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Comparative protein and folate content among canola cultivars and other cruciferous leafy vegetables

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Abstract
Numerous research data support the recommendation of an increased intake of a wide variety of dark green leafy vegetables, which provide major dietary benefits in the human diet. The potential of canola (*Brassica napus* L.) leafy greens as a food source for human consumption is being considered to increase the variety of nutritious vegetables available to consumers. Five canola cultivars (ca), (Abilene, Jetton, Kronos, Virginia and Wichita) grown at the Alabama A&M University Winfred Thomas Agricultural Research Station in Hazel Green, Alabama, were analyzed for protein and folate composition of raw canola greens and compared to store-purchased collard greens (*Brassica oleracea* var. *acephala*), kale (*Brassica oleracea* var. *viridis*) and cabbage (*Brassica oleracea* var. *capitata*). Results showed no significant difference in the mean protein content of kale (24.85%) and the canola cultivar Kronos (22.70%). Cabbage, however, had the lowest protein content of all the vegetables tested in this study (10.15%). Among the three harvest stages (rosette, pre-bolting and blooming), the pre-bolting stage showed significantly higher protein (23.69%) compared to the rosette (20.52%) and blooming (22.27%) stages. No significant differences were observed in the mean folate content of canola and the store-purchased vegetables. Although not statistically significant, the trend from highest to lowest folate content among the *Brassica* species in this study was: Kale>Virginia-(ca)>Kronos-(ca)>Jetton-(ca)>Abilene-(ca)>Wichita-(ca)>Cabbage>Collard. The distribution of folate among all the samples showed variations from 0.02 mg/100 g in collard greens to 0.148 mg/100 g and 0.149 mg/100 g in canola (cultivar Jetton) and kale, respectively. Results indicated that canola greens could be a nutritionally acceptable substitute for traditional leafy green vegetables.

Key words: *Brassica*, cabbage, canola, collard, folate, kale, protein.

Introduction

There are many underutilized green vegetables of promising nutritive value that have not been extensively investigated because of lack of awareness and popularization of technologies for utilization [14]. According to Flyman and Afolayan [5], many researchers are advocating the use of a food-based strategy to achieve optimal dietary requirements. Guerrero *et al.* [8] reported that plant greens were important foods in some traditional diets, but today they are no longer generally considered for nutrition. The beneficial value of neglected vegetables is regarded worldwide as an important area of nutritional research, and more scientists are investigating various underutilized plant species to determine how they may contribute to the health of the population [2, 14]. The presence of a large number of inexpensive edible green leafy vegetables, their abundance and their attributive qualities create interest in nutritional value of selected green leafy vegetables [9].

In many parts of the world, many species of the cruciferous family (especially *Brassica)* are highly consumed as vegetables [1, 6]. Some of the most common cruciferous vegetables in the United States are Brussels sprouts, broccoli, cabbage, kale, turnips, collard greens and cauliflower [7]. The regular consumption of vegetables, specifically the dark green leafy vegetables is highly recommended because of their potential in reducing the risks of chronic diseases [15]. These vegetables are important food crops because they provide adequate amounts of dietary vitamins and minerals for humans [14]. Cruciferous vegetables of the genus *Brassica* are also known to provide dietary proteins [3] and folate [10].

Canola (also known as rapeseed) is a member of the cruciferous family of vegetables and is closely related to cabbage, kale and collard greens. *Brassica* production has become increasingly popular in North America and other regions of the world, and is an important plant source of oil and protein for human and animal nutrition, respectively [6]. Though canola is grown predominately in Canada, it has gained acceptance in the United States as a cash crop. In several countries rapeseed/canola has long been consumed as a green vegetable [1, 6, 4]. However, in the United States, canola is produced primarily for vegetable oil which is widely used in cooking, salad dressings, margarines and shortenings. After the oil is extracted from the canola seed, the remaining protein meal is used as protein supplement to feed livestock.

Significant information exists on the nutrient content of common cruciferous vegetables. However, there is little documentation on canola leafy greens as a potential nutritious vegetable. Therefore, this study was designed to (1) determine the protein and folate composition of raw canola greens at different growth stages; (2) examine variations in protein and folate content among different canola cultivars and (3) compare protein and folate content of canola with that of store-purchased cabbage, collard greens and kale.

