



Research article

Effect of drying methods on chemical composition and antioxidant activity of underutilized stinging nettle leaves

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ABSTRACT

Stinging nettles provide low-cost quality nutrition for alleviating malnutrition. Previous research on stinging nettles focused mainly on the nutritional quality of fresh leaves. In this study, the effect of drying method on macronutrients, mineral content, ascorbic acid, β -carotene content and total phenols content and antioxidant activity were investigated. The contribution of fresh, oven dried or freeze dried stinging nettle leaves to the required daily value for the nutrients were also determined. Oven drying of nettle leaves resulted in a higher loss of β -carotene and ascorbic acid content compared to freeze drying. In contrast, the total phenols content and total antioxidant activity were higher in oven dried stinging nettle leaves compared to freeze dried leaves. Overall, freeze dried and oven dried nettle leaves can be considered as a rich source of Ca, Mg and vitamin A; a good source of vitamin C, Fe, and Mn; and a source for Mg and K. Stinging nettle leaves could potentially be used as a cheap natural source of antioxidants and for addressing micronutrient malnutrition.

1. Introduction

Stinging nettle, *Urtica dioica* L., provides vitamins and minerals needed to maintain health in humans (Kavalali, 2003). The plant may help to combat malnutrition or nutrition-related health problems due to its bioactive compounds (Adhikari et al., 2016). Fresh nettle leaves contain phenolic compounds (Orčić et al., 2014; Farag et al., 2013; Otlés and Yalcin, 2012; Pinelli et al., 2008) and polyunsaturated fatty acids (Rutto et al., 2013; Guil-Guerrero et al., 2003), essential amino acids (Hughes et al., 1980; Rutto et al., 2013) and ascorbic acid (Ioana et al., 2013). Nettle leaves contain nine carotenoids of which lutein and lutein isomers, and β -carotene are the basic carotenoids (Guil-Guerrero et al., 2003). Nettle leaves are good sources of minerals such as calcium, iron, magnesium, manganese, zinc, phosphorus, potassium, copper and selenium (Pytlakowska et al., 2012; Kara, 2009; Özcan et al., 2008). Popov et al. (2020) investigated the application of contemporary extraction techniques for elements and minerals from stinging nettle leaves. The authors found that microwave and ultrasonically-assisted extraction techniques could possibly be used for obtaining extracts as a secondary source of minerals. Nettle leaves are good sources of protein and dietary fiber (Hughes et al., 1980).

Fresh stinging nettle leaves are reported to contain phenolic acid (e.g. hydroxycinnamic acid, hydroxybenzoic acid), tannins and flavonoids (e.g. flavones, flavonols, iso-flavonols, anthocyanins, catechins, lignin) (Pinelli et al., 2008; Farag et al., 2013; Orčić et al., 2014). Gião et al. (2007) evaluated the antioxidant and phenolic content of 48 Portuguese herbal plants for their dependence on extraction features [e.g. powder infusion (dried leaves), fresh leaf infusion and fresh leaf boiling (decoction)]. The authors recommended infusion as the most effective mode of extraction of antioxidants from medicinal plants.

Stinging nettle leaves add variety to the menu, thus could be used as a supplementary, spinach-like vegetable in human diet (Hughes et al., 1980). Naude (2013a, b) also emphasized that the need to include dark green vegetables at least one serving per day to reduce the burden of nutrition-related disease. Nettle plant is widely used as food in early spring when young leaves are added to soups, salads, herbal tea or decocted tea as well as in dried form for winter use (Guil-Guerrero et al., 2003).

Drying of stinging nettle leaves not only grants their use when the plants are not physiologically active but also extends their consumption period. Additionally, the irritating contents of the stinging hairs are dissipated upon drying (Upton, 2013).

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The drying method chosen can have a major impact on nutrient degradation and retention (Shilton, 2003). Ambient air-drying (such as well-ventilated air drying and sun drying) was mentioned as a common method of drying stinging nettle leaves (Maanda and Bhat, 2010; Upton, 2013). However, the slow drying process involved in ambient air-drying methods may lead to a loss of quality of the leaves (e.g. colour changes, losses of ascorbic acid and carotenoids) (Harbourne et al., 2009). Freeze-drying is the most common method of drying nettle for medicinal purposes such as for the production of encapsulated nettle products (Dey, 2013; Ait Haj Said et al., 2015). During freeze-drying, very few chemical changes occur whereas oven drying at 45 °C–140 °C can cause faster degradation of colour and loss of primary metabolites (Shilton, 2003).

Drying processes involving high temperature such as oven drying result in protein denaturation, ascorbic acid and β -carotene degradation (Shilton, 2003) and might affect antioxidants in food products (Chang et al. 2006; Rodrigues et al. 2009). When drying herbs the amount of phenolic compounds and antioxidant activity of herb extracts increased (Suhaj, 2006; Hossain et al., 2010). Differences in aroma, flavour and colour of leaf infusions and cooked leaves were noted when oven-dried leaves used compared to fresh leaves (Shonte and de Kock, 2017). Colour changes during oven drying of stinging nettle leaves were also reported (Alibas, 2006; Shonte and de Kock, 2017). Ascorbic acid and β -carotene are better retained in freeze-dried food products compared to oven-dried (Gupta et al., 2013; Abascal et al., 2005).

In general, the low temperature of the freeze drying process more likely slow down degradation reactions and preserves the nutrient content of food more efficiently than oven or solar drying (Ratti, 2001; Shilton, 2003). However, the cost of freeze drying equipment limits its application to pharmaceutical products and production of highly valued healthy products such as nettle leaves, nettle leaf powder; nettle leaf tea bags, etc. These products are expensive and therefore only affordable to high economic end consumers (developed market, rich) where such consumers demand a higher value and natural products.

Oven drying is used more often in food processing industries due to its lower production costs leading to products that are more affordable to consumers at the lower end of the market (developing market, poor).

