Sensory and nutritional properties of stinging nettle (*Urtica dioica* L.) leaves and leaf infusions

by

Tigist Tadesse Shonte

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DECLARATION

I, Tigist Tadesse Shonte, declare that the thesis, which I hereby submit for the degree of PhD in Food Science at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Tigist Tadesse Shonte

27/11/2017

ABSTRACT

Sensory and nutritional properties of stinging nettle (*Urtica dioica* L.) leaves and leaf infusions

By

Tigist Tadesse Shonte

Study leaders: Prof H.L.de Kock

Prof K.G Duodu

Stinging nettle/common nettle (*U. dioica* L.) is known since ancient times as a wild source of food and a herbal medicine, but the plant remains underutilized. Drying of stinging nettle leaves not only allow their use when the plants are not physiologically active but also extend their consumption period and utilization at times of food shortage and for addressing micronutrient malnutrition. However, drying could result in decomposition of heat sensitive metabolites such as fatty acids, amino acids, carotenoids, ascorbic acid and insoluble phenolic compounds present in the fresh stinging nettle leaves. These changes might lead to production of volatile compounds, non-volatile compounds, soluble phenolic compounds etc. The systematic description of the aroma, flavour and colour of cooked stinging nettle leaves and leaf infusions, prepared from fresh or dried leaves has not been published. With this study, the effects of using fresh or oven-dried leaves to cook a relish or to prepare an infusion on sensory and nutritional properties were measured. In addition, the effect of two infusion cycles on the sensory properties of leaf infusions was determined.

Although the colour changed during heat processing, most of the characteristic green-related aroma and flavour notes of fresh nettle leaves were preserved in cooked leaves and leaf infusions prepared from dried leaves. When cooking the leaves, the use of dried leaves resulted in an increase in fermented aroma, earthy, burnt flavour, bitter and also salty taste compared to fresh leaves. In leaf infusions, a decrease of grassy, earthy and mint aromas as well as seafood and green-herblike aroma and flavour notes were observed. The first two brewed infusions from fresh or dried leaves provided similar aroma and flavour intensities. Further, the ΔE (total colour difference) value, showed variation in colour of fresh leaves compared to oven dried leaves. The ΔE value also showed variation in colour between the two infusion cycles as well as in uncooked and cooked leaves.

The change in aroma, flavour and colour of leaf infusions and cooked leaves when oven dried leaves were used compared to fresh leaves, prompted an investigation into the effect of drying methods (i.e. freeze-drying and oven drying) on nutritional properties of stinging nettle leaf food products and food ingredient components. Oven drying of stinging nettle leaves resulted in a higher loss of β-carotene and ascorbic acid content compared to freeze drying. A typical serving portion of either fresh, freeze dried or oven dried nettle leaves could provide more than 20 % of the daily value of vitamin A (e.g. 870 µg per day); therefore, nettle leaves in all these forms are rich sources of vitamin A. In contrast, freeze dried and oven dried nettle leaves were found to be a good source of vitamin C while fresh leaves can be considered as a rich source of vitamin C. In general, dried stinging 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 of Mg and K. In contrast to a decrease in β-carotene and ascorbic acid content, an increase in total phenol content and antioxidant activity were observed in oven dried leaves compared to fresh stinging nettle leaves.

Dried stinging nettle leaves or leaf powder are used to make infusions and decoctions for human medicinal and nutritional purposes due to the antioxidant properties of its constituent vitamins A and C, and phenolic compounds. This led to further investigation into the effect of the type of extraction (i.e. infusion and decoction) on the ascorbic acid, β-carotene, total phenol content, antioxidant activity of stinging nettle leaf powder manufactured using freeze drying or oven drying. β-carotene and ascorbic acid was found to be higher in infusions compared to decoctions. The total phenol content and antioxidant activity of decoction samples were significantly higher compared to infusions ($p < 0.01$).

This study provides evidence that stinging nettle leaf food products could potentially contributes to dietary intakes of minerals (i.e. Ca, Mg, Fe, Zn, Mn, Mg and K), protein, vitamins (i.e. A and C) and antioxidants and can potentially be incorporated in the diet for overcoming micronutrient malnutrition. Further consumer research is needed to determine which sensory characteristics of the products from stinging nettles drive liking or disliking by target consumers. All in all, this study contributes to the understanding of the potential of stinging nettle for addressing food and nutrition security.

DEDICATION

This Thesis is dedicated to the Almighty God, his mother (Saint Virgin Mariam) S/Michael S/Gebriel S/Urael and all the angels and Saints, for the blessing and guidance that I received from Him to undertake the research work, successfully compile this manuscript and get to this level. To my beloved families Mrs Simegn Alemayehu, Mrs Meskerem Tadesse and Mr Workalemaw Tegenu, Mrs Meselu Tadesse and Mr Dawit, Mrs Mulualem Tadesse and Mr Belete Kasa, Mrs Meseret Tadesse, Selamawit Tadesse and H/Michael Alelign for their affection, unreserved encouragement and constant support to reach higher heights.

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1: INTRODUCTION

Stinging nettle, *Urtica dioica* L., is an undervalued wild green vegetable (Khatiwada et al., 2011) distinguished by stinging hairs (Kavalali, 2003; Moskovitz, 2009). The plant require less care and is drought tolerant but it is regarded as a weed in agricultural production systems (Di Virgilio et al., 2015; Khatiwada et al., 2011). Upton (2013) stated that 'let food be your medicine', which add value to the potential of incorporating traditional knowledge of stinging nettle.

Seasonality, the fear of the stinging hairs, lack of commercial availability and the stigma related to stinging nettles being associate with famine/poor man's food are some of the reasons for its limited utilization as a source of food. The irritating contents of the stinging hairs are dissipated upon drying (Upton, 2013), blanching and cooking of the leaves (Rutto et al., 2013). As a vegetable, stinging nettle leaves are utilized in the preparation of many dishes such as salads, pies, soups, herbal tea or decocted tea in and combination with noodles, omelets or scrambled eggs (Bisht et al., 2012; Guarrera and Savo, 2013; Guil-Guerrero et al., 2003; Orčić et al., 2014; Sansanelli et al., 2014).

Nettle leaves, are eaten like spinach, prepared as cooked leaves, the leaves boiled or added to soups and sauce are eaten as famine food in many parts of the world (Davidson and Jaine, 2006; Khatiwada et al., 2011). Nettle leaves have been used, particularly in rural areas of Africa, as a potherb, soup and herbal infusions (Kavalali, 2003; Moskovitz, 2009; Roberts, 2011). In Nepal, nettle leaves are added to soups or cooked as a potherb (Adhikari et al., 2016). In Ethiopia, a sauce is prepared from young nettle leaves and barley powder and served with injera (Shonte and WoldeTsadik, 2013). Stinging nettles are used as a wild source of vegetables in South Africa (Bhat et al., 2002; Jimoh et al., 2010; Maanda and Bhat, 2010). Infusion and decoction made from medicinal plant (e.g. lavender, chamomile, thyme, sage, fennel, nettle leaves etc.) leaves and leaf powder is the most traditionally used form of drinks in Portugal (Gião et al., 2007).

Several studies reported the presence of a wide range of bioactive compounds in fresh stinging nettle leaves, especially chlorophylls (chlorophyll a and b) (Alibas, 2007; Dey, 2013), nine forms of carotenoids (including β-carotene, neoxanthin, violaxanthin, lutein and lycopene) (Guil-Guerrero et al., 2003; Rutto et al., 2013).

Fresh stinging nettle leaves are reported to contain essential fatty acids (especially dominant palmitic, cis-9,12 linoleic and a-linolenic acid) (Farag et al., 2013; Guil-Guerrero et al., 2003; Rutto et al., 2013), essential amino acids (Hughes et al., 1980; Rutto et al., 2013), different polyphenolic compounds (Orčić et al., 2014; Otles and Yalcin, 2012; Pinelli et al., 2008), ascorbic acid (Ioana et al., 2013; Rutto et al., 2013) and minerals (especially K, Ca, Mg, Fe, Mn and Zn) (Kara, 2009; Musa Özcan et al., 2008; Pytlakowska et al., 2012). These nutrients reported to be present in fresh nettle leaves may be degraded during drying, cooking, infusion and decoction processes.

Drying of stinging nettle leaves not only grant their use when the plants are not physiologically active but also extend their consumption period and utilization at times of food shortage and help for addressing micronutrient malnutrition. Drying processes involving high temperature such as oven drying result in protein denaturation, ascorbic acid degradation, β-carotene losses (Shilton, 2003) and might affect antioxidants in food products (Chang et al., 2006; Rodrigues et al., 2009). The temperature difference of preparation involved in decoctions (extraction by boiling) and infusions (extraction by steeping in boiled water) (Courtine, 1984), might affect the chemical compositions of the product differently. Lee (2009) reported that the physicochemical properties and flavour of green tea were influenced by many factors such as the water to tea ratio, type of water used, water temperature, brewing time, brewing equipment, number of brewing cycle etc. In Ethiopia and elsewhere, for economic reasons an infusion from the leaves is often brewed multiple times. Lee et al. (2013) reported that loose green teas can be brewed up to four times.

As a result of such circumstances, there is a need for further research looking into the sensory and nutritional properties of stinging nettle leaves and leaf infusions, which was the main goal of this research. This enable consumers and processors to choose the drying, cooking, infusion or decoction techniques that favour retention of sensory and nutritional properties of stinging nettle leaves for maximum health benefits in addition to overcoming micronutrient malnutrition.

To sum up, stinging nettle leaves could potentially contribute to a sustainable supply of healthy and nutritious food as well as add value to local cuisines (Mithril and Dragsted, 2012) and industrially processed foods. Maanda and Bhat (2010) emphasized that nutritional value of stinging nettle should be determined to encourage its utilization.

Therefore, this research contributes to the understanding of the potential of stinging nettle for addressing food and nutrition security to contribute to the well-being of consumers.

This study provides first a literature overview of the stinging nettle plant, including its botanical properties, growth characteristics, uses, chemical composition and safety, selected processing methods, nutritional properties and sensory characteristics of stinging nettle leaves as well as the chemistry of the formation of flavour compounds during thermal processing (chapter 2). Chapter 3 will provide hypotheses with its scientific justifications and the associated specific objectives of the study. Chapter 4 will focus on the main research chapters whereby in each sub chapters structured and contained its own sections for abstract, introduction, methodology, result, discussion and conclusions. Chapter 5 will provide critical review of experimental design and methodologies used in this study, research findings and identifies future research needs. Chapter 6 deals with conclusions and recommendations emanating from this research project. Chapter 7 provides detailed biography of in text cited references. Chapter 8 details publications and conference oral or poster presentations based on this research and the last chapter provides supporting information, (appendices) summarized as tables for better understanding of the findings from the research.

2: LITERATURE REVIEW

This review will first present an overview of the stinging nettle plant, including its botanical properties, growth characteristics, uses, chemical composition and safety with a focus on the leaves (2.1). The next part (2.2) will highlight selected processing methods of stinging nettle leaves including cooking, infusion, decoction and drying. Part three (2.3) will provide an overview of the nutritional properties of stinging nettle leaves. Further, the chemistry behind the changes in carotenoids, ascorbic acid, total phenol content and antioxidant activity during thermal processing will be discussed. The final part (2.4) will deal with the sensory characteristics of stinging nettle leaves including colour, aroma, flavour and mouthfeel and the chemistry of the formation of flavour compounds during thermal processing.

2.1 Stinging nettle plant

Urtica L., the stinging nettle, is an annual and perennial herb distinguished by stinging hairs (Kavalali, 2003). Among the Urticaceae family that comprises 40 genera and greater than 700 species; *U. dioica* is the most common, followed by *U. urens* (lesser nettle), *U. pilulifera* (roman nettle) and *U. membranacea*, had a wide range of distribution extending from Europe, N. Africa, to Asia, up to North and South America, and South Africa.

U. dioica L., common nettle/stinging nettle, is a flowering perennial herb with 30-150 cm height and the leaves are dull green (Kavalali, 2003). The plant is a very easy plant to grow and widely distributed and often considered as undesired invasion plant or a weed. As a cover crop it protects the soil structure, and is a beneficial companion plant and highly viable for organic farming (Bacci et al., 2011). Once planted it produces viable seeds within five weeks of germination and can be also propagated vegetatively using rhizome, stem or shoot cuttings under favourable conditions (DiTomaso and Healy, 2007).

The leaves and stems of the plant has stinging hairs called trichomes (Figure 2.1). The trichomes contain chemicals such as histamine, 5- hydroxytryptamine and acetylcholine and when touched by humans and other animals it produces a stinging sensation (Bisht et al., 2012; Mithril and Dragsted, 2012). The root, however, lacks stingers (Upton, 2013). Therefore, hand gloves and leg protection should be used to avoid the stings when harvesting the leaves (DiTomaso and Healy, 2007).

Studies reported that blanching or cooking of stinging nettle leaves will distruct the trichomes and deactivate the stinging chemicals (Hughes et al., 1980; Rutto et al., 2013).

Figure 2.1: Stinging hairs on stems and leaves of nettle plant

The plant is well known as a herbal remedy and wild vegetable since ancient times (Bhat et al., 2002; Jimoh et al., 2010; Kavalali, 2003; Khatiwada et al., 2011; Maanda and Bhat, 2010; Moskovitz, 2009). Mithril and Dragsted (2012) reported that nettle plants have been consumed without any report of serious adverse effects. Most of the bioactive compounds in stinging nettle are also found in other food plants such as spinach, lettuce, green tea or coffee and are present at a safe level (Table 2.1). The leaves are eaten like spinach; can be freshly cooked after harvest (Guil-Guerrero et al., 2003). In Ethiopia cooked samma leaves "the leaves of stinging nettle, *U. simensis*" is most commonly used during fasting (strictly vegetarian diet periods); at times of food shortage, when other vegetables such as spinach or cabbages are not available at the farm (as rain fed agriculture mostly practiced) and as a herbal remedy for stomach-ache (Shonte and WoldeTsadik, 2013) and diabetics (Tsegaye et al., 2009). Stinging nettles are also widely used among Western herbalists predominantly as a blood nourishing tonic and for seasonal rhinitis (Kavalali, 2003; Roberts, 2011; Upton, 2013).

Table 2.1: List and content of bioactive compounds in a 50 g portion of stinging nettle (Mithril and Dragsted, 2012)

Bioactive compounds	Compound group	Content in stinging nettle (mg/kg)	Estimated Content in 50 g of stinging nettle (mg)	Estimated safe level (mg/day)
Arsenic	Mineral	0.02 - 0.11	0.01	0.01 (1 L water)
Kaempferol	Flavonoid	20	1.00	100 (EDI)
Histamine	Aliphatic amine	2.12	0.11	100 g spinach
Isoquercitrin	Flavonoid	200	10.00	100 (EDI)
Glucoside	Flavonoid	200	10.00	100 (EDI)
Isorhamnetin-3-O-rutinoside	Flavonoid	50	2.50	100 (EDI)
Nitrate	Inorganic salt	184-199	9.90	222 (ADI)
Rutin	Flavonoid	500-6000	300	100 (EDI)
Sitosterol-glucoside	Phytosterol	500	25.00	100 (EDI)
Titanium	mineral	27	1.35	200 (EDI)
Hyperin (hyperoside)	Flavonoid	500	25.00	100 (EDI)
Oxalic acid	Dicarboxylic acid	3000	150	1336 (100 g rhubarb)

ADI = acceptable daily intake and EDI = estimated daily intake

2.2 Selected methods of preparing and processing stinging nettle leaves

2.2.1 Cooking

Stinging nettle leaves based dishes are used in the diet of many countries, particularly among low socioeconomic populations. As an example, nettle soup or potherb in Nepal (Adhikari et al., 2016), boiled nettle and barely flour 'samma sauce' in Ethiopia (Shonte and WoldeTsadik, 2013), and nettle leaves added to soups or stews in Nordic countries (e.g. Denmark, Finland, Iceland, Norway and Sweden) (Mithril and Dragsted, 2012). In the areas around the Black sea, nettles are traditional foods consumed for health purposes. It is used in the form of a sour soup in Romania, as a nettle walnut sauce in Georgia and as a herb in Ukraine (Danesi et al., 2013). Maanda and Bhat (2010) reported that stinging nettle has a flavour similar to spinach when cooked, and it is used as a side dish to flavour the meal or used to add a bitter taste to meals.

The aforementioned cooking (boiling) and preparation methods can probably improve the palatability and make stinging nettle leaves more appetizing. By raising the temperature of foods (including leaves) through cooking one could induce thermal degradation and matrix softening which influence the flavour, texture, appearance, nutrient content, and safety of foods (Saikia and Mahanta, 2013; Rosenthal, 2001; Palermo et al., 2014).

2.2.2 Infusion and decoction

Decoction is the extraction of constituents accomplished by constant boiling where the plant material is exposed to boiling temperature for a particular time period (Courtine, 1984; Gião et al., 2007). Decoction can be used to make herbal teas to prepare broth, soup or stock from meats and vegetables (Courtine, 1984). With infusion the plant material is suspended/soaked/steeped in hot or boiled water for a specified time with a gradual drop in temperature over time (Courtine, 1984). The temperature difference of preparation of decoctions and infusions might result in products with differing chemical properties. Infusions and decoctions are prepared either from fresh or dried stinging nettle leaves (Ait Haj Said et al., 2015; Upton, 2013). A regular supply of fresh stinging nettle leaves is a practical limitation due to seasonality and limited availability in some regions. Fortunately, utilization of dried stinging nettle could make it possible to have nettle leaves available all year round.

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 infusions made from leaf powder as the most effective mode of extraction of antioxidants from medicinal plants.

Infusions and decoctions extracts prepared from stinging nettle leaves are a natural source of antioxidants for use in human medicine and nutrition in many countries (Ait Haj Said et al., 2015; Kavalali, 2003; Moskovitz, 2009; Roberts, 2011; Upton, 2013). The number of brewing cycle, water temperature and brewing time were reported to influence the physicochemical properties and flavour of green tea (Lee et al., 2013; Lee, 2009). Investigating the sensory properties of nettle leaf infusions brewed multiple times can help determine how many times nettle leaves can be brewed without changing the characteristics properties of nettle tea.

2.2.3 Drying

Nettle leaves perish rapidly after harvest and therefore they are mainly consumed when in season (Kavalali, 2003). Maanda and Bhat (2010) reported that drying wild vegetables such as stinging nettle and storing for use when the plants are not physiologically active would extend the consumption period and utilization at times of shortages. However, aroma and flavour changes could occur during drying. The drying process also changes appearance of leaves. The loss of green colour is due to degradation of chlorophylls and carotenoids during thermal processing (Di Cesare et al., 2004; Kidmose et al., 2001; Shilton, 2003).

The drying method chosen can have a major impact on the 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). The slow drying process involved in ambient air-drying methods may lead to loss of quality of the leaves (e.g. colour changes, losses of ascorbic acid, carotenoids etc.) (Harbourne et al., 2009). Freeze-drying is the most common method of drying nettle for medicinal purposes (Ait Haj Said et al., 2015; Dey, 2013). During freeze drying very few chemical changes occur whereas oven drying can cause faster degradation of colour and loss of primary metabolites as it runs between 45 °C to 140 °C (Shilton, 2003).

The high 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 leaves tea bags etc. These products are expensive and therefore only affordable to high economic end consumers (developed market, rich) where such consumers demand 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 low economic end of the market (developing market, poor).

The high thermal treatment during oven drying could make the leaves more convenient for handling during subsequent preparation. It was reported that during freeze drying heat-labile antioxidants (e.g. ascorbic acid, carotenoids and soluble phenolics) are preserved compared to oven drying (Abascal et al., 2005; Gupta et al., 2013; Shilton, 2003; Shofian et al., 2011).

It has been reported that drying changes the chemical composition of food products via enzymatic, redox, or pyrolytic reactions of amino acids (Arimura et al., 2001), fatty acids (Owuor, 2003), and carotenoids (Giada, 2013; Goff and Klee, 2006; Owuor, 2003; Rodriguez-amaya, 1997), chlorophyll a and b (Di Cesare et al., 2004; Kidmose et al., 2000). Fresh stinging nettle leaves are reported to contain chlorophyll a and b (Alibas, 2007; Dey, 2013), β-carotene (Guil-Guerrero et al., 2003; Rutto et al., 2013), fatty acids (Farag et al., 2013; Guil-Guerrero et al., 2003; Rutto et al., 2013) and amino acids (Hughes et al., 1980; Rutto et al., 2013).

Secondary metabolites and flavour compounds may be produced from these metabolites of stinging nettle leaves during drying, cooking, infusions and decoctions processes. The effect of oven drying or freeze drying of stinging nettle leaves on sensory and nutritional properties of cooked leaves, leaf infusions and decoctions have not been published.

2.3 Nutritional properties of stinging nettle leaves and changes during processing

Stinging nettle leaves, not only add variety to the menu, but they are also valuable sources of nutrients where they contribute protein, minerals, vitamins, fiber and phytonutrients (e.g. phenolic compounds). Consumption of numerous types of wild edible plants especially green leafy vegetables has been described as potentially beneficial for food and nutrition security as well as to alleviate nutrition-related health problems (Naude, 2013a, 2013b). The nutritional properties of stinging nettle leaf products and the chemistry behind the changes in nutritional properties during thermal processing shall be discussed next.

2.3.1 Macronutrients

Table 2.2 shows the proximate composition of fresh stinging nettle leaves, leaf flour and cooked leaves as reported by different authors. Fresh nettle leaves can be used as a rich-protein and dietary fiber source in vegetarian, diabetic, or other specialized diets (Hughes et al., 1980). The authors emphasized that in terms of vitamins (Rutto et al., 2013), fibre and protein (Hughes et al., 1980), young nettle foliage has much to offer and could be utilized as a supplementary, spinach-like vegetable in the human diet. This is because as a source of essential amino acids stinging nettle leaves are comparable to common bean (*Phaseolus vulgaris*) and chicken (*Gallus gallus*) (Hughes et al., 1980).

Adhikari et al. (2016) compared the nutritional properties of flour from stinging nettle leaves with wheat and barley flours. The authors indicated that nettle leaf powder contained higher protein compared to wheat and barley flours.

Table 2.2. Proximate composition (g/100 g) of stinging nettle leaves in different formats and as reported by different authors

	Fresh leaves		Leaf flour	Cooked leaves
Nutrients	dry basis	as is	dry basis	as is
Moisture	NR	89	NR	87.7
Crude protein	29.7	3.7	33.8	3.6
Fiber	49.4[§]	6.4[y]	9.1[y]	3.5[y]
Crude fat	5.6	0.6	3.6	0.4
Ash	14.8	2.1	16.2	1.5
Carbohydrates*	0.5	7.1	37.4	6.3
References	(Hughes et al., 1980)	(Rutto et al., 2013)	(Adhikari et al., 2016)	(Rutto et al., 2013)

§ = dietary fiber, y = crude fibre, * = carbohydrate value by difference, NR = not reported

2.3.2 Mineral composition and anti-nutrients

Stinging nettle leaves are rich in minerals, especially with respect to the nutritionally important ones such as Fe, Ca, K, Zn, Cu, P, Mg, Mn and Na as summarised in Table 2.3. It was reported that fresh nettle leaves are a source of Ca and Fe (Rutto et al., 2013) and Mg, Ca, Fe, Mn, Zn (Kara, 2009; Musa Özcan et al., 2008; Pytlakowska et al., 2012). Rutto et al. (2013) reported that Ca and Fe contents of nettle leaves was affected by cooking.

Concerning anti-nutrients, Jimoh et al. (2010) reported nettle leaves (*U. urens*) contained alkaloids at 0.6 mg/100 g, phytates (4.39 mg/100 g) and saponins (3.25 mg/100 g). The phytates in the leaves can easily be detoxified by soaking, boiling or frying (Akubugwo and Obasi, 2007). In fact, there is a need for further research looking into the bioavailability of proteins and iron in stinging nettle leaves, during drying and cooking processes.

Table 2.3. Mineral content of stinging nettle leaf products (mg/100 g) as reported in different studies

	Fresh leaves				Dried leaf powder	Cooked leaves
Minerals	dry basis	dry basis	as is	as is	dry basis	as is
Ca	3840	865.4	90.3 - 142.6	278	168.8	376
Fe	99.9	10.7	1.44 -2.21	1.2	227.9	2.6
Zn	2.2	0.3	6.39 - 7.31	NR	NR	NR
Mg	732.4	14.6	45.7 - 55.2	NR	NR	NR
Cu	1.12	NR	NR	NR	NR	NR
Mn	6.65	1.1	0.69 - 1.02	NR	NR	NR
Na	12.8	22.7	16.8 - 20.6	5.7	NR	6.5
P	336.5	141.8	511.5 - 573.1	NR	NR	NR
K	1747.2	2139.0	243.3 - 278.2	NR	NR	NR
References	(Kara, 2009)	(Musa Özcan et al., 2008)	(Pytlakowska et al., 2012)	(Rutto et al., 2013)	(Adhikari et al., 2016)	(Rutto et al., 2013)

NR = not reported

2.3.3 Carotenoids

Carotenoids are C40 isoprenoid compounds composed of C=C conjugated double bonds (Rodriguez-amaya, 1997), which comprises carotenes and xanthophylls as the two main groups. Carotenes are related to hydrocarbons (contain no oxygen) while the xanthophylls include the corresponding oxidized derivatives (hydroxy, epoxy and keto compounds) and are frequently esterified (Eskin and Hoehn, 2013; Furr and Clark, 2003; Rodriguez-amaya, 1997). Xanthophylls are more polar than carotenes because they contain at least one hydroxyl group (Krinsky and Johnson, 2005).

Carotenoids have provitamin A activity and are natural pigments contributing to the red, yellow, orange, and purple colours of a variety of fruits and vegetables (Rodriguez-amaya, 1997). Nine carotenoids were identified in fresh nettle leaves, of which lutein and lutein isomers and all trans-β-carotene was the dominant carotenoids in nettle leaves as shown in Figure 2.2 (Guil-Guerrero et al., 2003). Total carotenoid content of different clones collected from Latvia ranged between 520 µg/g - 630 µg/g (Zeipiņa et al., 2015) and was in the range 220 µg/g - 320 µg/g in Serbia leaf samples collected at different times during vegetation period (Kukrić et al., 2012), on fresh weight basis.

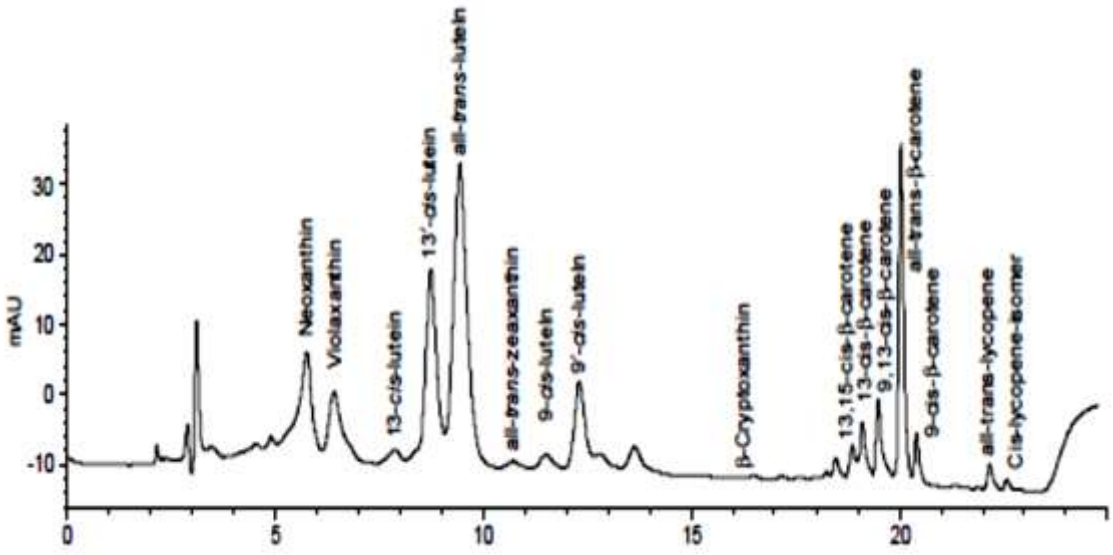


Figure 2.2. The carotenoids profile of fresh stinging nettle leaves (i.e. plotted at 450 nm) (Guil-Guerrero et al., 2003)

β-carotene has the highest provitamin A activity of the 50 different carotenoids (Furr and Clark, 2003; Krinsky and Johnson, 2005; Rodriguez-amaya, 1997). The carotenes (e.g α-carotene, γ-carotene and xanthophylls (e.g. α-cryptoxanthin and β-cryptoxanthin) have also vitamin A acivity due to the β-ionone ring (Rodriguez-amaya, 1997). All other carotenoids (e.g. phytofluene, ζ-carotene, lycopene, zeaxanthin, lutein, violaxanthin, and astaxanthin) that do not have a β-ring and thus do not have vitamin A activity. Of course these carotenoids may have antioxidant activity.

The retinol activity equivalent (RAE) of β-carotene is calculated using a RAE conversion factor of 12 µg β-carotene to 1 µg retinol (Furr and Clark, 2003; Joint FAO/WHO, 2001; Nishida et al., 2004).

The concentration of β-carotene in fresh nettle leaves was reported as 30.2 µg/g, as is basis (Rutto et al., 2013), whereas 3.8 µg/g all-trans-β-carotene and 3.2 µg/g β-carotene cis-isomers, on dry basis (Guil-Guerrero et al., 2003) (Table 2.4).

In green vegetables, the yellow colour of β-carotene is masked by the chlorophylls (Kidmose et al., 2000), which is due carotenoid-protein-complexes in the chloroplasts (Schieber and Carle, 2005). Cooking, boiling and drying might increase the release of β-carotene content of the vegetable products by destructing the cellular matrix and allowing its release from the protein complexes.

Although the fresh stinging nettle leaves may contain high amount of β-carotene, the contribution to vitamin A intake will definitely depend on the amount that has been retained after drying or cooking. Bunea et al. (2008) reported 20 % - 40 % loss of β-carotene in boiled spinach compared to raw spinach. Loss of β-carotene was also reported during drying and cooking of Amaranthus and spinach (Yadav and Sehgal, 1995), cooking of kale, spinach and Swiss chard (Miti et al., 2013) and green vegetables (Acho et al., 2014). Rutto et al. (2013) reported a decrease in β-carotene content of stinging nettle leaves from 30.21 µg/g in fresh leaves to 27.29 µg/g in cooked leaves. Therefore, investigation looking into how β-carotene content of stinging nettle leaves changes during drying, infusion and decoction processes is important.

2.3.4 Ascorbic acid

In fresh vegetables, ascorbic acid content varied between 6.5 - 105 mg/100 g while the boiled vegetables contained 4 - 85 mg/100 g (Waheed Uz et al., 2013). In spinach, loss of ascorbic acid was, on average, reported to be 60 % through boiling (Rumm-Kreuter and Demmel, 1990). Cooking leads to loss of ascorbic acid in leafy vegetables (Waheed Uz et al., 2013; Acho et al., 2014). In stinging nettle (*U. dioica*) leaves, Rutto et al. (2013) reported a loss of ascorbic acid from 1.1 mg/100 g in fresh leaves to 0.6 mg/100 g in cooked leaves, as is basis (Table 2.4). The type of drying also has a major influence on ascorbic acid loss or retention, with a higher loss observed when the drying temperature was high (Santas et al., 2008; Shilton, 2003). Freeze-drying preserves the ascorbic acid content more efficiently than processes such as oven drying or solar drying (Shilton, 2003). During the freeze-drying process, the temperature of the product is low (- 45 °C), which reduces degradation reactions (Ratti, 2001; Santas et al., 2008).

Table 2.4. Total phenolic content, antioxidant activity, β-carotene and ascorbic acid content of stinging nettle leaf products

Stinging nettle leaf products		β-carotene (µg/g)	Ascorbic acid (mg/100 g)	Total phenol content	Antioxidant activity	References
Fresh leaves		NR	NR	367 mg GAE/serving[a]	NR	(Danesi et al., 2013)
		NR	NR	7.62 mg GAE/g	76.1 % DPPH	(Hudec et al., 2007)
		7.0	NR	NR	NR	(Guil-Guerrero et al., 2003)
		30.21	1.1	NR	NR	(Rutto et al., 2013)
		NR	NR	151 - 1941 mg GAE/g	61 - 320 mg GAE/g	(Otles and Yalcin, 2012)
		NR	NA	10 - 95 mg GAE/g	NR	(Farag et al., 2013)
Dried leaves	Powder	3497	NR	128.75 mg GAE/g	66.3 % DPPH	(Adhikari et al., 2016)
Cooked leaves	Sauce	NR	NR	253 mg GAE/serving[b]	NR	(Danesi et al., 2013)
	Soup	NR	NR	309 mg GAE/serving[c]	NR	(Danesi et al., 2013)
	leaves	27.29	0.6	NR	NR	(Rutto et al., 2013)
Infusion	Leaves	NR	NR	23.4 mg GAE/100ml	NR	(Almajano et al., 2008)
	Powder	NR	NR	0.149 mg GAE/L	0.083 g AAE/L	(Gião et al., 2007)
	Leaves	NR	NR	0.163 mg GAE/L	0.113 g AAE/L	(Gião et al., 2007)
Decoction	Leaves	NR	NR	0.141 mg GAE/L	0.113 g AAE/L	(Gião et al., 2007)

a = 100g fresh leaves, b = 200 g sauce, c = 350 g soup, AAE = ascorbic acid equivalent, GAE = Gallic acid equivalent, DPPH = 2, 2-diphenyl-1-picrylhydrazyl, NR = not reported

The loss of ascorbic acid during thermal processing (e.g. boiling, cooking and drying) could be linked to its high water solubility because of two hydroxyl groups in its structure as shown in Figure 2. 3 . These structures are vulnerable to enzymatic degradation of protein-ascorbic acid aggregates and partial oxidation to dehydroascorbic acid (Ajayi et al., 1980; Harbourne et al., 2013; Kall, 2003; Sanmartin et al., 2000; Shilton, 2003; Waheed Uz et al., 2013). The effect of drying on ascorbic acid content of stinging nettle leaf infusions and decoctions has not been published previously.

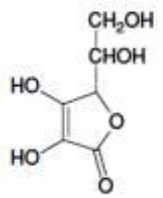


Figure 2. 3: Structure of ascorbic acid (Rincón-León et al., 2003)

2.2.5 Phenolic compounds and antioxidant activity

Phenolic compounds are generated as a secondary metabolites in response to cellular disruption (Giada, 2013) and cleavage of phenolic-sugar glycosidic bonds during thermal processes (Singleton et al., 1999; Turkmen et al., 2005). The main groups of phenolic compounds include phenolic acids, flavonoids and tannins (Do et al., 2014; Ferreira and Pinho, 2012; Giada, 2013; Khoddami et al., 2013; Tsao, 2010). Fresh stinging nettle leaves were reported to contain phenolic acid (e.g. hydroxycinnamic acid, hydroxybenzoic acid), tannins and flavonoids (e.g. flavones, flavonols, iso-flavonols, anthocyanins, catechins, lignin) (Farag et al., 2013; Orčić et al., 2014; Pinelli et al., 2008) (Table 2.5).

The main functions of phenolic compounds in foods are as colourants, natural flavourings and antioxidants. Dark green leafy vegetables are high in phenolic compounds and antioxidant activity (Giada, 2013). In a study to analyse the phenolic compounds of roots, stalks and leaves of nettle by Otles and Yalcin (2012), the total phenol content of nettle leaves ranged from 151 - 1001 mg GAE/g. Further, the total phenol content of fresh nettle leaves was reported by different authors as; 229 mg GAE\100 g (Turkmen et al., 2005), 176 mg GAE/100 g (Adhikari et al., 2016), 762-3996 mg GAE/100 g (Hudec et al., 2007), 5960 mg GAE /100 g (Kaledaite et al., 2011) and 736.4 mg GAE /100 g by (Pinelli et al., 2008) (Table 2.4).

In another study, Song et al.(2010) reported total phenolic content ranging from 0,12 to 5943 mg GAE/100 g, with large differences between the plants of up to 495-fold. According to (Ioana et al., 2013), total phenolic content decreased with plant growth and they indicated that the decrease in total phenolic derivatives was due to the decrease of non-tannin phenols (carboxylic acid and flavonoids). They reported total phenol content of 700 mg tannic acid/100 g (as is) in young nettle leaves to 145 mg tannic acid/100 g, (as is) in mature leaves.

Drying of herbs (Hossain et al., 2010; Suhaj, 2006) and cooking of spinach (Turkmen et al., 2005) were reported to increase the amount of phenolic compounds and antioxidant activity of those products. The increase in total phenol content could be linked with extraction of the insoluble phenolics during thermal processing such as condensed tannins and phenolic acids bound to cellular polysaccharides or proteins (Singleton et al., 1999; Giada, 2013).

The increase in total phenol content could also probably be due to suppression of oxidation by antioxidants (Yamaguchi, Mizobuchi and Kajikawa, 2001) during thermal processing. Owuor (2003) reported that polyphenol oxidase activity decreased while caffeine levels increased during drying of tea leaves.

The increase in antioxidant activity could be also linked to the antioxidants such as polyphenols/phenolic acid, carotenoids, ascorbic acid and amino acid (Rincón-León et al., 2003). Studies reported a strong correlation between total phenol content and antioxidant activity (Alarcón et al., 2008). Flavonoids, tannins and phenolic acids (e.g. cinnamic acids, benzoic acids, esters of cinnamic acids) have antioxidant activity (Giada, 2013; Rincón-León et al., 2003).