Materials and Methods

Canola production, sample collection and preparation: Five canola cultivars (Abilene, Kronos, Jetton, Wichita and Virginia) were grown at the Alabama A&M University (AAMU) Winfred Thomas Agricultural Research Station (WTARS), located in Hazel Green, Alabama. Each plot, which consisted of 6 rows, 6 m long and 18 cm apart, was replicated four times. Seeds were sown in October 2005 on Decatur silty clay loam soil type using a Hege 1000 Series Drill/Planter (Heston, KS) at seeding rate of approximately 6 kg ha^{-1} [11]. All plots were fertilized with 30 kg ha^{-1} of N-P-K (13-13-13) fertilizer after crop establishment, and split application of 100 kg ha^{-1} of nitrogen (34-0-0) was applied at budding (50 kg ha^{-1}) and flowering/blooming (50 kg ha^{-1}) stages. The first sample of plants was harvested on February 27, 2006 at the rosette stage, 144 days after planting (DAP). At this stage, the plants were approximately 15-25 cm in height with older leaves at the base and younger leaves developing in the center. The second harvest was done at the budding stage on March 17, 2006 (162 DAP). At this stage, flower buds were present on the panicle. The final harvest was done at the flowering/blooming stage on March 23, 2006 (168 DAP). At this stage, the buds of the plant began to open as the plant continued to grow and develop new buds. At each stage of harvest, samples were taken at approximately 5 cm above ground from plants within a randomly selected 1 m section of each plot.

Following field collection, the samples were weighed, washed in tap-water and patted dry with paper towels and then stored in a -80°C freezer. Frozen samples were later transferred from -80°C to a Consul 24 Virtis freeze-dryer (The Virtis Company, Gardiner, NY) for 5 days. Freeze-dried samples were milled using a Robot Coupe, Blixer RS1 BX3 Food Processor (Robot Coupe U.S.A. Inc., Ridgeland, Miss.). The traditional cruciferous leafy greens (cabbage, collard greens and kale) were obtained from a local food store and were then prepared as the canola samples.

Protein analysis of samples: Dry matter total nitrogen was determined according to the Dumas protein method. Using a CNS Elementar Analyzer (Variomax CNS, Elementar Americas, Inc. NJ), approximately 0.5 g of each freeze-dried ground sample was transferred into a crucible to which tungsten oxide (oxidation catalyst) was added. Crucibles were placed on a carousel from which robotic arms lifted the crucibles and lowered them in a combustion tube. Samples were combusted at approximately 1200°C. Protein was determined using the nitrogen to protein conversion factor of 6.25*N, where N = total nitrogen. Four replicates were prepared for each sample which included 5 canola cultivars at 3 harvest stages (60 analytical canola samples) and 4 replications of the traditional leafy greens: cabbage, collard greens and kale (12 analytical samples).

Folate analysis: Samples were shipped to Eurofins Scientific Laboratory in Des Moines, Iowa, for folate analysis. Eurofins reported the use of a modified microbiological assay method [16] to determine total folate. Three replicates were prepared for each sample, including 5 canola cultivars at 3 harvest stages (45 analytical canola samples) and the 3 traditional leafy greens, 3 replications each (9 analytical samples).

Statistical analyses: All statistical analyses were carried out using SAS (2006) General Linear Model (GLM), to determine significance among treatments. Duncan Multiple Range Test ($\alpha = 0.05$) was used for mean separation.

Results

Protein content: The protein content in freeze-dried leaves (FDL) of all the *Brassica* vegetables tested indicated significant differences among purchased traditional vegetables (kale, cabbage and collard greens) and canola from different growth stages (Table 1). Kale had the highest mean protein content (24.85% FDL sample weight). Protein in the canola cultivar Kronos (22.70%) was not significantly different from kale; however, the amount of protein in both species was significantly higher than that of cabbage (10.15% of the FDL sample weight). Protein content of collard greens was not significantly different from the other four canola cultivars tested in this study. Mean protein among *Brassica* species ranked kale>canola >collard>cabbage.

Analysis of mean protein content in FDL samples for canola at different physiological growth stages as indicated by harvesting period (Table 1), revealed that canola leaves at pre-bolting stage have significantly higher protein content (23.69%) compared to that of the rosette and blooming growth stages (20.52% and 22.27% of the FDL sample weight, respectively).

Protein content varied among canola cultivars at the different harvest periods and among store purchased kale, collard greens and cabbage (Fig. 1). The highest range of protein content (18.17% to 30.23% of the FDL sample weight) was among canola cultivars at the pre-bolting stage. The rosette growth stage ranged between 17.98 and 24.56% protein, while the lowest, 16.34 to 24.17% of the FDL sample weight, was during blooming. Similar protein values for canola were reported by Ahmad *et al.* [1], in a study indicating that protein content increased with nitrogen fertilization.