However, information on the effect of oven drying and freeze drying on vitamins, total phenolics content and antioxidant activity of nettle leaves are lacking. This study was carried out to determine the effect of oven drying and freeze drying of nettle leaves on macronutrient, minerals, β -carotene, ascorbic acid and total phenolics content, as well as antioxidant activity. The findings of this study could enable the consumers or food industry to choose either an oven drying or freeze drying method for maximum retention of nutritional properties of stinging nettle leaves.

2. Materials and methods

2.1. Drying processes

Stinging nettle young and tender shoots were produced and harvested in October (spring season) 2014 at the University of Pretoria Experimental Farm Station, South Africa. Spinach leaves (as a control) were purchased from a supermarket. Twenty units (500 g each) of young nettle and spinach leaves were sorted and washed. Treatments, each replicated three times, were prepared as follows. Oven dried leaves (70 °C for 15 h) and freeze dried leaves (-40 °C for 5 days) were prepared. Dried leaves were ground to a powder using a coffee grinder and sealed in polyethylene bags. All samples were kept frozen (-4 °C) until analysis.

2.2. Nutritional properties

2.2.1. Proximate composition

AACC International (2000) methods were used to determine moisture (method 44-15A), fats (method 30-25), proteins content (N x 6.25) by

Dumas combustion (method 46-30), ash (method 08-01) and crude fibre (method 32-10.01) while available carbohydrate content was calculated by difference.

2.2.2. Mineral analyses

AACC International (2000) method No.40–70.01 was used for mineral analyses (Ca, Fe, Mg, Mn, Zn, P, K, and Na) using Ion Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) (SpectroAcros, SPEC-TRO Analytical Instruments GmbH, Kleve, Germany).

2.2.3. β -Carotene content

The quantitative analysis of β -carotene content of fresh, freeze dried, and oven dried stinging nettle leaves was done using an auto-sampler Shimadzu Ultra-Fast Liquid Chromatography. Sample extraction and mobile phase preparation were done following the method of Rodriguez-Amaya and Kimura (2004).

The detection of β -carotene was performed at 450 nm and UV/Vis spectra of between 200 to 600 nm, 25 °C on a YMC carotenoid-C30 column (250 × 4.6 mm i.d., 5 μ m pore size) through isocratic elution with a methanol:methyl-*tert*-butyl ether (80:20, v/v) mobile phase at a flow rate of 0.8 ml/min (Kimura et al., 2007). Identification of the all-*trans*- β -carotene was carried out through the combined use of the retention time, visible absorption spectrum obtained with a photodiode array detector and co-injection with an all-*trans*- β -carotene standard at four different concentration levels (calibration curve of the all-*trans*- β -carotene standard, Figure 1). The chromatogram is presented in Figure 2, and all-*trans*- β -carotene (peak 1) was identified by comparing the spectrum (λ max) (Figure 3) with those given in literature (Mercadante et al., 1997; Rodriguez-Amaya and Kimura, 2004) and retention time of the all-*trans*- β -carotene standard. Vitamin A content of fresh and dried stinging nettle leaves was calculated using retinol activity equivalents (RAE) conversion factor of 12 μ g β -carotene to 1 μ g retinol (Joint FAO/WHO, 2001).

2.2.4. Ascorbic acid content

The quantitative analysis of ascorbic acid content of fresh, freeze-dried, and oven-dried leaves was carried out using high-performance liquid chromatography-HPLC (Waters Alliance, Milford, Massachusetts, USA) equipped with 1525 Binary pump system, 2487 Dual λ Absorbance detector (operated at 254 nm) and manual injection valve with 20- μ L sample loop.

Sample extraction and mobile phase preparation was done following Maia et al. (2007). The components were filtered through 0.45 μ m Nylon filters (Millipore) before analysis by the HPLC.

The separation of ascorbic acid was performed on a 4.6 mm × 250 mm i.d., 5 μ m pore size Phenomenex-C18 column by isocratic elution with a mobile phase consisting of 0.2 % metaphosphoric acid/methanol/acetonitrile (90:8:2, v/v/v) at a flow rate of 0.9 ml/min. The quantification of ascorbic acid was done using a calibration curve of L-ascorbic acid standard (Figure 4). The determination of ascorbic acid content was carried out by HPLC through the combined use of the retention time and co-injection with L-ascorbic acid standard (Figure 5).

2.2.5. Total antioxidant activity and phenols content

Sample extraction was performed as described by Otlés and Yalcin (2012). Fresh, freeze-dried, and oven-dried leaves (1 g each) were extracted in covered test tubes in a drying oven (PROLAB, Model: IDS 160, Switzerland) for 1 h at 50 °C using 10 mL of 80 % methanol-water mixture. The extracts were centrifuged at 3000 rpm for 10 min and the supernatants were recovered for analysis. Total phenolics content was determined using the Folin-Ciocalteu (FC) method (Singleton et al., 1999). Total antioxidant activity of fresh, freeze-dried, and oven-dried leaves were determined using the DPPH Radical Scavenging Activity Assay according to Brand-Williams et al. (1995).

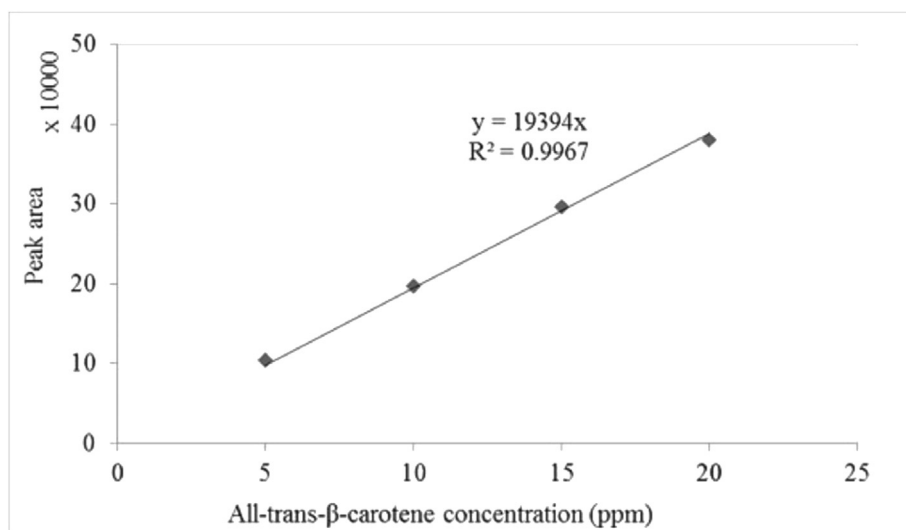


Figure 1. Standard curve of the all-trans-β-carotene standard.

