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Mineral micronutrient and prebiotic carbohydrate profiles of USA-grown kale (*Brassica oleracea* L. var. *acephala*)

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ABSTRACT

Kale (*Brassica oleracea* L. var. *acephala.*) is a leafy green brassica vegetable, the production levels of which have increased over the past 10 years due to increased demand by North American consumers. Kale is perceived to be highly nutritious leafy green vegetable even though its nutrient profile has not been well characterized to date. The objective of this study was to determine the nutritional composition (energy, protein, mineral, and prebiotic carbohydrate concentrations) of 25 different kale genotypes grown in Pelion, South Carolina, USA, and assess its potential as a whole food source of daily essential minerals and dietary fiber. The results of this study show a single 100 g serving of fresh kale can provide a significant percentage of the recommended daily intake of mineral micronutrients (188–873 mg K; 35–300 mg Ca; 20–100 mg Mg) identified by the 2015 Dietary Guidelines Advisory Committee as being underconsumed by Americans. This serving of kale can also provide approximately 0.4–6.7 g of prebiotic carbohydrates, including sugar alcohols (45.4–59.8 mg), simple sugars (0.4–3348 mg), and hemicellulose (245–703 mg). Fresh kale is a low calorie food (36–98 kcal/100 g) with moderate levels of protein (1.6–5.9 g/100 g). Among the genotypes studied, 'Frizzy Lizzy', 'Dauro' and 'Fizz' have significantly high levels of essential minerals and prebiotic carbohydrates, moderate protein content, and low caloric value. These data confirm that kale is a whole food that can provide significant quantities of daily essential minerals and prebiotic carbohydrates. By genetic and location sourcing, the nutritional quality of kale could be further enhanced to benefit North American consumers.

1. Introduction

More than 50 nutrients are essential for human physiological functions and general health. However, billions of people worldwide do not receive sufficient daily quantities of micronutrients due to their low bioavailability in commonly eaten foods. Inadequate intake of these essential minerals leads to "hidden hunger", also known as micronutrient malnutrition. Among these, potassium (K), calcium (Ca), and magnesium (Mg) deficiencies are common in the USA, while deficiencies in other essential minerals including iron (Fe) and zinc (Zn) are prevalent in Southeast Asia and Africa (USDA, 2015a,b; WHO, 2015). Cruciferous green leafy vegetables including kale (*Brassica oleracea* L. var. *acephala*) are major sources of these essential micronutrients, however, few Americans consume the daily recommended intake of leafy vegetables. In 2015, the Dietary Guidelines Advisory Committee (USDA, 2015a,b) urged Americans to increase their vegetable intake as a strategy to prevent mineral deficiencies, obesity, and overweight. According to the Center for Disease Control (CDC, 2013), no state's population is meeting the nationally recommended vegetable consumption level of 5–6 servings per day. In fact, the average American consumes only ~59% of the recommended intake of vegetables (USDA, 2015a,b). The result is widespread under consumption of several nutrients (e.g., K, Ca, Mg) and dietary fiber relative to nutritional needs (USDA, 2015a,b).

Kale has received recent attention from the health and nutrition sectors due to its nutrient profile. However, current kale nutritional quality data are based on a very narrow sampling base conducted more than 10 years ago and are lacking with respect to information on prebiotic carbohydrates (USDA, 2015a,b). The available data

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support the consumer perception that kale places high on lists of healthy foods. On average, kale contains a total of 8% carbohydrates and 3.6% dietary fiber (USDA, 2015a,b); however, the levels of phytonutrients in kale genotypes have not been well characterized.

Several studies show prebiotic-rich, micronutrient-rich, low-calorie diets to play important roles in supporting intestinal health, with potential to prevent obesity and promote gut health (Ley et al., 2005; Dumas et al., 2006; Wu et al., 2011). Prebiotic-rich diets promote intestinal microbial diversity, stimulate the immune system, promote mineral micronutrient absorption, decrease the risk of developing colon cancer, reduce excessive blood glucose and cholesterol levels, and improve insulin sensitivity (Johnston et al., 2010; Lee and Mazmanian, 2010; Caselato et al., 2011). As such, products enriched with prebiotics are becoming more popular, in the belief that they may also have probiotic effects.

Naturally occurring prebiotic carbohydrates include dietary fiber and sugar alcohols (Roberfroid, 2007). Dietary fiber is comprised of non-digestible starch polysaccharides including resistant starch (RS) and non-starch polysaccharides. Sugar alcohols (e.g., sorbitol, mannitol) are derived from simple sugars (Roberfroid, 2007). Americans 20 years of age and over consume less than half of the recommended amounts of prebiotic carbohydrates (e.g., 10 g/d of fructooligosaccarides; 7 g/d of galactooligosaccharide). Legumes, cereals, and vegetables are rich in these prebiotic carbohydrates. For example, a single 100 g serving of lentils (*Lens culinaris* Midik) provides 13 g of prebiotics, the level of which can as much as double after cooking/processing (Johnson et al., 2013, 2015). However, no prebiotic data are available for commonly consumed leafy green vegetables and none are available for kale. Therefore, this study was undertaken to determine the nutritional composition (energy, protein, minerals, and prebiotic carbohydrate concentration) of 25 different kale genotypes grown in Pelion, South Carolina (SC), USA, to the end of developing an evidence-based understanding of the nutritional value of kale.

