable traits such as low preparation time are often sought out in order to minimize the amount of time and energy needed to prepare beans for consumption.

Differences in water absorption kinetics among heirloom beans could be related to physical characteristics of the seed [15] or to compositional traits, such as total phenolics content [16, 17]. Pulses with a thin, amorphous seed coat tend to have higher rates of water absorption during the initial hydration phase versus those with thicker seed coats. Additionally, smaller beans tend to hydrate at faster rates due to higher surface area to volume ratios [15]. For phenolic compounds, higher rates of water uptake during soaking have been linked to lower total phenolics content in the seed coat [16, 17]. This relationship was also found in some of the heirloom varieties tested here. Hutterite Soup had one of the fastest hydration rates while also having the lowest amount of GAE (Figure 3). On the contrary, the bean with the fastest hydration rate was Tiger's Eye, which had high GAE (Figure 3).

As regards cooking time, Cichy et al. [18] reported that beans with lighter seed coats had lower cooking times than those with red or purple seed coat colors. Despite these findings, the opposite effect was observed here as Hutterite Soup had the lightest seed coat color while having the longest recorded cooking time. Indeed, other traits should be taken into consideration, such as surface area to volume ratio, seed coat thickness and permeability, and starch granule structure [18]. In a previous study, Garretson and Marti [6] evaluated the physicochemical properties of pigmented beans in terms of firmness and pasting properties. No significant correlation was found between these parameters and total phenols and flavonoids measured in the present study. Further study might consider a large number of varieties.

A considerable amount of the commercial appeal heirloom beans carry is attributable to their vibrant colors and distinct seed coat patterns. In addition, colored commodity beans already are regarded as rich sources of antioxidants [6]. In raw beans, the results of the Folin-Ciocalteu assay (i.e., the GAE) of a variety significantly correlated with results in the DPPH ($r = 0.914$; $P < 0.05$) as well as the total flavonoids assay ($r = 0.98$; $P < 0.05$), in agreement with the other literature on bean-derived products [19]. However, processing treatments such as soaking and cooking can affect antioxidant levels and activity. As regards processing, although after soaking the amount of GAE was still significant correlated to the flavonoids content ($r = 0.921$; $P = 0.027$), no significant correlation was detected after cooking.

While ample literature exists on the overall change in antioxidants between raw and cooked beans [7, 20], information is lacking regarding the effects of soaking and cooking separately and no information is available for heirloom varieties specifically.

All varieties were affected by processing; however, cooking generally caused greater loss than soaking. Pinto showed the highest amount of loss in antioxidants and antioxidant activity, while among heirlooms, Hutterite Soup had the lowest percent loss throughout soaking and cooking. However, it also contained the lowest amount total phenolics and flavonoids, which in some cases decreased to below the limit of quantification after processing. Alternatively, it is possible that Hutterite Soup had a different distribution of AOs than the other varieties, that is, more in the inner seed parts (such as the testa or cotyledon), which may prevent them from leaching, as proposed by Xu and Chang [9].

In addition to differences between varieties, not all antioxidants types showed the same behavior. The GAE of all varieties seemed to be more heavily affected by soaking than the DPPH or total flavonoid values, suggesting that the flavonoids contributed more to the DPPH results than nonflavonoid phenolics and were less prone to leaching. In general, the activities observed in the DPPH assay are likely a combination of flavonoids and other nonflavonoid phenolic compounds, perhaps even constituents with low activities in either the total flavonoids or the Folin-Ciocalteu assay.

Similar to our results, Moreno-Jiménez et al. [21] highlighted that the changes in individual phytochemical classes did not follow the exact same trend as the results of their antioxidant assays, which however involved different methods than the ones used here. Chen et al. [22] investigated cranberry beans and found that the total phenolics and total flavonoids level (as measured by the same methods we used) decreased as well. However, a more detailed analysis via HPLC revealed that different classes of phenolic compounds were affected in different ways. It is possible that certain antioxidants were more tightly bound to the cell walls in certain varieties and therefore were less susceptible to leaching.