Table 2.5. Phenolic compounds reported to be present in fresh stinging nettle leaves

The three groups of dietary phenolics		Bioactive components	References
Flavonoids		Daidzein Apigenin Genistein Baicalein Naringenin Luteolin Kaempferol Quercetin Isorhamnetin Myricetin Vitexin Baicalin Kaempferol 3-O-glucoside Luteolin 7-O-glucoside Isoquercitrin Epigallocatechin gallate Hyperoside Amentoflavone Rutin	(Orčić et al., 2014)
		Quercetin dihexoside Megastigmane hexoside Isorhamnetin hexoside Rutin p-coumaroyl malate Isoquercetrin Kaempferol	(Farag et al., 2013)
		Myricetin Quercetin Rutin	(Otles and Yalcin, 2012)
		Kaempferol 3-O-rutinoside Isorhamnetin Rutin Quercetin	(Pinelli et al., 2008)
Phenolic acids	Benzoic acid and derivetives	Hydroxybenzoic acid Vanillic acid Gallic acid, Syringic aci	(Orčić et al., 2014)
	Cinnamic acid and derivetives	Cinnamic acid p-coumaric acid o-coumaric acid Esculetin Caffeic acid Scopoletin, Ferulic acid, 3,4-dimethoxy Cinnamic acid Sinapic acid	(Orčić et al., 2014)
		Caffeoyl malic acid dicaffeoyl quinic acid	(Farag et al., 2013)
		2-O-caffeoylmalic acid	(Pinelli et al., 2008)
		Quinic acid	(Orčić et al., 2014)
		3-(4-Hydroxycinnamoyl) quinic acid Neoolivil-4-O-glucopyranoside O-Feruloyl quinic acid	(Farag et al., 2013)
		Chlorogenic acid	(Otles and Yalcin, 2012) (Pinelli et al., 2008)
Polymers		Tannin (Catechin, Epicatechin) Liginin	(Orčić et al., 2014)

2.3.6 The potential contribution of stinging nettle leaves to dietary intakes of nutrients in human diet

Micronutrient malnutrition or deficiency remains a serious problem in Sub-Saharan Africa (Rodriguez-amaya, 1997). In line with this issue, the 2005 Dietary Guideline Advisory Committee recommended an increase in dietary intake of antioxidant vitamins (e.g. vitamins A and C) and dietary fibre from vegetables and fruits in order to reduce the risk of cardiovascular disease, stroke, and cancer. The Advisory Committee recommended that fruits and vegetables intake should increase to 5 - 13 servings per day depending on individuals calorie needs (Thompson and Veneman, 2005). Naude (2013) stated that at least one serving each of dark-green leafy vegetables could provide the daily requirements for vitamins and minerals as well as reduce the burden of nutrition-related disease.

According to US FDA (2013), the Dietary Reference Intakes (DRIs) reports vary with age, sex and life stage which limits its use in nutrition labelling of food products. The FDA used DRIs values to create a single number that represents the daily requirement for each nutrient, known as daily value (DV). The daily value is based on the amount of each nutrient needed for a 2,000-calorie-a-day diet for adults and children four or more years of age (Table 2.6).

The percent daily value (Equation 1) tells what percentage of each nutrient that an individual will get from one serving of the product and help for identifying the dietary value of the product. As described by US FDA (2013), for example, 5 % DV or less is considered low while 20 % DV or more indicates a rich source (Table 2.6). The percentage daily value (% DV) is calculated as the ratio of the amount of the nutrient in a serving of food and the DV for the nutrient (Equation 1).

$$\% \text{ DV} = \frac{\text{Nutrient content in a serving of food}}{\text{The daily value for the nutrient}} \text{ X } 100 \qquad (1)$$

Table 2.6. Daily values (DVs*) and conditions for nutrient content claims in food products as described by US FDA (2013)

Nutrients	DVs	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

* DVs based on a caloric intake of 2,000 calories, for adults and children four or more years of age

In many developing countries, for example, to meet the daily requirements of vitamin A in deficient populations identifying and inclusion of carotenoid-rich plants in the diet is the best option (Tanumihardjo, 2008). This because in developing countries up to 82 % of the total vitamin A intake is sourced from plant foods (van den Berg et al., 2000).

Stinging nettle is an underutilized wild vegetable, yet contains valuable sources of nutrients where contributing to protein, minerals, vitamins, fiber and antioxidants requirements for a healthy diet (Ait Haj Said et al., 2015; Kavalali, 2003; Kavtaradze et al., 2001; Upton, 2013). Hughes et al. (1980) reported that young stinging nettle leaves can potentially be used as a supplementary food to spinach-like vegetables in the human diet. Rutto et al. (2013) reported that a 100 g serving of fresh nettle leaves, blanched or cooked leaves can supply 90 - 100 % to the daily requirement of vitamin A and found to be a good source for dietary intake of Ca, Fe and protein.

2.4 Sensory properties of stinging nettle leaves and changes during processing

The overall sensation of flavour perception of a product is a combined effect of taste and aroma, appearance, texture and mouthfeel (Ruther and Kleier, 2005). Colour, aroma and flavour, and mouthfeel characteristics of stinging nettle leaves will be highlighted in the following sections.

2.4.1 Colour

Colour affects marketability and perception pleasantness of food products. However, it can also interfere with the overall flavour perception, such as sweetness, bitterness and saltiness of foods (Clydesdale, 1993). Green colour is an index for freshness, nutritional value and marketability of leafy vegetables (Haisman and Clarke, 1975; Kidmose et al., 2000). The leaves of the stinging nettle contain ample β-carotene (Guil-Guerrero et al., 2003; Rutto et al., 2013) and chlorophyll a and b (Alibas, 2007; Dey, 2013) as shown in Table 2.4 and Table 2.7, respectively.

Total chlorophyll content of fresh stinging nettle leaves was reported to be from 1.87- 2.51 mg/g by Zeipiņa et al. (2015) and 2.50 mg/g by Hojnik et al. (2007), on fresh weight basis. A decrease in chlorophyll a from 38 mg/L to 30 mg/L and chlorophyll b from 53 mg/L to 49 mg/L was reported during freeze drying by Dey (2013). During processing, the chlorophylls (green) degraded to pheophytins and pheophorbides (brownish) (Sai et al., 2011). The phytol degradation products contribute to the aroma of the food product (Di Cesare et al., 2004; Kidmose et al., 2000; Owuor, 2003). As described by Kidmose et al. (2000) and Di Cesare et al. (2004), degradation of chlorophylls during thermal processing changes the colour of the food product from a bright green to the olive brown (e.g. pheophytins a and b) due to the loss of magnesium ions.

Dey (2013) and Therdthai and Zhou (2009) reported that the lower temperature of the drying, the better colour retention. Alibas (2006) reported that the total colour difference (ΔE) between fresh nettle and oven dried leaves ranged from 5.9 - 11.2; which shows great variation of colour difference among samples; whereas lower variability between fresh nettle leaves and the freeze-dried leaves with total colour difference of 4.95 (Alibas, 2007).

Table 2.7. A review of literature reporting on the colour, aroma, flavour and mouthfeel of fresh and dried stinging nettle leaf products

Colour	Fresh leaves	Freeze dried leaves	Convection Dried leaves	Reference
L	27.6	32.3	NR	(Dey, 2013)
	27.6	NR	24.2	(Alibas, 2007)
a	- 4.7	- 6.0	NR	(Dey, 2013)
	- 6.9	NR	- 5.0	(Alibas, 2007)
b	8.5	9.7	NR	(Dey, 2013)
	9.7	NR	8.1	(Alibas, 2007)
ΔE	NR	5.0	NR	(Dey, 2013)
Chlorophyll a (mg/L)	38.0	30.4	NR	(Dey, 2013)
Chlorophyll b (mg/L)	53.3	48.7	NR	(Dey, 2013)
Odour/Aroma	Intense fresh vegetable Pine Spice Herbaceous Balsamic	Infusion-like Hay-like Earthy Woody	NR	(Dey, 2013)
Flavour	Odourless Earthy Bitter	NR	Dry faint Fishy Earthy	(Upton, 2013)
Mouthfeel	Burning Unpleasant stinging sensation	NR	NR	(Upton, 2013)

NR = not reported

2.4.2 Aroma and flavour

Research to distinguish the aroma/odour of fresh and freeze dried uncooked nettle leaves by Dey (2013), intense fresh vegetable, piney, spicey, herbaceous and balsamic odour notes were reported in fresh nettle leaves while the odour of freeze dried leaves was described as infusion-like, hay-like, earthy and woody (Table 2.7). Upton (2013) described fresh uncooked nettle leaves as odourless or earthy with a slight bitter taste while dried leaves had a dry faint and mildly fishy or earthy aroma. The presence of these particular flavour notes in stinging nettle leaves could be compared with a number of volatile and/or non-volatile compounds that may have a similar odour based on literature as described by (Di Donfrancesco et al., 2012). Table 2.8 provides a review of these related flavour descriptors and associated volatile/non-volatile compounds in different food products. For example, King et al. (2006) reported that green-related notes such as asparagus, green-herblike, grassy, and spinach characteristics were associated with hexenal aromatic compounds.

Of hundreds of volatiles produced by plants, only a few generates the flavour fingerprint that helps humans to recognize food products (Goff and Klee, 2006). Such flavour volatile compounds (see Table 2.8) are generated during thermal processing via enzymatic, redox, or pyrolytic reactions of amino acids (Arimura et al., 2001), fatty acids (Owuor, 2003), and carotenoids (Giada, 2013; Goff and Klee, 2006; Owuor, 2003; Rodriguez-amaya, 1997). Stinging nettle leaves are a rich sources of essential amino acids (Hughes et al., 1980; Rutto et al., 2013) and fatty acids (Guil-Guerrero et al., 2003; Rutto et al., 2013), therefore, these compounds could be associated with the specific aroma and flavour of stinging nettle leaf products.

Accordingly, the first class of volatiles could come from fatty acid hydrolysed to free fatty acid and then oxidized to produce green notes (green or grassy) (Owuor, 2003) as shown in Figure 2.4. Aldehydes and alcohols derived from omega-3-linolenic acid were reported to be responsible for the flavours of bay leaves and tea leaves (Goff and Klee, 2006).

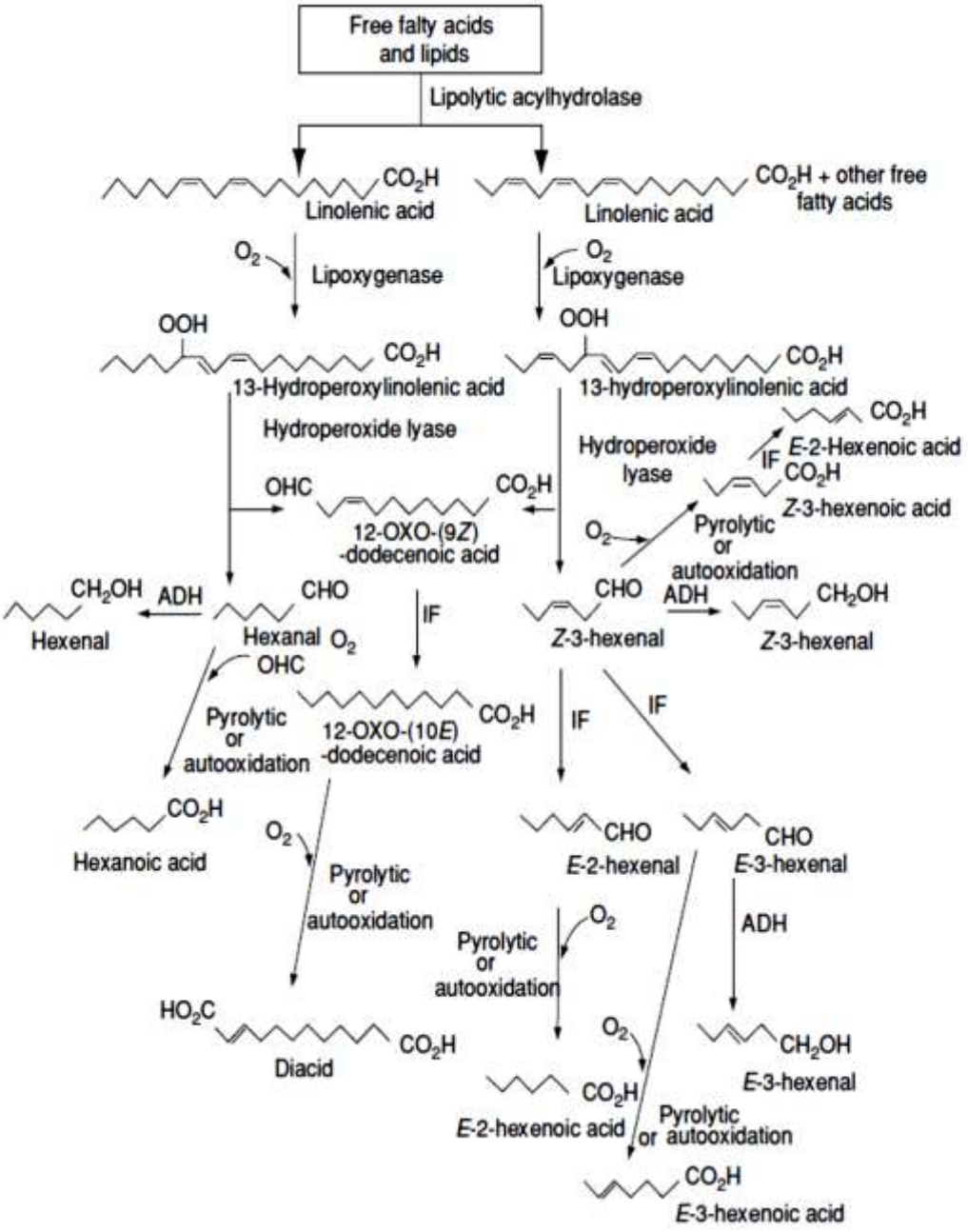


Figure 2.4. A flow chart representing volatile flavour compounds formation from linoleic and linolenic acid (Owuor, 2003)

Table 2.8. A review of flavour descriptors and associated volatile/non-volatile compounds in different food products that could be used to compare with similar flavour notes in stinging nettle leaves.

Flavour descriptors*	Flavour volatile/non-volatile compounds	Food products	References
Green-related notes	Hexenal	Green leaf volatiles used in beverage flavours	(King et al., 2006)
	decanal	Japanese green tea	(Hattori et al., 2005)
	(E)-2-Hexenal, 1-Hexanol, 1-Penten-3-ol	Chinese green and black teas	(Qin et al., 2013)
	(Z)-3-hexenol, (E)-2-nonenal, 3-methylnonane-2,4-dione	Infusions from different green tea	(Kumazawa and Masuda, 1999)
	Hexanal, (E)-2-hexenal, 1-heptanol	Cooked Black Rice	(Yang et al., 2008)
	1,4-heptadiene, 3-methyl, hexanal, (E,E)-2,4-hexadienal	Fish oil enriched milk emulsion	(Venkateshwarlu et al., 2004)
Grassy	Hexanal	Chinese green and black teas	(Qin et al., 2013)
Woody	(E)-2-penten-1-ol	Fish oil enriched milk and in a emulsion	(Venkateshwarlu et al., 2004)
	2-propylpyridine, 2, 3-diethyl-5-methylpyrazine, (E)-2-phenyl-2-butenal	Dried and roasted nori (*Porphyra yezoensis*)	(Shu and Shen, 2012)
	Cadinene, β-Lonone and Cedrol	Chinese green and black teas	(Qin et al., 2013)
Minty	Hexyl esters such as hexyl proprionate and hexyl tiglate	Green odour or flavour chemicals	(Hongsoongnern and Chambers IV, 2008)
	B-Cyclocitral and α-Terpineol	Chinese green and black teas	(Qin et al., 2013)
Citrus	Limonene	Chinese green and black teas	(Qin et al., 2013)
	Octanal		
	nonanal		
	decanal	Cooked black rice	(Yang et al., 2008)
		Fish oil enriched milk emulsion	(Venkateshwarlu et al., 2004)
Earthy/musty	2-isobutyl-3-methoxypyrazine	Infusions from different green tea varieties	(Kumazawa and Masuda, 1999)
	2, 3-Dimethylnaphthalene	Dried and roasted nori	(Shu and Shen, 2012)
	(E)-2-heptenal	Fish oil enriched milk emulsion	(Venkateshwarlu et al., 2004)

Table 2.9. *(continued)*

Flavour descriptors*	Aromatic compounds	Food product	References
Cucumber	(E, Z)-2, 6-nonadienal	Infusions from different green tea varieties	(Kumazawa and Masuda, 1999)
	(E)-2-nonenal	Cooked Black Rice	(Yang et al., 2008)
	(E)-2-Nonenal and (E,E)-2,6-nonadienal	Dried and roasted nori (*Porphyra yezoensis*)	(Shu and Shen, 2012)
		Fish oil enriched milk emulsion	(Venkateshwarlu et al., 2004)
Burnt	Guaiacol	Infusions from different green tea varieties	(Kumazawa and Masuda, 1999)
	3-methyl-2-thiophenecarbaldehyde 2, 5-dimethyl-3-ethylpyrazine	Dried and roasted nori (*Porphyra yezoensis*)	(Shu and Shen, 2012)
	Heptanal, (E)-2-octenal and (E)-2-nonen-1-olfor	Fish oil enriched milk emulsion	(Venkateshwarlu et al., 2004)
Fermented	3-methylbutanoic acid	Dried and roasted nori (*Porphyra yezoensis*)	(Shu and Shen, 2012)
Herbal	2-ethylhexanoic acid		
Fishy	Trimethylamine and dimethylamine	Marine fish and seafood	
	(Z)-1, 5-octadien-3-one	Dried spinach leaves	(Masanetz et al., 1998)
Bitter	Caffeine and saponin	Green tea	(Lee et al., 2013)
Astringent	Epicatechin and epigallocatechin	Green tea	(Owuor, 2003; Wang and Ruan, 2009)
	Tannins	Green tea	(Troszynska et al., 2003)
	Polyphenol, Theaflavins, Thearubigin		(Sai et al., 2011)

*Sensory lexicons for green vegetable (Talavera-Bianchi et al., 2010), green tea (Lee et al., 2007) and green odour (Hongsoongnern and Chambers IV, 2008) were the basis for selected flavour descriptors.

Threonine, valine, leucine, isoleucine, and phenylalanine were the dominant amino acids found in fresh stinging nettle leaves (Hughes et al., 1980; Rutto et al., 2013) (Table 2.9). A second class of volatiles (e.g. 2-methylpropanal, 2-methylbutanal, pentanal, and phenyl acetaldehyde) are generated from valine, leucine, isoleucine, and phenylalanine as outlined in Figure 2.5. Aspartic and glutamic acid are found in relatively high concentration in stinging nettle leaves (Hughes et al., 1980), therefore, these amino acid can potentially contribute to umami taste of the leaves. Owuor (2003) reported that threonine is associated with a 'brothy' taste in green tea.

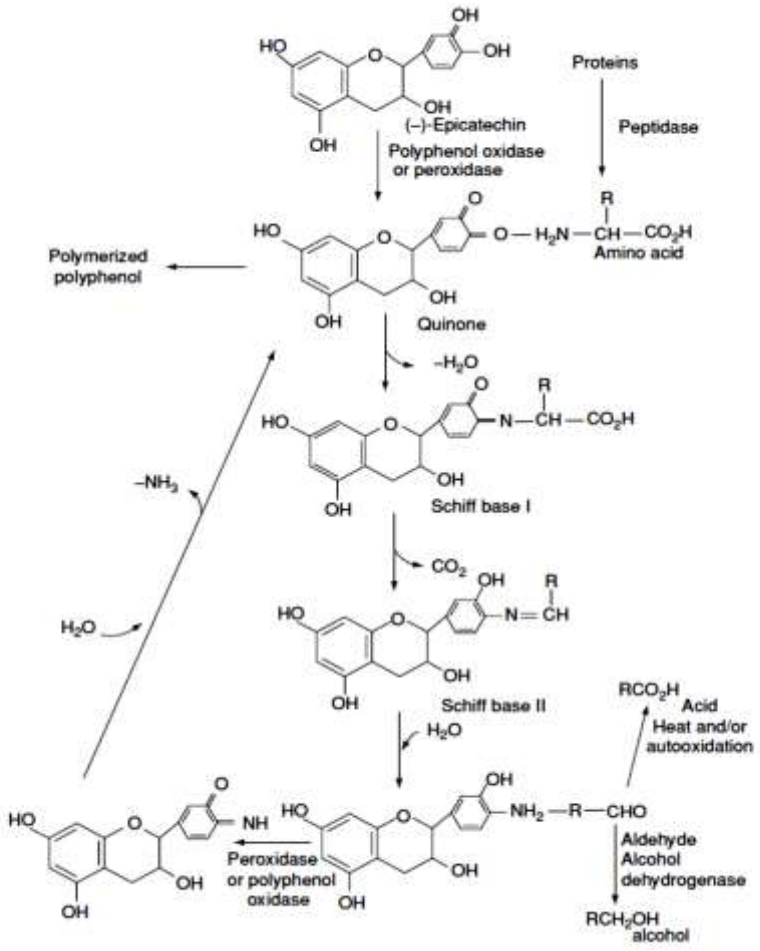


Figure 2.5. A flow chart representing aldehydes, alcohols and carboxylic acid formation from amino acids (Owuor, 2003)

Table 2.9. A review of literature reporting on the amino acid and fatty acid profile present in stinging nettle leaves

Amino acid composition (g/100 g)	Fresh leaves	Cooked leaves		Fresh leaves	Fresh leaves	Cooked leaves
Aspartic acid	0.85	0.88	Saturated fatty acids (%)	NR	35.50	23.60
Threonine	1.00	1.12	Monounsaturated fatty acid (%)	NR	2.70	4.80
Serine	0.85	1.14	Polyunsaturated fatty acids (%)	NR	61.80	71.60
Glutamic acid	1.69	1.97	Moisture (g/100 g)	82.10	NR	NR
Proline	0.90	1.31	Saponifiable oil (g/100 g)	3.40	NR	NR
Glycine	0.92	1.26	Fatty acid (%)			
Alanine	1.20	1.54	16:0	20.20	17.06	14.80
Valine	1.11	1.60	16:1n-7	4.00	2.54	3.50
Methionine	0.24	0.33	18:0	2.00	1.86	1.60
Isoleucine	0.90	1.30	18:1n-9	2.90	2.18	1.90
Leucine	1.65	2.37	18:2n-6	18.20	23.30	210
Tyrosine	0.75	1.11	18:3n-3	29.70	49.56	55.50
Phenylalanine	1.03	1.43	20:0	NR	0.83	0.70
Lysine	1.11	1.37	20:1n-9	0.80	0.03	0.00
Histidine	0.42	0.64	22:0	NR	1.37	1.10
Arginine	1.22	1.79	22:1n-9	0.60	0.06	0.10
Total essential amino acid	8.23	11.26	24:0	NR	1.23	0.90
Total amino acid	17.46	22.87	n-3/n-6	1.60	NR	NR
Dry matter (g/100 g edible portion)	11.00	12.3	Total fat (%)	NR	3.15	4.65
References	(Rutto et al., 2013)	(Rutto et al., 2013)		(Guil-Guerrero et al., 2003)	(Rutto et al., 2013)	(Rutto et al., 2013)

NR = not reported

The third class of volatiles, could be derived from oxidative cleavage of carotenoids (e.g. safranal in green tea is derived from zeaxanthin) (Goff and Klee, 2006).

Lastly, phenolic compounds are also an important contributor of flavour eg. caffeine and saponins are responsible for bitterness (Lee et al., 2013) whereas epicatechin and epigallocatechin (Owuor, 2003; Wang and Ruan, 2009) and tannins (Troszynska et al., 2003) contribute to astringency in green tea. In fresh nettle leaves catechin, tannins, caffeic acid, chlorogenic acid, and naringin were found to be abundant (Farag et al., 2013; Otles and Yalcin, 2012; Pinelli et al., 2008). Therefore, catechin and tannins in nettle leaves could be responsible for an astringent taste while caffeic acid, chlorogenic acid, and naringin may be responsible for bitterness.

2.4.3 Mouthfeel

Mouthfeel is a physical characteristic that arises from the structural elements (physical) sensed by the palate during chewing of food (Bourne and Szczesniak, 2003). Upton (2013) reported that fresh stinging nettle leaves initiated a burning/unpleasant stinging mouthfeel sensation (chemosensory). However, the urticating sensation of the fresh leaves could be neutralized by thermal processing such as drying or steaming of the leaves (Roberts, 2011) and during juice extraction processes involving destruction, homogenization or blending of the leaves.

Shonte and WoldeTsadik (2013) reported on perception and use of the stinging nettle plant in Ethiopia that the participating consumers associated consumption of cooked nettle leaves with a smooth mouthfeel sensation and considered as appetizing. These reponses confirm that the urticating sensation was lost during cooking. A detailed description of the aroma, flavour, mouthfeel and colour of cooked stinging nettle leaves and leaf infusions, either prepared from fresh or dried leaves, has not been published previously.

2.5 Conclusions

Stinging nettle (*U. dioica* L.) is an undervalued wild vegetable distinguished by stinging hairs with a high potential use in food industries. Seasonality, the fear of the stinging hairs, lack of commercial availability and the stigma related to stinging nettles being associate with famine/poor man's food might be reasons for its limited utilisation. Drying stinging nettle leaves not only dissipate irritating contents of the stinging hairs but also extend the consumption period, marketing and utilization at times of shortages. Fresh or dried nettle leaves are added to soups or stews, and also cooked as a potherb in dishes in many countries, or infused in boiled water or decocted as a herbal remedy. For economic reasons infusions from the leaves is often brewed multiple times. Its leaves besides adding variety to the menu, they are also valuable sources of nutrients where they contribute protein, minerals, vitamins, fiber and phytonutrients (e.g. antioxidants and phenolic compounds).

A wide range of studies have been reported on the chemical composition of fresh stinging nettle leaves. Fresh stinging nettle leaves contain chlorophylls (chlorophyll a and b), nine forms of carotenoids (especially β-carotene, neoxanthin, violaxanthin, luteinand lycopene), essential fatty acids (especially dominant palmitic, cis-9, 12 linoleic and a-linolenic acid), essential amino acids, different phenolic compounds, ascorbic acid and minerals (especially K, Ca, Mg, Fe, Mn and Zn). The chlorophylls, carotenoids, fatty acids, amino acids, polyphenolic compounds and ascorbic acid present in the fresh stinging nettle leaves may be degraded during drying, cooking, infusion and decoction processes. The degradation of these metabolites may result in the production of different volatile compounds and non-volatile compounds that might bring a change in the sensory and nutritional characteristics of the product. However, no literature was found indicating how the sensory and nutritional properties change when stinging nettle leaves are dried, especially regarding when dried nettle leaves are cooked, infused or used for decoction.

3: HYPOTHESES AND OBJECTIVES

3.1 Hypotheses

Hypothesis 1

The sensory properties (e.g. aroma, flavour and colour) of cooked leaves and infusions prepared from oven dried nettle leaves will be different compared to the same products prepared from fresh nettle leaves.

Oven drying of stinging nettle leaves could bring change in aroma, flavour and colour of the fresh leave; due to enzymatic, redox, or pyrolytic reactions of carbohydrates, chlorophylls, protein/amino acids, fatty acids and carotenoids (Goff and Klee, 2006; Pichersky et al., 2006). Oven drying of the leaves could degrade the chlorophyll a and b of fresh nettle leaves (Alibas, 2007; Dey, 2013) and that might be responsible for colour changes from a bright green colour to the olive brown colour of pheophytins a and b, due to the loss of magnesium ions (Di Cesare et al., 2004; Kidmose et al., 2000; Owuor, 2003). Oven drying also promotes oxidation and cis-trans-isomerization of β-carotene (due to the C=C conjugated double bonds) (Kidmose et al., 2000; Di Cesare et al., 2004). Degradation of β-carotene produces β-ionone, 3-oxo-β-ionone, β-ionone, a-ionone, 3-hydroxy-β-ionone and other terpenoid aldehydes and ketones (Owuor, 2003), that might contribute to the change in aroma, flavour and colour (Rodriguez-amaya, 1997).

Hypothesis 2

The sensory properties (e.g. aroma, flavour and colour) of a first brewed infusion made from either fresh or oven dried leaves will be different from a second infusion of the same leaves.

Lee et al. (2013) reported that the intensity of the green flavour generally decreased as the samples were brewed repeatedly, for example, a decrease in the intensities of spinach flavour, bitterness, astringency and toothetch from the first to the second brews were noted. A decrease in the percentage of caffeine released in each brew of green tea leaf was observed as the leaves were brewed repeatedly (Hicks et al., 1996; Lee, 2009). When green tea leaves were brewed repeatedly, the colour of the infusion changed from deep green or olive brown colour to a lighter form of the respective colours from the first to the second brew due to heating and pigment dilution (Kidmose et al., 2000).

Hypothesis 3

The ascorbic acid and β-carotene content of fresh and freeze dried nettle leaves will be higher compared to oven dried leaves.

Oven drying utilizes high drying temperatures (> 50 °C) (Shilton, 2003), whereas during the freeze-drying the temperature of the product is low (- 45 °C), which reduces thermal degradation reactions of heat-labile nutrients (Ratti, 2001; Santas et al., 2008). The heat labile nature of ascorbic acid cause it to oxidize easily to dehydro-ascorbic acid during heating (Ajayi et al., 1980; Sanmartin et al., 2000; Waheed Uz et al., 2013). The C=C conjugated double bonds of the β-carotene structure makes it susceptible to geometric isomerization (isomerization of trans-carotenoids to the cis-form) with exposure to heat and light (Bernhardt and Schlich, 2006; Di Cesare et al., 2004; Kidmose et al., 2000; Owuor, 2003; Rodriguez-amaya, 1997). Eskin and Hoehn (2013) reported that all-trans-β-carotene can be convered to 9-cis- and 13-cis-β-carotene due to thermal treatment and exposure to light.

In contrast to the ascorbic acid and β-carotene content, oven drying of stinging nettle leaves could result in an increase in total phenol content and total antioxidant activity compared to freeze drying and fresh leaves.

It was reported that drying of herbs result in an increase in the amount of phenolic compounds and antioxidant activity of the product (Hossain et al., 2010; Suhaj, 2006). During oven drying, besides suppression of the oxidation of antioxidants by thermal inactivation of oxidative enzymes (Yamaguchi, Mizobuchi and Kajikawa, 2001), the cleavage of phenolic-sugar glycosidic bonds can lead to the formation of phenolic aglycons that react better with the Folin–Ciocalteu reagent (Singleton et al., 1999).

Decoction prepared from either freeze dried or oven dried leaf powder would result in a higher total phenol content and total antioxidant activity compared to an infusion made from the same leaves.

Cleavage of phenolic-sugar glycosidic bonds and release of bound or insoluble phenolic compounds from cellular matrix (Giada, 2013), will be greater due to a constant higher temperature during decoction compared with steeping in hot water during infusion (Courtine, 1984).

Hypothesis 4

Ascorbic acid and β-carotene content will be higher in an infusion made from either freeze dried or oven dried leaf powder compared to decoction prepared from the same leaves.

The two hydroxyl groups of ascorbic acid makes it easily water soluble (Ajayi et al., 1980; Sanmartin et al., 2000; Waheed Uz et al., 2013). The constant boiling during decoction degrades protein-ascorbic acid aggregates on top of partial oxidation of ascorbic acid to dehydroascorbic acid because of its heat labile nature compared to a steeping in hot or boiled water processes involved in infusion (Courtine, 1984). The C=C conjugated double bonds of β-carotene makes it susceptible to oxidation and geometric isomerization upon exposure to heat (Bernhardt and Schlich, 2006; Di Cesare et al., 2004; Kidmose et al., 2000; Owuor, 2003; Rodriguez-amaya, 1997). During constant heating/boiling in decoction (Courtine, 1984), more denaturation of protein and release of β-carotene associated with low-density lipoproteins proteins (Rodriguez-amaya, 1997) will occur compared to steeping in hot water (infusion) (Courtine, 1984). Ascorbic acid and β-carotene molecules will be exposed to a constant high temperature during decoction whereby the respective degrading enzyme catalyse the reaction at constant rate. Whereas during infusion, the gradual decease in temperature will cause a concomitant decrease in the activity of the degrading enzymes.

3.2 Objectives

The aim of this study was to evaluate the sensory and nutritional properties of stinging nettle (*U. dioica* L.) leaves and leaf infusions.

1. To determine the effects of using fresh or oven-dried stinging nettle leaves to cook or to prepare an infusion on aroma, flavour and colour of the leaves.
2. To determine the effect of two infusion cycles on sensory properties of infusions prepared using fresh or oven dried stinging nettle leaves.
3. To determine the effects of freeze drying and oven drying on ascorbic acid, β-carotene, total antioxidant activity and total phenol content of stinging nettle leaves.
4. To evaluate the effect of type of extraction (i.e. infusion and decoction) on the ascorbic acid, β-carotene, total antioxidant activity and total phenol content of nettle leaf powder manufactured using freeze drying or oven drying.

4: RESEARCH

4.1 Descriptive sensory evaluation of cooked stinging nettle (*U. dioica* L.) leaves and leaf infusions: effect of using fresh or oven dried leaves

4.1.1 Abstract

Stinging nettles (*U. dioica* L.) is known since ancient times as a wild source of food and a herbal medicine, but the plant remains underutilized. The aroma, flavour and colour of cooked stinging nettle leaves and leaf infusions prepared from the fresh or dried leaves, has not been published. The effects of using fresh or oven-dried leaves to cook a relish or to prepare an infusion on sensory properties were measured. In addition, the effect of two infusion cycles on the sensory properties were determined. A trained descriptive sensory panel evaluated the sensory characteristics of cooked nettle and spinach leaves using 19 aroma and 26 flavour descriptors. Twenty aroma and 25 flavour descriptors were used for evaluating of the leaf infusions. The L, a*, b* and ΔE values of fresh, dried and cooked leaves were measured. Although the colour changed, most of the characteristic green type aroma and flavour notes of fresh nettle leaves were preserved in cooked leaves and leaf infusions prepared from dried leaves. The first two brewed infusions from fresh or dried leaves provided similar aroma and flavour intensities. Further consumer research will determine which sensory characteristics of the products from stinging nettles drive liking or disliking by target consumers. This research contributes to the understanding of the potential of stinging nettle for addressing food and nutrition security and well-being of consumers.

Keywords: Stinging nettle leaves, sensory, aroma, flavour, infusion cycles, total colour difference, oven dried

4.1.2 Introduction

In sub-Saharan Africa a large proportion of households is poor, food insecure and exists on a diet composed primarily of staple foods which are generally low in micronutrients (IFPRI, 2011). Stinging nettle, *U. dioica* L., is an edible wild green vegetable (Khatiwada et al., 2011) and medicinal plant distinguished by stinging hairs (Kavalali, 2003; Moskovitz, 2009; Roberts, 2011).

Nettle leaves have been used, particularly in rural areas of Africa, as a potherb, soup and herbal infusion (Kavalali, 2003; Moskovitz, 2009; Roberts, 2011). Nettle leaves are eaten like spinach; can be cooked fresh after harvest or dried and stored for later preparation. Young nettle shoots are eaten as famine food in many parts of the world (Davidson and Jaine, 2006; Khatiwada et al., 2011). In Ethiopia and elsewhere, an infusion is made by brewing the leaves. For economic reasons the infusion is often brewed multiple times.

Stinging nettle leaves represent an inexpensive but high quality source of essential amino acids (Hughes et al., 1980; Rutto et al., 2013), vitamin A (Guil-Guerrero et al., 2003), vitamin C (Ioana et al., 2013), minerals (Kara, 2009; Musa Özcan et al., 2008; Pytlakowska et al., 2012), polyphenols and antioxidants (Farag et al., 2013; Otles and Yalcin, 2012). However, the plant remains underutilized (Khatiwada et al., 2011). Seasonality, the wild nature, the fear of the stinging hairs, lack of commercial availability and marketing, the stigma related to stinging nettles being associated with famine/poor man's food, as well as potential undesirability of the sensory properties of nettles might be reasons for its limited utilisation.

Nettle leaves perishes rapidly after harvest and therefore is mainly consumed in season (Kavalali, 2003). Drying of the leaves would extend the consumption period and utilization. However, aroma and flavour changes could occur during drying. The drying process also changes appearance of leaves. The loss of green colour is mainly due to degradation of chlorophyll a and b, and carotenoid losses due to oxidation of the highly unsaturated carotenoid structure and cis-trans-isomerization during thermal processing (Di Cesare et al., 2004; Kidmose et al., 2001; Shilton, 2003).

Drying changes the aroma of food products through losses in volatile compounds or formation of new volatile compounds as a result of oxidation and esterification reactions (Dey, 2013; Díaz-Maroto et al., 2002; King et al., 2006; Orphanides et al., 2013). Oven drying compared to freeze drying results in more degradation of colour and flavour changes (Shilton, 2003), but it is a more cost effective drying method. Dey (2013) observed changes in aroma of uncooked nettle leaves during and after freeze drying with decreased fresh, vegetable, pine, herbaceous, balsamic, and spice odour notes and increased hay-like, sweet, earthy, woody and infusion odour notes.

The sensory characteristics of cooked nettle leaves and leaf infusions have not been published. The objective of this study was to determine the effects of using fresh or oven dried stinging nettle leaves to cook or to prepare an infusion on sensory properties. In addition, the effect of two infusion cycles was determined.