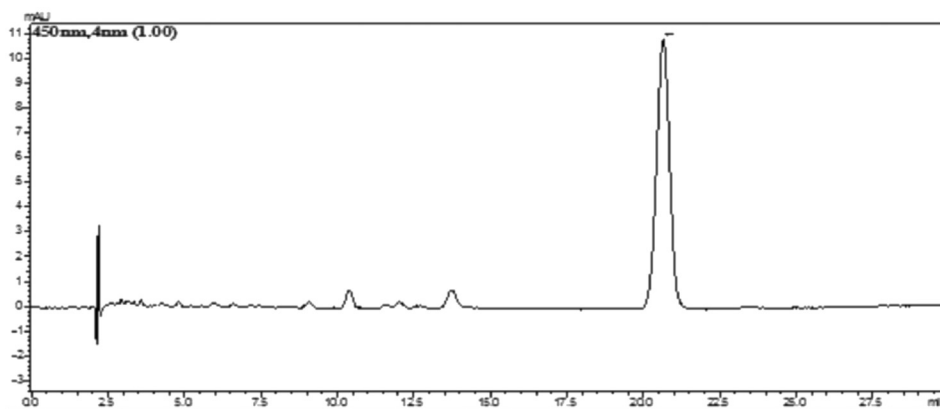


Figure 2. HPLC chromatograms of the all-trans-β-carotene standard

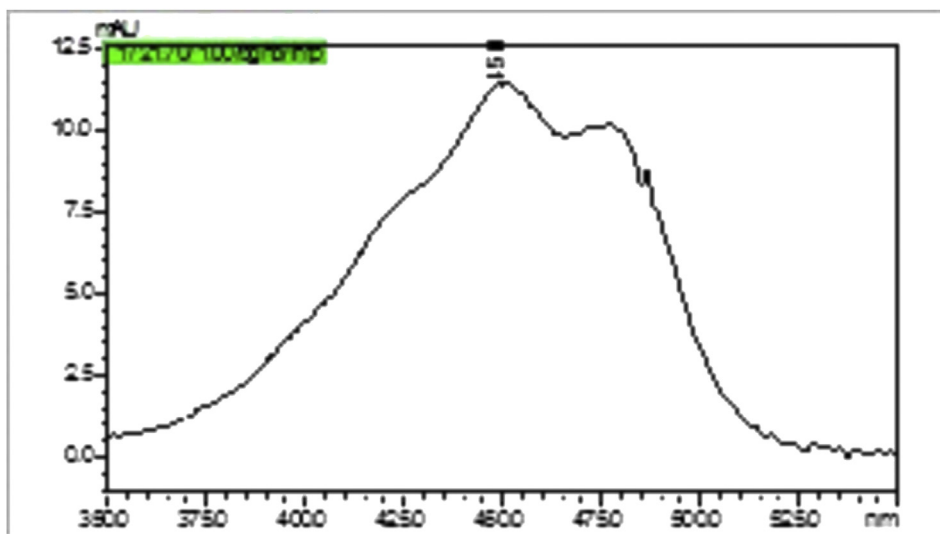


Figure 3. HPLC UV/Vis spectra of the all-trans-β-carotene standard.

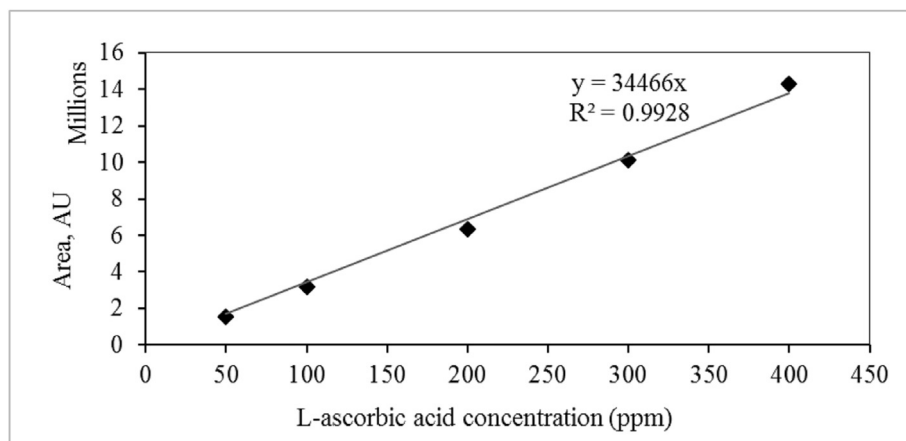


Figure 4. Standard curve of L-ascorbic acid standard.

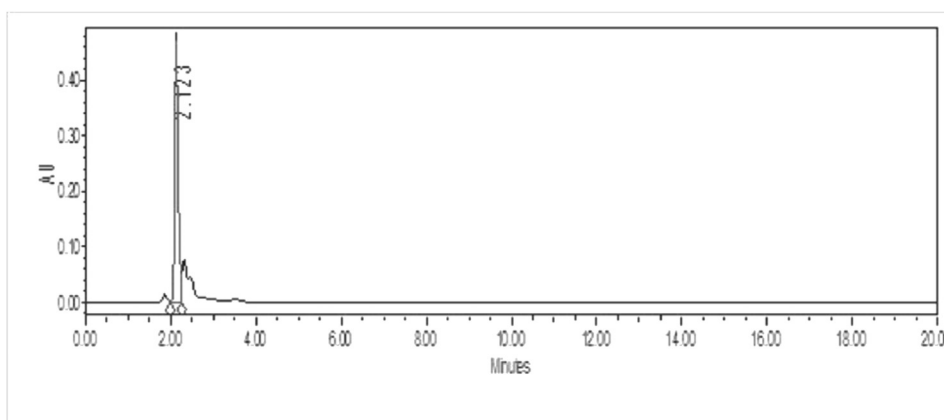


Figure 5. L-ascorbic acid HPLC chromatograms

2.2.6. The contribution of fresh or dried stinging nettle leaves to dietary intakes of protein, minerals and vitamins content