Among the traditional vegetables, protein content ranged from 9.02% (cabbage) to 27.07% (kale). Cabbage, whose protein content was lower than that of the other traditional vegetables studied,

Table 1. Mean protein content of freeze-dried canola leaves harvested at different stages of growth in comparison to locally purchased collard greens, cabbage and kale.

Cultivar	Rosette	Pre-bolting	Blooming	Grand† mean
Kronos	21.06 a	25.95 a	21.08 a	22.70^{wx}
Jetton	20.77 a	22.65 b	20.52 a	21.31^{xy}
Virginia	20.73 a	23.06 ab	20.70 a	21.50^{xy}
Wichita	20.72 a	23.36 ab	20.74 a	21.60^{xy}
Abilene	19.34 a	23.47 ab	18.31 a	20.37^{xy}
μ	20.52_B	23.69_A	22.27_B	
Traditional *Brassica* vegetables				
Kale	---	---	---	24.85^{w}
Collard green	---	---	---	19.59^{y}
Cabbage	---	---	---	10.15^{z}

† Means of protein by harvest with the same letter are not significantly different with LSD and $\alpha = 0.05$; values represent means of 4 replications

was reported in a study by Mosha and Gaga [12] to be normally lower in nutritional characteristics than other leafy greens they tested.

Folate content: Results from this study indicated no significant differences in mean folate content (Fig. 2) among canola greens and the store purchased traditional vegetables (kale, collard greens and cabbage). Although not statistically significant, the trend from highest to lowest folate content among the *Brassica* species in this study was: Kale>Virginia-(ca)>Kronos-(ca)>Jetton-(ca)>Abilene-(ca)>Wichita-(ca)>Cabbage>Collard, where (ca) = canola cultivar.

Mean folate content in the freeze-dried leaf samples ranged from 0.03 mg/100 g in collard greens to 0.12 mg/100 g in kale (Fig. 2). Folate content (Fig. 3) in canola samples harvested at different physiological growth stages indicated an increase from rosette to blooming (Rosette<Pre-bolting<Blooming). Variations among all samples show folate ranging from 0.03 mg/100 g in collard greens to 0.15 mg/100 g FDL for kale (Fig. 4).

Discussion and Conclusions

The findings from this study indicate that canola leafy greens can be an additional natural source of protein and folate for the diet. The variations observed in protein content of canola cultivars at different growth stages allow the determination of the best time to harvest canola leafy greens for protein benefits. The pre-bolting physiological stage represents a period of rapid growth development with the initiation of flower buds and increased biomass production. This stage offers the opportunity of harvesting the most biomass during the period when protein content reaches its peak and is not significantly different from the protein content of kale.

The range of folate found in canola is comparable to those of the traditional vegetables tested. The microbiological method used in this study provided a value for the total folate present and not the specific forms of folate. Future research should be done to analyze the various forms of folate and their levels in canola leafy greens.

This research provides the framework for additional studies on a crop that is fast becoming wide spread in North America. Additional work is needed to examine other nutrient capabilities and the bioavailability of different nutrients in this plant. Further studies should be carried out on other chemical components of the canola plant. Different cultivars, their genetic characteristics and growing conditions should be carefully examined to produce canola leafy greens that have acceptable and beneficial nutrient quality.

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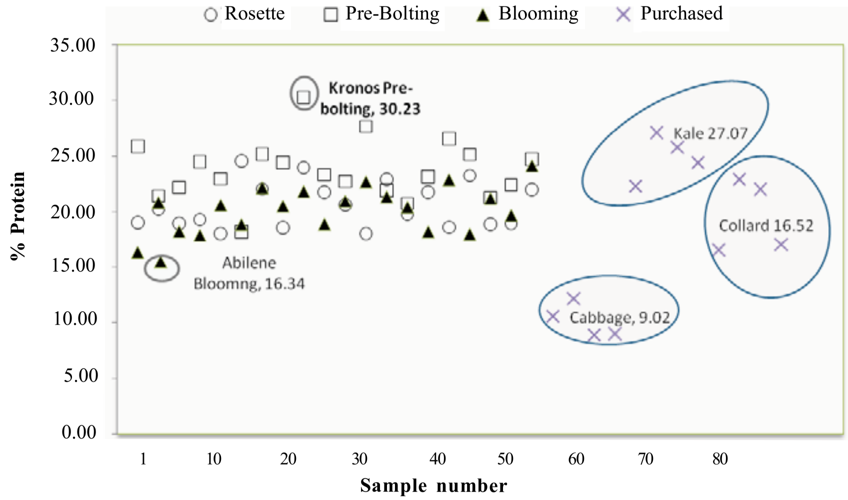

Figure 1. Distribution of protein content in individual samples of canola cultivars across harvest stages in comparison to store-purchased kale, collard greens and cabbage.