4.1.3 Materials and methods

4.1.3.1 Experimental design

The effect of two factors, species [nettle compared to spinach as a control (the product sold under this name in South Africa is actually 'swiss chard' *Beta vulgaris subsp. vulgaris*)] and state of the leaves used (fresh or oven dried) on descriptive sensory and colour characteristics of two products (cooked leaves and leaf infusions) were determined. For the leaf infusions an additional factor, the effect of two infusion cycles (first and second) was included.

4.1.3.2 Production and harvesting of nettle leaves

Stinging nettle plants were obtained from Margaret Roberts Herbal Centre, Hartebeespoort/De Wildt area (GPS: -25° 41' 03.25", +27° 55' 04.06"). Seedlings were propagated and transplanted on the experimental farm of University of Pretoria. The plot was ploughed, levelled and prepared. The plot size was 3 m x 9 m with a spacing of 1 m between rows and 1 m between plants. The spacing between adjacent replications was 0.5 m. Wonder 3:2:1(28)SR, (AGRO SERVE Pty Ltd Trading as WONDER™) slow release nitrogen organic fertilizer (nitrogen-140 g/kg, phosporus-91.5 g/kg, potassium-47 g/kg) was applied at a rate of 45 g/m^2 and repeated every 6 weeks.

Water was applied after every application to enhance effectiveness of absorption by the roots and prevent the burning of the roots. Other agronomic practices (weeding, cultivation, irrigation) were applied during the growth season. Plots were irrigated uniformly every other day for the first two weeks after transplanting and then twice a week. Young and tender shoots of nettle were harvested using scissors and wearing hand gloves after five weeks of planting. The leaves were thoroughly mixed and carefully handled to ensure that their quality was maintained.

4.1.3.3 Preparation of fresh and oven dried leaves

Fifteen kilograms of nettle were harvested in October (spring season) while thirty bunches (each weighing 500 g) spinach leaves were purchased from a supermarket, Hatfield, South Africa. The leaves were sorted, washed and surface air dried. Treatments, each replicated three times, were applied as follows: half of the raw leaves were oven dried (70 °C for 15 h) using a drying oven (PROLAB, Model: IDS 160, Switzerland) while the other half was packed in polyethylene bags (215 mm x 315 mm, 500 g/bag) and stored at 0 °C.

4.1.3.4 Cooking of leaves

The cooked leaves were prepared following directions by Francisco et al. (2009) with a few modifications. Dry leaves (100 g) were cooked in stainless steel pots (3.5 L, 18-10 Edelstahl, Rostfrei, Prochef) with 1100 ml boiling water (deionised water) added while 600 ml boiling water (deionised water) was added to fresh leaves (600 g). The leaves were cooked for 15 min on 2000 W single plate stove (STA001, ANVIL, South Africa) at power level 4. While cooking, the contents were mixed 10 times with a wooden spoon.

4.1.3.5 Leaf infusions

The infusions were prepared following directions by Lee (2009) with a few modifications. Ten gram of oven dried or 50 g fresh leaves were placed inside a 1L glass coffee plunger (CIRO® Taste the freshness coffee maker, PYREX), to which 300 ml of deionised water at 80 °C were added. The leaves were infused for 6 min while the pot was swirled 10 times clockwise.

After infusion, the plunger was pushed down and the infusion was poured into a pre-warmed thermos flask (1.02 L, Thermo Ltd, England). Then 50 ml of the infusion was poured into pre-warmed tea cups and presented to the sensory panel.

4.1.3.6 Descriptive sensory evaluation

The descriptive sensory properties of stinging nettle cooked leaves and leaf infusions were determined following the generic descriptive analysis method (Einstein, 1991) which involves recruitment and screening of the panel, panel training and product evaluation.

4.1.3.6.1. Training of the panel

Twelve panellists (i.e. 7 female and 5 male panellists, 22 - 40 years old) participated in this study. A number of sensory "lexicons" for green leafy vegetable (Talavera-Bianchi et al., 2010), green tea (Lee et al., 2007) and green odour (Hongsoongnern and Chambers IV, 2008) were the basis for lexicon development and preparation of reference samples. Training of the panel was done in 10 h including five 2 h per day sessions. During the training, each panellist identified words to describe the differences between cooked spinach and stinging nettle leaves prepared from fresh and dried leaves. Similarly, the differences in the sensory properties of infusions prepared from spinach and stinging nettle fresh and dried leaves, brewed once or twice were described. Term generation was repeated three times. Lexicon and scale anchors were developed, defined and agreed upon for evaluation as shown in Table 4.1.

4.1.3.6.2 Evaluation of cooked leaves and leaf infusions

During each evaluation session of 2 h, the panellists evaluated cooked leaves from the four treatments or the eight infusion samples. The evaluation of the cooked leaves was completed before the panel started with the evaluation of infusions. Cooked leaf samples were kept warm on a warming tray at 50 °C and 50 g of each was served in 90 ml glass ramekin bowls covered with aluminium foil. The panel had 15 min for each sample, typically 10 min to evaluate and 5 min to rest before the next sample. The panellists used cream crackers (Bakers Biscuits, Durban South Africa) and deionised water to cleanse their palates before the next sample.

Panellists evaluated the sensory properties of cooked nettle from fresh and dried leaves using 19 aroma, 24 flavour and two mouthfeel descriptors and leaf infusions from two subsequent brews using 20 aroma and 25 flavour attributes. Aroma was evaluated immediately after removing the foil cover using short sniffs, thereafter the product was tasted to evaluate the flavour and texture properties. Five-point category scales (1 = not perceived to 5 = extreme intensity) were used to measure the intensity of each attribute for a given sample. Leaf infusions were kept warm in thermos flasks and 50 ml of each was served in pre-warmed tea cups covered with aluminium foil at ≈ 64 °C, one sample at a time. The same methodology was used for the infusions as described for the cooked leaves.

4.1.3.7 Colour measurement

The L*, a* and b* colour values of raw leaves, cooked leaves and leaf infusions was measured using a Konica Minolta colorimeter (CR-400, made in Japan). A 5 cm diameter glass petri dish was filled with either leaves or infusions. All measurements were replicated thrice and the average value was recorded. In colour measurement, the coordinates show the degree of brightness (L), the degree of redness (+a*) or greenness (-a*), and the degree of yellowness (+b*) or blueness (-b*), respectively (León et al., 2006). The changes in colour of leaves from fresh to dried, and fresh to cooked were recorded as total colour difference (ΔE). In addition, the ΔE of leaf infusions (compared to first brew of fresh leaves) were calculated as follows (Equation 2):

$$\Delta E = \Delta L\hat{}2 + \Delta a\hat{}2 + \Delta b\hat{}2) \hat{} 0.5 \qquad (2)$$

Where, ΔL = L-L0, Δa = a-a0, Δb = b-b0; and where L, a, and b are the measured values of dried, cooked or infusion samples; L0, a0, b0 are the values for fresh uncooked leaves and/ or 1st brew infusion from fresh leaves.

Table 4.1: Lexicon used to describe sensory characteristics of stinging nettle cooked leaves and leaf infusions

Descriptors*	**Definition**	
	Aroma (perceived ortho-nasally by opening the foil cover and sniffing the content)	**Flavour** (perceived during consumption of the products including retro-nasally perceived aromatics, basic tastes and trigeminal nerve interaction effects)
Asparagus-woody	Green woody aromatics associated with cooked green asparagus	Perception of green woody odorants and tastes associated with cooked green asparagus
Beany	Aromatics associated with cooked legumes, beans, peas, peanuts and soybean	Green beany aromatics and taste associated with cooked legumes, beans, peas, peanuts and soybean
Beet	Musty, dusty, earthy aromatics of fresh beets	The dark, musty, dusty, earthy aromatics and tastes of fresh beets
Brown spice	Aromatics associated with a range of brown spices such as cinnamon, nutmeg and allspice	The combined sensation of aromatics and tastes associated with a range of brown spices such as cinnamon, nutmeg, allspice
Burnt	Aromatics associated with burned or scorched vegetables or grains such as roasted wheat	The somewhat sharp and acrid notes associated with burned or scorched vegetables or grains
Brussels Sprouts	Aromatics associated with Brussels sprouts and cauliflower	The somewhat sharp, slightly sour tastes, pungent aromatics associated with Brussels sprouts and cauliflower
Cabbage	Aromatics associated with raw cabbage, cooked cabbage	The green, somewhat sharp, slightly sulphurous, sweet and pungent aromatics and taste associated with raw cabbage or cooked cabbage
Celery	Aromatics associated with fresh or boiled celery leaves	The slightly sweet, green, slightly bitter taste and aromatics associated with celery leaves
Citrus	The aromatics associated with commonly known citrus fruits, such as lemons, limes oranges	The combined perception of sweet, sour taste and aromatics associated with commonly known citrus fruits, such as lemons, limes oranges, could also contain a peely note
Cooked-morogo	Aromatics associated with commonly cooked mixed green leafy vegetables such as spinach, amaranths, pumpkin leaves etc	The combined sensation of green leafy vegetable notes and aromatics associated with mixed cooked green leafy vegetables such as spinach, amaranths, pumpkin leaves etc
Cucumber	Typical aromatics associated with fresh or boiled cucumbers	Green notes, slightly sour-sweet taste and aromatics associated with fresh or cooked cucumbers
Earthy	Humus-like aromatics that may or may not include damp soil, decaying vegetation	Humus-like aromatics and taste that may or may not include damp soil, decaying vegetation
Fermented	The yeasty notes that are associated with fermented fruits or grains, wine etc	Yeasty notes that are associated with fermented fruits or grains, wine that may be sweet and sour tastes
Grassy	The green aromatics associated with newly cut-grass or leafy plants	Green notes, sweet taste and slightly pungent aromatics associated with newly cut-grass and leafy plants
Green-leafy	Green odorants typically associated with green plant/vegetable matter such as spinach, kale, Swiss chard etc	Sharp and slightly pungent aromatics, green flavour notes, and sweet taste associated with spinach, kale, Swiss chard etc

Table 4.1 ***(Continued)***

	Aroma (perceived ortho-nasally by opening the foil cover and sniffing the content)	**Flavour** (perceived during consumption of the products including retro-nasally perceived aromatics, basic tastes and trigeminal nerve interaction effects)
Green-herblike	The aromatics associated with green herbs such as bay leaves, thyme, basil	Green notes, sweet and slightly bitter tastes, slightly pungent and sweet aromatics associated with green herbs such as bay leaves, thyme, basil
Lettuce	Odorants typically associated with freshly cut lettuce	Green notes, slightly musty, sweet aromatics and bitter taste associated with lettuce
Mint	Green aromatics commonly associated with fresh mint leaves	Green flavour notes, sweet taste and aromatics associated with fresh mint leaves
Parsley	Green aromatics associated with fresh or cooked parsley leaves	The clean fresh green notes, sweet and bitter tastes, pungent aromatics associated with fresh or cooked parsley leaves
Seafood/fishy	Odorants commonly perceived in shellfish, fresh fish and ocean vegetation, tuna etc	Off odours and flavour notes, sharp and pungent aromatics associated with shellfish, fresh fish and ocean vegetation
Spinach	Green aromatics associated with cooked spinach	The green, sweet taste, slightly musty, earthy aromatics associated with cooked spinach
Hay-like	The dry, woody, slightly dusty aromatics with the absence of green; associated dry grain stems	The dry, woody, slightly dusty aromatics and tastes with the absence of green associated dry grain stems
Sweet aromatics	Aromatics associated with the impression of sweet substances such as fruit or flowers, or vanilla	The combine perception of aromatics associated with the impression of sweet substances such as fruit or flowers
		Basic tastes
Bitter		A basic taste of which caffeine in water is typical
Salty		The fundamental taste associated with a sodium chloride solution
Sweet		The fundamental taste associated with a sucrose in water solution
Umami		Flat, salty and brothy flavour of a monosodium glutamate solution, a basic taste
		Mouthfeel
Astringent		The drying mouth-feel, puckering sensation on the tongue and other mouth parts as salivary protein precipitates when exposed to water-soluble phenols from the food
Chewiness		Mouthfeel sensation of laboured mastication due to sustained, elastic resistance
Smoothness		Mouthfeel sensation of extent of smoothness while chewing the cooked leaves in the mouth

*A five-point category scale used to measure the intensity of each sensory descriptor with the following category values (none=1, slight=2, moderate=3, strong=4 and extreme=5).

4.1.3.8 Data analyses

For cooked leaves the main and interaction effects of species and state of leaves used on sensory properties and colour were determined using analysis of variance (ANOVA) by the Modelling data option (XLSTAT 2014 by AddinSoft™ SARL, Paris, France). For leaf infusions the main and the interaction effects of species, state of leaves used and infusion cycle on sensory properties and colour were analysed. Significant differences between means were determined using Fisher Least Significant Difference test (LSD) at 5 % probability level ($p < 0.05$). Principal Component Analysis (PCA) was conducted on the observations or variables table to show a visual interpretation of differences among species, state of leaves used and where appropriate, infusion cycles using a vector distance plot. PCA plots give visual information of data for easier understanding of overall differences or similarities among products.

4.1.4 Results

4.1.4.1 Effects of species and state of leaves used for cooking on aroma and flavour of cooked leaves

Nine aroma (Table 4.2), and 13 flavour (nine aroma attributes perceived retronasally, two basic tastes and two mouthfeel) (Table 4.3) descriptors differed significantly between species, whereas only one aroma, four flavour (one aroma perceived retronasally, two basic tastes and one mouthfeel) descriptors differentiated the products from fresh and oven dried leaves. There was no significant species x state of leaves interaction effect found for any of the sensory properties.

Figure 4.1 presents a summarised view of where the first two principal components (F1 and F2) explained 75 % of the total variability in sensory properties of cooked nettle and spinach leaves. F1 differentiates nettle leaves (FrN and ODN) on the left from spinach (FrS and ODS) on the right while F2 differentiated cooked leaves prepared from leaves that were oven dried (ODN and ODS) at the bottom from those that were prepared from fresh leaves (FrN and FrS) at the top.

Table 4.2: The mean (± standard deviation) intensities of aroma descriptors of cooked leaves from fresh and oven dried nettle and spinach leaves

	Species		p- values	State of leaves used		p - values	
						State of leaves	Species x State of leaves
Aroma descriptors*	Nettle	Spinach	Species	Fresh	Oven dried		
Asparagus-woody	3.1[a] (1.0)	2.1[b] (1.0)	0.00	2.6[a] (0.1)	2.7[a] (0.0)	0.65	0.11
Beany	2.4[b] (0.9)	2.8[a] (0.8)	0.03	2.5[a] (0.1)	2.8[a] (0.1)	0.09	0.69
Beet	1.8[a] (1.0)	1.6[a] (0.9)	0.48	1.6[a] (0.0)	1.8[a] (0.1)	0.48	0.80
Burnt	2.4[a] (1.1)	2.0[a] (1.1)	0.07	2.0[a] (0.3)	2.4[a] (0.1)	0.07	0.10
Cabbage	1.7[a] (0.8)	1.7[a] (0.8)	0.69	1.6[a] (0.0)	1.8[a] (0.0)	0.50	0.69
Celery	1.9[a] (1.0)	1.6[a] (0.8)	0.15	1.7[a] (0.1)	1.7[a] (0.2)	1.00	0.47
Citrus	1.3[a] (0.5)	1.0[b] (0.2)	0.01	1.1[a] (0.1)	1.2[a] (0.4)	0.36	0.13
Cucumber	1.9[a] (0.8)	1.7[a] (0.6)	0.37	1.8[a] (0.1)	1.8[a] (0.1)	0.76	0.14
Earthy	3.1[a] (0.9)	2.7[a] (1.2)	0.09	2.7[a] (0.0)	3.1[a] (0.2)	0.09	0.14
Fermented	1.6[a] (0.8)	1.1[b] (0.2)	0.00	1.2[b] (0.4)	1.5[a] (0.5)	0.02	0.12
Fishy	2.6[a] (1.1)	1.2[b] (0.4)	0.00	1.9[a] (0.6)	1.9[a] (0.5)	0.79	0.59
Grassy	3.7[a] (1.0)	2.9[b] (1.3)	0.00	3.2[a] (0.2)	3.4[a] (0.2)	0.34	0.57
Green-leafy	3.6[a] (1.0)	3.9[a] (0.9)	0.13	3.7[a] (0.2)	3.8[a] (0.1)	0.56	0.20
Lettuce	1.5[a] (0.8)	1.7[a] (1.0)	0.35	1.6[a] (0.2)	1.6[a] (0.1)	0.81	0.48
Mint	1.7[a] (0.8)	1.3[b] (0.6)	0.02	1.5[a] (0.2)	1.6[a] (0.1)	0.63	0.42
Parsley	2.0[a] (1.1)	1.7[a] (0.7)	0.16	1.9[a] (0.2)	1.9[a] (0.3)	0.81	0.55
Seafood	2.5[a] (1.1)	1.4[b] (0.6)	0.00	1.9[a] (0.4)	2.0[a] (0.3)	0.80	0.32
Spinach	2.5[b] (1.3)	3.8[a] (1.2)	0.00	3.2[a] (0.3)	3.1[a] (0.1)	0.79	0.33
Sweet aromatics	1.7[a] (0.9)	1.6[a] (0.8)	0.79	1.7[a] (0.1)	1.6[a] (0.0)	0.43	0.60

[ab] Means for a specific main effect (species, state of leaves used), within a row not sharing a superscript letter are significantly different ($p < 0.05$)

*A five-point category scale (none=1, slight=2, moderate=3, strong=4 and extreme=5).

Table 4.3: The mean (± standard deviation) intensities of flavour and mouthfeel descriptors of cooked leaves from fresh and oven dried nettle and spinach leaves

	Species		p-values	State of leaves used		p-values		
Flavour descriptors*	Nettle	Spinach	Species	Fresh	Oven dried	State of leaves	Species x State of leaves
Perceived aroma retro-nasally							
Asparagus-woody	3.1[a] (1.0)	2.4[b] (1.0)	0.00	2.6[a] (0.0)	2.9[a] (0.0)	0.22	0.74
Beany	1.9 [b] (0.9)	2.3[a] (0.9)	0.05	2.2[a] (0.1)	2.0[a] (0.1)	0.22	0.62
Beet	1.7[a] (0.9)	1.5[a] (0.9)	0.54	1.6[a] (0.0)	1.6[a] (0.1)	0.90	0.71
Burnt	2.5[a] (1.2)	2.4[a] (1.2)	0.71	2.2[b] (0.0)	2.8[a] (0.1)	0.03	0.71
Cabbage	1.6[a] (0.9)	1.6[a] (0.6)	0.88	1.7[a] (0.2)	1.6[a] (0.2)	0.47	0.88
Celery	2.0[a] (1.0)	1.8[a] (0.7)	0.25	1.9[a] (0.2)	1.9[a] (0.1)	0.70	0.90
Citrus	1.4[a] (0.7)	1.1[b] (0.3)	0.01	1.3[a] (0.3)	1.2[a] (0.3)	0.66	1.00
Cucumber	1.8[a] (0.8)	1.7[a] (0.8)	0.79	1.8[a] (0.0)	1.7[a] (0.1)	0.42	0.42
Earthy	3.0[a] (1.2)	2.7[a] (1.1)	0.17	2.6[a] (0.1)	3.1[a] (0.1)	0.05	1.00
Fermented	1.7[a] (0.9)	1.2[b] (0.4)	0.00	1.4[a] (0.5)	1.5[a] (0.3)	0.36	0.76
Fishy	2.2[a] (1.1)	1.2[b] (0.4)	0.00	1.6[a] (0.5)	1.9[a] (0.5)	0.14	0.34
Grassy	4.0[a] (0.9)	2.9[b] (1.2)	0.00	3.3[a] (0.2)	3.6[a] (0.2)	0.16	0.59
Green-leafy	3.9[a] (1.1)	3.6[a] (0.9)	0.16	3.7[a] (0.1)	3.7[a] (0.2)	0.91	0.59
Lettuce	1.5[a] (0.8)	1.8[a] (1.1)	0.17	1.7[a] (0.2)	1.5[a] (0.2)	0.49	0.82
Mint	1.7[a] (0.9)	1.4[b] (0.5)	0.03	1.6[a] (0.3)	1.5[a] (0.3)	0.75	0.75
Parsley	2.2[a] (1.2)	1.9[a] (0.9)	0.33	1.9[a] (0.4)	2.2[a] (0.1)	0.33	0.33
Seafood	2.4[a] (1.1)	1.3[b] (0.5)	0.00	1.7[a] (0.4)	1.9[a] (0.5)	0.22	0.14
Spinach	2.4[b] (1.3)	3.0[a] (1.0)	0.00	3.3[a] (0.4)	3.1[a] (0.1)	0.44	0.70
Sweet aromatics	1.6[a] (0.9)	1.4[a] (0.6)	0.14	1.6[a] (0.2)	1.3[a] (0.1)	0.08	0.55
Basic tastes							
Bitter	3.3[a] (0.9)	2.5[b] (1.0)	0.00	2.7[b] (0.2)	3.1[a] (0.1)	0.05	0.20
Salty	2.2[b] (1.0)	3.3[a] (1.1)	0.00	2.5[b] (0.2)	3.0[a] (0.2)	0.03	0.37
Sweet	1.8[a] (1.0)	1.6[a] (0.8)	0.38	1.8[a] (0.2)	1.6[a] (0.1)	0.17	0.90
Umami	2.2[a] (1.2)	2.7[a] (1.2)	0.07	2.4[a] (0.0)	2.5[a] (0.0)	0.72	0.86
Mouthfeel							
Astringent	2.8[a] (0.9)	2.1[b] (0.8)	0.00	2.3[a] (0.0)	2.5[a] (0.1)	0.35	0.89
Chewiness	3.0[a] (1.1)	3.4[a] (0.8)	0.07	3.3[a] (0.1)	3.0[a] (0.4)	0.26	1.00
Smoothness	1.8[b] (0.7)	3.3[a] (0.8)	0.00	2.9[a] (0.2)	2.3[b] (0.1)	0.00	1.00

[ab] Means for a specific main effect (species, state of leaves used) within a row not sharing a superscript letter are significantly different ($p < 0.05$).
*A five-point category scale (none=1, slight=2, moderate=3, strong=4 and extreme=5).

Figure 4.2 is a representation of aroma descriptors whereas Figure 4.3 represent the flavour, basic tastes and mouthfeel descriptors that differentiated significantly between cooked nettle and spinach leaves. On the plots the attributes are positioned clockwise from the top from highest to lowest intensity for nettle. In cooked nettle leaves grassy, asparagus-woody, seafood, fishy, fermented, mint and citrus aromas and flavours were more prominent compared to spinach leaves. In contrast spinach and beany aromas and flavours were more intense in cooked spinach leaves. The nettle leaves also tasted more bitter and had astringent mouthfeel than spinach leaves while spinach tasted more salty with a smoother mouthfeel. Fermented aroma, burnt flavour, and bitter and salty taste were more intense in the product from oven dried leaves compared to fresh leaves, whereas cooked products from fresh leaves had smoother mouthfeel.

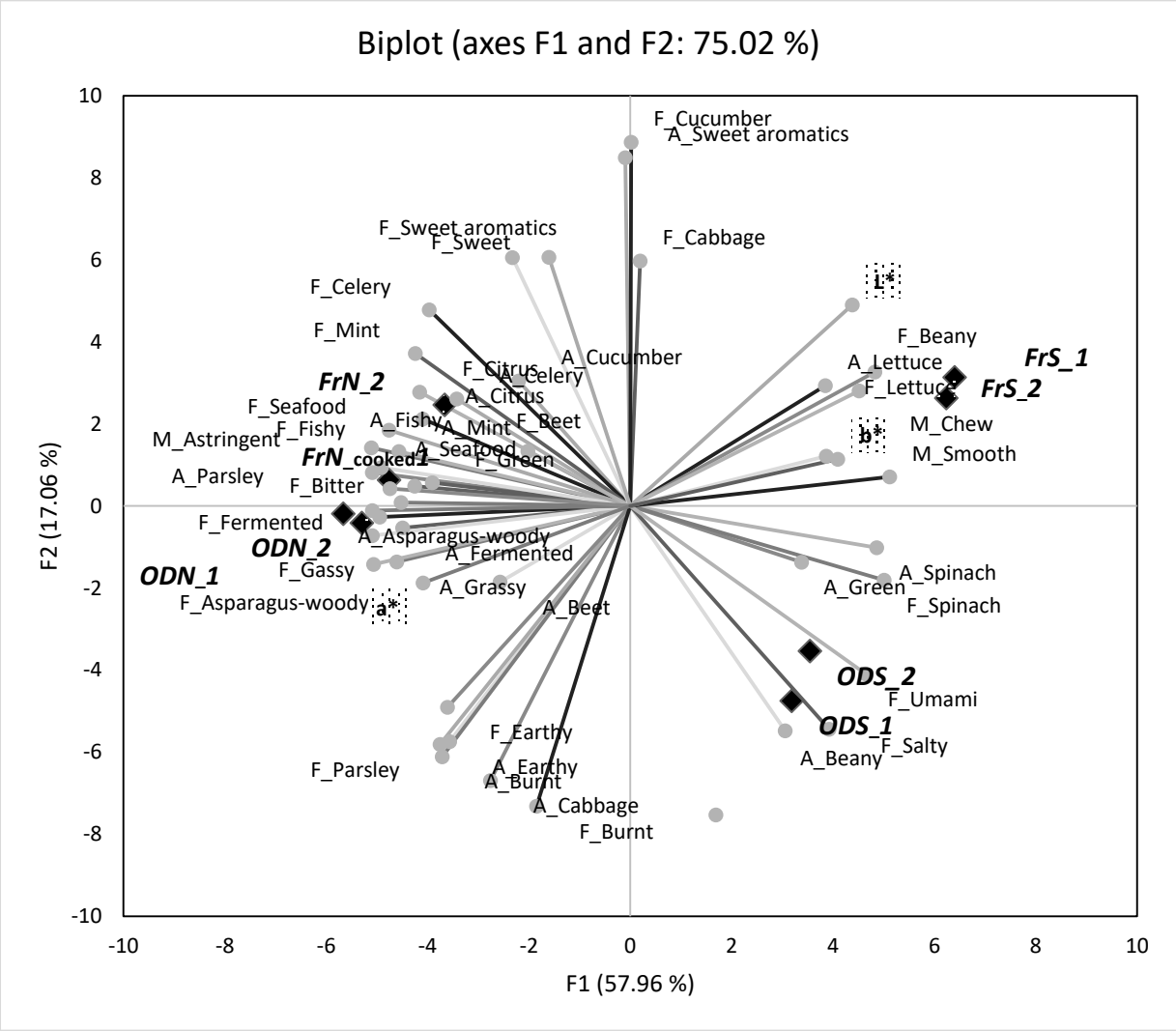


Figure 4.1: Principal component loadings and scores of aroma (A), flavour (F), and mouthfeel (M) descriptors as well as colour values [L a* b*] of cooked leaves from fresh (Fr) and oven dried (OD) nettle and spinach leaves. N-nettle leaves, S-spinach leaves, 1 and 2 are replicates

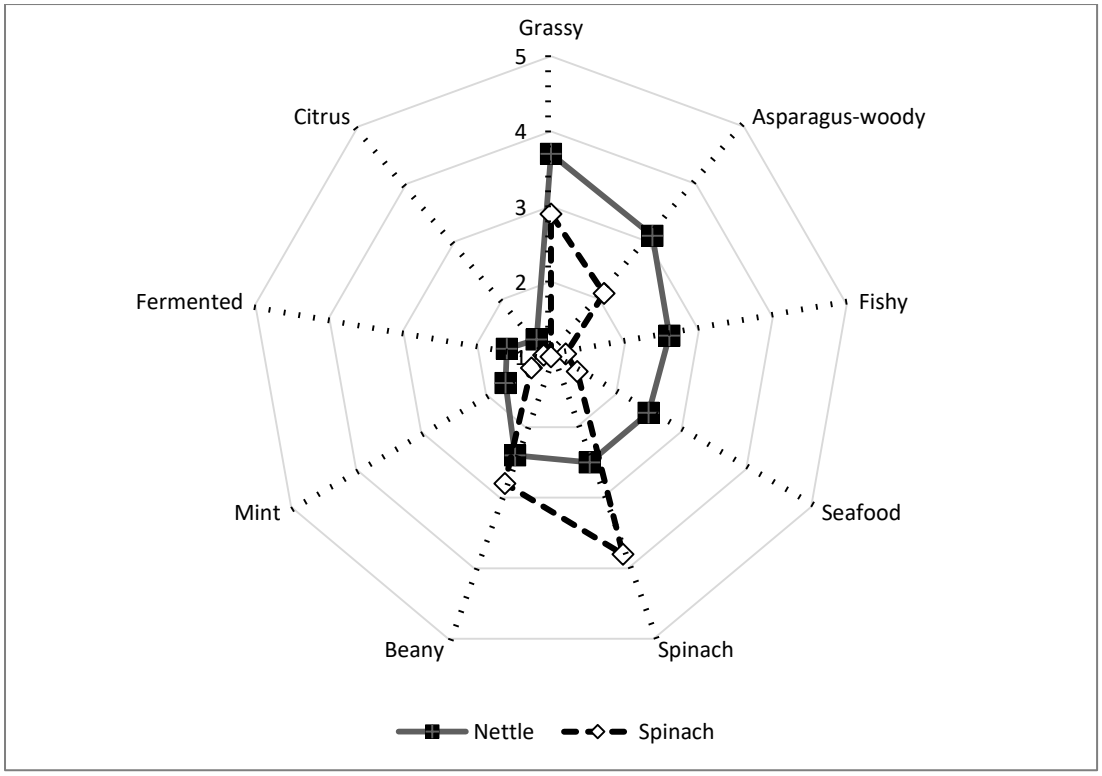


Figure 4.2: Spider plot representation of aroma descriptors that differentiate significantly between cooked nettle and spinach leaves. On the plot the attributes are positioned clockwise from the top from highest to lowest intensity for nettle (none=1, slight=2, moderate=3, strong=4 and extreme=5).

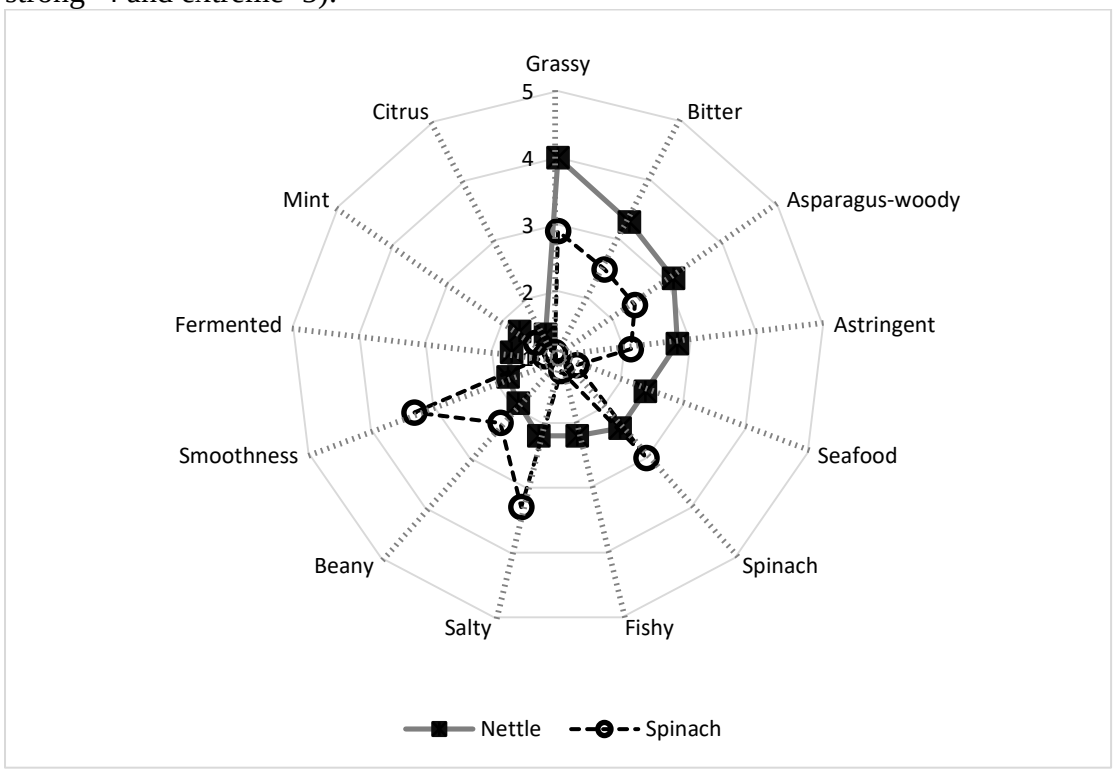


Figure 4.3: Spider plot representation of flavour and mouthfeel descriptors that differentiate significantly between cooked nettle and spinach leaves. On the plot the attributes are positioned clockwise from the top from highest to lowest intensity for nettle (no ne=1, slight=2, moderate=3, strong=4 and extreme=5).

4.1.4.2 The effect of species, state of leaves used and infusion cycles on aroma and flavour of infusion

Two aroma (burned and fishy) (Table 4.4) and three flavour (burnt, fishy and bitter) descriptors (Table 4.5) described differences between the species, whereas eight aroma and four flavour descriptors differentiated the infusions made from fresh and oven dried leaves. There was no significant species x state of leaves interaction effect found for any of the sensory properties with exception of 'burnt' and 'fishy' aroma descriptors. On the other hand, aroma and flavour of infusions was not significantly affected by the two infusion cycles, and also the interaction effects of species x infusion cycle except for 'cooked-morogo' aroma descriptor, state of the leaves x infusion cycle as well as species x state of the leaves x infusion cycle except for 'green-leafy' flavour descriptor were not significant.

The first two principal components (F1 and F2) explained 53 % of the variation in aroma, flavour, basic taste and mouthfeel of infusions (Figure 4.4). F1 indicates the effect of the state of the leaves used with infusions prepared from fresh leaves (FrS and FrN) on the right and those prepared from oven dried leaves on the left (ODN and ODS). F2 differentiated nettle leaves at the top (ODN and FrN) from spinach leaves at the bottom (ODS and FrS).

The aroma of the nettle leaf infusions were more fishy and burnt compared to spinach leaf infusions. Leaf infusions made from fresh leaves was typified by more grassy, green-herblike, earthy and mint aroma compared to that made from oven dried leaves. In contrast, leaf infusions from oven dried leaves had more spinach, beany, and cucumber aroma than infusions made from fresh leaves.

Nettle leaf infusion was typified by pronounced bitter taste and fishy and burnt flavour compared to spinach leaf infusion. Leaf infusions made from fresh leaves contained more green-herblike and seafood flavour compared to those made from oven dried leaves. Spinach flavour was stronger in leaf infusions made from oven dried leaves.

Table 4.4: The mean (± standard deviation) intensities of aroma descriptors of first and second infusion from fresh and oven dried nettle and spinach leaf infusion

	Species		p-values	State of leaves used		p-values					
Aroma descriptors*	Nettle	Spinach	Species	Fresh	Oven dried	State of leaves	Infusion cycle	Species X State of leaves	Species x Infusion cycle	State of leaves x Infusion cycle	Species x State of leaves x Infusion cycle
Asparagus-woody	1.9[a] (1.2)	2.0[a] (1.3)	0.54	2.0[a] (1.2)	2.0[a] (1.2)	0.90	0.27	0.46	0.46	1.00	0.90
Beany	2.2[a] (1.3)	2.4[a] (1.2)	0.23	2.1[b] (1.1)	2.6[a] (1.4)	0.02	0.81	0.34	0.72	0.34	0.90
Brown spice	1.3[a] (0.7)	1.4[a] (0.7)	0.67	1.3[a] (0.7)	1.4[a] (0.7)	0.67	0.40	0.83	0.83	0.29	0.67
Brussels sprouts	2.0[a] (1.3)	2.2[a] (1.2)	0.26	2.0[a] (1.1)	2.1[a] (1.4)	0.51	0.86	0.59	0.68	0.44	0.51
Burnt	1.6[a] (0.6)	1.2[b] (1.0)	0.00	1.5[a] (1.0)	1.3[b] (0.7)	0.05	0.37	0.02	0.37	0.59	0.37
Celery	1.8[a] (1.1)	1.9[a] (1.1)	0.73	1.9[a] (1.1)	1.8[a] (1.1)	0.38	0.54	0.63	0.73	0.54	0.95
Cooked-morogo	2.3[a] (1.5)	2.4[a] (1.4)	0.59	2.2[a] (1.4)	2.5[a] (1.4)	0.07	0.34	0.83	0.03	1.00	0.34
Cucumber	1.9[a] (1.2)	2.0[a] (1.1)	0.40	1.8[b] (1.1)	2.2[a] (1.2)	0.03	0.75	0.56	0.48	0.65	0.85
Earthy	1.7[a] (0.8)	1.4[a] (1.0)	0.11	1.7[a] (1.0)	1.4[b] (0.7)	0.04	0.68	0.36	0.56	0.80	0.16
Fermented	1.3[a] (0.9)	1.3[a] (0.8)	0.79	1.3[a] (0.9)	1.3[a] (0.8)	0.79	0.93	0.79	0.93	0.79	0.79
Fishy	2.3[a] (0.8)	1.5[b] (1.3)	0.00	1.8[a] (1.1)	2.0[a] (1.2)	0.20	0.73	0.04	0.63	0.73	0.12
Grassy	2.7[a] (1.5)	2.8[a] (1.4)	0.63	3.2[a] (1.5)	2.3[b] (1.3)	0.00	0.15	0.87	0.36	0.26	0.63
Green-leafy	2.9[a] (1.4)	3.1[a] (1.4)	0.30	3.1[a] (1.4)	2.8[a] (1.3)	0.18	0.48	0.79	0.87	0.42	0.55
Green-herblike	2.7[a] (1.4)	2.7[a] (1.4)	0.75	2.9[a] (1.4)	2.5[b] (1.4)	0.04	0.83	0.67	0.45	0.59	0.52
Hay-like	2.0[a] (1.1)	1.8[a] (1.1)	0.12	2.0[a] (1.1)	1.7[a] (1.0)	0.07	0.78	0.40	0.67	0.07	0.57
Mint	1.5[a] (0.9)	1.6[a] (0.9)	0.68	1.7[a] (1.0)	1.4[b] (0.8)	0.04	0.68	0.80	0.56	0.28	0.36
Parsley	2.1[a] (1.2)	2.3[a] (1.2)	0.41	2.3[a] (1.2)	2.2[a] (1.2)	0.49	0.95	0.75	0.95	0.85	0.49
Seafood	2.0[a] (0.9)	1.7[a] (1.2)	0.08	1.8[a] (1.0)	1.9[a] (1.1)	0.44	0.53	0.44	0.44	0.44	0.30
Spinach	2.5[a] (1.4)	2.9[a] (1.3)	0.05	2.5[b] (1.3)	2.9[a] (1.4)	0.02	0.73	0.21	0.65	0.91	0.65
Sweet aromatics	1.4[a] (0.8)	1.4[a] (0.8)	0.85	1.4[a] (0.7)	1.5[a] (0.8)	0.18	0.34	0.45	0.45	1.00	0.85

[ab] Means for a specific main effect (species, state of leaves used) within a row not sharing a superscript letter are significantly different ($p < 0.05$).
*A five-point category scale (none=1, slight=2, moderate=3, strong=4 and extreme=5).