The % contribution of a serving of fresh (100 g), freeze dried (15 g) or oven dried (15 g) leaves (see Table 1) to the daily value (DV) (i.e. Daily values of nutrients provided in Table 2) [based on a caloric intake of 2,000 Cal for adults and children four or more years of age as described by US FDA (2013)] of a specific nutrient including minerals (Ca, Fe, Mg, Mn, Zn, P, K, and Na), protein, vitamin A and vitamin C was determined as follows (1):

$$\% \text{ DV} = \frac{\text{Nutrient content in a serving}}{\text{Daily value for the nutrient}} \times 100 \quad (1)$$

2.3. Data analyses

Analysis of variance (ANOVA) in XLSTAT 2015 (AddinSoft™ SARL, Paris, France) was applied to assess the effects of species (viz. stinging nettle, spinach (*Spinacia oleracea*) included as a control) and drying method (viz. freeze drying and oven drying) on macronutrients, minerals

content, ascorbic acid, β -carotene content, total phenols content and antioxidant activity. Fisher's Least Significant Difference (LSD) test was applied to separate statistically significant means (at the 5 % level).

3. Results

3.1. Proximate composition

Results for the proximate composition of the fresh, freeze-dried and oven-dried leaves are presented in Table 3. The moisture, fat, crude fibre, ash, crude protein and available carbohydrate contents were significantly affected by species and drying method, whereas only moisture and available carbohydrates were significantly affected by the species and drying method interaction effect. For fresh leaves, crude fibre and crude protein content were higher ($p < 0.01$) in nettle leaves compared to spinach. For freeze-dried and oven-dried samples; fat, crude fibre, ash, and crude protein were higher ($p < 0.01$) in nettle leaves compared to spinach, whereas available carbohydrates and moisture content were higher ($p < 0.01$) in spinach.

Table 1. Recommended serving sizes for green leafy vegetables and stinging nettle leaf food products.

| Food products | Serving sizes (g) | References |
|------------------------|-------------------|--|
| Green leafy vegetables | 80–100 | (Joint WHO/FAO, 2003; Naude, 2013b, 2013a) |
| Fresh nettle leaves | 100 | (Danesi et al., 2013; Rutto et al., 2013) |
| Dried nettle leaves | 15–18 | (Ait Haj Said et al., 2015) |
| Infusion or decoction | 250 | (Gallaher et al., 2006) |

Table 2. Daily values (DV_s^{*}) and conditions for nutrient content claims in food products as described by US FDA (2013).

| Nutrients | DV _s | Unit | Conditions for nutrient contents | |
|------------|-----------------|------|----------------------------------|--------------------------|
| | | | Conditions (% DV per serving) | Claim |
| Vitamin A | 870 | µg | | |
| Vitamin C | 60 | mg | | |
| Calcium | 1000 | mg | ≤5% | Low |
| Iron | 18 | mg | 6–9 % | Source |
| Magnesium | 400 | mg | 10–19 % | Good source |
| Manganese | 2 | mg | ≥20 % | High source or 'rich in' |
| Phosphorus | 1000 | mg | | |
| Zinc | 15 | mg | | |
| Potassium | 3.5 | g | | |
| Protein | 50 | g | | |
| Sodium | 2.4 | g | 0.12 g per 100 g or per serving | Low |
| | | | 0.04 g per 100 g or per serving | Very Low |
| | | | 0.005 g per 100 g or per serving | Free |

* DV_s based on a caloric intake of 2,000 cal, for adults and children four or more years of age.

Table 3. Effect of drying method on the mean proximate composition (g/100 g ± SD) and percent daily value (% DV^{*}) of stinging nettle and spinach**leaves.

| Drying method (DM) | Fresh | | Freeze dried | | Oven dried | | p-values | | |
|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|--------------------------|-------------------------|----------|------|---------|
| | Nettle | Spinach | Nettle | Spinach | Nettle | Spinach | SP | DM | SP x DM |
| Moisture | 85.0 ^b (1.5) | 89.6 ^a (0.6) | 6.4 ^c (0.2) | 6.6 ^c (0.0) | 3.4 ^d (0.1) | 4.5 ^d (0.0) | 0.01 | 0.01 | 0.01 |
| Ash | 3.1 ^c (0.3) | 1.8 ^c (0.2) | 19.5 ^a (0.2) | 16.7 ^b (0.5) | 19.2 ^a (1.0) | 17.0 ^b (0.7) | 0.01 | 0.01 | 0.23 |
| Macronutrients | | | | | | | | | |
| Fats | 0.6 ^d (0.1) | 0.4 ^d (0.0) | 3.5 ^a (0.1) | 3.2 ^b (0.1) | 3.4 ^{ab} (0.1) | 2.8 ^c (0.1) | 0.01 | 0.01 | 0.15 |
| Available carbohydrates | 2.2 ^c (0.2) | 2.4 ^c (0.1) | 14.7 ^d (0.7) | 22.1 ^b (0.6) | 18.9 ^c (2.2) | 26.1 ^a (0.4) | 0.01 | 0.01 | 0.01 |
| Fibres | 4.6 ^d (0.5) | 2.9 ^c (0.2) | 27.5 ^a (0.5) | 25.4 ^b (0.3) | 26.3 ^{ab} (1.0) | 23.8 ^c (0.1) | 0.01 | 0.01 | 0.50 |
| Proteins | 4.6 ^c (0.4) | 2.9 ^d (0.1) | 28.3 ^a (0.4) | 26.0 ^b (0.4) | 28.8 ^a (0.4) | 25.8 ^b (0.1) | 0.01 | 0.01 | 0.08 |
| Proteins, g/serving | 4.6 | 2.9 | 4.2 | 3.9 | 4.3 | 3.9 | | | |
| % DV | 9.2 | 5.8 | 8.5 | 7.8 | 8.6 | 7.7 | | | |

^{a-d} Means within same row with different superscripts are different ($p < 0.05$) when analysed using analysis of variance.