While Pinto had a significantly longer soaking time than all heirloom beans, it was only for total flavonoids that Pinto had higher loss than the heirloom varieties, which had shorter soaking times. Other authors [7, 9, 10] observed more loss of phenolic compounds in beans that required longer soaking times. The phenomenon was attributed to longer soaking times allowing unbound phenolics to solubilize and diffuse into the soaking water. On the contrary, Siah et al. [8] observed little change in the phenolic profiles of soaked faba beans and attributed this to the fact that soaking at room temperatures was not enough to disrupt the physicochemical structure of the seed coats that house many antioxidant compounds. The majority of results point out loss of antioxidant compounds into the water; however, further study is needed on the isolated effect of soaking in order to elucidate the exact mechanisms of loss.

For beans other than Hutterite Soup, prolonged exposure to heat led to significantly more antioxidant loss than soaking at ambient temperatures. The extent of loss was so great that after cooking the differences among varieties that had been detected in raw and soaked samples were not detectable anymore. These results suggest that all the varieties of beans are subjected to significant loss. While many studies have investigated the effect of thermal processing, changes in antioxidant profiles are often attributed to a combination of thermal degradation and leaching of water soluble compounds into the cooking water. In addition to the isolated effect of soaking, analyzing antioxidants after thermal processing can provide a good indication of the nutritional value of beans as they would typically be consumed. While the major mechanism of loss during soaking is likely the aforementioned leaching of water soluble compounds into soaking water, boiling adds an additional aspect of thermal degradation that could lead to antioxidant loss.

## 5. Conclusions

The pigmented heirloom beans used in this study had higher levels of antioxidants and *in vitro* antioxidant activity compared to Pinto. This trend was observed at all stages of processing, implying the possibility that the dark and vibrant seed coat pigments that give heirlooms their remarkability may also come with the inherent health benefits attributed to food with high antioxidant levels. With such loss being likely due to both thermal degradation and pigment leaching into the cooking water, opportunity exists to expand study to include additional heirloom varieties in hope of identifying a variety that is both quick cooking and experiences a minimal amount of antioxidant loss before it is ready for consumption. Finally, the use of processing methods such as steaming or dry roasting should be explored, in hope of identifying methods that induce minimal degradation and loss into the surrounding environment.

## Additional Points

Results contribute to increase the general awareness of heirloom beans and thus to create new market opportunities. Aside from an attractive appearance and their connection with sustainable, small-scale farming practices, heirloom beans had specific compositional traits, whose potential health benefits should be investigated in future studies.

## Conflicts of Interest

The authors declare there are no conflicts of interest.

## References


[1] J. A. Foley, R. DeFries, G. P. Asner et al., "Global consequences of land use," *Science*, vol. 309, no. 5734, pp. 570–574, 2005.

[2] H. R. Swegarden, C. C. Sheaffer, and T. E. Michaels, "Yield stability of heirloom dry Bean (*Phaseolus vulgaris* L.) cultivars in midwest organic production," *HortScience*, vol. 51, pp. 8–14, 2016.

[3] B. O. Brouwer, K. M. Murphy, and S. S. Jones, "Plant breeding for local food systems: a contextual review of end-use selection for small grains and dry beans in Western Washington," *Renewable Agriculture and Food Systems*, vol. 31, no. 2, pp. 172–184, 2015.

[4] B. Brouwer, L. Winkler, K. Atterberry, S. Jones, and C. Miles, "Exploring the role of local heirloom germplasm in expanding western Washington dry bean production," *Agroecology and Sustainable Food Systems*, vol. 40, no. 4, pp. 319–332, 2016.

[5] C. Miles, K. A. Atterberry, and B. Brouwer, "Performance of Northwest Washington Heirloom dry bean varieties in organic production," *Agronomy*, vol. 5, no. 4, pp. 491–505, 2015.

[6] L. Garretson and A. Marti, "Pigmented heirloom beans: nutritional and cooking quality characteristics," *Cereal Chemistry Journal*, vol. 94, no. 2, pp. 363–368, 2017.

[7] J. Boateng, M. Verghese, L. T. Walker, and S. Ogutu, "Effect of processing on antioxidant contents in selected dry beans (*Phaseolus* spp. L.)," *LWT–Food Science and Technology*, vol. 41, no. 9, pp. 1541–1547, 2008.