Table 4.5: The mean (± standard deviation) intensities of flavour descriptors of first and second infusion from fresh and oven dried nettle and spinach leaf infusions

	Species		p-values								
				State of leaves used							
Flavour descriptors*	Nettle	Spinach	Species	Fresh	Oven dried	State of leaves	Infusio cycle	Species x State of leaves	Species x Infusion cycle	State of leaves x Infusion cycle	Species x State of leaves x Infusion cycle
Asparagus-woody	2.0[a] (1.2)	2.0[a] (1.1)	1.00	2.0[a] (1.2)	2.0[a] (1.2)	0.90	0.80	0.26	0.62	0.26	0.53
Beany	2.0[a] (1.2)	2.1[a] (1.2)	0.53	2.0[a] (1.2)	2.1[a] (1.2)	0.62	0.90	1.00	0.17	1.00	0.32
Brown spice	1.4[a] (0.7)	1.2[a] (0.5)	0.23	1.3[a] (0.6)	1.3[a] (0.6)	0.81	0.81	0.33	0.33	1.00	0.47
Brussels sprouts	2.1[a] (1.3)	2.0[a] (1.3)	0.40	2.0[a] (1.3)	2.1[a] (1.4)	0.40	0.47	0.87	0.61	0.20	0.96
Burnt	1.6[a] (1.0)	1.3[b] (0.7)	0.01	1.5[a] (0.9)	1.4[a] (0.8)	0.93	0.14	0.54	0.54	0.93	0.79
Celery	1.8[a] (1.1)	1.9[a] (1.1)	0.50	2.0[a] (1.2)	1.8[a] (1.0)	0.14	0.28	0.79	0.68	0.50	0.79
Citrus	1.2[a] (0.6)	1.2[a] (0.6)	0.91	1.2[a] (0.6)	1.2[a] (0.6)	0.91	0.91	0.72	0.91	0.91	0.72
Cooked-morogo	2.3[a] (1.3)	2.3[a] (1.4)	0.82	2.1[a] (1.3)	2.5[a] (1.4)	0.09	0.82	0.82	0.15	0.65	0.57
Cucumber	2.0[a] (1.4)	2.1[a] (1.2)	0.72	2.1[a] (1.3)	2.1[a] (1.2)	0.90	0.47	0.81	0.72	0.72	0.81
Earthy	1.4[a] (0.6)	1.5[a] (0.8)	0.22	1.6[a] (0.8)	1.4[a] (0.6)	0.07	0.68	0.84	0.42	0.54	0.84
Fermented	1.4[a] (1.0)	1.3[a] (0.9)	0.39	1.3[a] (1.0)	1.4[a] (0.9)	0.94	0.94	0.58	0.94	0.58	0.48
Fishy	1.8[a] (1.1)	1.5[b] (0.7)	0.02	1.5[a] (0.9)	1.7[a] (1.0)	0.19	0.87	0.63	0.33	0.51	0.42
Grassy	2.7[a] (1.4)	2.7[a] (1.4)	0.87	3.0[a] (1.4)	2.3[b] (1.3)	0.00	0.78	0.14	0.78	0.96	0.21
Green-leafy	2.8[a] (1.4)	3.0[a] (1.3)	0.24	3.0[a] (1.4)	2.8[a] (1.3)	0.19	0.87	0.28	0.40	0.24	0.04
Green-herblike	2.7[a] (1.4)	2.5[a] (1.4)	0.38	2.9[a] (1.4)	2.3[b] (1.3)	0.00	0.51	1.00	1.00	0.82	1.00
Hay-like	2.1[a] (1.1)	1.8[a] (1.0)	0.17	2.1[a] (1.2)	1.8[a] (1.0)	0.10	0.58	0.78	0.78	0.41	0.58
Mint	1.5[a] (0.9)	1.4[a] (0.8)	0.86	1.5[a] (0.9)	1.4[a] (0.9)	0.49	1.00	0.86	0.86	1.00	0.61
Parsley	2.2[a] (1.2)	2.3[a] (1.2)	0.42	2.4[a] (1.3)	2.1[a] (1.1)	0.09	0.66	0.19	0.85	0.85	0.19
Seafood	1.8[a] (1.0)	1.7[a] (0.8)	0.29	2.0[a] (1.0)	1.6[b] (0.8)	0.03	0.12	0.93	0.46	0.68	0.29
Spinach	2.5[a] (1.4)	2.6[a] (1.4)	0.54	2.3[b] (1.3)	2.8[a] (1.4)	0.02	0.70	0.29	0.78	0.35	0.78
Sweet aromatics	1.4[a] (0.9)	1.5[a] (0.9)	0.68	1.4[a] (0.9)	1.4[a] (1.0)	0.93	0.68	0.68	0.57	0.93	0.93
Bitter	2.4[a] (1.1)	2.0[b] (1.1)	0.03	2.2[a] (1.2)	2.1[a] (1.0)	0.54	0.64	0.64	0.54	0.16	0.84
Salty	1.8[a] (1.0)	1.8[a] (1.0)	0.94	1.7[a] (0.9)	1.9[a] (1.1)	0.16	0.12	0.16	0.60	0.82	0.26
Sweet	1.8[a] (1.1)	1.7[a] (1.1)	0.78	1.7[a] (1.1)	1.8[a] (1.1)	0.78	0.13	0.68	0.78	0.41	0.68
Astringent	2.3[a] (1.2)	2.0[a] (1.2)	0.21	2.2[a] (1.3)	2.1[a] (1.1)	0.53	0.70	0.37	0.37	0.31	0.61

[ab] Means for a specific main effect (species, state of leaves used) within a row not sharing a superscript letter are significantly different ($p < 0.05$).
*A five-point category scale (none=1, slight=2, moderate=3, strong=4 and extreme=5).

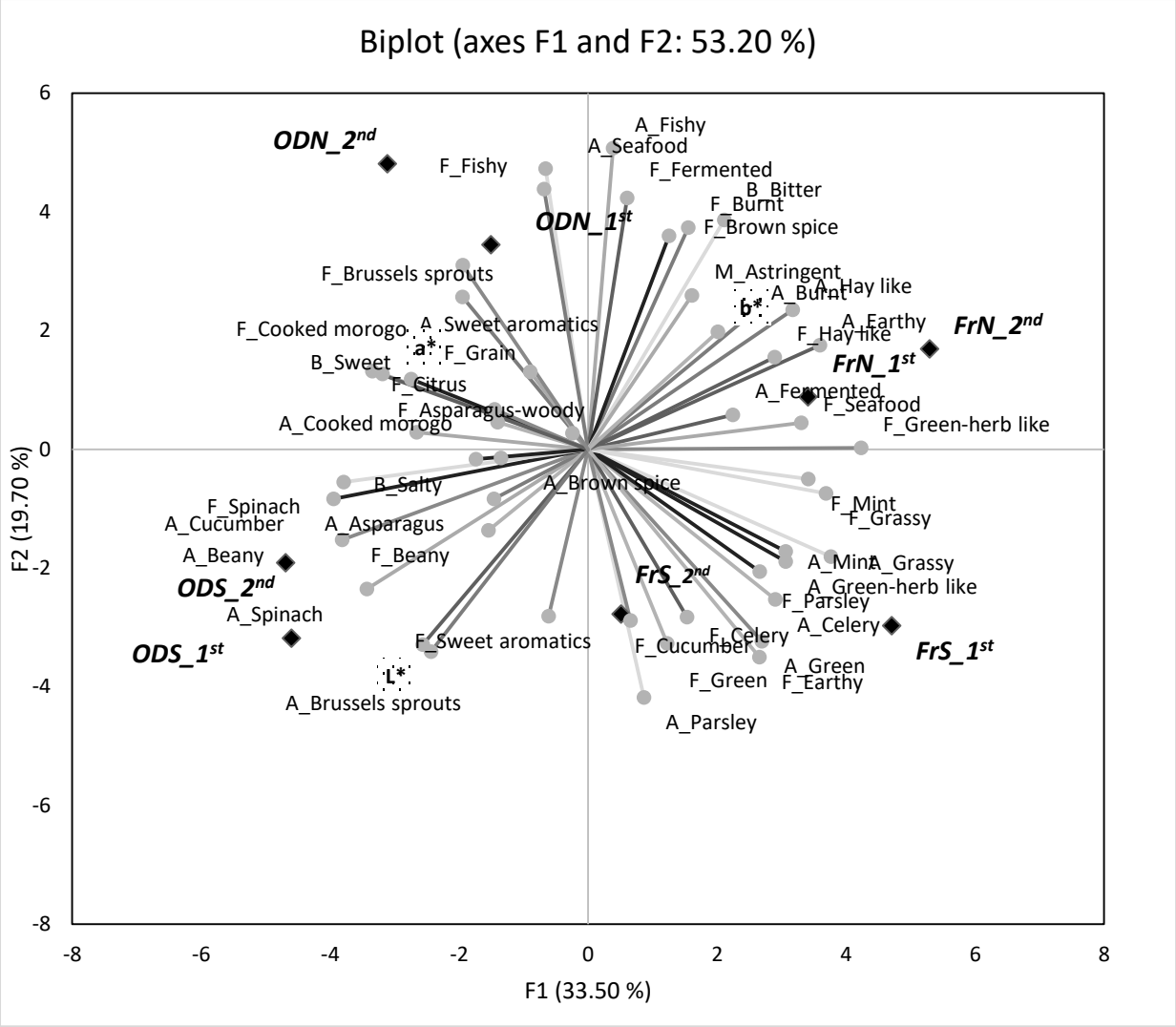


Figure 4.4: Principal component loadings and scores of aroma (A), flavour (F) and mouthfeel (M) descriptors as well as colour values [L a* b*] of 1st and 2nd brews from fresh (Fr) and oven dried (OD) nettle and spinach leaves. N-nettle leaves, S-spinach leaves.

4.1.4.3 Colour values

In leaves samples, L, a* and b* colour values were significantly affected by cooking and species while only L and b* were affected by the state of the leaves and the interaction between cooking x species (Table 4.6). Whereas the interaction between species x state of the leaves and cooking x species x state of the leaves were not significant for all colour values. Lightness (L) increased from fresh to oven dried leaves in uncooked samples while decreased from fresh to cooked leaves in both nettle and spinach. Nettle and spinach behaved differently for green (a*) and yellow (b*) values, a* and b* increased from fresh to oven dried in uncooked nettle leaves, but decreased from fresh to oven dried for spinach. Higher a* and b* values were observed in cooked leaves compared to fresh for both nettle and spinach.

In leaf infusions, L and a* colour values were significantly affected by infusion cycle, state of the leaves, and the interaction between species x state of the leaves, infusion cycle x species x state of the leaves (Table 4.7). While only a* was significantly affected by species. The interaction between infusion cycle x specie and infusion cycle x state of the leaves were not significant.

L was found to be higher in spinach infusion compared to nettle infusion. Lightness increased from the 1st brew to the 2nd brew in both leaf infusions from fresh and oven dried leaves. In contrast, a* increased from the 1st brew to the 2nd brew in both leaf infusion made from fresh and oven dried leaves.

4.1.5 Discussion

Grassy, asparagus-woody, mint, citrus, fermented, seafood and fishy aromas and flavours typified cooked leaves from nettle compared to spinach leaves. Nettle leaf infusion was more intense in fishy and burnt aromas and flavours while tasting bitterer compared to spinach. These differences might be attributed to variability in chemical composition of the two species due to variation in genotype and pre-harvest environmental conditions. Grassy, woody and bitter were attributes previously used to describe herbs such as parsley, bay leaf, spearmint, basil (Diaz-Maroto et al., 2004), rosemary (Díaz-Maroto et al., 2007) and fresh or freeze dried (uncooked) nettle leaves (Dey, 2013). Fishy aroma and bitter taste were reported by Upton (2013) to describe fresh and dried (uncooked) nettle leaves.

In contrast, cooked spinach tasted saltier with a smoother mouthfeel than nettle. The salty taste could be related to compositional difference of the leaves due to variability in pre-harvest conditions such as geographical location, soil conditions, fertilizer use and other cultural practices. The difference in mouthfeel could be because of differences in the consumable parts of the two plants and structure of the leaves. For nettle, young and tender shoots (including soft stem and leaves) were used whereas only the leave part of spinach was used.

Table 4.6: The mean (± standard deviation) colour values of uncooked and cooked leaves from fresh and oven dried nettle and spinach leaves

									p -values						
Species	Nettle				Spinach							Cooking x	Cooking x	Species x	Cooking x Species x
Cooking	Uncooked		Cooked		Uncooked		Cooked								
Drying	Fresh	Oven dried	Fresh	Oven dried	Fresh	Oven dried	Fresh	Oven dried	Cooking	Species	State of leaves	Species	State of leaves	State of leaves	State of leaves
L	44.2^{c} (0.2)	53.1^{a} (0.2)	24.1^{e} (0.4)	23.5^{e} (0.4)	47.6^{b} (0.5)	53.0^{a} (0.1)	27.2^{d} (1.5)	24.4^{e} (0.6)	0.00	0.00	0.00	0.50	0.00	0.00	0.22
a*	-8.5^{b} (0.0)	-9.7^{bc} (0.1)	-5.8^{a} (0.2)	-8.6^{b} (0.6)	-12.0^{de} (0.2)	-10.5^{cd} (0.0)	-12.2^{e} (2.6)	-9.0^{bc} (0.7)	0.01	0.00	0.66	0.14	0.90	0.00	0.06
b*	9.0^{c} (0.1)	15.5^{a} (0.2)	2.6^{e} (1.0)	5.8^{d} (0.5)	12.8^{b} (0.2)	14.8^{ab} (0.1)	9.2^{c} (3.7)	6.2^{d} (0.2)	0.00	0.00	0.00	0.11	0.00	0.00	0.47
ΔE		11.1	21.2	20.9		5.9	20.7	24.3							

Means within a row not sharing a superscript letter are significantly different ($p < 0.05$)

$\Delta E = \Delta L\hat{}2 + \Delta a\hat{}2 + \Delta b\hat{}2) \hat{} 0.5$

Where ΔL = L-L0, Δa = a-a0, Δb = b-b0; and where L, a, and b are the values for dried or cooked leaves; L0, a0, b0 are the values for fresh uncooked leaves.

Table 4.7: The mean (± standard deviation) colour values of first and second brew from fresh and oven dried nettle leaf and spinach infusion

									p-values						
Species	Nettle				Spinach							Infusion cycle x Species	Infusion cycle x State of leaves	Species x State of leaves	Infusion cycle x Species x State of leaves
Drying	Fresh		Oven dried		Fresh		Oven dried								
Infusion cycle	1st brew	2nd brew	1st brew	2nd brew	1st brew	2nd brew	1st brew	2nd brew	Infusion cycle	Species	State of leaves				
L	33.3^{d} (6.3)	52.1^{c} (6.0)	49.7^{c} (2.9)	52.7^{c} (4.0)	59.3^{bc} (11.9)	67.6^{ab} (2.7)	68.3^{ab} (1.0)	75.0^{a} (5.7)	0.00	0.00	0.00	0.49	0.09	0.97	0.16
a*	-7.4^{d} (1.2)	-3.4^{abc} (1.1)	-6.5^{cd} (3.3)	-2.5^{ab} (0.8)	-7.0^{d} (3.0)	-5.3^{bcd} (1.4)	-3.5^{abc} (0.6)	-2.2^{a} (1.0)	0.00	0.56	0.01	0.12	0.92	0.12	0.88
b*	12.9^{cd} (1.0)	21.6^{ab} (5.5)	25.7^{a} (8.9)	17.9^{abcd} (4.2)	20.6^{abc} (4.7)	16.7^{bcd} (3.2)	15.2^{bcd} (0.8)	12.2^{d} (3.3)	0.45	0.10	0.91	0.32	0.06	0.02	0.04
ΔE		21.1	20.7	20.6		9.3	11.1	18.4							

Means within a row not sharing a superscript letter are significantly different ($p < 0.05$).

$\Delta E = \Delta L\hat{}2 + \Delta a\hat{}2 + \Delta b\hat{}2) \hat{} 0.5$

Where ΔL = L-L0, Δa = a-a0, Δb = b-b0; and where L, a, and b are the values for dried or fresh leaf infusions; L0, a0, b0 are the values for fresh leaf first brew infusion.

When leaves were oven dried, all green type aromas and flavours were preserved in the cooked product with the exception of more pronounced fermented aroma and flavour, bitter and salty tastes. In contrast when oven dried leaves were infused, a decrease in grassy, green-herblike, earthy, and mint aromas, and green-herblike and seafood flavours were noted with an increase in spinach, beany and cucumber aromas, and spinach flavour. This could be related to a reduction or an increase in volatile and non-volatile compounds in the leaves.

Oven drying caused losses or formation of volatiles as a result of oxidation and/or esterification reactions (Dey, 2013; Díaz-Maroto et al., 2002; King et al., 2006; Orphanides et al., 2013). Volatile flavour compounds are produced during thermal processing via enzymatic, redox, or pyrolytic reactions of amino acid (Arimura et al., 2001), fatty acid (Owuor, 2003) and carotenoids(Giada, 2013; Goff and Klee, 2006; Owuor, 2003; Rodriguez-amaya, 1997).

In stinging nettle leaves flavour volatiles could potentially be generated from fatty acid (e.g. linoleic acid, α-linolenic acid, and linolenic acid) (Farag et al., 2013; Guil-Guerrero et al., 2003; Rutto et al., 2013); amino acid (e.g. valine, leucine, isoleucine, and phenylalanine) (Hughes et al., 1980; Rutto et al., 2013) and carotenoids (Guil-Guerrero et al., 2003) during oven drying. These flavour volatiles would be responsible for the perceived aroma and flavour of cooked stinging nettle leaves and leaf infusions. For example, linoleic acid is hydrolysed to hexanal while linolenic acid is hydrolysed to cis-3-hexenal, cis-3-hexenol, trans-2-hexenal via lipoxygenase activity and oxidized to produce green aromatics described as grassy (Owuor, 2003).

In fresh and cooked nettle leaves, polyunsaturated fatty acid are approximately two to three times more than saturated fatty acid (Rutto et al., 2013). This could be the reason for the fishy/seafood flavour of cooked nettle leaves and leaf infusions. Fishy aromatics are produced as a result of oxidation of polyunsaturated fatty acid to trimethylamine and dimethylamine in marine fish and seafood and to (Z)-1, 5-octadien-3-one in dried spinach leaves (Masanetz et al., 1998).

Lastly, bitter and astringent taste of the cooked leaves and leaf infusions could possibly be attributed to non-volatile phenolic compounds such as catechin, tannins, caffeic acid, chlorogenic acid and naringin present in stinging nettle leaves (Farag et al., 2013; Otles and Yalcin, 2012; Pinelli et al., 2008).

Caffeine and saponins are also responsible for bitterness (Lee et al., 2013) whereas epicatechin and epigallocatechin (Owuor, 2003; Wang and Ruan, 2009) and tannins (Troszynska et al., 2003) contribute to astringency in green tea. The decrease in bitterness during cooking of stinging nettle could be due to the loss of soluble phenolic compounds such as simple phenols, flavonoids and tannins of low and medium molecular weight not bound to membrane compounds (Giada, 2013), due to thermal and enzymatic degradation.

In general, flavours were perceived more intense compared to aroma of cooked leaves. This was probably due to more aromatic compounds being released during mastication of the leaves and perceived retronasally. The importance of oral processing to food flavour has been evidenced by many observations of positive correlations between mastication and flavour release from food (Neyraud et al., 2005, 2003). Laboured mastication destructs the cellular structure and exposes the macromolecules to saliva enzymes such as amylase (starch), lysozyme (breaks polysaccharides in the cell walls), lingual lipase (fats), and proteases (protein). This could release and solubilize the complex structural aromatic compounds and enhance retronasal perception. Saliva also plays a role in the perception of bitter, sour and salty tastes that are presumed to be derived from the concentration of free cations or anions dissolved in saliva (Neyraud and Dransfield, 2004) and enhancing the taste of the food (Humphrey and Williamson, 2001).

Interestingly, the intensity of all aroma and flavour descriptors in the first and the second brews made either from fresh or oven dried leaves were not statistically different, except for the colour. For green tea leaves, Lee (2009) and Lee et al. (2013) found similar aroma intensities of the first two brews. However, they noted an increase in bitterness and astringency from the first to the second brew, and the intensities of green and brown flavour notes decreased beyond the second brew. This could be because the concentrations of most aromatic flavour compounds were highest in the first two brews and then declined with repeated brewing (Hicks et al., 1996; Lee et al., 2013).

In contrast, the ΔE value, showed variation in colour between the two infusion cycles and in cooked leaves as well. The high ΔE between the brews could be due to the effect of dilution and duration of thermal processing. A high ΔE was also observed for leaves. The degree of colour change in green vegetables is linked to the thermal process, pigment dilution and oxygen level (Kidmose et al., 2000).

The loss of green colour in green vegetables is due to degradation of chlorophyll a and b, and oxidation of carotenoids (Kidmose et al., 2000; Di Cesare et al., 2004). It is probable that the chlorophyll a and b (Alibas, 2007; Dey, 2013) and β-carotene (Guil-Guerrero et al., 2003; Rutto et al., 2013) in stinging nettle leaves, were degraded during oven drying, cooking and the infusion processes.

The high temperature of oven drying could lead to the replacement of magnesium in the chlorophyll by hydrogen, thereby converting the green chlorophylls to brown pheophytins (Baritaux et al., 1992). Previous research on different herbs reported that the colour change was more pronounced as the temperature of thermal processing increased (Alibas, 2006; Dey, 2013; Ozkan et al., 2007). For example, a higher change in L, a*, and b* values was observed in convection dried swiss chard leaves with ΔE of 5.9-11.2 (Alibas, 2006) than freeze-dried samples ΔE of 4.95 (Alibas, 2007).

4.1.6 Conclusions

A total of 19 aroma and 26 flavour descriptors for cooked leaves and 20 aroma and 25 flavour descriptors for leaf infusions from two subsequent brews were developed and used to characterize the sensory properties of nettle products from fresh and oven dried leaves. Cooked nettle leaves are differentiated from spinach, a popular vegetable, due to its grassy, asparagus-woody, seafood, fishy, fermented, mint and citrus aroma and flavours, as well as higher bitterness and astringency. Similarly, the aroma and flavour of nettle leaf infusions are more burnt and fishy and it tastes bitterer than spinach. Drying the leaves results in more intense fermented aroma, burnt flavour, and bitter and salty tastes. Drying nettle leaves reduces the overall aroma and flavour of infusions. Nettle leaves can be brewed twice without much difference in aroma and flavour. It is important to note however that drying of leaves changes the colour of the cooked product and infusions.

This baseline sensory information could be utilized to describe, compare, and differentiate the characteristics of various nettle food products around the world. Further research should determine which sensory properties of the products from the nettle plant drives liking or disliking by target consumers. This research contributes to the understanding of the potential of stinging nettle for addressing food and nutrition security, and well-being of consumers.

4.2 Oven drying and freeze drying of stinging nettle (*U. dioica* L.) leaves: effects on nutritional properties

4.2.1 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 species (viz. stinging nettle, spinach (*Spinacia oleracea*) included as a control) and drying of leaves (viz. freeze dried and oven dried) on macronutrients, mineral content, ascorbic acid, β-carotene content and total phenol content as well as antioxidant activity, were investigated. The % contribution of fresh, oven dried or freeze dried stinging nettle leaves to the required daily value (% DV) for the nutrients and nutrient retention were also determined. Nettle leaves were found to contain significantly more Fe, K, Mg, Mn, Ca, Zn compared to spinach leaves. The drying processes did not affect the mineral content of both nettle and spinach leaves. However, oven drying of nettle leaves resulted in a higher loss of β-carotene and ascorbic acid content compared to freeze drying. Oven dried stinging nettle leaves retained less ascorbic acid and β-carotene (72 % and 90 %) compared to freeze dried leaves (88 % and 97 %). In contrast, the total phenol content and total antioxidant activity was higher in oven dried stinging nettle leaves compared to freeze dried leaves. A serving of either fresh, freeze dried or oven dried nettle leaves could provide more than 20 % of the daily value of vitamin A (e.g. 870 µg per day); therefore, nettle leaves in all forms are "rich sources" of vitamin A. 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 sources of antioxidant and for addressing micronutrient malnutrition.

Keywords: Stinging nettle leaves, ascorbic acid, β-carotene, total antioxidant activity, total phenol content, dietary value, oven dried, freeze dried

4.2.2 Introduction

Stinging nettle, *U. 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 nettles contain phenolic compounds (Farag et al., 2013; Orčić et al., 2014; Otles and Yalcin, 2012; Pinelli et al., 2008) and polyunsaturated fatty acids (Guil-Guerrero et al., 2003; Rutto et al., 2013), 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, 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 (Kara, 2009; Musa Özcan et al., 2008; Pytlakowska et al., 2012). Nettle leaves are also good sources of protein and dietary fiber (Hughes et al., 1980).

Stinging nettle leaves add variety to the menu, thus could be used to advantage as a supplementary, spinach-like vegetable in the human diet (Hughes et al., 1980). Naude (2013) also emphasized the need to include dark green vegetables such as wild vegetables at least one serving to reduce the burden of nutrition-related disease. The plant is widely used as food in early spring where young leaves are added to soups, salads, herbal tea or decocted tea as well as dried 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).

The change in aroma, flavour and colour of leaf infusions and cooked leaves when oven dried leaves are used compared to fresh leaves (Research chapter 4.1), prompted an investigation into the effect of drying (e.g. freeze-drying and oven drying) on nutritional properties of stinging nettle leaves. The type of drying chosen can have a major impact on the nutrient degradation and retention (Shilton, 2003). 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). Colour changes during oven dying of stinging nettle leaves was also reported (Research chapter 4.1) (Alibas, 2006).

Drying processes involving high temperature such as oven drying result in protein denaturation, ascorbic acid and β-carotene degradation (Shilton, 2003). It was reported that drying of herbs increased the amount of phenolic compounds and antioxidant activity of herb extracts (Hossain et al., 2010; Suhaj, 2006).

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 leaves tea bags etc. These products are expensive and therefore only affordable to high economic end consumers (developed market, rich) where such consumers demand higher value and natural products. Oven drying is used more often in food processing industries due to its lower production costs leading to products which are more affordable to consumers at the low economic end of the market (developing market, poor).

However, information on the effect of oven drying and freeze drying on vitamins, total phenol content and antioxidant activity of nettle leaves is 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 phenol content as well as antioxidant activity. The findings of this study could enable consumers and processors to choose the drying techniques that favour retention of nutritional properties of stinging nettle leaves for maximum health benefits in addition to overcoming micronutrient malnutrition.

4.2.3 Materials and methods

4.2.3.1 Drying processes

Stinging nettle young and tender shoots were produced and harvested from University of Pretoria Experimental Farm Station harvested as described in research chapter 4.1. Whereas the 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. Fresh leaves and oven dried leaves (70 °C for 15 h) were prepared as described in research chapter 4.2.3.

Fresh leaves were freeze dried at -40 °C in a Instruvac Lyophilizer model 13 KL (Air and vacuum technologies (Pty) Ltd, Midrand, Gauteng, South Africa) for 5 d. Dried leaves were ground to a fine flour using a coffee grinder and sealed in polyethylene bags (215 mm x 315 mm, 500 g/bag). All samples were kept frozen (- 4 °C) until analysis.

4.2.3.4 Nutritional properties

4.2.3.4.1 Proximate composition

AACC International (2000) methods were used to determine moisture (method 44-15A), fat (method 30-25), protein content (N x 6.25) by Dumas combustion (method 46-30), ash (method 08-01) and crude fibre (method 32-10.01) while total carbohydrate content was calculated by difference.

4.2.3.4.2 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, SPECTRO Analytical Instruments GmbH, Kleve, Germany).

4.2.3.4.3 β-carotene content

The quantitative analysis of β-carotene content of fresh, freeze dried, and oven dried leaves was carried out using a Shimadzu Ultra-Fast Liquid Chromatograph (Shimadzu, Tokyo, Japan) equipped with a SIL-20A Prominence auto-sampler, a DGU-20A3 Shimadzu degasser, a CTO-10AS VP Shimadzu column oven and a SPD-M20A Shimadzu diode array detector. Sample extraction and mobile phase preparation was done following the method of Rodriguez-Amaya and Kimura (2004). The detection was done at 450 nm and UV/Vis spectra of carotenoids were recorded between 200 to 600 nm.

The separation of β-carotene was performed at 25 °C on a C30 YMC carotenoid column (250 x 4.6 mm, i.d., 5 µm particle size) by isocratic elution with a mobile phase consisting of methanol:methyl-tert-butyl ether (80:20, v/v) at a flow rate of 0.8 ml/min (Kimura et al., 2007). The quantification of carotenoids was done using a calibration curve of β-carotene standard. Vitamin A content of fresh and dried leaves were calculated as retinol activity equivalents (RAE) using an RAE conversion factor of 12 µg β-carotene to 1 µg retinol (Joint FAO/WHO, 2001).

4.2.3.4.4 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 (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-µL sample loop.

Sample extraction and mobile phase preparation was done following Maia et al. (2007). The components were filtered through 0.45-µm 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-µm 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.

4.2.3.4.5 Total antioxidant activity and phenol content

Sample extraction was performed as described by Otles 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 phenolic content was determined using the Folin-Ciocalteau (FC) method (Singleton et al., 1999). Total antioxidant activity of fresh, freeze dried, and oven dried leaves was determined using the DPPH Radical Scavenging Activity Assay according to Brand-Williams et al. (1995).

4.2.3.4.6 The contribution of fresh or dried stinging nettle leaves to dietary intakes of fibre, protein, minerals and vitamins

The % contribution of a serving of fresh (100 g), freeze dried (15 g) or oven dried (15 g) leaves (see

Table 4.8) to the daily value (DV) (i.e. Daily values of nutrients provided in Table 2.6) [based on a caloric intake of 2000 calories 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), fibre, protein, vitamin A and vitamin C was determined as follows (Equation 1):

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

Table 4.8: 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, 2013a, 2013b)
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)

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

4.2.4 Results

4.2.4.1 Proximate composition

Results for proximate composition of the fresh, freeze dried and oven dried leaves are presented in Table 4.9. Fat, crude fibre, ash, crude protein and total carbohydrate were significantly affected by species. The drying methods significantly affected fat, crude fiber and total carbohydrate content, whereas ash and crude protein were not significantly affected by the drying methods. No significant species x drying method interaction effects were found. Stinging nettle leaves had significantly higher fat, crude fibre, ash and crude protein compared to spinach leaves, but total carbohydrate was higher in spinach. Fresh and freeze dried leaves contained significantly ($p < 0.01$) higher fat and crude fibre compared to oven dried leaves. In contrast, total carbohydrate was significantly higher in oven dried leaves compared to fresh and freeze dried leaves.

4.2.4.2 Mineral 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 4.9. Nettle leaves were found to contain significantly higher ($p < 0.01$) Fe, K, Mg, Mn, Ca, Zn compared to spinach leaves. The drying processes did not affect the mineral content of leaves.

4.2.4.3 The effects of drying methods on β-carotene, ascorbic acid, total phenol content and antioxidant activities

The result for the effects of drying on β-carotene, ascorbic acid, total phenol content and total antioxidant activities of nettle and spinach leaves can be found in Table 4.9. Ascorbic acid, β-carotene, total phenol content and total antioxidant activities were significantly ($p < 0.01$) affected by species and state of the leaves. Stinging nettle leaves contained significantly ($p < 0.01$) more ascorbic acid, β-carotene, total phenol 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.

4.2.4.4 The contribution of fresh or dried stinging nettle leaves to dietary intakes of fibre, protein, minerals and vitamins

The percent daily values of protein from a serving of fresh (100 g) or dried (15 g) nettle leaves were found to be 9.2 % and 8.5 %, respectively (Table 4.10). 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 4.10) . The percent daily value of β-carotene (calculated as RAE, vitamin A) and vitamin C as a function of 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 %), respectively (Table 4.10).

4.2.5 Discussion of results

Fat, crude fibre, ash and crude protein content of freeze dried and oven dried nettle leaves was 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 (Farag et al., 2013; Otles and Yalcin, 2012). 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; Joint WHO/FAO, 2003; Naude, 2013b), 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.

Table 4.9: Effect of drying methods on the mean (± standard deviation) proximate composition (g/100 g), mineral content (mg/100 g), β-carotene (µg/100 g), ascorbic acid (mg/100 g), total phenol content (TPC, mg GAE/g), and total antioxidant activity (TAA, % DPPH inhibition) of stinging nettle and spinach leaves

Drying methods (DM)	Fresh		Freeze dried		Oven dried		p-values		
Species (SP)	Nettle	Spinach	Nettle	Spinach	Nettle	Spinach	SP	DM	SP x DM
Moisture (as is) %	85.0	89.6	6.4	6.6	3.4	4.5			
Proximate composition, db									
Fat	4.0^{a}(0.1)	3.8^{ab}(0.1)	3.8^{ab}(0.1)	3.4^{c}(0.1)	3.5^{bc}(0.1)	3.0^{d}(0.2)	0.00	0.00	0.29
Crude Fibre	30.2^{a}(0.1)	27.8^{b}(0.4)	29.4^{a}(0.5)	27.2^{b}(0.3)	27.2^{b}(1.0)	24.9^{c}(0.1)	0.00	0.00	0.86
Ash	20.5^{a}(0.0)	17.1^{b}(1.0)	20.9^{a}(0.2)	17.9^{b}(0.5)	19.9^{a}(1.1)	17.8^{b}(0.8)	0.00	0.48	0.48
Crude Protein	30.8^{a}(0.4)	28.2^{b}(0.8)	30.3^{a}(0.4)	27.8^{bc}(0.4)	29.8^{a}(0.4)	27.0^{c}(0.1)	0.00	0.05	0.85
Total carbohydrates	14.5^{d}(0.1)	23.2^{b}(0.4)	15.7^{d}(0.8)	23.7^{b}(0.6)	19.5^{c}(2.3)	27.4^{a}(0.4)	0.00	0.00	0.84
Mineral content, db									
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
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
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
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
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
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
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
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
β-carotene, db	58059^{a}(243)	44160^{d}(256)	56341^{b}(453)	43537^{d}(62)	52504^{c}(447)	40847^{e}(479)	0.01	0.01	0.01
Ascorbic acid, db	93.8^{a}(3.3)	76.3^{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
TPC, db	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, db	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-e] Means within the same row with different superscripts are different ($p < 0.05$) when analysed using analysis of variance.

db = dry basis

Table 4.10: The contribution of a serving of fresh or dried stinging nettle or spinach leaves to the percent daily value (% DV, as is basis) of protein, minerals and vitamins (A and C).

Drying methods (DM)	Fresh		Freeze dried		Oven dried	
Species (SP)	Nettle	Spinach	Nettle	Spinach	Nettle	Spinach
Protein						
g/serving	4.6	2.9	4.2	3.9	4.3	3.9
% DVs	9.2	5.8	8.5	7.8	8.6	7.7
Ca						
mg/serving	323	52	320	75	299	78
% DVs	32.3	5.2	32	7.5	29.9	7.8
Fe						
mg/serving	2.5	0.8	2.5	1.1	2.6	1.2
% DVs	14	4.3	14	6.3	14.2	6.6
Mg						
mg/serving	104	45	104	64	105	59
% DVs	26	11.2	26	16.1	26.3	14.7
Mn						
mg/serving	0.4	0.2	0.4	0.3	0.4	0.3
% DVs	19.0	11.9	18.9	17.2	18.5	16.8
Zn						
mg/serving	0.5	0.2	0.5	0.4	0.6	0.4
% DVs	3.5	1.7	3.5	2.4	3.7	2.4
P						
mg/serving	82.6	56.7	82.5	81.5	84.6	80
% DVs	8.3	5.7	8.2	8.2	8.5	8.0
K						
g/serving	0.2	0.1	0.2	0.1	0.2	0.1
% DVs	5.4	1.8	5.4	2.6	5.3	2.7
Na						
g/serving	0.0	0.0	0.0	0.0	0.0	0.0
% DVs	0.0	0.5	0.0	0.7	0.0	0.6
K/Na ratio	419	5.5	415	5.5	381	6.2
Vitamin A*						
µg/serving	728	383	659	508	634	488
% DVs	83.7	44.0	75.7	58.4	72.9	56.0
Vitamin C						
mg/serving	14.2	8.0	11.8	10.6	9.9	9.4
% DVs	23.7	13.3	19.6	17.7	16.5	15.6

% DVs = (Nutrient content in a serving)/ (Daily value for the nutrient) x 100

Where,

Serving (100 g for fresh leaves or 15 g for dried leaves)

*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).