** Spinach leaves were used as an external reference sample.

* represent percent contribution from a serving of 100 g fresh or 15 g dried stinging nettle and spinach leaves to the daily value of proteins (50 g) was calculated as described by US FDA (2013) using the following formula:

$$\% \text{ DV} = \frac{\text{Nutrient content in a serving}}{\text{Daily value for the nutrient}} \times 100$$

3.2. Minerals content

The concentrations of eight minerals (Ca, Fe, Mg, Mn, P, Zn, K, and Na) determined in fresh, freeze dried and oven dried stinging nettle and spinach leaves are shown in Table 4. Nettle leaves were found to contain significantly higher ($p < 0.01$) Fe, K, Mg, Mn, Ca and Zn compared to spinach leaves. The drying processes did not affect the mineral content of the leaves.

3.3. The effects of drying methods on β -carotene, ascorbic acid, total phenols content and antioxidant activities

The result for the effects of drying on β -carotene, ascorbic acid, total phenols content and total antioxidant activities of nettle and spinach leaves can be found in Table 5. Ascorbic acid, β -carotene, total phenols content and total antioxidant activities were significantly ($p < 0.01$) affected by species and drying methods.

Stinging nettle leaves contained significantly ($p < 0.01$) more ascorbic acid, β -carotene, total phenols content and total antioxidant

activities compared to spinach. Oven drying of nettle leaves resulted in a higher loss of β -carotene and ascorbic acid content compared to freeze drying.

3.4. The contribution of fresh or dried stinging nettle leaves to dietary intakes of proteins, minerals and vitamins

The percent daily value of proteins from a serving of fresh (100 g) or dried (15 g) nettle leaves was found to be 9 % (Table 3). Similarly, a typical serving of fresh or dried nettle leaves provided approximately 30–32 % Ca, 14 % Fe, 26 % Mg, 19 % Mn, 4 % Zn, 9 % P, and 5 % K to the daily values of the respective mineral elements (e.g. 1000 mg Ca, 18 mg Fe, 400 mg Mg, 2 mg Mn, 15 mg Zn, 1000 mg P and 3.5 g K per day) (Table 4). The percent daily value of β -carotene (calculated as RAE, vitamin A) and vitamin C to the daily requirement of these nutrients (870 µg/day for vitamin A and 60 mg/day for vitamin C) were found to be higher in fresh (83.7 %, 23.7 %), followed by freeze-dried (75.7 %, 19.6 %) leaves compared to oven-dried (72.9 %, 16.5 %) leaves, respectively (Table 5).

Table 4. Effect of drying method on the mean minerals content (mg/100 g, db \pm SD) and percent daily value (% DV^{*}, as is basis) of stinging nettle and spinach**leaves.

| Drying method (DM) | Fresh | | Freeze dried | | Oven dried | | p-values | | |
|--------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|----------|------|---------|
| | Nettle | Spinach | Nettle | Spinach | Nettle | Spinach | SP | DM | SP X DM |
| Ca | 2136 ^a (182) | 500 ^b (4) | 2283 ^a (198) | 536 ^b (4) | 2065 ^a (133) | 543 ^b (26) | 0.01 | 0.31 | 0.32 |
| Ca, mg/serving | 323 | 52 | 320 | 75 | 299 | 78 | | | |
| % DV | 32.3 | 5.2 | 32 | 7.5 | 29.9 | 7.8 | | | |
| Fe | 16.7 ^a (0.7) | 7.5 ^b (1.8) | 17.9 ^a (0.7) | 8.0 ^b (1.9) | 17.6 ^a (1.8) | 8.3 ^b (1.6) | 0.01 | 0.57 | 0.93 |
| Fe, mg/serving | 2.5 | 0.8 | 2.5 | 1.1 | 2.6 | 1.2 | | | |
| % DV | 14 | 4.3 | 14 | 6.3 | 14.2 | 6.6 | | | |
| Mg | 692 ^a (13) | 430 ^b (95) | 740 ^a (14) | 460 ^b (102) | 726 ^a (32) | 411 ^b (93) | 0.01 | 0.60 | 0.80 |
| Mg, mg/serving | 104 | 45 | 104 | 64 | 105 | 59 | | | |
| % DV | 26 | 11.2 | 26 | 16.1 | 26.3 | 14.7 | | | |
| Mn | 2.5 ^{ab} (0.1) | 2.3 ^c (0.1) | 2.7 ^a (0.1) | 2.5 ^{bc} (0.1) | 2.6 ^{ab} (0.0) | 2.3 ^{bc} (0.2) | 0.01 | 0.09 | 0.97 |
| Mn, mg/serving | 0.4 | 0.2 | 0.4 | 0.3 | 0.4 | 0.3 | | | |
| % DV | 19.0 | 11.9 | 18.9 | 17.2 | 18.5 | 16.8 | | | |
| Zn | 3.5 ^a (0.1) | 2.4 ^b (0.6) | 3.8 ^a (0.1) | 2.6 ^b (0.7) | 3.8 ^a (0.2) | 2.5 ^b (0.7) | 0.01 | 0.71 | 0.94 |
| Zn, mg/serving | 0.5 | 0.2 | 0.5 | 0.4 | 0.6 | 0.4 | | | |
| % DV | 3.5 | 1.7 | 3.5 | 2.4 | 3.7 | 2.4 | | | |
| P | 550 ^a (31) | 543 ^a (62) | 588 ^a (33) | 582 ^a (66) | 584 ^a (47) | 558 ^a (53) | 0.60 | 0.44 | 0.93 |
| P, mg/serving | 82.6 | 56.7 | 82.5 | 81.5 | 84.6 | 80 | | | |
| % DV | 8.3 | 5.7 | 8.2 | 8.2 | 8.5 | 8.0 | | | |
| K | 1266 ^a (60) | 610 ^b (73) | 1354 ^a (62) | 653 ^b (78) | 1278 ^a (43) | 663 ^b (91) | 0.01 | 0.30 | 0.58 |
| K, g/serving | 0.2 | 0.1 | 0.2 | 0.1 | 0.2 | 0.1 | | | |
| % DV | 5.4 | 1.8 | 5.4 | 2.6 | 5.3 | 2.7 | | | |
| Na | 3.1 ^b (0.8) | 110.9 ^a (25) | 3.3 ^b (0.9) | 118.7 ^a (26) | 3.4 ^b (0.9) | 107.9 ^a (26) | 0.01 | 0.87 | 0.87 |
| Na, g/serving | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | | | |
| % DV | 0.0 | 0.5 | 0.0 | 0.7 | 0.0 | 0.6 | | | |

^{ab} Means within same row with different superscripts are different ($p < 0.05$) when analysed using analysis of variance.