2. Materials and methods

2.1. Materials

Standards, reagents, acids, and high-purity solvents used for sample and reagent preparation were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) and VWR International (Radnor, PA, USA) and used without further purification. Water, distilled and deionized (ddH_2O) to a resistance of $\geq$18.2 MΩ (Milli-Q Water System, Millipore, Milford, MA, USA), was used for sample and reagent preparation.

2.2. Kale samples

Field-grown fresh kale samples were collected from variety trials conducted by Walter P. Rawls and Sons, Inc. in Pelion, SC, USA in the fall of 2014. Twenty-five commonly grown kale genotypes were selected based on market class, consumer demand, disease resistance, biological yield, and preference for future breeding and selection research (Table 1). Kale plants were harvested at physiological or horticultural maturity (ready to eat). Fresh leaf samples (250 g) were taken randomly from the entire harvested plant of each of four independent replicated field plots and subjected to energy, protein, mineral, and carbohydrate analyses. A total of 100 replicate kale leaf samples were collected. Moisture content of fresh sub-samples (105 °C for 16 h) and the remaining samples were immediately freeze- dried and stored at −40 °C until analysis. Prior to each analysis, the freeze dried samples were finely ground using a mortar and pestle. Nutrient composition data are reported on a fresh weight basis (85% moisture).

2.3. Energy and protein analysis

Finely ground freeze-dried kale samples (1 g) were compressed into a pellet, and then ignited in an oxygen-rich closed environment using an oxygen bomb calorimeter (Parr Instrument Company, Moline, Illinois 61265). Quality control and calibration was accomplished using benzoic acid as the standard (Schmidt-Rohr, 2015). Samples were analyzed in duplicate, and a NIST standard (Typical diet SRM 1548A; NIST Gaithersburg, MD 20899, USA) was used as a reference. Total nitrogen was determined on freeze dried kale samples using a LECO FP3000 CNS analyzer; protein content was calculated (N × 6.25).

2.4. Mineral concentrations

Minerals were determined by inductively coupled argon plasma emission spectroscopy (ICP) using a Thermo 6500 Duo instrument (Thermo Fisher Scientific, [no city], PA, USA) after HNO_3—H_2O_2 digestion (Thavarajah et al., 2009). Aliquots (500 mg) of freeze-dried samples were digested with 6 mL of 70% HNO_3 overnight at room temperature. They were then heated to 90 °C for 1 h, after which 3 mL of 30% of 30% H_2O_2 were added and the sample was held at 90 °C for 15–20 min. Finally, 3 mL of 6 M hydrochloric acid was added and samples were held at 90 °C for another 5 min. Samples were then filtered and made up to 10 mL in Milli-Q water. Detection limits were 30 μg/L for K, Ca, and Mg and 5 μg/L for Fe, Zn, Mn, Cu, and Se. Analytical quality assurance was accomplished using authentic calibration standards and the NIST standard reference material peach leaves 1547. Results were used to calculate the percentages of recommended daily allowance values (%RDA, National Academy of Sciences, 2004) provided by a 100 g portion size of fresh kale

2.5. Carbohydrate analysis

Freeze-dried kale samples (~500 mg) were extracted as described by Muir et al. (2009). Briefly, this method involved extraction with distilled water at 80 °C for 1 h. The resulting extract was passed through a 13 mm × 0.45 μm nylon syringe filter (Chromatographic Specialties, Brockville, ON, Canada). Analyses for sugar alcohols, monosaccharides, disaccharides, and oligosaccharides were performed by high-performance liquid–liquid partition chromatography (ICS-5000 Dionex, Sunnyvale, CA,

Table 1
Kale genotypes grown in Pelion, SC, USA in the fall of 2014.

Market Class	Genotype
Curly	Darkibor, Dwarf Green Curled Afro, Pentlang Brig, Red Russian, Redbor, Reflex, Ripbor, Scarlet, Star & Stripes, Starbor, Vates, Winterbor, *withheld* (cultivar name withheld by grower), Blue Ridge, Blue Knight, Maribor
Portuguese	Beira, Dauro
Dinosaur	Black Magic, Bonanza, Italian Kale, Lacinato
Ornamental	Fizz
Mustard	Frizzy Joe, Frizzy Lizzy