Daily value for the nutrient (protein = 50 g Ca = 1000mg, Fe = 18 mg, Mg = 400 g, Mn = 2 mg, P = 1000 g, Zn = 15 mg, K = 3.5 g, Na = 2.4 g, vitamin A = 870 µg and vitamin C = 60 mg)

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 (see section 2.3.6, Table 2.6). Fresh or dried stinging nettle leaves were found to be a source of protein, 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, Zn compared to spinach leaves. This could be attributed to differences in fertility status of the soil during production (Lee and Kader, 2000a; 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. Nettle leaves 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 (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, Table 4.10) 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 phytate content and concomitant increase in bioaccessability 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 phytate content and increase the bioaccessibility 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 vitamins that the plant accumulate is a function of pre-harvest factors such as genotypic differences, climatic conditions of the region (e.g. temperature and sunlight) and fertilizers used (natural or artificial). For example, the higher the intensity of light during the growing season and less frequent irrigation, the greater the vitamin C content in plant tissues, whereas nitrogen fertilizers at high rates tend to decrease the vitamin C content 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, 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 °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; Rodriguez-amaya, 1997). 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 dehydro-ascorbic 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 bioaccessibility is influenced by a complex interplay of several components in dark green leafy vegetables, including protein, ascorbic acid, β-carotene and total polyphenols. The authors reported that the high levels of ascorbic acid and β-carotene in moringa leaves compared with other leafy green vegetables increased iron bioaccessibility from that source.

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 phenol 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 phenol content (151 - 1001 mg GAE/g) and antioxidant activity (ranging from 60.62 to 320.38 mg GAE/g) of fresh stinging nettle leaves collected from the Mediterranean, Aegean, Black sea and Marmara coastal parts in Turkey.

The higher total phenol 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 acid (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).

The high total phenol 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, teriary 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.

4.2.6 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 nettle leaves increases the total antioxidant activity and phenol content compared to freeze drying. Overall, fresh stinging nettle leaves can be considered as a rich sources of antioxidants, Ca, Mg, vitamin 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 sources of antioxidants, Ca, Mg, and vitamins A while a good source of vitamin C, Fe and Mn. These benefits present possible avenues for utilization of dried nettle leaves or in leaf powder form by the food industry and consumers for addressing micronutrient deficiency and for providing healthy diet.

4.3 Infusion and decoction of stinging nettle (*U. dioica* L.) leaf powder manufactured using freeze drying or oven drying: effects on nutritional properties

4.3.1 Abstract

Stinging nettle has a long history of use as a food source and is widely used among Western herbalists. Oven dried nettle leaves are a rich sources of antioxidants and a good sources of β-carotene and ascorbic acid. Infusions and decoctions prepared from nettle leaf powder is a natural source of antioxidants in human medicine and nutrition. The temperature difference of preparation involved in decoctions (extraction by boiling) and infusions (extraction by steeping in boiled water), might affect the chemical compositions of the product differently. The present study was undertaken to evaluate the ascorbic acid, β-carotene, antioxidant activity and dietary value of infusions and decoctions from stinging nettle leaf powder manufactured using freeze drying or oven drying. Contribution of infusions and decoctions prepared from nettle leaf powder to the dietary intakes of vitamins expressed as percent daily value (% DV) was also determined. Decoction was found to be a more efficient method of extraction for high total antioxidant activity and phenol content whereas infusion was the more efficient mode of extraction for ascorbic acid and β-carotene. Infusions and decoctions made from nettle leaf powder manufactured using oven drying or freeze drying can be considered as sources of dietary vitamin A. In order to meet the 10 % daily value of vitamin C, an individual needs to consume at least three cups (250 g each) of infusions and decoctions. Overall, infusions and decoctions from stinging nettle leaf powder can potentially be used as cheap natural sources of antioxidant to reduce the burden of nutrition-related disease such as cardiovascular diseases.

Keywords: Nettle, Spinach, oven dried, freeze dried, infusion, decoction, leaf powder, daily value, extraction efficiency, β-carotene, ascorbic acid, total phenol content, total antioxidant activity

4.3.2 Introduction

Stinging nettle, *U. dioica L,* has been used as a leafy vegetable for preparation of many dishes such as salads, pies, soups, omelettes, scrambled eggs, herbal tea and decocted tea (Bisht et al., 2012; Guarrera and Savo, 2013; Guil-Guerrero et al., 2003; Orčić et al., 2014; Sansanelli et al., 2014). Infusions and decoctions are prepared either from fresh or dried stinging nettle leaves (Ait Haj Said et al., 2015; Upton, 2013). However, a regular fresh stinging nettle leaves supply is a practical limitation due to its seasonality and limited availability in some regions. Inevitably, utilization of dried stinging nettle leaves could help in the utilization of the plant. Although the colour changed, most of the characteristic green-related aroma and flavour notes of fresh nettle leaves were preserved in cooked leaves and leaf infusions prepared from oven dried leaves (Chapter 4.1). Freeze dried and oven dried nettle leaves were found to be a rich source of Ca, Mg and vitamin A; a good source of vitamin C, fibre, Fe, and Mn; and a source for Mg and K (Chapter 4.2).

Gião et al. (2007) evaluated antioxidant capacity and phenol content of 48 Portuguese medicinal plants prepared by different extraction methods (e.g. infusion, decoction). The authors recommended infusions prepared from leaf powder, as it was the most effective mode of antioxidants extraction from herbal plants. Ait Haj Said et al. (2015) reported that infusion and decoction extracts prepared from stinging nettle leaf powder are natural sources of antioxidants in human medicine and nutrition. The temperature difference of preparation involved in decoctions and infusions might affect the chemical compositions of the product differently. Extraction of active compounds in decoction is accomplished through constant boiling while with infusion the plant material is steeped in boiled water for a specified time (Courtine, 1984).

This study was undertaken to evaluate the effect of type of extraction (infusion and decoction) applied to nettle leaf powder manufactured using freeze drying or oven drying on the contents of ascorbic acid, β-carotene, total phenol and antioxidant activity. Additionally, the contribution of infusions and decoctions from nettle leaf powder to the dietary intakes of vitamins expressed as percent daily value (% DV) was also evaluated. This type of information will help to choose the extraction method that favours highest vitamin and antioxidants retention for maximum health benefits.

4.3.3 Materials and methods

4.3.3.1 Sample preparation

Stinging nettle young and tender shoots were produced and harvested from University of Pretoria Experimental Farm Station harvested as described in research chapter 4.1. Whereas the spinach leaves (as a control) were purchased from a supermarket. Freeze drying and oven drying of the leaves were carried out as described in Research chapter 4.2. Dried leaves were ground to a fine flour using a IKA basic mill (IKA@ A11B, Germany). The leaf 'powder' was sealed in polyethylene bags (215 mm x 315 mm, 500 g/bag) and kept frozen (- 4 °C). Infusions and decoctions from nettle leaf powders manufactured using freeze-drying or oven drying were prepared as described by Gião et al. (2007).

4.3.3.1.1 Infusions

To prepare an infusion, 100 mL boiled water (96 °C) was added to 1 g of freeze-dried or oven dried leaf (at room temperature) powder in a 500 ml graduated borosilicate glass beaker (GLASSCO, India); mixed and infused for 5 min at room temperature and then cooled to 4 °C in a cold room.

4.3.3.1.2 Decoctions

For decoction, 100 mL boiled water (96 °C) was added to 1 g of freeze-dried or oven dried powder (at room temperature) in a 500 ml graduated borosilicate glass beaker (GLASSCO, India); the mixture was boiled for 5 min on a 2000 W single plate stove (STA001, ANVIL, South Africa) and then cooled to 4 °C in a cold room.

4.3.3.2 Nutritional properties

β-carotene content, ascorbic acid content, total antioxidant activity and phenol content of infusion and decoction (unfiltered, containing both the solid and liquid part) from nettle leaf powder manufactured using freeze drying or oven drying were determined as described in Research chapter 4.2.

4.3.3.3 The contribution of infusion and decoction from stinging nettle leaf powder to dietary intakes of vitamins

The percentage contribution to the daily value (% DV) of a specific nutrient in a serving of 250 g infusions or decoctions to the daily value of each nutrient (e.g. vitamin A and C) for adults and children four or more years of age was determined as described by US FDA (2013) using the following formula (Equation 1):

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

4.3.3.4 Data analyses

Analysis of variance (ANOVA) in XLSTAT 2015 (AddinSoft™ SARL, Paris, FRANCE) was applied to all experimental results produced, in attempts to assess the effects of species (viz. nettle, spinach), drying method (viz. freeze dried and oven dried), and type of extraction (viz. infusions and decoctions) on ascorbic acid, β-carotene content, total antioxidant activity and phenol content were investigated. Fisher's Least Significant Difference test (LSD) was also applied to all experimental results, with the goal of pinpointing statistically significant differences (at the 5 % level).

4.3.4 Results

4.3.4.1 Ascorbic acid and β-carotene

The concentration of ascorbic acid and β-carotene extracted in decoctions and infusions from freeze dried and/or oven dried nettle or spinach leaf powder are shown in Table 4.11. Ascorbic acid and β-carotene contents showed a significant difference between species, drying process, extraction type and among all other possible interactions (extraction types x species, extraction types x drying process, species x drying process, and extraction types x species x drying process). Significantly higher ascorbic acid and β-carotene were recorded in infusions and decoctions made from nettle leaf powder compared to similar products made from spinach powder.

Infusions made either from freeze dried or oven dried leaf powders contained significantly higher ascorbic acid and β-carotene content compared to decoctions samples. Ascorbic acid and β-carotene were extracted significantly higher from freeze dried leaf powder into either infusion or decoctions compared to those made from oven dried leaf powder.

To sum up, high β-carotene content was detected in infusion from freeze dried samples while considerably lower β-carotene were measured in decoction from oven dried leaves. β-carotene content varied from 41244 µg/100 g in infusion from freeze dried nettle leaf powder to 21188 µg/100 g in decoction from oven dried spinach. Similarly, ascorbic acid content varied from 77.7 mg/100 g in infusion from freeze dried nettle leaf powder to 35.3 mg/100 g in decoction from oven dried spinach.

4.3.4.2 The contribution of infusion and decoction from stinging nettle leaf powder to dietary intakes of vitamins

The contribution of nettle and spinach leaf powder decoction and infusion to the daily intake of vitamin A and vitamin C can be found in Table 4.12. Percentage contribution to the daily value (% DV) was calculated based on the vitamins concentration in the infusion or decoction and the assumption that a person consumes one cup per serving (250 g per cup) (Gallaher et al., 2006). A serving of a cup of nettle leaf powder decoction provided 7.4 % (from freeze dried) and 6.6 % (from oven dried) of the daily value of vitamin A. Whereas a cup of nettle leaf powder infusion provided 10.8 % (from freeze dried) and 8.1 % (from oven dried) to the daily value of vitamin A.

Similarly, a cup of decoction provided from 2.3 % (from oven dried leaf powder) to 3.0 % (from freeze dried powder) to the daily value of Vitamin C. A cup of infusion provided from 2.5 % (from oven dried) to 2.9 % (from freeze dried) to the daily value of vitamin C.

4.3.4.3 Total antioxidant activity and phenol content

Total phenol content and antioxidant activity of decoctions and infusions from freeze dried and/or oven dried nettle leaf powder can be found in Table 4.11. Total phenol content was significantly different between species, drying method, extraction types and among all possible pair wise interactions with exception of extraction types x drying method.

Table 4.11: Effect of extraction types on the mean values (± standard deviation, dry basis) for β-carotene (µg/100g), ascorbic acid (mg/100 g), total phenol content (TPC, mg GAE/g) and total antioxidant activity (TAA, % DPPH) of stinging nettle and spinach leaf powder manufactured using freeze drying or oven drying

Extraction types (ET)	Decoction				Infusion				p-values						
Drying method (DM)	Freeze dried		Oven dried		Freeze dried		Oven dried					ET x	ET x	DM x	ET x
Species (SP)	Nettle	Spinach	Nettle	Spinach	Nettle	Spinach	Nettle	Spinach	ET	DM	SP	DM	SP	SP	DM x SP
β-carotene	34334^{d} (122)	26723^{f} (243)	29912^{e} (73)	21188^{h} (76)	41244^{a} (98)	36406^{c} (88)	37444^{b} (185)	25023^{g} (11)	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Ascorbic acid	65.7^{bc}(1.0)	55.5^{e}(1.7)	60.2^{d}(0.2)	35.3^{f}(2.2)	77.7^{a}(2.3)	68.3^{b}(1.4)	65.6^{bc}(0.7)	63.7^{c}(0.2)	0.01	0.01	0.01	0.01	0.01	0.03	0.01
TPC	191.1^{b}(3.8)	121.8^{e}(0.7)	199.4^{a}(1.3)	156.3^{d}(4.7)	169.8^{c}(4.8)	111.5^{f}(2.8)	195.8^{ab}(2.2)	126.8^{e}(0.5)	0.01	0.01	0.01	0.76	0.01	0.01	0.01
TAA	79.0^{b}(1.3)	65.1^{f}(1.6)	86.8^{a}(0.5)	75.3^{d} (0.4)	71.1^{e}(0.5)	64.5^{f}(0.5)	77.3^{c}(0.4)	70.2^{e}(1.6)	0.01	0.01	0.01	0.01	0.01	0.20	0.09

$^{a-g}$ Means within a row not sharing a superscript letter are significantly different ($p < 0.05$).

Table 4.12: Effect of extraction types on the mean values for percent daily value (% DV, as is basis) for vitamin A and C of stinging nettle and spinach leaf powder manufactured using freeze drying or oven drying

Extraction types (ET)	Decoction				Infusion			
Drying method (DM)	Freeze dried		Oven dried		Freeze dried		Oven dried	
Species (SP)	Nettle	Spinach	Nettle	Spinach	Nettle	Spinach	Nettle	Spinach
Vitamin A*								
µg/ serving	64.4	55.8	57.0	44.7	93.5	67.7	70.6	59.3
% DVs	7.4	6.4	6.6	5.1	10.8	7.8	8.1	6.8
Vitamin C								
mg/serving	1.8	1.4	1.4	1.0	1.8	1.7	1.5	1.5
% DVs	3.0	2.4	2.3	1.7	2.9	2.9	2.5	2.4

% DVs = (Nutrient content in a serving)/ (Daily value for the nutrient) x 100

Where,
Serving (250 g of infusions or decoctions)
*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).
Daily value for the nutrient (vitamin A = 870 µg and vitamin C = 60 mg)

Total antioxidant activity showed a significant variation between species, drying method, extraction types and among all possible pairs of interactions with exception of species x drying. Significantly higher total antioxidant activity and phenol content were recorded in infusions and decoctions from nettle leaf powder compared to spinach. Total phenol content and antioxidant activity was found to be significantly higher in decoction samples compared to infusion samples. Significantly lower total phenol content and antioxidant activity were observed in infusion and decoction samples made from freeze dried leaves compared to those made from oven dried leaves. In general, high total antioxidant activity values corresponded to a high total phenol content. Extracts with low antioxidant activity also had relatively low total phenol content. Total phenol content and antioxidant activity varied from 199.4 mg GAE/g and 86.8 % DPPH inhibition in decoction made from oven dried nettle leaf powder to 111.5 mg GAE/g and 64.5 % DPPH inhibition in infusion from freeze dried spinach, respectively

4.3.5 Discussion of results

Ascorbic acid and β-carotene were found to be higher in infusions compared to decoctions. Although the boiling process involved in decoctions could help to release oil-soluble carotenoids such as β-carotene from the lipoprotein complexes and enable them to be readily extracted (Cadenas and Packer, 2002), this could also expose the β-carotene molecules to enzymatic degradation and non-enzymatic oxidation and geometric isomerization (Rodriguez-amaya, 1997; Kidmose et al., 2000). Similarly, decoctions could also promote enzymatic degradation of protein-ascorbic acid aggregates. However, further heat exposure during the boiling process of decoctions compared to steeping in hot water in infusions (Courtine, 1984) might lead to a loss of ascorbic acid due to its heat labile nature and high water solubility because of two hydroxyl groups in its structure and partial oxidation to dehydroascorbic acid (Kall, 2003; Sanmartin et al., 2000; Waheed Uz et al., 2013).

β-carotene and ascorbic acid are well known for their dietary value (World Health Organization, 2003). Recommended serving sizes was reported to be 250 g for infusions or decoctions from stinging nettles (Gallaher et al., 2006). Accordingly, the potential contribution of a serving size of 250 g of infusion or decoctions to the dietary intakes of vitamin A and C were determined.

As a whole, the findings from this study indicate that infusions and decoctions prepared from freeze-dried or oven-dried nettle leaf powder could be considered as a source of vitamin A. This is because a cup (250 g) per serving of decoctions and infusions can contribute from 6-9 % of the daily value (870 µg/day for vitamin A) necessary for a food to be considered a ‘‘a source’’ of that nutrient (US FDA, 2013; Joint FAO/WHO, 2007). However, a cup of infusions or decoctions provided less than 5 % of the daily value for vitamin C (60 mg). To get at least the 10 % daily value of vitamin C, a person needs to consume at least three cups of infusions or decoctions per day.

In contrast to β-carotene and ascorbic acid, it was observed that decoctions yielded higher total phenol content and antioxidant activity compared to infusions. This probably happened due to the boiling processes involved in decoctions that might have resulted in cleavage of more phenolic-sugar glycosidic bonds and release of bound or insoluble phenolic compounds from the cellular matrix compared to infusions. Heat treatments by disrupting the cell membrane and release membrane-bound polyphenols, that may result in an increase in extractability of phenolic compounds (Giada, 2013).

Conversely, it was reported that the ability of β-carotene and ascorbic acid to scavenge free radicals is due to the fact that ascorbic acid can regenerate antioxidants such as alpha-tocopherol (Halliwell and Gutteridge, 2015). Total phenol content was positively correlated (r = 0.8) to total antioxidant activity in both infusions and decoctions. Many studies have reported a strong positive correlation between total phenol content and antioxidant activity in different herbs and vegetables. For example, in Kale extracts (Ayaz et al., 2008) and 23 basil accessions (Javanmardi et al., 2003), a high positive correlation between antioxidant activity and total phenolic compounds was observed. This finding also confirms that total antioxidant activity of stinging nettle leaf food products is highly dependent on the phenolic content, as already suggested by Lutz et al. (2011) and Danesi et al. (2013). The high antioxidant activity of stinging nettle leaf powder infusions and decoctions found in this study could also enhance its health and nutrition benefits (Chrubasik et al., 2007).

4.3.6 Conclusions

Decoction is most efficient if high total antioxidant activity and phenol content is sought whereas infusion is the most efficient mode of extraction for ascorbic acid and β-carotene. It can be concluded that infusions and decoctions from stinging nettle leaf powder can be considered as a sources of dietary antioxidants which can be due to its high total phenol content. All in all, this type of information on top of providing unique insight about the vitamin and antioxidant activity of stinging nettle leaf powder infusions and decoctions, it will encourage further studies and production of new functional products utilizing stinging nettle leaf powder. It is necessary to determine the specific phenolic compounds responsible for the antioxidant activity and sensory properties of infusions and decoctions made from stinging nettle leaf powder.

5: GENERAL DISCUSSION

This study investigated the sensory and nutritional properties of stinging nettle leaves and leaf infusions with the aim to: (a) determine the effects of using fresh or oven-dried leaves to cook a relish or to prepare an infusion on sensory properties, (b) investigate the effects of oven drying and freeze drying of stinging nettle leaves on nutritional properties, and (c) to compare the effect of infusion and decoction of stinging nettle leaf powder manufactured using freeze drying or oven drying of the leaves on nutritional properties. This chapter will first provide a critical review of methodologies used (5.1), discuss the main findings of this study (5.2) and then finally identify future research needs (5.3).

5.1 Methodological considerations

5.1.1 Stinging nettle production

A crucial aspect of this study was the on-farm introduction of stinging nettle plants which involved a chain of production practices including field establishment, crop management and harvesting. The on-farm introduction was started with propagation of stinging nettle planting materials. Four plants were obtained from one location namely, the Margaret Roberts Herbal Centre. The centre which is located west of Pretoria was chosen as the plant material collection site based on the availability of the plants. The collection of plants from only one location could however be a limitation of the study. This is due to the fact that chemical composition of stinging nettle plants might be influenced by agroecological factors such as temperature, soil conditions and light. For example, Lee and Kader (2000a) reported that the higher the intensity of light the greater is the vitamin C content in many vegetables while high soil nitrogen level, irrigation and high temperature tend to decrease the vitamin C content. Otles and Yalcin (2012) also reported varation in phenolic profile, total phenol content and antioxidant activities of stinging nettle leaves collected from different agroecological conditions from the coastal part of Turkey (e.g. Mediterranean, Aegean, Black sea and Marmara). Farag et al. (2013) emphasized that for better understanding of the chemical diversity of stinging nettle and to compare different species of Urtica, samples representing broad agroecological locations should rather be considered.

Stem, rhizomes and shoots cuttings were taken from the four stinging nettle plants and were successfully propagated into hundreds of seedlings. The seedlings were then transplanted into three plots as shown in Figure 5.1. The details of all the production practices can be found in section 4.1.3.2. Harvesting season and maturity stages of the leaves can affect the nutritional quality of the leaves therefore, for this study, young and tender shoots (leaves and soft stem) and spring harvesting season were selected. Rutto et al. (2013) reported more variation in protein, iron, calcium, β-carotene and ascorbic acid content as well as amino acid and fatty acid profiles in fresh, cooked or blanched stinging nettle leaves harvested during fall season compared to spring season.

A number of studies have reported that young and tender shoots represent the consumable part of the stinging nettle plant as these have higher nutritional value compared to mature leaves (Ioana et al., 2013; Kavalali, 2003). Spinach leaves were selected as a control [the product sold under this name in South Africa is actually 'swiss chard' *Beta vulgaris* subsp. *vulgaris*]. Spinach is a popular green leafy vegetable and in high market demand in South Africa. It has wide availability ranging from road side retailers to the big supermarkets at affordable price. For the study spinach leaves were bought in bulk from a supplier. However, no details about growing conditions, maturity etc. were obtained. The lower nutrient content of the spinach might be explained by the fact that the samples are older and poor postharvest handling may have degraded the nutrients in the leaves as purchased due to prolonged exposure to light.

Figure 5.1: Production of stinging nettle leaves at the experimental farm of University of Pretoria

5.1.2 Sample preparation

Fresh nettle leaves are widely used in early spring and dried for winter use whereby the leaves are cooked as potherb or added to soups, salads, herbal tea or decocted tea (Guil-Guerrero et al., 2003). Although dried stinging nettle leaves can be prepared using sun drying, solar drying and freeze drying (Upton, 2013), oven drying and freeze drying were chosen as method of drying the leaves. Oven drying appears to be the most common method of drying used in the food processing industry due to its lower production cost compared to freeze drying, leading to products which are more affordable to consumers at the lower economic end of the market.

Drying temperatures ranging from 55 to 75 °C and drying time not longer than 48 h for drying of fruits and vegetables in a forced oven dryer is preferred (Deinum and Maassen, 1994; Kakade and Neeha, 2014). This is because drying of fruits and vegetables with temperature of greater than 75 °C in a forced air oven dryer for long periods (e.g. > 48 h), some amino acids reacting with reducing sugars can give rise to formation of Maillard reaction products, or some protein can be denatured (Alomar et al., 1999). The higher drying temperatures for long periods during oven drying might also affect phenolics composition and antioxidant activity of foods (Rodrigues et al., 2009). This is probably due to the fact that high temperature treatments during oven drying can bring about changes in chemical compositions of food products due to enzymatic and redox reactions of various components such as carbohydrates, chlorophylls, protein/amino acids, fatty acids and carotenoids (Goff and Klee, 2006; Pichersky et al., 2006).

Freeze-drying is generally recommended for preserving heat-sensitive antioxidant components such as ascorbic acid, carotenoids and soluble phenolics (Shofian et al., 2011), as this drying process is accomplished by sublimation of ice from the food. However, Abascal et al. (2005) emphasized that there may be a decline in nutritional quality (when compared to the fresh material) during freeze-drying due to degradation of certain compounds such as polyphenols, carotenoids and loss of volatile compounds. This is probably due to the need for an extended drying time (≈ 5 d) because of lower vapour pressure driving force of the freeze-dryer. For this study, oven drying at 70 °C for 15 h and freeze drying at - 45 °C for 5 d (Figure 5.2) of fresh stinging nettle leaves was done as described in sections 4.1.3.3 and 4.2.3.3, respectively.

Figure 5.2: Fresh and oven dried stinging nettle leaves (from left to right)

Cooked stinging nettle leaves and leaf infusions for the study of the effects of using fresh or oven-dried leaves on the sensory properties were prepared as described by Francisco et al. (2009) with modifications (see section 4.1.3.4) (Figure 5.3). The authors cooked 1 part of turnip green leaves in 2 parts of water [1100 g of leaves in 2 L boiling water (no salt)] for 45 min on 1000 W heat-plates and the excess water was drained before serving. Fransisco's group used a combination of long cooking time with excess water for cooking of turnip greens. However, such extended duration of cooking in excess water and draining the excess water can cause high loss of nutrients (e.g. antioxidants) from green leafy vegetables (Mavhungu, 2011). Rutto et al. (2013) cooked (98–100 °C) 1 part of stinging nettle leaves in 200 parts of water [5 g of fresh stinging nettle leaves in 1L of water] for 7 min. The authors reported that cooking of stinging nettle leaves resulted in a significant loss of β-carotene, ascorbic acid, amino acids and fatty acids.

In this study, to prevent the loss of nutrients due to the use of excess cooking water and for sample uniformity (between fresh and dried leaves), a preliminary experiment was conducted to select the optimum amount of cooking water on the basis of moisture content of the leaves (fresh and dried leaves) and time of cooking. The average moisture content was 80 % (80 g in 100 g) in fresh and 6 % (6 g in 100 g) in dried nettle leaves. Dry matter content of the fresh and dried leaves were determined and used to decide how much water to be added for cooking and infusions. Accordingly, for this study, dry leaves (100 g) were cooked with 1100 ml boiling water (1 part of the leaves in 11 parts of water) whereas fresh leaves (600 g) were cooked with 600 ml boiling water (1 part of the leaves in 1 part of water). The same procedure was applied for cooking of fresh and dried spinach leaves.

Figure 5.3: Cooked leaves from fresh stinging nettle leaves

The leaves were cooked for 15 min on a 2000 W single plate stove (STA001, ANVIL, South Africa) at power level 4. A preliminary experiment was conducted to select the optimum cooking time to make the leaves palatable since young and tender shoots normally accounts for the edible portion of nettle leaves. The cooking time was also dependent on the amount of water used to boil the leaves on a hot plate, and there was no extra cooking water left at the end of the cooking time so no cooking water was discarded. Adams and Erdman (1988) stated that some vegetables need longer thermal processing to inactivate enzymes or to tenderize and enhance chewiness of the product.

Infusions from stinging nettle leaves are well known as a herbal remedy in many countries (Ait Haj Said et al., 2015; Kavalali, 2003; Moskovitz, 2009; Roberts, 2011; Upton, 2013). It was reported that loose black or green tea leaves can be brewed multiple times. For example, in Asia (e.g. China), multiple brewing is commonly applied for a high quality green tea (Lee et al., 2013). From the point of view of optimum physicochemical characteristics and sensory acceptability, loose green tea can be brewed up to four times (Lee, 2009). The authors also reported that the intensity of the green flavours generally decreased while no change in the intensities of brown flavours was noticed as the loose green tea was brewed four times. Therefore, the effect of two infusion cycles on the sensory properties of stinging nettle leaf infusion prepared from fresh or dried leaves was determined. The stinging nettle leaf infusions for this study was prepared as described in section 4.1.3.5 (Figure 5.4).

Figure 5.4: The first two tea cups represent leaf infusions from fresh and oven dried stinging nettle leaves followed by those made from fresh and oven dried spinach leaves, respectively (from left to right)

Infusions and decoctions are prepared either from fresh or dried stinging nettle leaves (Ait Haj Said et al., 2015; Upton, 2013). However, a regular supply of fresh stinging nettle leaves is a practical limitation due to its seasonality and limited availability in some regions. Inevitably, utilization of dried stinging nettle leaves could help in the utilization of the plant. Gião et al. (2007) also recommended infusion in the form of leaf powder prepared from dried leaves as this was the most effective mode of antioxidants extraction from medicinal plant e.g. lavender, chamomile, thyme, sage, fennel, stinging nettle leaves etc.). Ait Haj Said et al. (2015) also reported that infusion and decoction extracts prepared from stinging nettle leaf powder are natural sources of antioxidants in human medicine and nutrition. The temperature difference of preparation involved in decoctions and infusions might affect the chemical compositions of the product differently. This is probably due to the fact that with decoction, extraction of active compounds is accomplished through constant boiling while with infusion, the plant material is steeped in boiled water for a specified time (Courtine, 1984). Detailed methods of preparation of infusions and decoctions from stinging nettle leaf powder can be found in sections 4.3.3.1.1 and 4.3.3.1.2, respectively.

5.1.3 Sensory properties

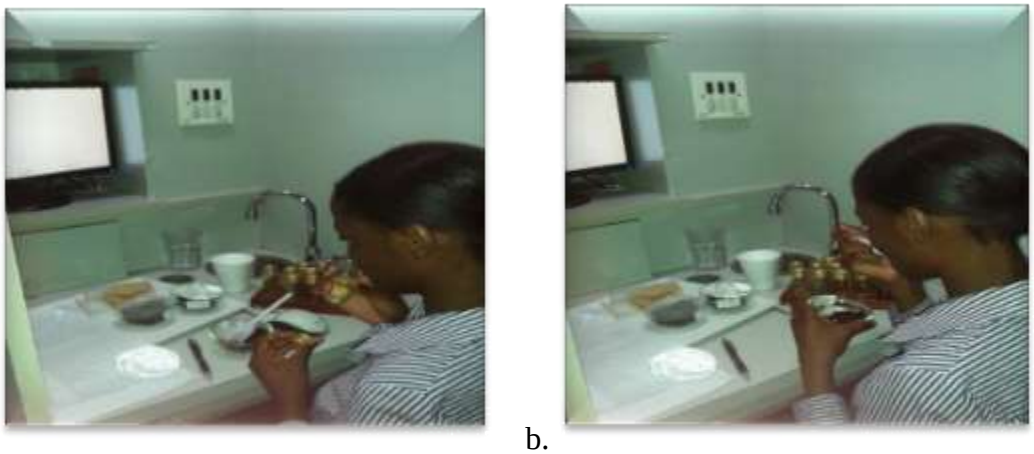
The descriptive sensory properties of stinging nettle cooked leaves and leaf infusions were determined following the generic descriptive analysis method (Einstein, 1991) which involves recruitment and screening of the panel, panel training and product evaluation as described in section 4.1.3.6. To minimize variation in the way individual panellists evaluated the products, in addition to the general sensory training, panellists completed product-specific training with the use of reference samples for each attribute (Figure 5.5; Figure 5.6 and Table 5.1).

Table 5. 1: Methods of preparation for the reference samples used to describe aroma, flavour and basic taste characteristics of cooked nettle leaves and leaf infusions

Descriptors	Reference samples**
Asparagus-woody	40 g of chopped fresh asparagus to which 300 mL of water added, covered and microwaved for 3 min. Then filtered, poured into a medium-size snifter and covered.
Astringent	0.15% "Alum solution" Alum
Beany	50 g of fresh peas to which 300 mL of water added, covered and microwaved for 3 min. Then filtered, poured into a medium-size snifter and screwed/covered.
Beet	100 g of fresh sliced beetroots to which 300 mL of water added and blended using a blender. Then 5ml of the juice poured into a medium-size snifter and screwed/covered.
Bitter	0.06% "Caffeine solution" Caffeine
Brown spice	0.2 g of dried mixed spices placed into a medium-size snifter and screwed/covered
Brussels Sprouts	20 g of sliced Brussels Sprouts to which 300 mL of water added, covered and microwaved for 3 min. Then filtered, 5 ml poured into a medium-size snifter and screwed/covered.
Burnt	0.5 g of roasted wheat seeds placed into a medium-size snifter and screwed/covered.
Cabbage	25 g of chopped cabbage to which 300 mL of water added and soaked for 15 min. Then filtered, 5 ml poured into a medium-size snifter and screwed/covered.
Celery	1.5 g of chopped fresh celery to which 300 mL of water added, covered and microwaved for 3 min. Then filtered, poured into a medium-size snifter and screwed/covered.
Citrus	0.5 g of fresh crushed lemon placed into a medium-size snifter to which 5ml of room temperature water added and screwed/covered.
Cooked-morogo	30g of chopped fresh spinach, nettle leaves, parsley and celery leaves to which 300 mL of water added, covered and microwaved for 3 min. Then filtered, poured into a medium-size snifter and screwed/covered.
Cucumber	20 g of chopped cucumber to which 300 mL of water added, covered and microwaved for 3 min. Then filtered, poured into a medium-size snifter and screwed/covered.
Earthy	0.5 g of fresh chopped mushrooms placed into a medium-size snifter and covered.
Fermented	1 part wine was diluted with 1 part water. Then 5 ml poured into a medium-size snifter covered.
Grassy	1 g of chopped fresh cut-grass placed into a medium-size snifter and screwed/covered.
Green-leafy	25 g of chopped fresh parsley leaves to which 300 mL of water added. Then set for 15 min, filtered, poured into a medium-size snifter and screwed/covered.
Green-herblike	0.5 g of dried herb mix, grinded using IKA basic miller (IKA@ A11B, made in Germany). 100 mL of water added, mixed well and 5 mL of herb water poured into a medium-size snifter and screwed/covered.
Hay-like	0.5 g of dried grass placed into a medium-size snifter and screwed/covered.
Lettuce	25 g of chopped fresh lettuce to which 300 mL of room temperature water added. Then set for 15 min, filtered, poured into a medium-size snifter and screwed/covered.
Mint	0.5 g of fresh crushed mint leaves placed into a medium-size snifter and covered.
Parsley	15 g of chopped fresh parsely to which 300 mL of water added, covered and microwaved for 3 min. Then filtered, poured into a medium-size snifter and covered.
Salty	0.60% NaCl solution
Seafood/fishy	5 g of dried fish to which 300 mL of water added and soaked for 10 min. Then filtered, 5 ml poured into a medium-size snifter and screwed/covered.
Spinach	35 g of chopped fresh spinach leaves to which 300 mL of water added, covered and microwaved for 3 min. Then filtered, poured into a medium-size snifter and covered.
Sweet	4% sucrose solution
Sweet aromatics	0.5 g of vanilla to which 250 mL of water added. Then 5 ml poured into a medium-size snifter and screwed/covered.
Umami	0.35% monosodium glutamate

The product specific training sessions were designed to illustrate the meaning of the descriptors and scoring procedures for the panellists to assit them in distinguishing among the products. Labbe et al. (2004) and Rossi (2001) emphasized that panellists should be carefully trained to increase reliability, discrimination and panel agreement.

Figure 5.5: Green and brown related flavour descriptor reference samples in covered medium-size snifter bottles



a. b.

Figure 5.6: Pictures showing sniffing of green and brown related flavour descriptors reference samples (a) followed by sample sensory evaluation by a panellist (b)

Verbal definitions of the descriptors were also agreed on during training. Additionally, the same panellists was used throughout the product evaluation sessions. Despite all, significant differences among individual panellists' ratings for some descriptors were found. As an example panellists showed significant differences for rating of the asparagus-woody aroma and flavour of cooked leaves and leaf infusions from stinging nettle and spinach leaves (Table 5.2).

This probably was due to variation in the perception of the sensory attributes by different panellists due to differences in the ability to detect small differences in attribute intensities or differences in the panellists' use of the scale for score values and location of rating values.