** Spinach leaves were used as an external reference sample.

* Percent contribution from a serving of 100 g fresh or 15 g dried stinging nettle or spinach leaves to the daily value of the specific mineral elements (e.g. 1000mg Ca, 18 mg Fe, 400 mg Mg, 2 mg Mn, 1000 g P, 15 mg Zn, 3.5 g K and 2.4 g Na) was calculated as described by US FDA (2013) using the following formula:

$$\% \text{ DV} = \frac{\text{Nutrient content in a serving}}{\text{Daily value for the nutrient}} \times 100$$

Table 5. Effect of drying method on the mean values of β -carotene ($\mu\text{g}/100 \text{ g}$), ascorbic acid (mg/100 g), total phenols content (TPC, mg GAE/g), total antioxidant activity (TAA, % DPPH inhibition) (\pm SD) and percent daily value (%DV^{*}, as is basis) of stinging nettle and spinach*** leaves.

| Drying method (DM) | Fresh | | Freeze dried | | Oven dried | | p-values | | |
|--|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|----------|------|---------|
| | Nettle | Spinach | Nettle | Spinach | Nettle | Spinach | SP | DM | SP x DM |
| β -carotene | 58059 ^a (243) | 44160 ^d (256) | 56341 ^b (453) | 43537 ^d (62) | 52504 ^c (447) | 40847 ^e (479) | 0.01 | 0.01 | 0.01 |
| Vitamin A, μg RAE**/serving | 728 | 383 | 659 | 508 | 634 | 488 | | | |
| % DV | 83.7 | 44.0 | 75.7 | 58.4 | 72.9 | 56.0 | | | |
| Ascorbic acid | 94.7 ^a (3.3) | 77.0 ^c (0.5) | 83.8 ^b (2.3) | 75.7 ^c (0.6) | 68.5 ^d (0.6) | 65.3 ^d (2.1) | 0.01 | 0.01 | 0.01 |
| Vitamin C, mg/serving | 14.2 | 8.0 | 11.8 | 10.6 | 9.9 | 9.4 | | | |
| % DV | 23.7 | 13.3 | 19.6 | 17.7 | 16.5 | 15.6 | | | |
| TPC | 118.4 ^b (0.8) | 87.3 ^d (3.9) | 121.5 ^b (3.8) | 111.0 ^c (1.5) | 128.7 ^a (1.3) | 117.8 ^b (0.2) | 0.01 | 0.01 | 0.01 |
| TAA | 65.1 ^b (1.6) | 52.9 ^c (1.6) | 66.6 ^b (1.7) | 55.1 ^c (0.5) | 70.6 ^a (0.9) | 65.1 ^b (0.6) | 0.01 | 0.01 | 0.01 |

^{a-d} Means within same row with different superscripts are different ($p < 0.05$) when analysed using analysis of variance.

Without 'db' represent dry basis.

*** Spinach leaves were used as an external reference sample.

** represent vitamin A content of fresh and dried leaves was calculated as retinol activity equivalents (RAE) using an RAE conversion factor of 12 μg β -carotene to 1 μg retinol (Joint FAO/WHO, 2001).

* represent percent contribution from a serving of 100 g fresh or 15 g dried stinging nettle or spinach leaves to the daily value of vitamin A and C was calculated as described by US FDA (2013) using the following equation:

$$\% \text{ DV} = \frac{\text{Nutrient content in a serving}}{\text{Daily value for the nutrient}} \times 100$$

4. Discussion of results

The fat, fibre, ash and protein content of freeze dried and oven dried nettle leaves were found to be significantly higher than spinach leaves. The variability in preharvest conditions (e.g. growth conditions, type of fertilizers, climatic conditions, and genotypic difference) could have an immense role leading to variability in nutrients accumulated by nettle leaves and spinach. For example, the same species of stinging nettle produced at different agro-ecological conditions were reported to contain different concentrations of nutrients (Otlés and Yalcin, 2012; Farag et al., 2013). According to Allen et al. (2006) and Nishida et al. (2004), a food may be described as a part of a healthy diet if the food carries a statement describing the conditions (e.g. rich source and good source) of the nutrient content claims per 100 g or per serving as provided in the dietary guidelines. For vegetables, the recommendation for school children and adults is at least 400 g/day of vegetables (80–100 g per serving or 4 to 5 servings) (World Health Organization, 2003). Recommended serving sizes was reported to be 80–100 g for green leafy vegetables (Danesi et al., 2013; Naude, 2013a; Joint WHO/FAO, 2003), 100 g for fresh nettle leaves (Danesi et al., 2013; Rutto et al., 2013) and 15–18 g for dried nettle leaves (Ait Haj Said et al., 2015).

Accordingly, a serving size of 100 g for fresh leaves and 15 g dried nettle leaves were used to determine the potential contribution of fresh or dried nettle leaves to the dietary intakes of the specific nutrient. As described by US FDA (2013), a food product with 5 % DV or less is considered low for that specific nutrient, 6–9 % DV a source, 11–19 % DV a good source and 20 % DV or more indicates a rich source (Table 2). Fresh or dried stinging nettle leaves were found to be a source of proteins; one will need to consume at least two servings of fresh or dried nettle leaves to reach 10 % of the DV (300 g/day for protein).