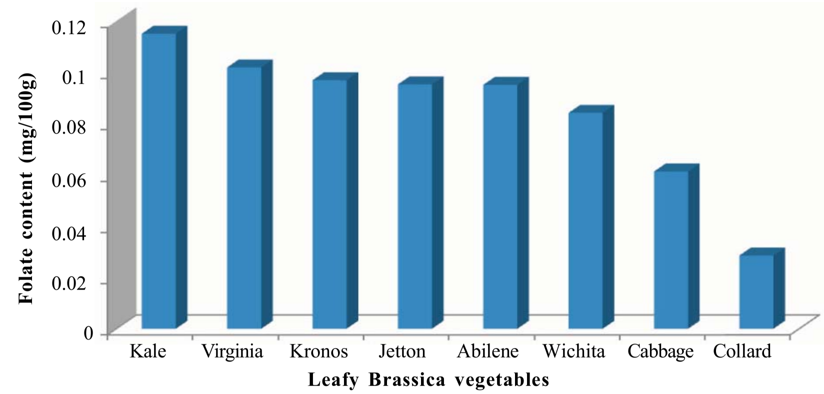

Figure 2. Distribution of folate among canola cultivars and store purchased kale, cabbage and collard greens.

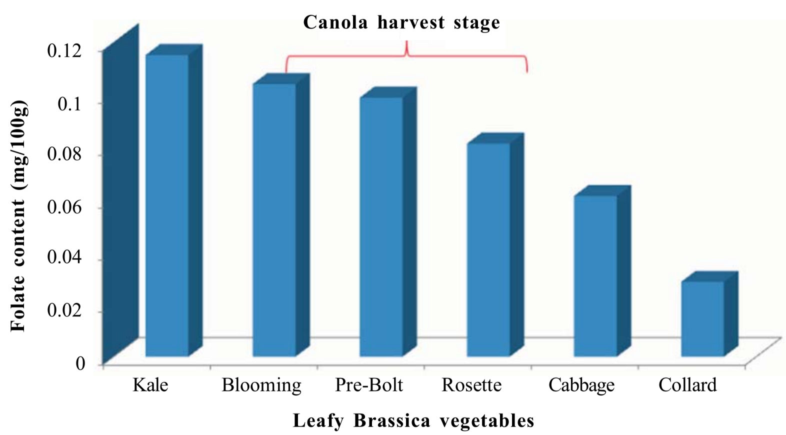

Figure 3. Distribution of folate in canola samples at different growth stages and store bought cabbage, collard greens and kale.

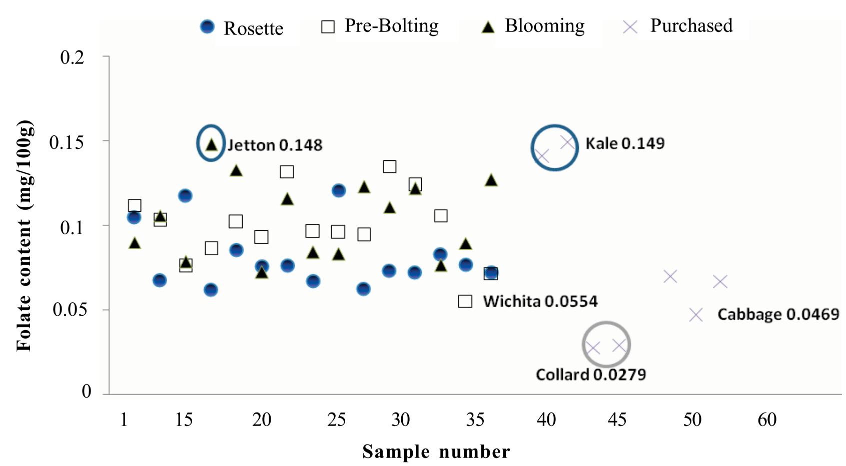

Figure 4. Distribution of folate content in individual samples of canola cultivars across harvest stages in comparison to store purchased kale, collard greens and cabbage.