Table 5.2: The effect of sessions and panellists on asparagus-woody aroma and flavour descriptor in cooked leaves and leaf infusions from stinging nettle and spinach leaves

		Cooked leaves		Leaf infusions	
		Aroma*	Flavour*	Aroma*	Flavour*
Sessions	1	2.7^{a} (1.2)	2.8^{a} (1.1)	2.1^{a} (1.2)	1.9^{a} (1.2)
	2	2.5^{a} (1.0)	2.7^{a} (1.0)	1.9^{a} (1.2)	2.1^{a} (1.1)
	p-values	0.56	0.56	0.35	0.30
Panellists	1	2.6^{bc} (0.7)	3.3^{ef} (0.9)	1.1^{a} (0.3)	1.0^{a} (0.0)
	2	2.9^{bc} (0.6)	2.8^{cde} (0.7)	1.0^{a} (0.0)	1.0^{a} (0.0)
	3	2.4^{b} (1.4)	2.0^{b} (1.1)	3.5^{e} (0.7)	3.6^{d} (0.6)
	4	2.5^{bc} (1.4)	2.5^{bcd} (1.1)	1.0^{a} (0.0)	1.0^{a} (0.0)
	5	2.5^{bc} (0.5)	2.3^{bc} (0.5)	2.2^{c} (0.7)	2.1^{b} (0.7)
	6	2.5^{bc} (0.5)	2.9^{def} (0.6)	1.3^{ab} (0.4)	1.3^{a} (0.4)
	7	3.9^{d} (0.6)	4.1^{g} (0.4)	4.0^{f} (0.0)	3.9^{e} (0.3)
	8	2.8^{bc} (1.4)	3.4^{f} (1.1)	2.1^{c} (1.2)	2.2^{b} (1.4)
	9	3.0^{c} (1.2)	3.0^{def} (0.5)	3.1^{d} (0.6)	2.6^{c} (0.7)
	10	1.4^{a} (0.5)	1.3^{a} (0.5)	1.5^{b} (1.0)	2.1^{b} (1.0)
	11	2.3^{b} (1.2)	2.6^{cd} (1.1)	1.0^{a} (0.0)	1.0^{a} (0.0)
	12	2.8^{bc} (1.0)	3.3^{ef} (0.7)	2.5^{c} (0.8)	2.3^{bc} (0.7)
	p-values	0.00	0.00	0.00	0.00

[ab] Means for a specific main effect (sessions, panellists), within a column not sharing a superscript letter are significantly different ($p < 0.05$)

[*]A five-point category scale (none=1, slight=2, moderate=3, strong=4 and extreme=5).

It was reported that differences in motivation, sensitivity and psychological response behaviours (LunDahl and McDaniel, 1991) and lack of agreement among panellists in descriptive sensory evaluations (Kermit and Lengard, 2005) were some of the reasons for uneven results stemming from trained panellists.

For example, agreement errors within a sensory panel during descriptive sensory evaluation were experienced when a panellist: rates the product using a wider or lesser range of the scale (magnitude error) compared to other panellists, rates a product or set of products in the opposite direction (crossover error), rates all the products in a set as similar (non-discriminator error) and does not perceive an attribute and scores all the products at '0 or not detected' (non-perceiver error) when the rest of the panel rated them as different (Figure 5.7).

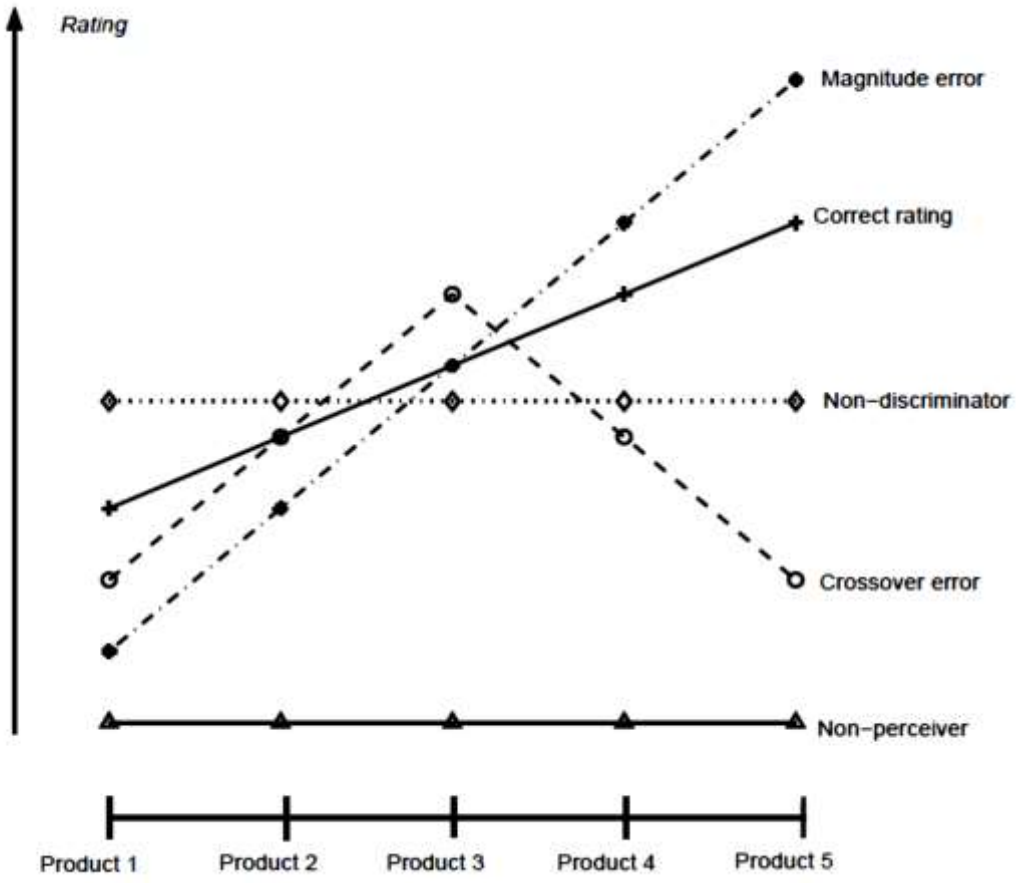


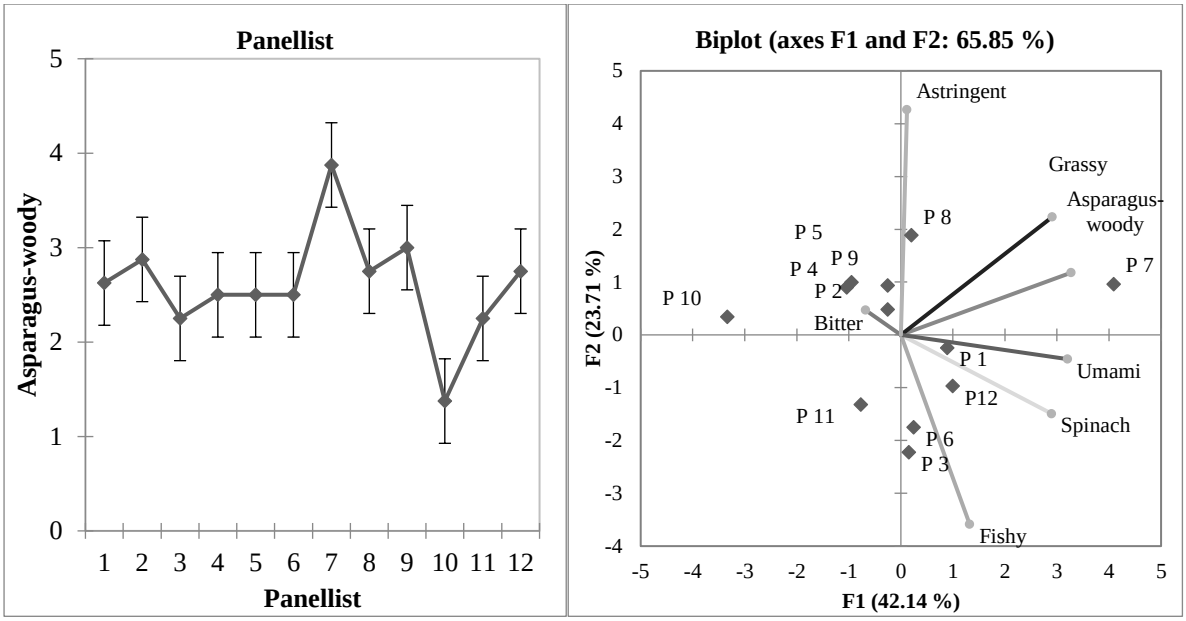
Figure 5.7: The different four types of panel agreement errors in descriptive sensory evaluation (Kermit and Lengard, 2005)

Figure 5.8a visualises the performance of individual panellists. Average ratings for asparagus-woody flavour descriptor in cooked leaves for the different panellists are presented. Panellist 7 clearly stands out from the other panellists because of a higher intensity score whereas panellist 10 gave a much lower intensity score for the tested samples. This possibly indicates magnitudinal error whereby panellist 7 used a broader range of the scale whereas panellist 10 used the smaller range of the scale than the rest of the panellists.

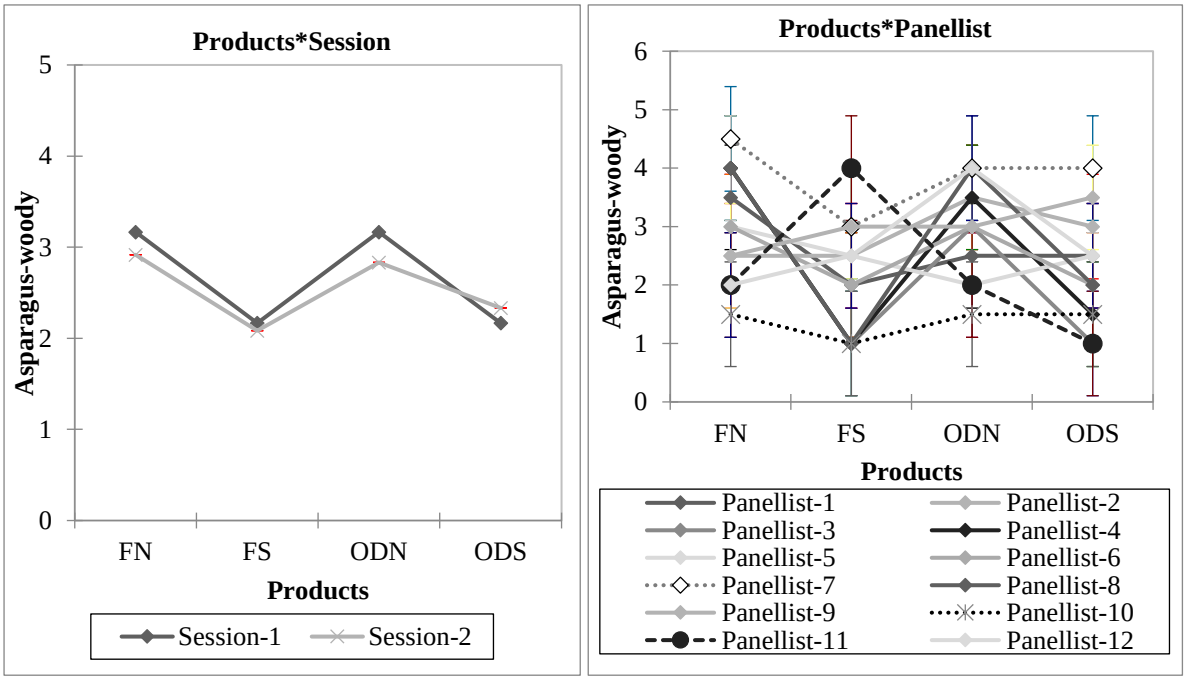
Figure 5.8d demonstrates panellist-by-product interactions to graphically examine the agreement among the panellists on the intensity of asparagus-woody flavour descriptor to discriminate cooked leaves. For example, panellist 10 used lower intensity scores and stand out as a none-perceiver or low perceiver of asparagus-woody aroma in all the cooked samples where as panellist 11 rated the samples differently compared to the rest of the panel (see Figure 5.8d). Kermit and Lengard (2005) noted that although non-perceiver errors are not crucial to the panel, leaving non-perceiver data in the analysis may affect the mean score for that attribute. In this study the intensity score from all panellists were utilized during analysis.

To explore the agreement among the panellists further, principal component analysis (PCA) was applied to illustrate how individual panellists used some of the flavour attributes to describe cooked stinging nettle and spinach leaves (Figure 5.8b). Various researchers showed that PCA is a useful tool for identifying dissimilar panellists (King, Hall and Cliff, 2001; Kermit and Lengard, 2005).

The PC chart identified panellist 7 and 10 as possible outliers suggesting their evaluations were dissimilar from the other panellists. For example, the PC plot suggests that panellist 7 may have used higher scores for the attributes (asparagus-woody, grassy, spinach, fishy, bitter, umami and astringent) loading positively on PC1 while panellist 10 gave lower scores compared to the rest of the panel for these attributes. Figure 5.8c visualizes the product-by-session interaction and indicates that the panel scores were reproducible between sessions. All in all, the panel as a whole was reproducible between sessions to discriminate cooked leaves and leaf infusion food products (Table 5.2).



(a) (b)



(c) (d)

Figure 5.8: (a) A line plot of average ratings by individual panellists' for flavour descriptor 'asparagus-woody' in cooked leaves where the vertical axes represent intensity scores and horizontal axes represent the effect of the 12 panellists, (b) PCA scores loadings plot showing how the 12 panellists scored some of the selected flavour descriptors of cooked leaves, (c) Line plot representing the interaction effect of sessions x products for Asparagus-woody flavour descriptor of cooked leaves, and (d) Profile plot of flavour descriptor 'asparagus-woody' in cooked leaves representing the interaction between panellists x products. 'P' reperesent panellist, 'FN' represent cooked leaves from fresh nettle, 'FS' represent cooked leaves from fresh spinach, 'ODN' represent cooked leaves from oven dried nettle, and 'ODS' represent cooked leaves from oven dried spinach.

5.1.4 Nutritional properties

Linearity, accuracy and recovery performance of blank or control samples and certified reference materials are used to proof the validation of analytical methods (Food and Drug Administration, 2016; van Reeuwijk, 1998). The validation techniques used for the nutrient analytical methods in this study will be discussed as follows.

5.1.4.1 β-carotene analysis

The quantitative analysis of β-carotene was performed using a reversed-phase HPLC coupled with a photodiode array detector at 450 nm. The method was optimized and validated with the use of a solvent (acetone) all-trans-β-carotene calibration standard (Merck,South Africa). The separation of the all-trans-β-carotene standard during this study was done on a C30 column due to its ability to separate different types of carotenoids (Rodriguez-Amaya and Kimura, 2004). Identification of the all-trans-β-carotene was carried out by HPLC 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. The HPLC chromatogram is presented in Figure 5.11a, and all-trans-β-carotene (peak 1) was identified by comparing the spectrum (λ max) with those given in literature (Mercadante et al., 1997; Rodriguez-Amaya and Kimura, 2004) and retention time of the all-trans-β-carotene standard. The mass spectrum showed the same characteristic peaks as those given in the literature.

The peak areas from all-trans-β-carotene standards series of four concentration levels (5, 10,15 and 20 ppm) with two repeated injections were found to fall within one-standard deviation from the mean (13.3×10^4 to 36.9×10^4) (Figure 5.9 and Table 9.4). There was no significant difference between the two repeated injections (Figure 5.11b_2). Examples of a typical control chart can be viewed in Figure 5.10. The basic assumption is that when a control result falls within a distance of 1-2 standard deviation (±s - ±2s) from the mean [within warning limits-Upper Warning Limit (UWL) and Lower Warning Limit (LWL)], the system was under control and the results of the batch as a whole have acceptable accuracy (Briggs, 1996; van Reeuwijk, 1998). However, if the readings from the standard samples were above three standard deviation (±3s) [control limits-Upper Critical Limit (UCL) and Lower Critical Limit (LCL)] from the mean, then the assay needs control measures.

For this study, it can be deduced that the β-carotene analytical method was under control and had acceptable accuracy.

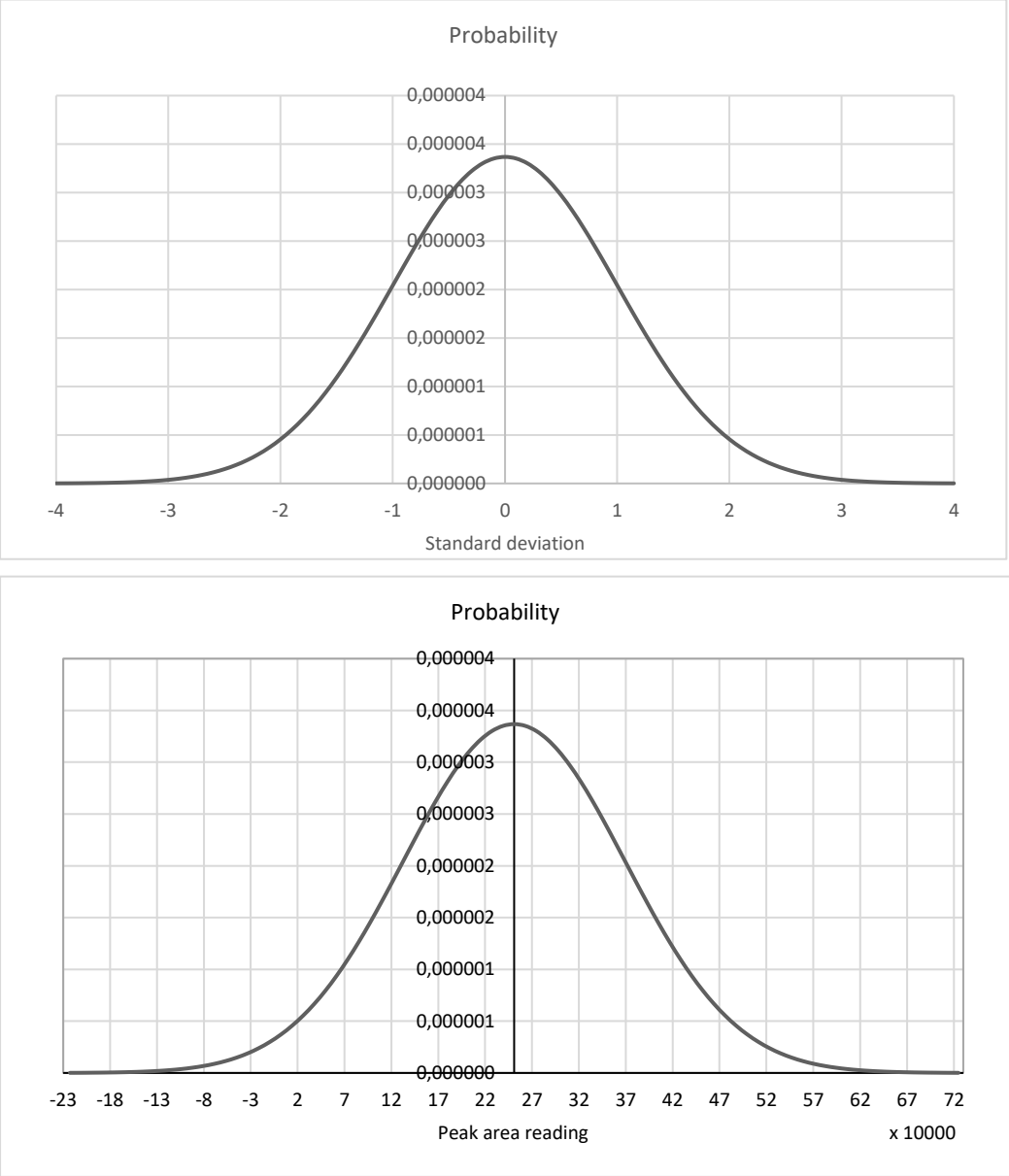


Figure 5.9: Control chart using all-trans-β-carotene standards (Mean= 250956, Standard deviation=118456) normal distribution data showing the accuracy and validity of β-carotene analytical method

Within these quantification limits (peak area ranging from 13.3 x 10^4 to 36.9 x 10^4), the measurement results have acceptable accuracy, therefore, the concentrations from the lowest to the highest was regarded as the range of the corresponding method for all-trans-β-carotene assay from stinging nettle and spinach leaf products. The quantification of all-trans-β-carotene in stinging nettle leaf products was done using a calibration curve standard as described in Equation 4.

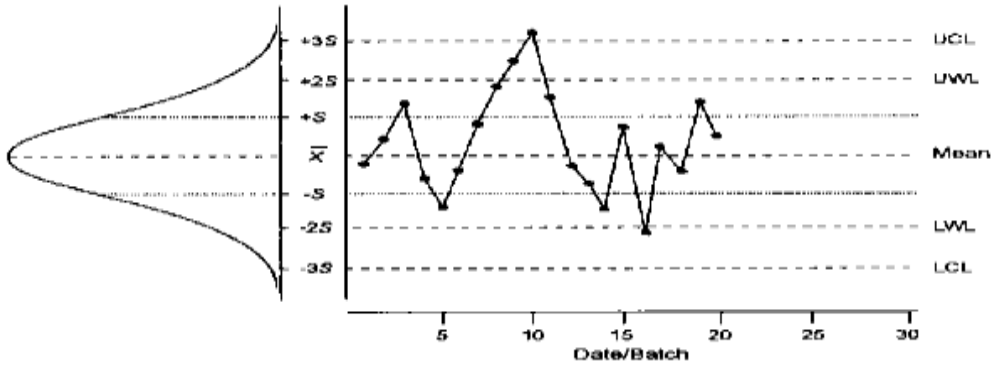
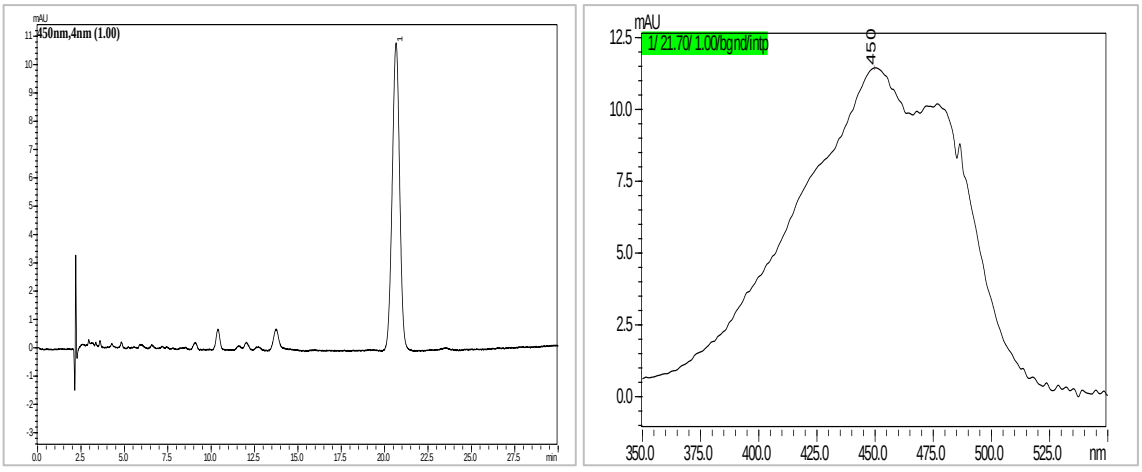


Figure 5.10: The principle of a Control Chart. UCL = Upper Control Limit (or Upper Action Limit). LCL = Lower Control Limit (or Lower Action Limit). UWL = Upper Warning Limit. LWL = Lower Warning Limit (van Reeuwijk, 1998).

(a)



(b)

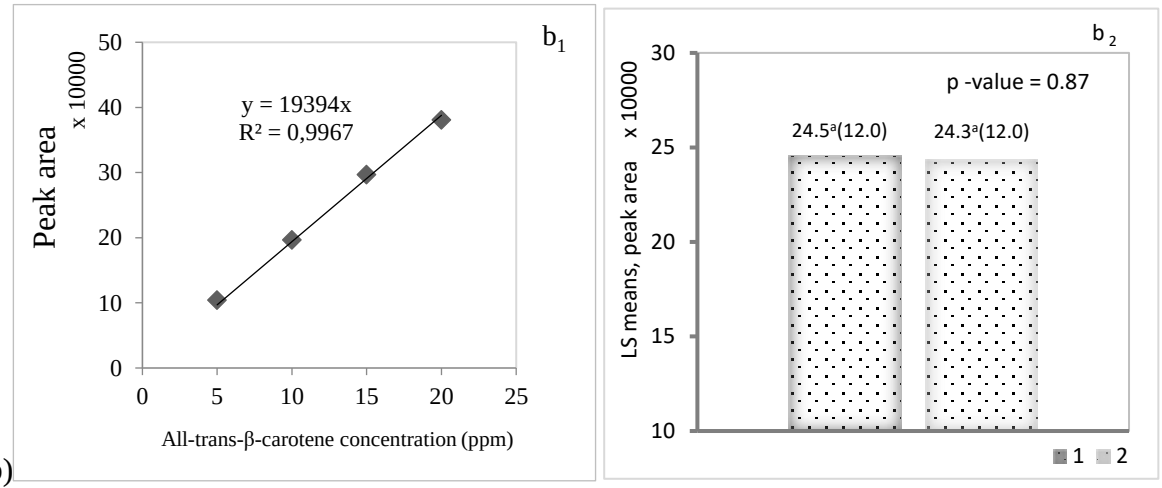


Figure 5.11: HPLC chromatograms and UV/Vis spectra (a), standard curve (b_1) and LS means chart (± standard deviation) of repeated injections (b_2) of the all-trans-β-carotene standard.

Once measurements of the calibration for all-trans-β-carotene standards were performed, the relationship between the equipment response (y) and the concentration (x) was determined ($y = \alpha_i + \beta_i$). For each series of all-trans-β-carotene concentrations, a straight-line response function was estimated based on the results of the calibration standards (Equation 3).

$$y = 1.9394x \quad (3)$$

Validity checking was done first by calibration check (i.e. during the whole analysis, standard solutions were analyzed from time to time within the required range of concentration). According to Food and Drug Administration (2016) the ideal calibration curve should be linear within its most useful range, with a regression coefficient of 0.99 or greater. Accordingly, the calibration curve for the all-trans-β-carotene had a regression coefficient ($R^2 = 0.9984$) and was found to be linear (y = 1.9394x) (Figure 5.11b_1), therefore, the analytical method was valid.

The amount of all-trans-β-carotene for the validation standards was estimated using Equation (4) (Table 5.3). Shewiyo et al. (2013) indicated that the concentration (x_m) of validation standards (for m = 1: v) can be estimated using the following formula (Equation 4):

$$x_m = \frac{y_m - \alpha_i}{\beta_i} \quad (4)$$

Where ˛ α_i and $\beta_i$are the true intercept and the true slope of the line, respectively, which are estimated for each series i = 1: s from the results of the calibration standards.

Estimation of accuracy

The accuracy of an analytical method expresses the closeness of agreement between the trial result x_{im} and the accepted reference value μ_{im} for each measured validation standard (i = 1:s, m = 1:v) (Food and Drug Administration, 2016; Shewiyo et al., 2013). The authors also provided an equation for accuracy (Equation 5), % accuracy (Equation 6) and % recovery (Equation 7) as follows:

$$Accuracy_{im} = x_{im} - \mu_{im} \quad (5)$$

$$\%\ Accuracy_{im} = \frac{x_{im} - \mu_{im}}{\mu_{im}}\ x\ 100 \quad (6)$$

$$\%\ Recovery_m = \frac{\bar{x}_m}{\hat{\mu}_m}\ x\ 100 \quad (7)$$

Where, $\bar{x}_m$ is the average of the back-calculated concentrations, and $\hat{\mu}_m$ is the average of the introduced amounts of the validation standards at each concentration level (m = 1: v).

The validation of the β-carotene analytical method was expressed as accuracy (Equation 5), % accuracy (Equation 6) and % recovery (Equation 7) (Table 5.3). The % accuracy of all-trans-β-carotene at the studied concentration levels ranged between −1.90 and 7.09 %. These values are much lower than the limits set for active ingredients in food products (i.e. the mean value should be within 15 % of the actual value) by Food and Drug Administration (2016), which indicated a very low bias and the absence of matrix effects.

The % recovery values (98.10 to 107.09 %) validated the measurement of the all-trans-β-carotene standard within acceptance limits, and therefore the analytical method was found to be valid for the assay. As described by Food and Drug Administration (2016), an average recovery of 80 to 110% should be obtained when the designated concentration of standard is 0.1 ppm or greater.

Table 5.3: Accuracy, % accuracy and % recovery of all-trans-β-carotene standard concentrations.

All-trans-β-carotene concentrations (ppm)				
Accepted reference values	Measured validation standards	Accuracy	% Accuracy	% Recovery
5	5.35	0.35	7.09	107.09
10	10.14	0.14	1.40	101.40
15	15.29	0.29	1.96	101.96
20	19.62	-0.38	-1.90	98.10

Refer to Equation 5 for accuracy, Equation 6 for % accuracy and Equation 7 for % recovery.

Extraction protocols

Preliminary experiments indicated that 100 % acetone was an efficient solvent for extracting β-carotene from stinging nettle leaf food products (e.g. fresh leaves, dried leaves, infusions and decoctions). When β-carotene was extracted using 80 % methanol-water mixture, identification and quantification of β-carotene was not efficient. Sample extraction and mobile phase preparation for the analysis of β-carotene was done following Rodriguez-Amaya and Kimura (2004).

β-carotene extraction with the use of the organic solvent (100 % acetone) was efficient probably due to the fact that a water-miscible solvent such as acetone is needed for complete penetration of vegetable material with high water content (e.g. nettle leaves) (Kopec et al., 2012).

Extraction of β-carotene was carried out with acetone containing 0.1 % butylated hydroxytoluene (BHT) and the extraction with acetone was repeated for about 9 to 10 times until most of the pigments were removed from the residue. The need for 9 to 10 repeated extractions was due to the fact that for complete removal of the carotenoid pigments residues should be re-extracted until the filtrate and residue were colorless (Kimura et al., 2007; Kopec et al., 2012). Acetone is efficient for β-carotene extraction, but owing to its tendency to form peroxides, it is often used with antioxidants, such as BHT to prevent oxidative degradation (Kimura et al., 2007).

Guil-Guerrero et al. (2003) extracted carotenoids from fresh stinging nettle leaves by blending with acetone, shaking with diethyl ether and water, and the extract also underwent saponification to remove chlorophylls and hydrolyse carotenoid esters. Saponification is an effective means of removing chlorophylls and unwanted lipids, and simplifies the chromatographic separation, identification, and quantification. However, the saponification and the subsequent washing can result in losses of carotenoids, hence, it should be omitted from the analytical procedure whenever possible (Rodriguez-Amaya and Kimura, 2004). Therefore, for this study the saponification step was omitted. The highly conjugated structure of carotenoids makes them susceptible to degradation by heat, light, and oxygen (Kopec et al., 2012). Samples were handled under low light conditions in the laboratory by covering glassware with aluminium foil to minimize the degradation due to ultraviolet and visible light (Lee and Chen, 2001; Teow et al., 2007).

Carotenoids are less stable in extracts compared to food matrices (Kopec et al., 2012), and therefore, after evaporating the solvent (in a rotary evaporator) and dissolving the dried sample into the mobile phase containing 0.1 % BHT, the carotenoid solution was immediately transferred to amber vials (brown vials) which provided further protection against light and analysed as quickly as possible.

5.1.4.2 Ascorbic acid analysis

Waheed Uz et al. (2013) indicated that HPLC methods are preferred mostly because they provide superior selectivity than other methods (e.g. spectrophotometric, titration or enzymatic methods). Therefore, for this study, the quantitative analysis of ascorbic acid was done using HPLC (Waters Alliance, Milford, Massachusetts, USA) as described in section 4.2.3.4.4. The method was optimized and validated with the use of solvent-based calibration standards (L-ascorbic acid). Identification of the ascorbic acid was carried out by HPLC through the combined use of the retention time and co-injection with L-ascorbic acid standards as monitored by HPLC (Figure 5.13a).

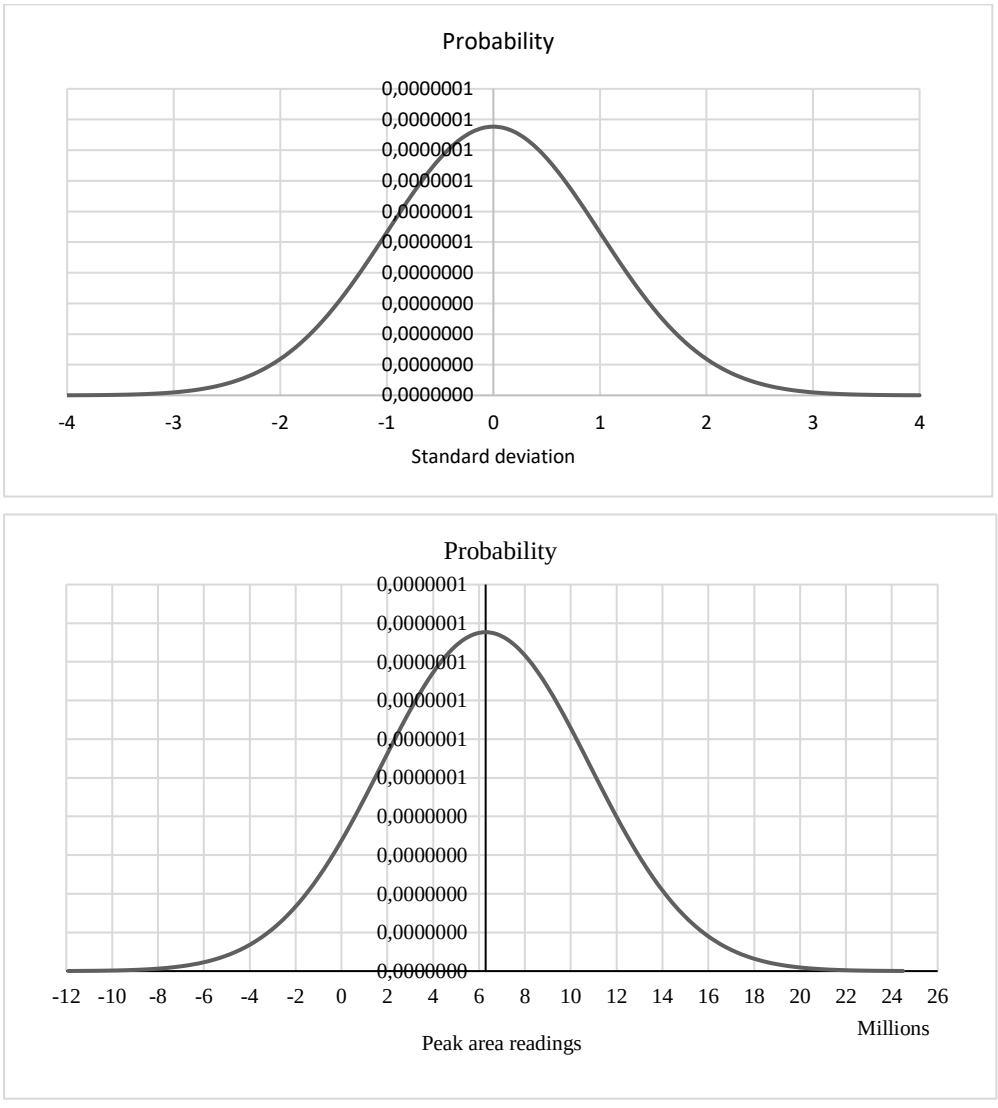
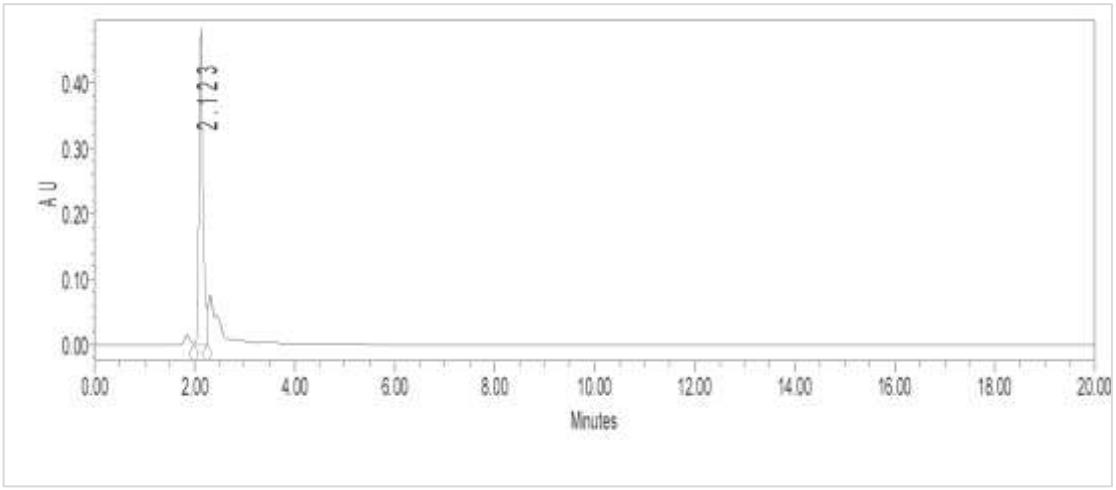
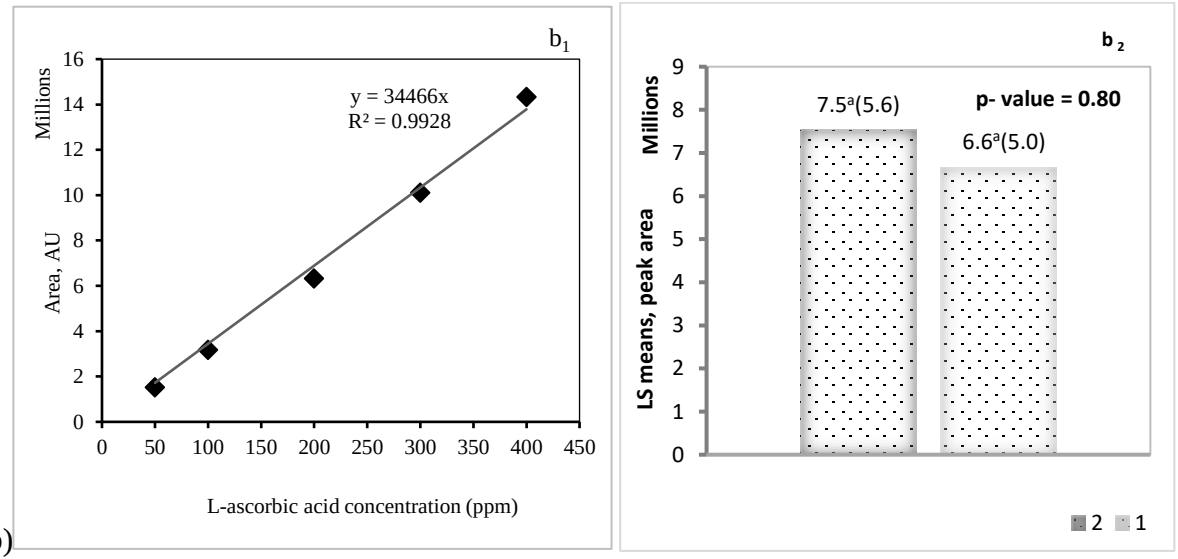


Figure 5.12: Control chart using L-ascorbic acid standards (Mean= 6287555, Standard deviation=4550415) normal distribution data showing the accuracy and validity of ascorbic acid analytical method.