The high ash content of nettle leaves explains their higher concentration of Fe, K, Mg, Mn, Ca and Zn compared to spinach leaves. This could be attributed to differences in cultural practices such as frequency of irrigation, fertilizer type, rate and time of application as well as fertility status of the soil during production (Lee and Kader, 2000; Walker et al., 2010). Pytlakowska et al. (2012) reported that medicinal plants (such as nettle, senna leaves) strongly vary in mineral elements concentration (e.g. Fe, Zn, Mn, Mg, K, Na, P, and Ca) because of differing absorption of mineral elements from the soil. Other authors reported variation in macro and micro mineral contents of moringa leaves sampled from different areas (Gyamfi et al., 2011).

Fresh and dried nettle leaves can be considered as a “good source” of Fe and Mn because the contribution from a serving of the leaves was more than 10 % of the DV for these nutrients and rich source for Ca and Mg (US FDA, 2013; Joint FAO/WHO, 2007). In developing countries, anaemia was reported to be a serious problem in pregnant woman and preschool children (World Health Organization, 2003). Anaemia also contributes to 20 % of all maternal deaths. A nettle leaf in both fresh and dried forms was found to be a good source of iron. Therefore, integrating stinging nettle leaves in the diet could help to combat anaemia which was reported to affect more than 60 % of children in Africa (Standing Committee on Nutrition, 2010).

A person will need to consume at least two to three servings of fresh or dried nettle to reach the 10 % DV for Zn, P and K. Fresh and dried stinging nettle leaves could be categorized as ‘free’ for sodium content of the leaves, as the contribution of the sodium per serving of the leaves to the daily value of sodium (e.g. 2.4 g per day) is not more than 0.005 g (US FDA, 2013; Joint FAO/WHO, 2007). The low level of sodium in stinging nettle leaves could be beneficial for persons on a restricted sodium diet. According to the World Health Organization (2014) an estimated 2.5 million deaths could be prevented each year if global salt consumption was reduced to the recommended level (2.4 g per day).

The high potassium/sodium ratio (K/Na ratio ranging from 381 to 494) of stinging nettle leaves could be also another potential indicator of the protective powers of the stinging nettle leaves foliage against

cardiovascular and neoplastic diseases (Kavalali, 2003; World Health Organization, 2014).

However, Jimoh et al. (2010) reported that stinging nettle leaves contained antinutrients such as alkaloids (0.6 mg/100 g), phytates (4.39 mg/100 g) and saponins (3.25 mg/100 g). The phytates in the green leafy vegetables can be reduced by soaking, boiling or frying (Akubugwo and Obasi, 2007). Kruger et al. (2015) reported that cooking maize meal fortified with green leafy vegetable (porridge) resulted in a decrease in phytates content and concomitant increase in bio-accessibility of Fe and Zn in the porridge. Bravo (2009) pointed out that tannins have the ability to chelate Fe and Zn by binding with their hydroxyl and carbonyl groups, and thereby reduces the bioavailability of these minerals. Therefore, cooking of stinging nettle leaves could potentially decrease the phytates content and increase the bio-accessibility of Fe and Zn in the cooked leaves.

The β -carotene concentrations determined in fresh stinging nettle leaves samples were higher than those reported by Guil-Guerrero et al. (2003) and Rutto et al. (2013) (for fresh nettle leaves) and Adhikari et al. (2016) (for dried nettle leaves). Similarly, the concentration of ascorbic acid found in fresh stinging nettle leaves samples was higher than those reported by Ioana et al. (2013) and Rutto et al. (2013) (in fresh nettle leaves). This is because the accumulation of vitamins and minerals by the plant tissues is a function of its genotype, pruning and thinning, frequency of irrigation, temperature, sunlight, fertilizers used and fertility status of the soil (Lee and Kader, 2000a). For example, the vitamin C content in plant tissues is highly correlated with the intensity of sunlight and whereas it is negatively correlated with the frequency of irrigation and the rates of nitrogen fertilizer during the growing season of vegetables (Lee and Kader, 2000a; Walker et al., 2010). Furthermore, the β -carotene and ascorbic acid content of stinging nettle leaves could be influenced by postharvest practices (e.g. drying and cooking). Drying conditions could have a great effect on heat and light labile β -carotene and ascorbic acid.

The ascorbic acid and β -carotene content of freeze-dried nettle leaves were higher compared to oven dried leaves. This can be attributed to the higher temperature of the oven drier ($>50^{\circ}\text{C}$). During the freeze-drying process the temperature of the product is low (-45°C), which limits degradation reactions (Ratti, 2001).

The higher loss of β -carotene during oven drying could be because of the highly unsaturated β -carotene structure which can lead to photooxidation and autooxidative reactions (Bernhardt and Schlich, 2006; Di Cesare et al., 2004; Kidmose et al., 2000; Owuor, 2003). Chang et al. (2006) reported heating causes increase in conversion of *trans*-isomers to *cis*-isomers of β -carotene by 50%. Previous research also confirmed that ascorbic acid and β -carotene are better retained in freeze dried food products compared to oven dried (Abascal et al., 2005; Gupta et al., 2013; Shilton, 2003).

The high loss of ascorbic acid during oven drying could be due to the two hydroxyl groups in its structure which could be oxidized to dehydroascorbic acid at high temperature (Ajayi et al., 1980; Sanmartin et al., 2000; Waheed Uz et al., 2013).

The loss of vitamin C in food products can range from 10 % to 50 % depending on temperature of drying processes (Shilton, 2003). This is in agreement with the findings of the present study where the loss of ascorbic acid was found to be 12 % in freeze dried and 22 % loss in oven dried nettle leaves.

Even though oven drying of stinging nettle leaves resulted in a higher loss of β -carotene and ascorbic acid content compared to freeze drying, a serving of either fresh, freeze dried or oven dried nettle leaves provided more than 20 % of the DV of vitamin A (870 μg per day). Therefore, nettle leaves in all forms can be considered as a “rich sources” of vitamin A. In contrast, oven dried nettle leaves found to be a ‘good source for vitamin C while fresh and freeze-dried nettle leaves can be considered as “rich sources” of vitamin C. This could imply that consumption of fresh or dried nettle leaves might help to reduce vitamin A deficiency, which has

been estimated to affect about 2.5 million preschool children in Africa (World Health Organization, 2009).