[8] S. Siah, J. A. Wood, S. Agboola, I. Konczak, and C. L. Blanchard, "Effects of soaking, boiling and autoclaving on the phenolic contents and antioxidant activities of faba beans (*Vicia faba* l.) differing in seed coat colours," *Food Chemistry*, vol. 142, pp. 461–468, 2014.

[9] B. Xu and S. K. C. Chang, "Effect of soaking, boiling, and steaming on total phenolic content and antioxidant activities of cool season food legumes," *Food Chemistry*, vol. 110, no. 1, pp. 1–13, 2008.

[10] B. J. Xu and S. K. C. Chang, "Total phenolic content and antioxidant properties of eclipse black beans (*Phaseolus vulgaris* L.) as affected by processing methods," *Journal of Food Science*, vol. 73, no. 2, pp. H19–H27, 2008.

[11] L. Garretson, "Pigmented heirloom beans: quality characteristics before and after cooking," University of Minnesota, Minneapolis, MN, USA, Thesis, 2016.

[12] S. Ragaee, I. Guzar, E.-S. M. Abdel-Aal, and K. Seetharaman, "Bioactive components and antioxidant capacity of Ontario hard and soft wheat varieties," *Canadian Journal of Plant Science*, vol. 92, no. 1, pp. 19–30, 2012.

[13] B. J. Xu and S. K. C. Chang, "A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents," *Journal of Food Science*, vol. 72, no. 2, pp. S159–S166, 2007.

[14] L. Mojica, A. Meyer, M. A. Berhow, and E. G. de Mejía, "Bean cultivars (*Phaseolus vulgaris* L.) have similar high antioxidant capacity, in vitro inhibition of $\alpha$-amylase and $\alpha$-glucosidase while diverse phenolic composition and concentration," *Food Research International*, vol. 69, pp. 38–48, 2015.

[15] B. K. Tiwari and N. Singh, *Pulse Chemistry and Technology*, Royal Society of Chemistry, London, UK, 2012.

[16] K. A. Ross, L. Zhang, and S. D. Arntfield, "Understanding water uptake from the induced changes occurred during processing: chemistry of pinto and navy bean seed coats," *International Journal of Food Properties*, vol. 13, no. 3, pp. 631–647, 2010.

[17] A. S. M. Golam Masum Akond, L. Khandaker, J. Berthold et al., "Anthocyanin, total polyphenols and antioxidant activity of common bean," *American Journal of Food Technology*, vol. 6, no. 3, pp. 385–394, 2011.

[18] K. A. Cichy, J. A. Wiesinger, and F. A. Mendoza, "Genetic diversity and genome-wide association analysis of cooking time in dry bean (*Phaseolus vulgaris* L.)," *Theoretical and Applied Genetics*, vol. 128, no. 8, pp. 1555–1567, 2015.

[19] Y. Ma and H. Huang, "Characterisation and comparison of phenols, flavonoids and isoflavones of soymilk and their correlations with antioxidant activity," *International Journal of Food Science & Technology*, vol. 49, no. 10, pp. 2290–2298, 2014.

[20] B. Xu and S. K. C. Chang, "Total phenolic, phenolic acid, anthocyanin, flavan-3-ol, and flavonol profiles and antioxidant properties of pinto and black beans (*Phaseolus vulgaris* L.) as affected by thermal processing," *Journal of Agricultural and Food Chemistry*, vol. 57, no. 11, pp. 4754–4764, 2009.

[21] M. R. Moreno-Jiménez, V. Cervantes-Cardoza, J. A. Gallegos-Infante et al., "Phenolic composition changes of processed common beans: their antioxidant and anti-inflammatory effects in intestinal cancer cells," *Food Research International*, vol. 76, pp. 79–85, 2015.

[22] P. X. Chen, J. H. Dupuis, M. F. Marcone et al., "Physicochemical properties and in vitro digestibility of cooked regular and nondarkening cranberry beans (*Phaseolus vulgaris* L.) and their effects on bioaccessibility, phenolic composition and antioxidant activity," *Journal of Agricultural and Food Chemistry*, vol. 63, no. 48, pp. 10448–10458, 2015.