The peak area from a calibration standard series of five concentration levels (50, 100, 200, 300 and 400 ppm) with two repeated injections were found to fall within one standard deviation from the mean (1.7 x 10^6 to 10.8 x 10^6) (Figure 5.12 and Table 9.2). There was also no significant difference between the repeated injuctions (Figure 5.13b_2).



(a)



(b)

Figure 5.13: L-ascorbic acid HPLC chromatograms (a) and standard curve (b_1)and LS mean (± standard deviation) of repeated injections chart (b_2) of L-ascorbic acid standard.

Within these quantification limits (peak area ranging from 1.7 x 10^6 to 10.8 x 10^6), the measurements have acceptable accuracy, therefore, the concentrations from the lowest to the highest were regarded as the range of the corresponding method for ascorbic acid determination from stinging nettle and spinach leaf products. The quantification of ascorbic acid in stinging nettle leaf products was done using a calibration curve of L-ascorbic acid standard (Figure 5.13b_1) as described in Equation 4.

From time to time validity checking was done through calibration of different concentrations of L-ascorbic acid . The values for the ascorbic acid analytical method validation was expressed as accuracy (Equation 5), % accuracy (Equation 6) and % recovery (Equation 7) (Table 5.4).

The % accuracy of L-ascorbic acid standards at the studied concentration levels ranged between −11.45 and 3.95 % and falls within the acceptable limits as described by Food and Drug Administration (2016). Additionally, the % recovery values 88.55 to 103.95 % confirmed that for the measurement of L-ascorbic acid fell within the acceptance limits provided by Food and Drug Administration (2016) and Mercadante et al. (1997), and therefore the analytical method is valid for the ascorbic acid assay.

Table 5.4: Accuracy, % accuracy and % recovery of L-ascorbic acid standard concentrations.

L-ascorbic acid concentrations (ppm)				
Accepted reference values	Measured validation standards	Accuracy	% Accuracy	% Recovery
50	44.27	-5.73	-11.45	88.55
100	92.36	-7.64	-7.64	92.36
200	183.54	-16.46	-8.23	91.77
300	293.44	-6.56	-2.19	97.81
400	415.78	15.78	3.95	103.95

Refer to Equation 5 for accuracy, Equation 6 for % accuracy and Equation 7 for % recovery.

Extraction protocols

Sample extraction and mobile phase preparation for the analysis of ascorbic acid was done following Maia et al. (2007), after trials of different types of extraction methods [e.g. (Maia et al., 2007; Sawant et al., 2010)]. When ascorbic acid was extracted using 80% methanol-water mixture with a gradient prepared from 0.1 % (v/v) acetic acid in HPLC-grade water (component A) and methanol (component B) following Sawant et al. (2010) directions, identification and quantification of ascorbic acid was not efficient. This might be because of the hydrophilic nature of ascorbic acid, so organic solvents such as methanol could not completely solubilize the ascorbic acid from the nettle leaves samples.

However, the use of 0.2 % metaphosphoric acid/methanol/acetonitrile (90:8:2, v/v/v) as an extraction solvent was very efficient for both identification and quantification of ascorbic acid from the samples. This probably occurred due to increased solvation of ascorbic acid provided by the presence of water accompanied by the acid buffer (metaphosphoric acid/methanol/acetonitrile) which were effective in reducing the degradation of the ascorbic acid. Metaphosphoric acid is often added to the solvent due to its capacity to rapidly precipitate proteins and reduce the pH of the matrix, stimulating the stability of ascorbic acid (Campos et al., 2009; Wechtersbach and Cigić, 2007).

5.1.4.3 Total phenol content analysis

Total phenolic content of stinging nettle leaf food products (i.e fresh leaves, freeze dried leaves, oven dried leaves, decoctions and infusions) was determined using the Folin-Ciocalteau (FC) method. The FC method is based on detection of phenolic hydroxyl groups including those in the extractable proteins present in the sample (Singleton et al., 1999). The method was optimized and validated with the use of solvent (methanol)-based calibration standards (gallic acid) (Figure 5.15a). The absorbance readings from the calibration standards' series of four concentration levels (50, 100, 150 and 200 ppm) with four repetitions fell within one standard deviation from the mean (0.79 to 2.15) (Figure 5.14 and Table 9.5).There was also no significant difference between the four repeatitions (Figure 5.15b). Within these quantification limits (absorbance readings from 0.79 to 2.15), the FC analytical method was found to be valid for the total phenol content assay. Therefore, the concentrations from the lowest to the highest was regarded as the range for total phenol content determination from stinging nettle and spinach leaf products. The calibration curve of gallic acid standard was used for quantification of the total phenol content in the stinging nettle leaf food products using the formula described in Equation 4.

The values for the FC analytical method validation for determination of total phenol content was expressed as accuracy (Equation 5), % accuracy (Equation 6) and % recovery (Equation 7) (Table 5.5). The % accuracy of gallic acid at the studied concentration levels ranged between −9.16 and 3.94 % and falls within the acceptable limits as described by Food and Drug Administration (2016). The % recovery values 90.84 to 103.94 % confirmed the validity of the method (Table 5.5), according to the acceptance limits described by Food and Drug Administration (2016) and Mercadante et al. (1997).

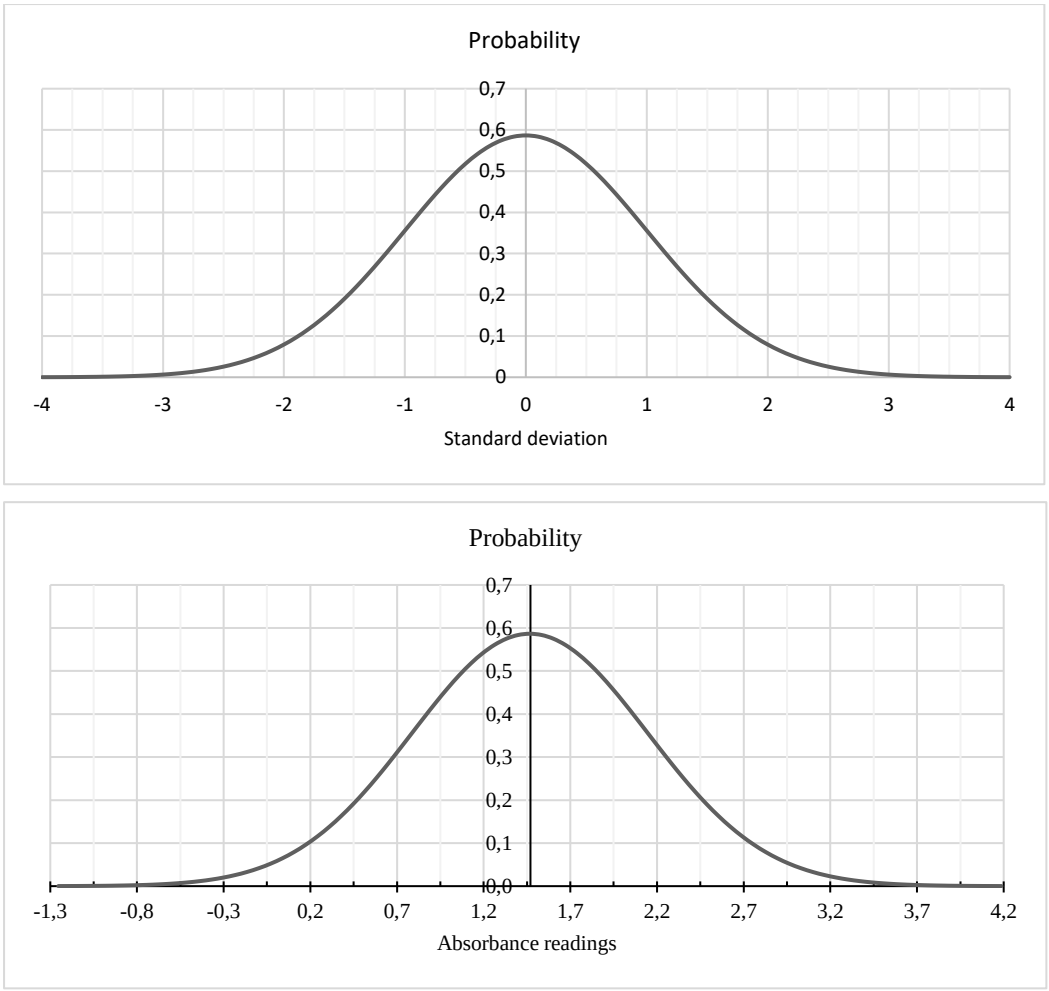


Figure 5.14: Control chart gallic acid standards (Mean= 1.467, Standard deviation=0.680) normal distribution data showing the accuracy and validity of the total phenol content analytical method.

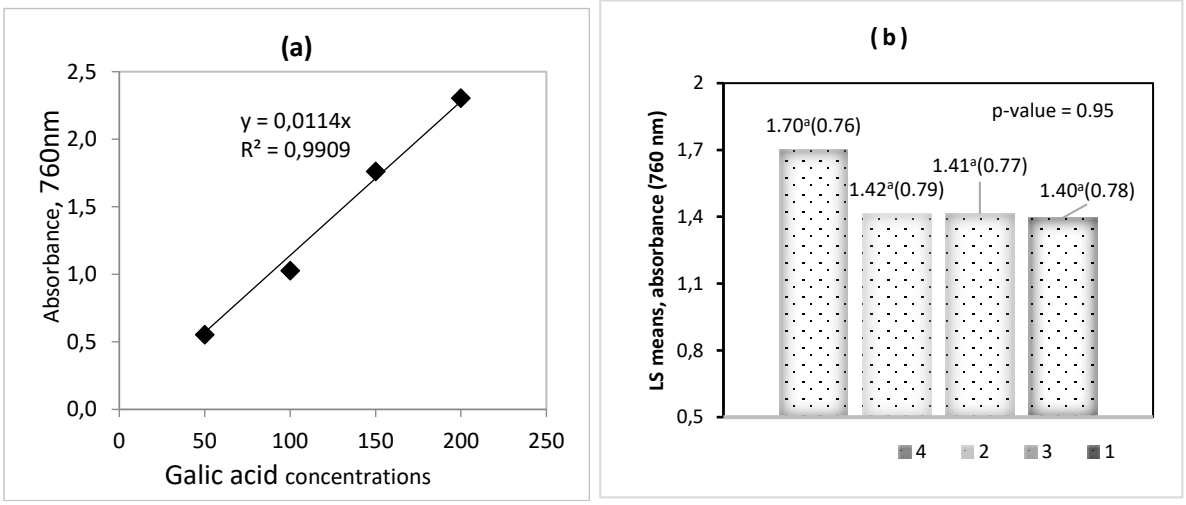


Figure 5.15: Gallic acid standard curve and LS means of the four repeated absorbance readings (± standard deviation) chart of gallic acid standard.

Table 5.5: Accuracy, % accuracy and % recovery of gallic acid standard concentrations.

Gallic acid concentrations (ppm)				
Accepted reference values	Measured validation standards	Accuracy	% Accuracy	% Recovery
50	48.85	-1.15	-2.30	97.70
100	90.84	-9.16	-9.16	90.84
150	155.91	5.91	3.94	103.94
200	203.81	3.81	1.90	101.90

Refer to Equation 5 for accuracy, Equation 6 for % accuracy and Equation 7 for % recovery.

5.1.4.4 Total antioxidant activity analysis

The DPPH radical scavenging activity assay as described by Brand-Williams et al. (1995) was used in this study to determine the total antioxidant activity. The DPPH method is based on the ability of antioxidants (i.e. antioxidants present in the stinging nettle leaf food products) to donate hydrogen atoms to the DPPH radical (Rincón-León et al., 2003).

Pure methanol was used to calibrate the spectrophotometer. Blank or control readings are used to monitor the quality of reagents, proficiency of the analytical method and to determine the concentration of the specific nutrient in the sample (Briggs, 1996; van Reeuwijk, 1998). The authors emphasised that in order to detect the limits of the method, blanks or controls should be analysed with each batch of samples (e.g. between every 10 or 20 samples). In this study, a total of four control sample readings (800 uL of methanol + 200 uL of 0.5 mM DPPH), one per batch analysed in duplicate, were used to monitor the quality of reagents and proficiency of the analytical method. The absorbance readings fell within one standard deviation from the mean (0.989 to 1.029) (Figure 5.16 and Table 9.3). There was no significant difference between the four control sample readings (Figure 5.17). It can be deduced that the DPPH radical scavenging activity technique was under control and valid. Total antioxidant activity of the samples were expressed as % DPPH inhibition using the following formula (Equation 8) (Turkmen et al., 2005):

$$\%\ \text{DPPH inhibition} = \left(1 - \frac{\text{Absorbance of the sample (517nm)}}{\text{Absorbance of the control (517nm)}}\right) x\ 100 \qquad (8)$$

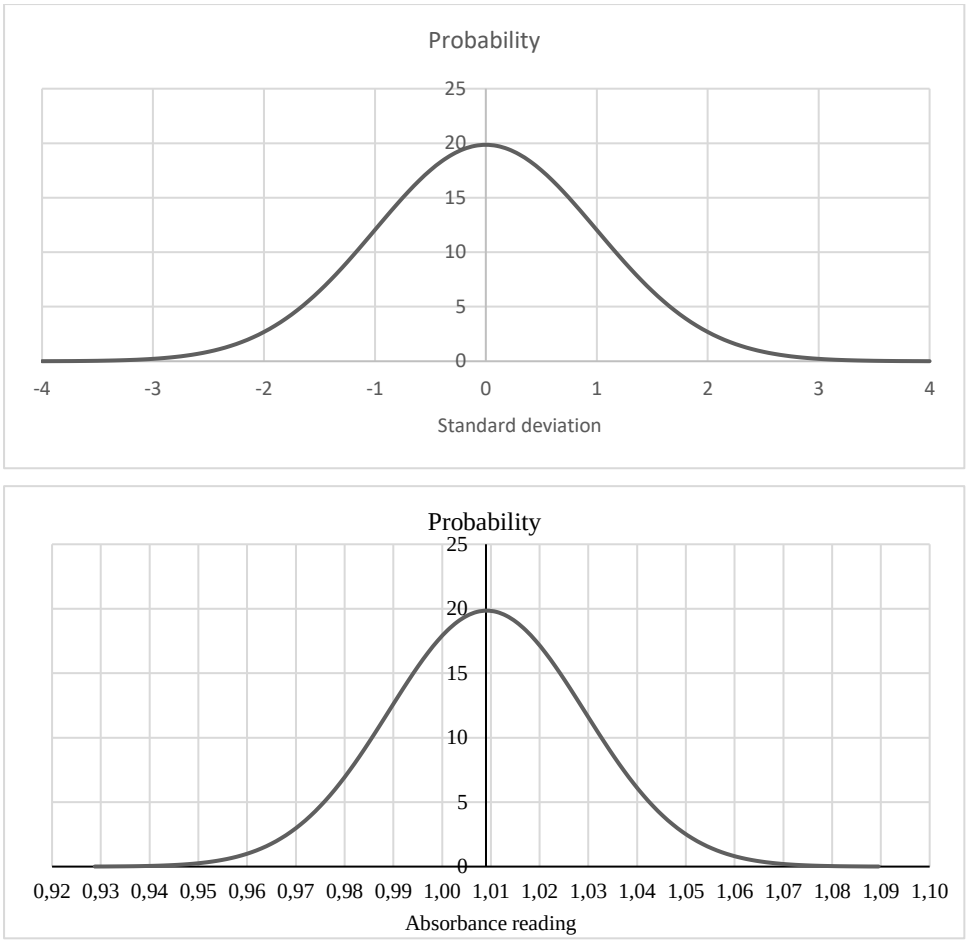


Figure 5. 16: Control chart using DPPH control (Mean= 1.009, Standard deviation=0.020) normal distribution data showing the accuracy and validity of total antioxidant activity/DPPH analytical method

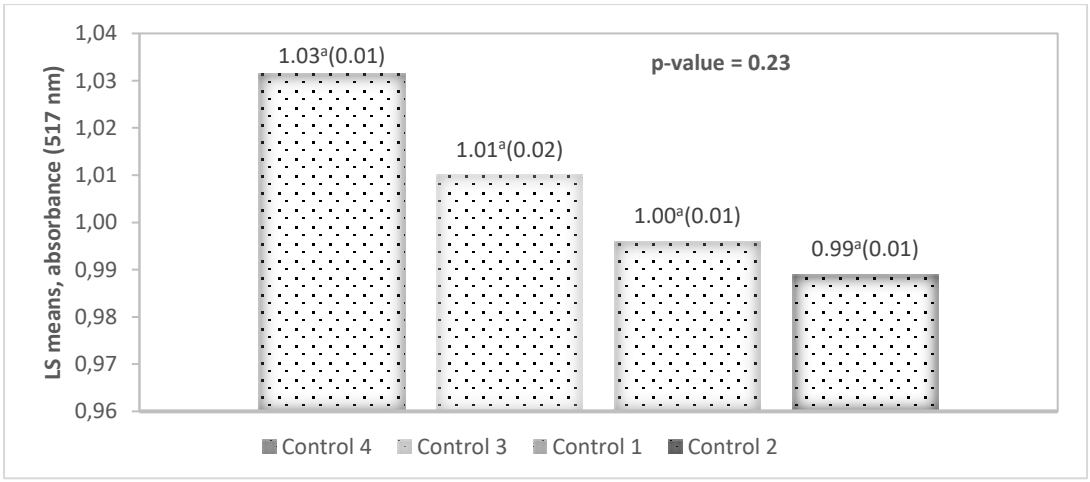


Figure 5.17: LS means chart (± standard deviation) using the duplicate absorbance readings of the DPPH control samples.

Extraction protocols

In general, preliminary experiments indicated that a mixture of methanol/water (80/20, v/v) was the most efficient solvent for the extraction of phenolic compounds and presented the highest antioxidant capacities. This is because of the variability in chemical characteristics and polarities of various phenolic and antioxidant compounds. Research findings reported that methanol is the most efficient solvent for extraction of antioxidant compounds or phenolic compounds from plant material, followed by water, ethanol and acetone (Bunea et al., 2008; Do et al., 2014; Khoddami et al., 2013; Michiels et al., 2012; Naczk and Shahidi, 2006).

Do et al. (2014) and Wu et al. (2004) emphasized that the use of a mixture of organic solvent with water is more efficient for extraction of phenolic compounds and antioxidants. Through preliminary investigations the mixture of methanol and water was found to be the most efficient solvent for the extraction of phenolics and antioxidants from stinging nettle leaves. This could have been due to the better solvation of phenolic and antioxidant compounds present in stinging nettle leaves as a result of interactions (hydrogen bonds) between the polar sites of the antioxidant molecules and the solvent.

5.1.4.5 Mineral analyses

Ca, Fe, Mg, Mn, Zn, P, K, and Na content of fresh, oven dried and freeze dried stinging nettle or spinach leaves were determined using an inductively Ion Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) after microwave digestion (AACC International, 2000) -analysis was done at the Dept of Soil Science University of Pretoria. The mean concentration of Ca, Fe, Mg, Mn, Zn, P, K, and Na from a total of four sets of blanks fell within one standard deviation (-0.11 to 0.44) from the overall mean (Figure 5.18 and Table 9.1). The mean value of the blank readings were also not significantly different (Figure 5.19). Therefore, it can be deduced that the mineral analytical technique was under control and valid. Sample results were calculated by subtracting blank readings from the sample readings.

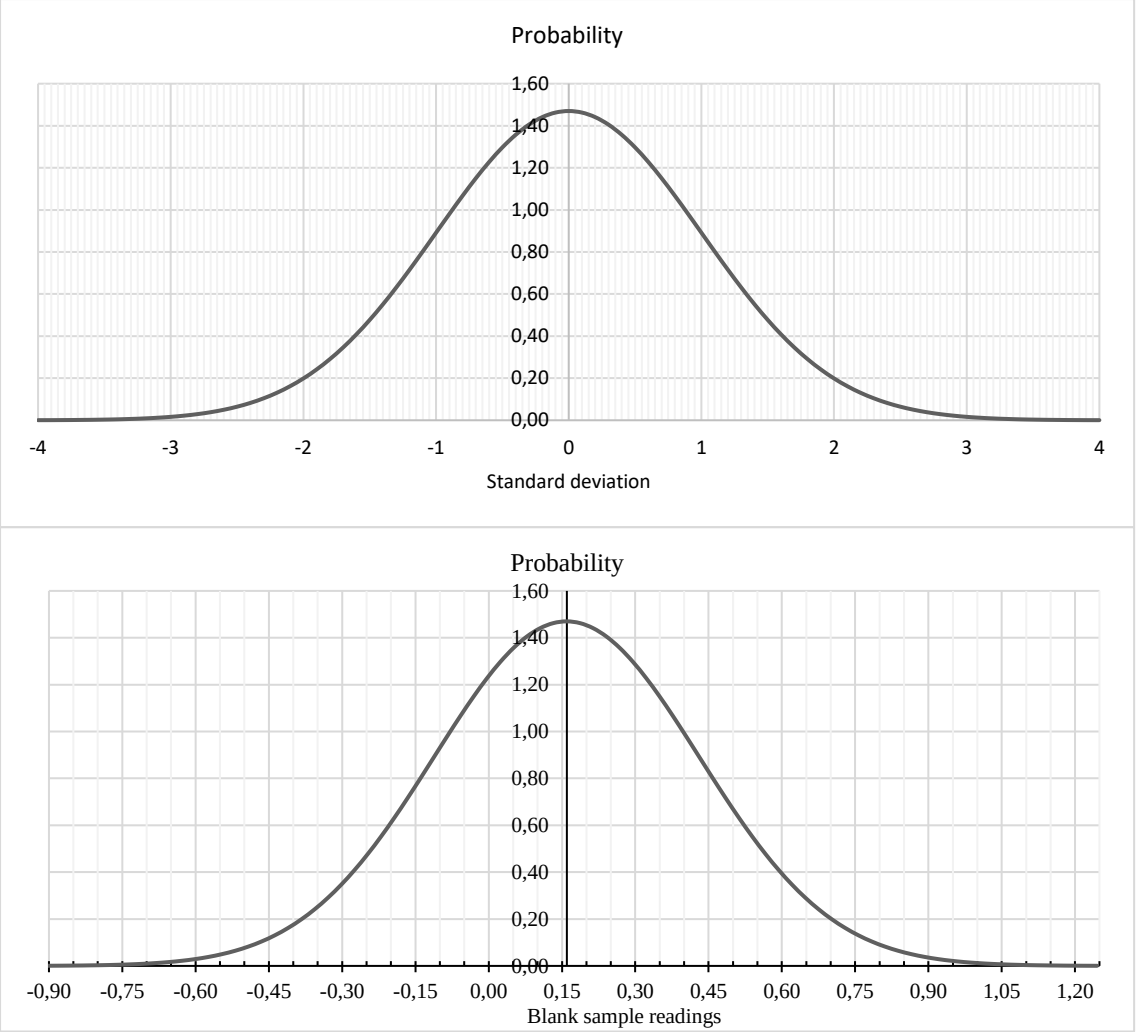


Figure 5. 18: Control chart using blanks (Mean= 0.1595, Standard deviation=0.2714) normal distribution data showing the accuracy and validity of mineral analytical method.

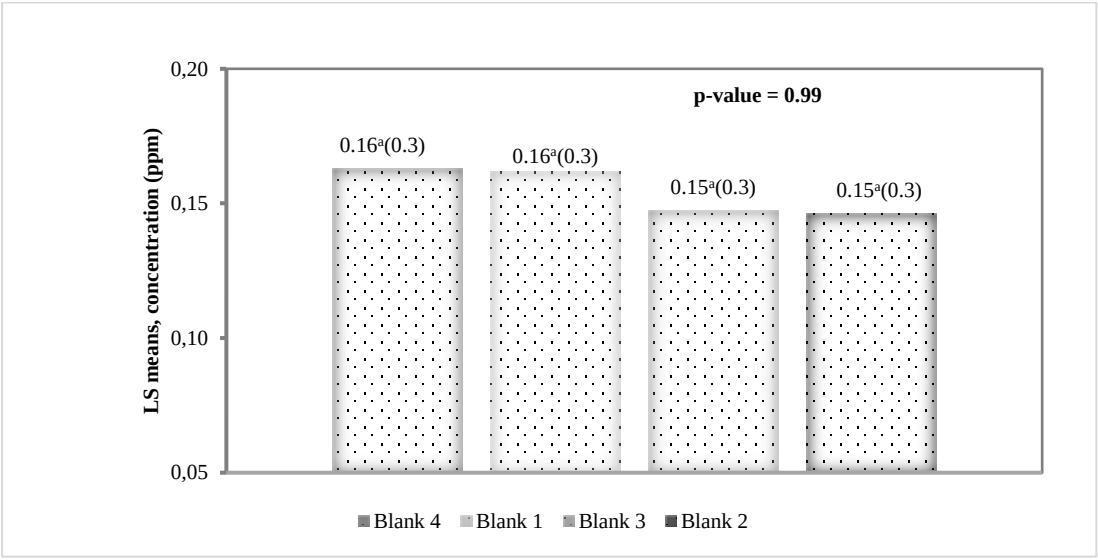


Figure 5.19: LS means (Ca, Fe, Mg, Mn, Zn, P, K, and Na) chart (± standard deviation) using the four repeated readings of the blank samples.

5.2 Research findings

Stinging nettle leaves are taken through some kind of processing (e.g. cooking, infusions, decoctions, drying) to improve their palatability. However, such processing affects levels of nutrients and other components such as phenolic compounds and can also bring about changes in quality aspects such as flavour and texture of the product. Chemical changes during drying and cooking can result in sensorial, nutritional and textural alterations of the food product (Otavio Minatel et al., 2017). For example, cooking can result in either formation or dissociation of the bond between the glycosyl residue and the flavonoid aglycone, leading to the different flavonoid derivatives that affect the sensorial properties (e.g. colour, aroma, taste and astringency) of the food product (Giada, 2013).

Change in sensory properties were observed when fresh or oven-dried stinging nettle leaves were cooked as relish or infused (Research chapter 4.1). Although the colour changed, most of the characteristic green type aroma and flavour notes of fresh nettle leaves were preserved in cooked leaves and leaf infusions prepared from dried leaves with exception of a few. This is possibly due to decomposition of heat sensitive metabolites such as fatty acids, amino acids, carotenoids and ascorbic acid during oven drying processes. This leads to variation in volatile compounds, non-volatile compounds, soluble phenolic compounds and sugars content of cooked leaves and leaf infusions made from oven dried stinging nettle leaves compared to the ones made using fresh leaves.

In the investigation about the effect of drying on nutritional properties of stinging nettle leaves (Research chapter 4.2), it was observed that the ascorbic acid and β-carotene content of fresh nettle leaves was higher compared to oven dried leaves. In contrast to the ascorbic acid and β-carotene content, oven drying of stinging nettle leaves led to an increase in total phenol content and total antioxidant activity compared to fresh leaves. This is probably because heating during oven drying could cause either loss or formation of new volatiles as a result of oxidation and/or esterification reactions of the bioactive compounds in food products (Díaz-Maroto et al., 2002; King et al., 2006; Orphanides et al., 2013; Shilton, 2003).

Volatile compounds or non-volatile componds can be generated during thermal processing (e.g oven drying and cooking) via enzymatic, redox, or pyrolytic reactions of amino acids (Arimura et al., 2001), fatty acids (Owuor, 2003), and carotenoids (Giada, 2013; Goff and Klee, 2006; Owuor, 2003; Rodriguez-amaya, 1997). Lee (2009) stated that the interactions of a wide range of volatiles as well as non-volatile compounds (e.g. phenolic compounds such as catechins and tannins, as well as other compounds such as caffeine, amino acids, and sugars) contribute to the flavour of green tea.

Studies have reported that fresh stinging nettle leaves contain fatty acids (e.g. linoleic acid and linolenic acid) (Farag et al., 2013; Guil-Guerrero et al., 2003; Rutto et al., 2013); amino acids (e.g. valine, leucine, isoleucine, and phenylalanine) (Hughes et al., 1980; Rutto et al., 2013); and carotenoids (Guil-Guerrero et al., 2003). These compounds could be decomposed during oven drying of nettle leaves and might be responsible for the noted variability in aroma and flavour of cooked leaves and leaf infusions. This could possibly explain why fermented aroma, burnt flavour, and bitter and salty taste were more intense in cooked leaves from oven dried leaves compared to fresh leaves. For example, volatiles (e.g. 2-methylpropanal, 2-methylbutanal, pentanal, and phenyl acetaldehyde) are generated from amino acids such as valine, leucine, isoleucine, and phenylalanine (Owuor, 2003). Fermented aroma note is related with methylbutanal in dried and roasted nori (*Porphyra yezoensis*) (Shu and Shen, 2012).

Phenolic compounds are also an important contributor to flavour. For example, caffeic acid, chlorogenic acid and naringin are responsible for bitterness in green tea (Lee et al., 2013). In fresh nettle leaves cinnamic acid derivatives such as caffeic acid and chlorogenic acid were found to be abundant (Farag et al., 2013; Otles and Yalcin, 2012; Pinelli et al., 2008). However, cinnamic acid derivatives such as chlorogenic acid are mostly in the form of esters (e.g. isochlorogenic acid, neochlorogenic acid and cripto chlorogenic acid) (Bravo et al., 2013; Bravo, 2009; Yang et al., 2001). For example, the cleavage of cinamic acids-methyl bonds in 4-Methoxycinnamic acid [the bond between the -OH (hydroxyl) group of cinamic acid and -O-methyl (methoxy) group] with heat treatment such as oven drying can lead to the formation of free forms of cinnamic acid derivatives such as caffeic acid and chlorogenic acid (see Figure 5.19). This probably explains why cooked leaves and leaf infusions made from oven dried leaves were more bitter than those made from fresh leaves.

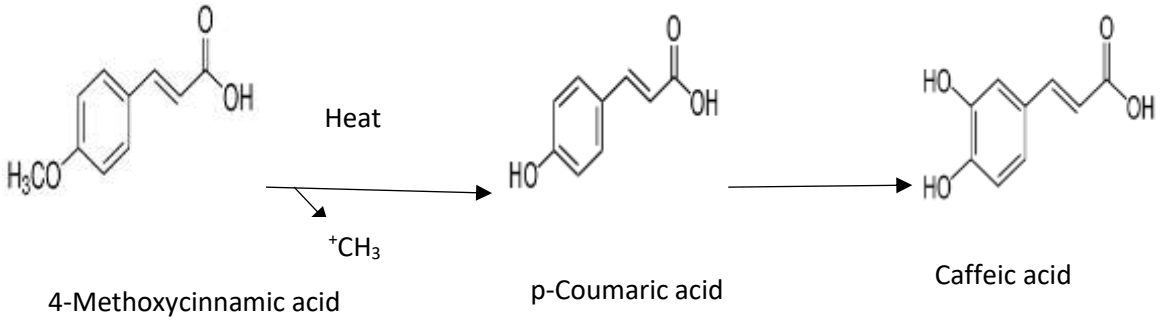


Figure 5.20: The cleavage of cinnamic acids-methyl bonds in 4-Methoxycinnamic acid [the bond between the -OH (hydroxyl) group of cinnamic acid and -O-methyl (methoxy) group] with heat treatment

In general, flavours and aroma were perceived more intense in cooked stinging nettle leaves compared to leaf infusions. This can be clearly viewed from the principal component analysis (PCA) chart (Figure 5.21). The PC plot suggested that cooked leaves from stinging nettle and spinach leaves had higher scores for the attributes (asparagus-woody, grassy, spinach, fishy, bitter and astringent) loading positively on PC1 and PC2 compared to lower scores for leaf infusions for these attributes.

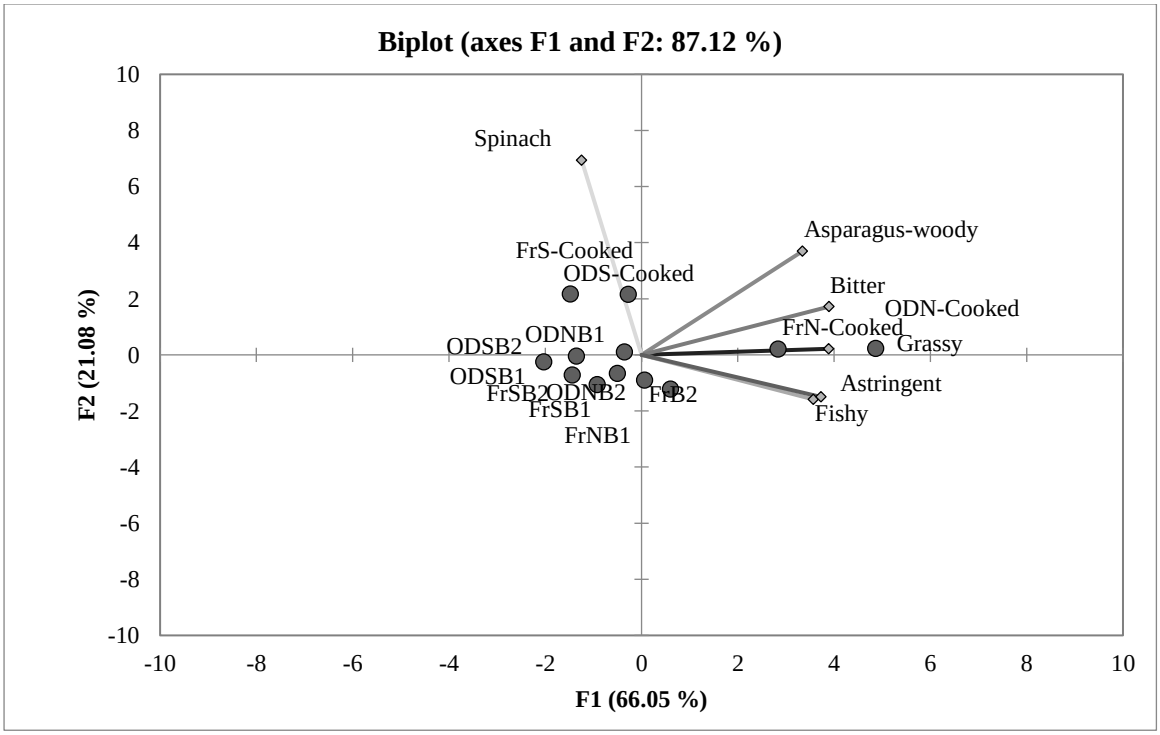


Figure 5.21: PCA scores loadings plot showing of the selected flavour descriptors in cooked leaves and leaf infusions from fresh (Fr) and oven dried (OD) nettle and spinach leaves. N-nettle leaves, S-spinach leaves, B1 and B2 are 1st and 2nd brews.

The effect of oral processing and the extent of heat involved in the preparation of the products could be a reason for such variability in sensory perception between cooked leaves and leaf infusions. The importance of oral processing to food flavour has been evidenced by many observations of positive correlations between mastication and flavour release from food (Neyraud et al., 2005, 2003). This was probably due to more aromatic compounds being released during mastication of the cooked leaves and perceived retronasally. Laboured mastication destroys the cellular structure and exposes the macromolecules to salivary enzymes such as amylase (for starch hydrolysis), lysozyme (breaks down polysaccharides in the cell walls), lingual lipase (for fat hydrolysis), and proteases (for protein hydrolysis). This could release and solubilize the complex structural aromatic compounds and enhance retronasal perception. This could probably be due to the role of saliva in the perception of bitter, sour and salty tastes during mastication (Neyraud and Dransfield, 2004) and enhancing the taste of the food (Humphrey and Williamson, 2001).

Cooking is accomplished by constant boiling where the plant material is exposed to boiling temperature for a particular time period, while with infusions, the plant material remains suspended in hot water for a specified time with a gradual drop in temperature (Courtine, 1984). This possibly results in decomposition of more bioactive compounds leading to formation or loss of volatile or non-volatile compounds during cooking of stinging leaves compared to leaf infusions. Rutto et al. (2013) reported that cooking of stinging nettle leaves resulted in a significant loss of β-carotene, ascorbic acid, amino acids and fatty acids.

In the investigation about the effect of type of extraction (e.g. infusion, decoction) on the ascorbic acid, β-carotene, total phenol content, antioxidant activity stinging nettle leaf powder manufactured using freeze drying or oven drying (Research chapter 4.3), it was observed that the extraction efficiency of ascorbic acid and β-carotene were significantly higher in infusions compared to decoctions (Table 5.6). The extraction efficiency of ascorbic acid and β-carotene was found to be lower than 100 % whereas it was greater than 100 % for total phenol content and antioxidants. An extraction efficiency lower than 100 % could indicates a loss of nutrients during oven drying processes. This could mean that there was a higher loss of ascorbic acid and β-carotene during the boiling processes of decoction compared to the steeping processes involed in infusion.