Furthermore, inclusion of β -carotene-rich food in the daily diets, instead of costly synthetic vitamin A supplementation, may be a more successful strategy for improving vitamin A status of at risk or malnourished populations (Gopalan, 1992). Hence, integrating either fresh or dried stinging nettle leaves in the diet or utilization of dried nettle leaves for fortifying cereal-based foods would help address vitamin A and C deficiencies. The high β -carotene content of fresh and dried stinging nettle leaves could also help to address health related problems due to their antioxidant activity (Guo et al., 2008).

Interestingly, the consumption of stinging nettle leaves could provide a double impact as a provitamin A, protein and vitamin C dietary source, and also as an enhancer of Fe absorption. Amagloh et al. (2017) emphasized that Fe bio-availability in dark green leafy vegetables is influenced by protein, ascorbic acid, β -carotene and total polyphenols content in the plant tissues. The authors reported that the high content of ascorbic acid and β -carotene in moringa leaves increased its Fe bio-availability.

The higher antioxidant activity of nettle leaves compared to spinach may be attributed to its β -carotene, phenolic compounds, ascorbic acid, Mn and Zn. Phenolic compounds, carotenoids and ascorbic acid (Rincón-León et al., 2003; Velioglu et al., 1998), Mn and zinc (Caballero et al., 2015) are nutrient antioxidants.

Additionally, the higher total antioxidant activity and phenols content of nettle leaves compared to spinach leaves found in this study could also be due to variability in genotype and environmental conditions (Vagiri, 2014). Although genetic factors are the main determinants for the content of phenolics and antioxidants, these contents can also be affected by light and temperature conditions of the environment (Tiwari and Cummins, 2013).

Genetic diversity in content of phenolic compounds and antioxidant activity was also reported in nettle leaves. For example, Otles and Yalcin (2012) reported a wide variability in total phenols content (151–1941 mg GAE/g DM) and antioxidant activity (ranging from 60.62 to 320.38 mg GAE/g DM) of fresh stinging nettle leaves collected from the Mediterranean, Aegean, Black Sea and Marmara coastal parts in Turkey.

The higher total phenols content of oven-dried compared to freeze-dried leaves could be linked to more efficient extraction of the insoluble phenolic compounds such as condensed tannins, and phenolic acids (Farag et al., 2013; Komes et al., 2011; Pinelli et al., 2008) bound to cell wall polysaccharides or proteins (Giada, 2013; Singleton et al., 1999). Because during oven drying processes phenolic-sugar glycosidic bonds may be cleaved with heat treatment leading to the formation of phenolic aglycons, which react better with the Folin-Ciocalteu reagent leading to higher values of total phenolics (Singleton et al., 1999).

Similarly, the higher total antioxidant activity in oven-dried nettle leaves compared to fresh and freeze dried leaves could probably be due to: 1) release of antioxidant nutrients by thermal (heating effects of the oven dryer) destruction of cell walls and subcellular compartments; 2) formation of antioxidants by thermal chemical reaction; 3) suppression of the oxidation of antioxidants by thermal inactivation of oxidative enzymes. As an example, the increase in total antioxidant activity after heat treatment could be due to the increased release of phytochemicals, such as lycopene, from the matrix (Gahler et al., 2003). Various studies reported that fresh stinging nettle leaves contain phenolic acid (e.g. hydroxycinnamic acid, hydroxybenzoic acid), tannins and flavonoids (e.g. flavones, flavonols, iso-flavonols, anthocyanins, catechins, lignin) using HPLC method (Farag et al., 2013; Orčić et al., 2014; Pinelli et al., 2008). To this end, HPLC method could enable to accurately track the phenolic compounds as well as the changes in total phenol content and antioxidant activity of dried stinging nettle leaves using different parameters of the selected drying techniques.

The high total phenols content and antioxidant activity in both freeze-dried and oven-dried leaves could present dried nettle leaf powders as good natural antioxidants for application in health-promoting foods and

as a food preservative. For example, natural sources of antioxidants are replacing synthetic antioxidants (such as butylated hydroxy anisole, butylated hydroxy toluene, tertiary butyl hydroquinone, and propyl gallate) to reduce toxicological and carcinogenic effects (Kumar et al., 2015). Lindsey, Motsei and Jäger (2002) suggested that nettle leaves may not only be a good dietary source but could also be used as a natural antioxidant in the food industry. Applications of the use of extracts from herbs like rosemary and oregano (Rojas and Brewer, 2008) and sage (Mariutti et al., 2011) in meat and poultry products have been well demonstrated.

A voucher specimen of the plant materials used was not included in this research. Given that the focus was on investigating the effect of the drying methods on response variables and not on botanical aspects (such as identification of the taxon and location of the stinging nettle), priority was given to including an external reference sample (spinach). Nonetheless, in future research of this nature, it would be useful to include a stinging nettle voucher specimen if available.

5. Conclusions

Even though, oven drying of nettle leaves results in a higher loss of β -carotene and ascorbic acid content compared to freeze drying, approximately 90 % and 72 %, respectively, of the nutrients are retained in the oven-dried leaves. In contrast, oven drying increases the total antioxidant activity and phenol content of nettle leaves more than freeze drying does. Overall, fresh stinging nettle leaves can be considered as a rich source of antioxidants, Ca, Mg, vitamins A and C, a good source of Fe and Mn and a source of P and K. Whereas, freeze-dried and oven-dried stinging nettle leaves can be considered as a rich source of antioxidants, Ca, Mg, and vitamin A, while it is a good source of vitamin C, Fe and Mn. These benefits present possible avenues for utilization of dried nettle leaves or leaf powder by the food industry and consumers for addressing micronutrient deficiencies and for providing a healthy diet. Further research should consider more drying methods and/or different parameters of the selected drying techniques and determine/track the change in TPC and antioxidant activity using the HPLC method.

Declarations

Author contribution statement

Tigist T. Shonte: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Geybi K. Duodu, Henriëtte L. de Kock: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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