Table 5.6: Effect of extraction types on the percent extraction efficiency (% EE[*]± standard deviation) of β-carotene, ascorbic acid, total phenol content, and total antioxidant activity of stinging nettle and spinach leaf powder manufactured using freeze drying or oven drying

Extraction types (ET)	Decoction				Infusion				p-values						
Drying method (DM)	Freeze dried		Oven dried		Freeze dried		Oven dried								
Species (SP)	Nettle	Spinach	Nettle	Spinach	Nettle	Spinach	Nettle	Spinach	ET	DM	SP	ET x DM	ET x SP	DM x SP	ET x DM x SP
β-carotene	60.9^{d}(0.7)	61.4^{d}(0.6)	57.0^{e}(0.6)	51.9^{f}(0.8)	73.2^{b}(0.4)	83.6^{a}(0.3)	71.3^{c}(0.3)	61.3^{d}(0.7)	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Ascorbic acid	79.2^{e}(0.9)	74.0^{f}(2.9)	88.7^{d}(0.5)	54.5^{g}(1.6)	93.6^{bc}(0.2)	91.0^{cd}(2.5)	96.6^{ab}(0.1)	98.4^{a} (3.5)	0.01	0.01	0.91	0.01	0.01	0.01	0.01
TPC	157.3^{a}(2.3)	109.7^{d}(2.1)	155.0^{a}(1.6)	132.7^{c}(4.2)	139.8^{b}(6.7)	100.4^{e}(2.0)	152.1^{a}(3.1)	107.6^{d}(0.4)	0.01	0.01	0.01	0.02	0.84	0.01	0.01
TAA	118.8^{ab}(3.3)	118.2^{b}(4.0)	122.9^{a}(1.1)	115.7^{b}(0.6)	106.9^{c}(2.8)	117.0^{b}(1.7)	109.4^{c}(1.8)	107.9^{c}(2.3)	0.01	0.85	0.22	0.01	0.06	0.01	0.22

[ab] Means within a row not sharing a superscript letter are significantly different ($p < 0.05$).

[*] Percentage extraction efficiency (% EE) of the nutrients from stinging nettle or spinach leaf powder into infusions or decoctions was calculated as described by Ammar et al. (2015) using the folloing formula (Equation 9):

$$\% EE = \frac{Nutrient\ content\ in\ infusions\ or\ decoctions(b)}{Nutrient\ contnet\ in\ the\ dried\ leaves\ (a)}\ x\ 100 \qquad (9)$$

Where. a = The nutrient content of freeze dried and oven dried nettle or spinach leaves (Research chapter 4.2, see Table 4.9)

b = The nutrient content of infusions and decoctions (Research chapter 4.3, see Table 4.11)

The decoction process could result in higher degradation of β-carotene compared to infusions to produce volatile compounds such as β-ionone, 3-oxo-β-ionone, β-ionone, α-ionone, 3-hydroxy-β-ionone and other terpenoid aldehydes and ketones (Owuor, 2003), probably due to its highly unsaturated structure (Bernhardt and Schlich, 2006; Di Cesare et al., 2004; Kidmose et al., 2000; Owuor, 2003; Rodriguez-amaya, 1997). Similarly, decoction probably resulted in a higher oxidation of ascorbic acid to dehydro-ascorbic acid compared to infusions because of the two hydroxyl groups in its structure, enzymatic degradation of protein-ascorbic acid aggregates and heat labile nature (Ajayi et al., 1980; Harbourne et al., 2009; Sanmartin et al., 2000; Shilton, 2003; Waheed Uz et al., 2013). It is reported that the ability of ascorbic acid to scavenge free radicals is due to the fact that ascorbic acid can regenerate antioxidants such as alpha-tocopherol (Halliwell and Gutteridge, 2015). This is probably also one of the reasons why the extraction efficiency of total phenol content and antioxidants were higher in decoctions compared to infusions.

Additionally, the high extraction efficiency of total phenol content and antioxidant activity ($p < 0.01$) in decoctions compared to infusions, could be due to the breakdown of proteins complexes containing polyphenolic compounds. Several research studies have reported an increase of phenolic compound levels and antioxidant activity after heating treatments in different vegetables (Drinkwater et al., 2015; Leong and Oey, 2012; Murador et al., 2016; Palermo et al., 2014). The high temperatures involved during cooking or drying processes can cause the release of phenolics found linked to the cell membranes/walls (e.g. condensed tannins, phenolic acids) (Giada, 2013; Otavio Minatel et al., 2017). This could result in an increase in total phenol content due to cleavage of phenolic-sugar glycosidic bonds due to heating and the inhibition of oxidative enzymes (Yamaguchi, Mizobuchi and Kajikawa, 2001). If the phenolic compound under consideration is a flavonoid glycoside, the cleavage of phenolic-sugar glycosidic bonds with heat treatment can lead to the formation of flavonoid aglycones which have high reactivity with the Folin–Ciocalteu reagent leading to increased values of total phenolics (Singleton et al., 1999).

The antioxidant activity of phenolic compounds can be explained by their possession of a benzene ring, a carboxylic group and one or more hydroxyl and/or methoxyl groups in the molecule (Yang et al., 2001).

However, the effectiveness of the phenolic compounds as antioxidants decreases with the substitution of hydroxyl groups with sugars (glycosyl residue), proteins, oligosaccharides, lipids, amines, carboxylic acids and organic acids and forming complexes (Duthie et al., 2017; Rice-Evans et al., 1996). For example, tannins due to the large number of hydroxyl groups contained therein are capable of forming strong complexes with proteins, starch, lipids and other molecules (Giada, 2013). Tannins have the ability to chelate Fe and Zn by joining with their hydroxyl and carbonyl groups and thereby reduces the bioavailability of these minerals (Bravo, 2009). This also reduces the number of hydroxyl and carbonyl groups from tannins available to exert antioxidant effects. Studies have reported a strong correlation between total phenol content and antioxidant activity. Flavonoids, tannins chalcones and coumarins as well as phenolic acid (cinnamic and benzoic acid, esters of caffeic acid, chlorogenic acid and ferulic acid) have antioxidant activity (Giada, 2013; Rincón-León et al., 2003; Ma et al., 2013; Marja et al., 1999; Wang et al., 1996).

Although oven drying of stinging nettle leaves resulted in a higher loss of β-carotene and ascorbic acid content compared to freeze drying, appreciable amounts of ascorbic acid and β-carotene were retained in both oven dried leaves (72 %, 90 % retention) and freeze dried leaves (88 %, 97 % retention), respectively as shown in Table 5.7. The high retention of β-carotene might help to improve vitamin A intake as it was reported that β-carotene has 100 % vitamin A activity (Furr and Clark, 2003; Krinsky and Johnson, 2005; Rodriguez-amaya, 1997). This has implications for the potential contribution of stinging nettle leaf food products to dietary intakes of nutrients in the human diet. All in all, fresh stinging nettle leaves can be considered as a rich source of antioxidants, Ca, Mg, vitamin A and C; a good source of Fe and Mn; and a source of P and K. Freeze dried and oven dried stinging nettle leaves can be considered as rich sources of antioxidants, Ca, Mg, and vitamin A and good sources of vitamin C, Fe and Mn. Interestingly, the retention of total phenol content and antioxidant activity was found to be greater than 100 %. This probably mean that drying of stinging nettle leaves increased the extractability of assayable phenolic compounds and antioxidants (e.g. formation of more free or unbound phenolic compounds). Infusions and decoctions from stinging nettle leaf powder can be considered as sources of dietary antioxidants which could be due to high total phenol content.

Table 5.7: Effect of drying method on the apparent retention (% AR*± standard deviation) of β-carotene, ascorbic acid, total phenol content, total antioxidant activity of stinging nettle and spinach leaves

Drying method (DM)	Freeze dried		Oven dried		p-values		
Species (SP)	Nettle	Spinach	Nettle	Spinach	SP	DM	SP x DM
β-carotene	97.0 [b](0.4)	98.6[a](0.4)	90.4[d](0.4)	92.5[c](0.5)	0.01	0.01	0.46
Ascorbic acid	88.5[b](0.7)	98.3[a](1.5)	72.4[c](3.2)	84.8[b](2.1)	0.01	0.01	0.42
TPC	102.7[b](3.8)	127.3[a](4.3)	108.7[b](1.8)	135.1[a](6.4)	0.01	0.02	0.73
TAA	102.4[b](5.2)	104.2[b](4.1)	108.6[b](1.4)	123.2[a] (3.7)	0.01	0.01	0.02

[a-d] Means within same row with different superscripts are different ($P < 0.05$) when analysed using analysis of variance.

[*] Percent apparent retention of β-carotene, ascorbic acid, total phenol content and total antioxidant activity in dried leaves was calculated as described by Murphy et al. (1975) using the following formulas (Equation 10)

$$\% \text{ AR} = \frac{\text{Amount of the nutrient in dried leaves (dry basis)}}{\text{Amount of the nutrient in fresh leaves (dry basis)}} \text{ x } 100 \qquad (10)$$

i.e. The calculation of % AR was done using the nutrient content of fresh, freeze dried and oven dried nettle or spinach leaves (Research chapter 4.2, Table 4.9)

5.3 Areas for further research

Stinging nettle leaves (underutilised and indigenous wild leafy vegetable in Africa) have unique sensory properties as discussed in Research chapter 4.1 and nutritional properties with health-promoting potential (Research chapter 4.2 and Research chapter 4.3). Potential use of the leaves as a natural fortificant offers opportunities for increasing the micronutrient content of food products. The observed antioxidant properties also offers opportunities for inhibiting lipid peroxidation in oils and fat containing food products. Nevertheless, there are problems in commercialization of stinging nettle, mainly because of lack of sufficient experimental data.

For example, Kumar et al. (2015) reported that antioxidants from natural sources are preferred over synthetic antioxidants (such as butylated hydroxy anisole, butylated hydroxy toluene, teriary butyl hydro quinone, and propyl gallate) in food industries because of reported negative health consequences. Lindsey, Motsei and Jäger (2002) suggested that nettle leaves may not only be a good dietary source of nutrients but could also be used as a natural antioxidant in the food industry. Applications of the use of extracts from herbs like oregano in meat and poultry products have been well demonstrated (Rojas and Brewer, 2008).

Inclusion of β-carotene-rich food in the daily diets, instead of costly synthetic vitamin A supplementation, may also be a more successful strategy for improving vitamin A status of at risk or malnourished populations (Gopalan, 1992). In sub-Saharan Africa a large proportion of households is poor, food insecure and depend on a diet composed primarily of starchy staples (e.g. cereals such as sorghum, cassava, teff, maize, rice) which are generally low in micronutrients (IFPRI, 2011).

Integrating either fresh or dried stinging nettle leaves in the diet or utilization of dried nettles leaves for fortifying cereal based foods would help address micronutrient deficiencies. However, integrating stinging nettle leaves in food products may affect sensory quality attributes positively or negatively and ultimately affect consumer acceptability of the product. For example, it has been shown that incorporating herbs in meat products caused changes in the colour, flavour, texture and emulsion properties of the end products (Kumar et al., 2015)

For stinging nettle leaves as a new source of natural antioxidant and micronutrients for use at small, medium, or commercial level, the following should be considered:

1. The effect of incorporating stinging nettle leaves in food products on colour, nutritional and sensory properties and acceptability by consumers should be evaluated. For example, further research should determine which sensory properties of the products from the nettle plant drives liking or disliking by target consumers as well as profiling of the volatile compounds in stinging nettle leaves and leaf infusions.
2. Stinging nettles have antimicrobial effects due to the phenolic contents (Gülçin et al., 2004). Application of stinging nettles leaf extracts to inhibit microorganisms as *Clostridium botulinum* and *Staphylococcus aureus* in low acid processed foods could also be another area of research.

In general, the potential contribution of stinging nettle to food and nutrition security, and to the well-being of consumers should be considered in the face of growing environmental and socio-economic changes.

6: CONCLUSIONS AND RECOMMENDATIONS

A total of 19 aroma and 26 flavour descriptors for cooked leaves and 20 aroma and 25 flavour descriptors for leaf infusions from two subsequent brews were developed and used to characterize the sensory properties of nettle products from fresh and oven dried leaves. Cooked nettle leaves are differentiated from spinach, a popular leafy vegetable, due to its grassy, asparagus-woody, seafood, fishy, fermented, mint and citrus aroma and flavours, as well as higher bitterness and astringency. Similarly, the aroma and flavour of nettle leaf infusions are more burnt and fishy and it tastes bitterer than spinach. Drying the leaves results in products with a more intense fermented aroma, burnt flavour, and bitter and salty tastes. Drying nettle leaves reduces the overall aroma and flavour of infusions. Nettle leaves can be brewed twice without much difference in aroma and flavour. It is important to note however that drying of leaves changes the colour of the cooked product and infusions. This baseline sensory information could be utilized to describe, compare, and differentiate the characteristics of various nettle food products around the world. Further research should determine which sensory properties of the products from the nettle plant drives liking or disliking by target consumers as well as profiling of the volatile compounds in stinging nettle leaves and leaf infusions.

In terms of nutritional properties, β-carotene, ascorbic acid, total antioxidant activity and total phenol content are affected significantly by drying, infusion and decoction processes. 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 still retained in the oven dried leaves. In contrast, oven drying increases the total antioxidant activity and phenol content of nettle leaves compared to freeze drying. Overall, freeze dried and oven dried nettle leaves can be considered rich sources of Ca, Mg, vitamin A, total phenol content and total antioxidant activity and good sources of Fe, Mn and vitamin C. Decoction is most efficient if high total antioxidant activity and phenol content is sought whereas infusion is the most efficient mode of extraction for ascorbic acid and β-carotene. These benefits present possible avenues for utilization of oven dried nettle leaves by the food industry and consumers for addressing micronutrient deficiency and for providing healthy diet from natural underutilised sources. This research contributes to the understanding of the potential of stinging nettle for addressing food and nutrition security.

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8: PUBLICATIONS AND CONFERENCE PRESENTAIONS BASED ON THIS RESEARCH

Publication

Shonte, T.T. and De Kock, H.L., 2017. Descriptive sensory evaluation of cooked stinging nettle (*Urtica dioica* L.) leaves and leaf infusions: Effect of using fresh or oven-dried leaves. South African Journal of Botany, 110, pp.167-176.

Oral presentations

Shonte, T.T, de Kock, H.L., 2016. Sensory properties of cooked leaves and leaf infusions from stinging nettle. 14th SAAFFI seminar "One Africa" held on 3rd March 2016 at the Bytes Conference Centre in Midrand, South Africa.

Shonte, T.T, de Kock, H.L., 2015. Sensory properties of cooked leaves and leaf infusions from stinging nettle. 1st AfroSense conference of new techniques and trends for sensory characterization & consumer profiling held from 23 to 26 November 2015 at STIAS, Stellenbosch University, South Africa.

Shonte, T.T, de Kock, H.L., 2015. Sensory properties of cooked leaves and leaf infusions from stinging nettle. "The 21st SAAFoST (South Africa Association of Food Science and Technology) Biennial International Congress and Exhibition" from 6-9 September 2015, Durban.

Poster presentation

Shonte, T.T, de Kock, H.L., 2016. Nutritional Properties of Stinging Nettle (*U. diocea* L) Cooked Leaves and Leaf Infusions: A Potential Food Source to Address Malnutrition. The 2016 Autumn International Scientific Conference on Food Safety and Security Conference (FSaS), University of Johannesburg, South Africa from 16th- 18th May 2016.

9: APPENDICES

Table 9.1: Blanks (Mean= 0.1595, Standard deviation=0.2714) normal distribution data to proof the accuracy and validity of the mineral analytical method

Standard deviation	Concentration, ppm	Probability	Cumulative	%
4	1.25	0.00	1.00	100.00
3.9	1.22	0.00	1.00	100.00
3.8	1.19	0.00	1.00	99.99
3.7	1.16	0.00	1.00	99.99
3.6	1.14	0.00	1.00	99.98
3.5	1.11	0.00	1.00	99.98
3.4	1.08	0.00	1.00	99.97
3.3	1.06	0.01	1.00	99.95
3.2	1.03	0.01	1.00	99.93
3.1	1.00	0.01	1.00	99.90
3	0.97	0.02	1.00	99.87
2.9	0.95	0.02	1.00	99.81
2.8	0.92	0.03	1.00	99.74
2.7	0.89	0.04	1.00	99.65
2.6	0.87	0.05	1.00	99.53
2.5	0.84	0.06	0.99	99.38
2.4	0.81	0.08	0.99	99.18
2.3	0.78	0.10	0.99	98.93
2.2	0.76	0.13	0.99	98.61
2.1	0.73	0.16	0.98	98.21
2	0.70	0.20	0.98	97.72
1.9	0.68	0.24	0.97	97.13
1.8	0.65	0.29	0.96	96.41
1.7	0.62	0.35	0.96	95.54
1.6	0.59	0.41	0.95	94.52
1.5	0.57	0.48	0.93	93.32
1.4	0.54	0.55	0.92	91.92
1.3	0.51	0.63	0.90	90.32
1.2	0.49	0.72	0.88	88.49
1.1	0.46	0.80	0.86	86.43
1	0.43	0.89	0.84	84.13
0.9	0.40	0.98	0.82	81.59
0.8	0.38	1.07	0.79	78.81
0.7	0.35	1.15	0.76	75.80
0.6	0.32	1.23	0.73	72.57
0.5	0.30	1.30	0.69	69.15
0.4	0.27	1.36	0.66	65.54
0.3	0.24	1.41	0.62	61.79
0.2	0.21	1.44	0.58	57.93
0.1	0.19	1.46	0.54	53.98
0	**0.16**	**1.47**	**0.50**	**50.00**
-0.1	0.13	1.46	0.46	46.02
-0.2	0.11	1.44	0.42	42.07
-0.3	0.08	1.41	0.38	38.21
-0.4	0.05	1.36	0.34	34.46
-0.5	0.02	1.30	0.31	30.85
-0.6	0.00	1.23	0.27	27.43
-0.7	-0.03	1.15	0.24	24.20
-0.8	-0.06	1.07	0.21	21.19

-0.9	-0.08	0.98	0.18	18.41
-1	-0.11	0.89	0.16	15.87
-1.1	-0.14	0.80	0.14	13.57
-1.2	-0.17	0.72	0.12	11.51
-1.3	-0.19	0.63	0.10	9.68
-1.4	-0.22	0.55	0.08	8.08
-1.5	-0.25	0.48	0.07	6.68
-1.6	-0.27	0.41	0.05	5.48
-1.7	-0.30	0.35	0.04	4.46
-1.8	-0.33	0.29	0.04	3.59
-1.9	-0.36	0.24	0.03	2.87
-2	-0.38	0.20	0.02	2.28
-2.1	-0.41	0.16	0.02	1.79
-2.2	-0.44	0.13	0.01	1.39
-2.3	-0.46	0.10	0.01	1.07
-2.4	-0.49	0.08	0.01	0.82
-2.5	-0.52	0.06	0.01	0.62
-2.6	-0.55	0.05	0.00	0.47
-2.7	-0.57	0.04	0.00	0.35
-2.8	-0.60	0.03	0.00	0.26
-2.9	-0.63	0.02	0.00	0.19
-3	-0.65	0.02	0.00	0.13
-3.1	-0.68	0.01	0.00	0.10
-3.2	-0.71	0.01	0.00	0.07
-3.3	-0.74	0.01	0.00	0.05
-3.4	-0.76	0.00	0.00	0.03
-3.5	-0.79	0.00	0.00	0.02
-3.6	-0.82	0.00	0.00	0.02
-3.7	-0.84	0.00	0.00	0.01
-3.8	-0.87	0.00	0.00	0.01
-3.9	-0.90	0.00	0.00	0.00
-4	-0.93	0.00	0.00	0.00

Table 9.2: L-ascorbic acid standards (Mean= 6287555, Standard deviation=4550415) normal distribution data to proof the accuracy and validity of the ascorbic acid analytical method

Standard deviation	Peak area	Probability	Cumulative	%
4	24489214	0.0000000000	1.000	100.00
3.9	24034173	0.0000000000	1.000	100.00
3.8	23579131	0.0000000001	1.000	99.99
3.7	23124090	0.0000000001	1.000	99.99
3.6	22669048	0.0000000001	1.000	99.98
3.5	22214007	0.0000000002	1.000	99.98
3.4	21758965	0.0000000003	1.000	99.97
3.3	21303924	0.0000000004	1.000	99.95
3.2	20848882	0.0000000005	0.999	99.93
3.1	20393841	0.0000000007	0.999	99.90
3	19938799	0.0000000010	0.999	99.87
2.9	19483758	0.0000000013	0.998	99.81
2.8	19028716	0.0000000017	0.997	99.74
2.7	18573675	0.0000000023	0.997	99.65
2.6	18118633	0.0000000030	0.995	99.53
2.5	17663592	0.0000000039	0.994	99.38
2.4	17208550	0.0000000049	0.992	99.18
2.3	16753509	0.0000000062	0.989	98.93
2.2	16298467	0.0000000078	0.986	98.61
2.1	15843426	0.0000000097	0.982	98.21
2	15388384	0.0000000119	0.977	97.72
1.9	14933343	0.0000000144	0.971	97.13
1.8	14478301	0.0000000174	0.964	96.41
1.7	14023260	0.0000000207	0.955	95.54
1.6	13568218	0.0000000244	0.945	94.52
1.5	13113177	0.0000000285	0.933	93.32
1.4	12658135	0.0000000329	0.919	91.92
1.3	12203094	0.0000000377	0.903	90.32
1.2	11748052	0.0000000427	0.885	88.49
1.1	11293011	0.0000000479	0.864	86.43
1	10837969	0.0000000532	0.841	84.13
0.9	10382928	0.0000000585	0.816	81.59
0.8	9927886	0.0000000637	0.788	78.81
0.7	9472845	0.0000000686	0.758	75.80
0.6	9017804	0.0000000732	0.726	72.57
0.5	8562762	0.0000000774	0.691	69.15
0.4	8107721	0.0000000809	0.655	65.54
0.3	7652679	0.0000000838	0.618	61.79
0.2	7197638	0.0000000859	0.579	57.93
0.1	6742596	0.0000000872	0.540	53.98
0	**6287555**	**0.0000000877**	**0.500**	**50.00**
-0.1	5832513	0.0000000872	0.460	46.02
-0.2	5377472	0.0000000859	0.421	42.07
-0.3	4922430	0.0000000838	0.382	38.21
-0.4	4467389	0.0000000809	0.345	34.46
-0.5	4012347	0.0000000774	0.309	30.85
-0.6	3557306	0.0000000732	0.274	27.43
-0.7	3102264	0.0000000686	0.242	24.20
-0.8	2647223	0.0000000637	0.212	21.19
-0.9	2192181	0.0000000585	0.184	18.41

-1	1737140	0.0000000532	0.159	15.87
-1.1	1282098	0.0000000479	0.136	13.57
-1.2	827057	0.0000000427	0.115	11.51
-1.3	372015	0.0000000377	0.097	9.68
-1.4	-83026	0.0000000329	0.081	8.08
-1.5	-538068	0.0000000285	0.067	6.68
-1.6	-993109	0.0000000244	0.055	5.48
-1.7	-1448151	0.0000000207	0.045	4.46
-1.8	-1903192	0.0000000174	0.036	3.59
-1.9	-2358234	0.0000000144	0.029	2.87
-2	-2813275	0.0000000119	0.023	2.28
-2.1	-3268317	0.0000000097	0.018	1.79
-2.2	-3723358	0.0000000078	0.014	1.39
-2.3	-4178400	0.0000000062	0.011	1.07
-2.4	-4633441	0.0000000049	0.008	0.82
-2.5	-5088483	0.0000000039	0.006	0.62
-2.6	-5543524	0.0000000030	0.005	0.47
-2.7	-5998566	0.0000000023	0.003	0.35
-2.8	-6453607	0.0000000017	0.003	0.26
-2.9	-6908649	0.0000000013	0.002	0.19
-3	-7363690	0.0000000010	0.001	0.13
-3.1	-7818732	0.0000000007	0.001	0.10
-3.2	-8273773	0.0000000005	0.001	0.07
-3.3	-8728815	0.0000000004	0.000	0.05
-3.4	-9183856	0.0000000003	0.000	0.03
-3.5	-9638898	0.0000000002	0.000	0.02
-3.6	-10093939	0.0000000001	0.000	0.02
-3.7	-10548980	0.0000000001	0.000	0.01
-3.8	-11004022	0.0000000001	0.000	0.01
-3.9	-11459063	0.0000000000	0.000	0.00
-4	-11914105	0.0000000000	0.000	0.00

Table 9.3: DPPH control (Mean= 1.009, Standard deviation=0.020) normal distribution data to proof the accuracy and validity of the total antioxidant activity/DPPH analytical method

Standard deviation	Absorbance reading , 517 nm	Probability	Cumulative	%
4	1.090	0.01	1.00	100.00
3.9	1.088	0.01	1.00	100.00
3.8	1.086	0.01	1.00	99.99
3.7	1.083	0.02	1.00	99.99
3.6	1.081	0.03	1.00	99.98
3.5	1.079	0.04	1.00	99.98
3.4	1.077	0.06	1.00	99.97
3.3	1.075	0.09	1.00	99.95
3.2	1.073	0.12	1.00	99.93
3.1	1.071	0.16	1.00	99.90
3	1.069	0.22	1.00	99.87
2.9	1.067	0.30	1.00	99.81
2.8	1.065	0.39	1.00	99.74
2.7	1.063	0.52	1.00	99.65
2.6	1.061	0.68	1.00	99.53
2.5	1.059	0.87	0.99	99.38
2.4	1.057	1.11	0.99	99.18
2.3	1.055	1.41	0.99	98.93
2.2	1.053	1.77	0.99	98.61
2.1	1.051	2.19	0.98	98.21
2	1.049	2.69	0.98	97.72
1.9	1.047	3.27	0.97	97.13
1.8	1.045	3.93	0.96	96.41
1.7	1.043	4.68	0.96	95.54
1.6	1.041	5.52	0.95	94.52
1.5	1.039	6.45	0.93	93.32
1.4	1.037	7.45	0.92	91.92
1.3	1.035	8.53	0.90	90.32
1.2	1.033	9.66	0.88	88.49
1.1	1.031	10.84	0.86	86.43
1	1.029	12.04	0.84	84.13
0.9	1.027	13.24	0.82	81.59
0.8	1.025	14.42	0.79	78.81
0.7	1.023	15.54	0.76	75.80
0.6	1.021	16.58	0.73	72.57
0.5	1.019	17.52	0.69	69.15
0.4	1.017	18.33	0.66	65.54
0.3	1.015	18.98	0.62	61.79
0.2	1.013	19.46	0.58	57.93
0.1	1.011	19.75	0.54	53.98
0	**1.009**	**19.85**	**0.50**	**50.00**
-0.1	1.007	19.75	0.46	46.02
-0.2	1.005	19.46	0.42	42.07
-0.3	1.003	18.98	0.38	38.21
-0.4	1.001	18.33	0.34	34.46
-0.5	0.999	17.52	0.31	30.85
-0.6	0.997	16.58	0.27	27.43
-0.7	0.995	15.54	0.24	24.20
-0.8	0.993	14.42	0.21	21.19

-0.9	0.991	13.24	0.18	18.41
-1	0.989	12.04	0.16	15.87
-1.1	0.987	10.84	0.14	13.57
-1.2	0.985	9.66	0.12	11.51
-1.3	0.983	8.53	0.10	9.68
-1.4	0.981	7.45	0.08	8.08
-1.5	0.979	6.45	0.07	6.68
-1.6	0.977	5.52	0.05	5.48
-1.7	0.975	4.68	0.04	4.46
-1.8	0.973	3.93	0.04	3.59
-1.9	0.971	3.27	0.03	2.87
-2	0.969	2.69	0.02	2.28
-2.1	0.967	2.19	0.02	1.79
-2.2	0.965	1.77	0.01	1.39
-2.3	0.963	1.41	0.01	1.07
-2.4	0.961	1.11	0.01	0.82
-2.5	0.959	0.87	0.01	0.62
-2.6	0.957	0.68	0.00	0.47
-2.7	0.955	0.52	0.00	0.35
-2.8	0.953	0.39	0.00	0.26
-2.9	0.951	0.30	0.00	0.19
-3	0.949	0.22	0.00	0.13
-3.1	0.947	0.16	0.00	0.10
-3.2	0.945	0.12	0.00	0.07
-3.3	0.943	0.09	0.00	0.05
-3.4	0.941	0.06	0.00	0.03
-3.5	0.939	0.04	0.00	0.02
-3.6	0.937	0.03	0.00	0.02
-3.7	0.935	0.02	0.00	0.01
-3.8	0.933	0.01	0.00	0.01
-3.9	0.931	0.01	0.00	0.00
-4	0.929	0.01	0.00	0.00

Table 9.4: All-trans-β-carotene standards (Mean= 250956, Standard deviation=118456) normal distribution data to proof the accuracy and validity of the β-carotene analytical method

Standard deviation	Peak area	Probability	Cumulative	%
4	724782	0.00000000	1.00	100.00
3.9	712937	0.00000000	1.00	100.00
3.8	701091	0.00000000	1.00	99.99
3.7	689245	0.00000000	1.00	99.99
3.6	677400	0.00000001	1.00	99.98
3.5	665554	0.00000001	1.00	99.98
3.4	653708	0.00000001	1.00	99.97
3.3	641863	0.00000001	1.00	99.95
3.2	630017	0.00000002	1.00	99.93
3.1	618171	0.00000003	1.00	99.90
3	606326	0.00000004	1.00	99.87
2.9	594480	0.00000005	1.00	99.81
2.8	582634	0.00000007	1.00	99.74
2.7	570789	0.00000009	1.00	99.65
2.6	558943	0.00000011	1.00	99.53
2.5	547098	0.00000015	0.99	99.38
2.4	535252	0.00000019	0.99	99.18
2.3	523406	0.00000024	0.99	98.93
2.2	511561	0.00000030	0.99	98.61
2.1	499715	0.00000037	0.98	98.21
2	487869	0.00000046	0.98	97.72
1.9	476024	0.00000055	0.97	97.13
1.8	464178	0.00000067	0.96	96.41
1.7	452332	0.00000079	0.96	95.54
1.6	440487	0.00000094	0.95	94.52
1.5	428641	0.00000109	0.93	93.32
1.4	416795	0.00000126	0.92	91.92
1.3	404950	0.00000145	0.90	90.32
1.2	393104	0.00000164	0.88	88.49
1.1	381259	0.00000184	0.86	86.43
1	369413	0.00000204	0.84	84.13
0.9	357567	0.00000225	0.82	81.59
0.8	345722	0.00000245	0.79	78.81
0.7	333876	0.00000264	0.76	75.80
0.6	322030	0.00000281	0.73	72.57
0.5	310185	0.00000297	0.69	69.15
0.4	298339	0.00000311	0.66	65.54
0.3	286493	0.00000322	0.62	61.79
0.2	274648	0.00000330	0.58	57.93
0.1	262802	0.00000335	0.54	53.98
0	**250956**	**0.00000337**	**0.50**	**50.00**
-0.1	239111	0.00000335	0.46	46.02
-0.2	227265	0.00000330	0.42	42.07
-0.3	215419	0.00000322	0.38	38.21
-0.4	203574	0.00000311	0.34	34.46
-0.5	191728	0.00000297	0.31	30.85
-0.6	179883	0.00000281	0.27	27.43
-0.7	168037	0.00000264	0.24	24.20
-0.8	156191	0.00000245	0.21	21.19

-0.9	144346	0.00000225	0.18	18.41
-1	132500	0.00000204	0.16	15.87
-1.1	120654	0.00000184	0.14	13.57
-1.2	108809	0.00000164	0.12	11.51
-1.3	96963	0.00000145	0.10	9.68
-1.4	85117	0.00000126	0.08	8.08
-1.5	73272	0.00000109	0.07	6.68
-1.6	61426	0.00000094	0.05	5.48
-1.7	49580	0.00000079	0.04	4.46
-1.8	37735	0.00000067	0.04	3.59
-1.9	25889	0.00000055	0.03	2.87
-2	14044	0.00000046	0.02	2.28
-2.1	2198	0.00000037	0.02	1.79
-2.2	-9648	0.00000030	0.01	1.39
-2.3	-21493	0.00000024	0.01	1.07
-2.4	-33339	0.00000019	0.01	0.82
-2.5	-45185	0.00000015	0.01	0.62
-2.6	-57030	0.00000011	0.00	0.47
-2.7	-68876	0.00000009	0.00	0.35
-2.8	-80722	0.00000007	0.00	0.26
-2.9	-92567	0.00000005	0.00	0.19
-3	-104413	0.00000004	0.00	0.13
-3.1	-116259	0.00000003	0.00	0.10
-3.2	-128104	0.00000002	0.00	0.07
-3.3	-139950	0.00000001	0.00	0.05
-3.4	-151795	0.00000001	0.00	0.03
-3.5	-163641	0.00000001	0.00	0.02
-3.6	-175487	0.00000001	0.00	0.02
-3.7	-187332	0.00000000	0.00	0.01
-3.8	-199178	0.00000000	0.00	0.01
-3.9	-211024	0.00000000	0.00	0.00
-4	-222869	0.00000000	0.00	0.00

Table 9.5: Gallic acid standards (Mean= 1.467, Standard deviation=0.680) normal distribution data to proof the accuracy and validity of the total phenol content analytical method

Stadard deviation	Absorbance readings, 760 nm	Probability	Cumulative	%
4	4.19	0.0002	1.00	100.00
3.9	4.12	0.0003	1.00	100.00
3.8	4.05	0.0004	1.00	99.99
3.7	3.98	0.0006	1.00	99.99
3.6	3.91	0.0009	1.00	99.98
3.5	3.85	0.0013	1.00	99.98
3.4	3.78	0.0018	1.00	99.97
3.3	3.71	0.0025	1.00	99.95
3.2	3.64	0.0035	1.00	99.93
3.1	3.57	0.0048	1.00	99.90
3	3.51	0.0065	1.00	99.87
2.9	3.44	0.0088	1.00	99.81
2.8	3.37	0.0116	1.00	99.74
2.7	3.30	0.0153	1.00	99.65
2.6	3.23	0.0200	1.00	99.53
2.5	3.17	0.0258	0.99	99.38
2.4	3.10	0.0329	0.99	99.18
2.3	3.03	0.0417	0.99	98.93
2.2	2.96	0.0522	0.99	98.61
2.1	2.89	0.0647	0.98	98.21
2	2.83	0.0794	0.98	97.72
1.9	2.76	0.0965	0.97	97.13
1.8	2.69	0.1161	0.96	96.41
1.7	2.62	0.1383	0.96	95.54
1.6	2.55	0.1631	0.95	94.52
1.5	2.49	0.1905	0.93	93.32
1.4	2.42	0.2202	0.92	91.92
1.3	2.35	0.2520	0.90	90.32
1.2	2.28	0.2855	0.88	88.49
1.1	2.21	0.3203	0.86	86.43
1	2.15	0.3558	0.84	84.13
0.9	2.08	0.3913	0.82	81.59
0.8	2.01	0.4260	0.79	78.81
0.7	1.94	0.4592	0.76	75.80
0.6	1.87	0.4900	0.73	72.57
0.5	1.81	0.5177	0.69	69.15
0.4	1.74	0.5415	0.66	65.54
0.3	1.67	0.5608	0.62	61.79
0.2	1.60	0.5750	0.58	57.93
0.1	1.53	0.5837	0.54	53.98
0	**1.47**	**0.5866**	**0.50**	**50.00**
-0.1	1.40	0.5837	0.46	46.02
-0.2	1.33	0.5750	0.42	42.07
-0.3	1.26	0.5608	0.38	38.21
-0.4	1.19	0.5415	0.34	34.46
-0.5	1.13	0.5177	0.31	30.85
-0.6	1.06	0.4900	0.27	27.43
-0.7	0.99	0.4592	0.24	24.20
-0.8	0.92	0.4260	0.21	21.19

-0.9	0.85	0.3913	0.18	18.41
-1	0.79	0.3558	0.16	15.87
-1.1	0.72	0.3203	0.14	13.57
-1.2	0.65	0.2855	0.12	11.51
-1.3	0.58	0.2520	0.10	9.68
-1.4	0.51	0.2202	0.08	8.08
-1.5	0.45	0.1905	0.07	6.68
-1.6	0.38	0.1631	0.05	5.48
-1.7	0.31	0.1383	0.04	4.46
-1.8	0.24	0.1161	0.04	3.59
-1.9	0.17	0.0965	0.03	2.87
-2	0.11	0.0794	0.02	2.28
-2.1	0.04	0.0647	0.02	1.79
-2.2	-0.03	0.0522	0.01	1.39
-2.3	-0.10	0.0417	0.01	1.07
-2.4	-0.17	0.0329	0.01	0.82
-2.5	-0.23	0.0258	0.01	0.62
-2.6	-0.30	0.0200	0.00	0.47
-2.7	-0.37	0.0153	0.00	0.35
-2.8	-0.44	0.0116	0.00	0.26
-2.9	-0.51	0.0088	0.00	0.19
-3	-0.57	0.0065	0.00	0.13
-3.1	-0.64	0.0048	0.00	0.10
-3.2	-0.71	0.0035	0.00	0.07
-3.3	-0.78	0.0025	0.00	0.05
-3.4	-0.85	0.0018	0.00	0.03
-3.5	-0.91	0.0013	0.00	0.02
-3.6	-0.98	0.0009	0.00	0.02
-3.7	-1.05	0.0006	0.00	0.01
-3.8	-1.12	0.0004	0.00	0.01
-3.9	-1.19	0.0003	0.00	0.00
-4	-1.25	0.0002	0.00	0.00