

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

Tigist T. Shonte, Henriëtte L. de Kock

*Department of Food Science and Institute for Food, Nutrition and Wellbeing, University of Pretoria,  
Private Bag X20, Hatfield, Pretoria 0028, South Africa*

Corresponding author

Tel.: +27 12 420 3238; fax: +27 12 420 2839.

E-mail address: riette.dekock@up.ac.za (H.L. de Kock).

## **Highlights**

- Sensory profiles for cooked nettle leaves and leaf infusions were developed.
- Grassy, asparagus-woody, fishy, fermented, mint and citrus describe nettle leaves.
- Nettle leaves can be brewed twice without much change in aroma and flavour.
- Drying of nettle leaves changes the colour of the cooked product and infusions.

## **ABSTRACT**

Stinging nettles (*Urtica dioica* L.) is known since ancient times as a wild source of food and a herbal medicine, but the plant remain underutilized. The aroma, flavour and colour of cooked stinging nettle leaves and leaf infusions prepared from the fresh or dried leaves, has not been researched. The effect of using fresh or oven-dried leaves in a cooked product or to prepare an infusion on sensory attributes were established. In addition, the effect of two infusion cycles on the

sensory quality was 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 the leaf infusions. The L, a\*, b\* and  $\Delta E$  values of fresh, dried and cooked leaves were also 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 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 consumer liking or disliking. 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 profile, aroma, flavour, infusion cycles, total colour difference, oven dried

## 1. 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, 2010). Stinging nettle, *Urtica dioica* L., is an edible wild green vegetable (Khatiwada et al., 2011) and medicinal plant distinguished by stinging hairs. Nettle leaves have been used, particularly in rural areas of Africa, as a potherb, soup and herbal infusion (Bhat and Moskovitz, 2009; Kavalali, 2004; Roberts, 2012). 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, 2013; 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 (Nencu et al., 2013), minerals (Kara, 2009; Ozcan et al., 2008), 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 attributes of nettles might be reasons for its limited utilisation.

Nettle leaves perishes rapidly after harvest (spring) and are therefore mainly consumed in season (Kavalali, 2004). 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., 2003; Kidmose et al., 2002; Shilton, 2016). 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; Diaz-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 (Shilton, 2016), but 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 researched. The objective of this study was to determine the effects of using fresh or oven dried stinging nettle leaves as a cooked product or to prepare an infusion on the sensory attributes. In addition, the effect of two infusion cycles on the sensory attributes was also determined.

## **2. Materials and methods**

### *2.1. Experimental design*

The effect of two factors were determined, i.e. 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 sensory profile and colour characteristics of two products (cooked leaves and leaf infusions). For the leaf infusions an additional factor, the effect of two infusion cycles (first and second) was included.

### *2.2. 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™, Benmore, South Africa ) slow release nitrogen organic fertilizer (nitrogen-140 g/kg,

phosphorus-91.5 g/kg, potassium-47 g/kg) was applied at a rate of 45 g/m<sup>2</sup> 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 until harvest. Young and tender shoots of nettle were harvested using scissors and wearing hand gloves after five weeks of transplanting. The leaves were thoroughly mixed and carefully handled to ensure that their quality was maintained.

### *2.3. 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.

### *2.4. 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 deionised boiling water added while 600 mL deionised boiling 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.

## *2.5. Leaf infusions*

The infusions were prepared following directions by Lee and Chambers (2009) with a few modifications. Ten grams of oven dried or 50 g fresh leaves were placed inside a 1L glass coffee plunger (CIRO® Taste the freshness coffee maker, PYREX, France), to which 300 mL of deionised water (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). Thereafter 50 mL of the infusion was poured into pre-warmed tea cups and presented to the sensory panel.

## *2.6. Descriptive sensory evaluation*

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

### *2.6.1. Training of the panel*

Twelve panellists participated in this study. A number of sensory “lexicons” for green leafy vegetables (Talavera-Bianchil et al., 2010), green tea (Lee and Chambers, 2007) and green odour (Hongsoongnern and Chambers IV, 2008) were used as basis for lexicon development and preparation of reference samples. Training of the panel was done in 10 h, five 2 h sessions per day. 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 attributes of infusions prepared from spinach and stinging nettle fresh and dried leaves, brewed once or twice were also described. Term generation was repeated three times. Lexicon and scale anchors were developed, defined and agreed upon (Table 1).

Table 1. Lexicon used to describe sensory characteristics of stinging nettle cooked leaves and leaf infusions

Descriptors*	Definition	
	Aroma (perceived orthonasally by opening the foil cover and sniffing the content)	Flavour (perceived during consumption of the products including retronasally 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 peel note
Cooked-morogo	Aromatics associated with commonly cooked mixed green leafy vegetables such as spinach, amaranths, pumpkin leaves	The combined sensation of green leafy vegetable notes and aromatics associated with mixed cooked green leafy vegetables such as spinach, amaranths, pumpkin leaves
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	Sharp and slightly pungent aromatics, green flavour notes, and sweet taste associated with spinach, kale, Swiss chard etc

**Table 1 (Continued)**

	<b>Aroma</b> (perceived orthonasally by opening the foil cover and sniffing the content)	<b>Flavour</b> (perceived during consumption of the products including retronasally 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
Bitter		<b>Basic tastes</b> 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
Astringent		<b>Mouthfeel</b> 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).

### *2.6.2. Descriptive analysis 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 attributes 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 attributes 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  $\approx 64$  °C, one sample at a time. The same methodology was used for the infusions as described for the cooked leaves.

### *2.7. 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 three times 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

(Leon et al., 2006). The changes in colour of leaves from fresh to dried, and fresh to cooked were recorded as total colour difference ( $\Delta E$ ). In addition, the  $\Delta E$  of leaf infusions (compared to first brew of fresh leaves) were calculated as follows:

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

Where  $\Delta L = L - L_0$ ,  $\Delta a = a - a_0$ ,  $\Delta b = b - b_0$ ; and where L, a, and b are the measured values of dried, cooked or infusion samples;  $L_0$ ,  $a_0$ ,  $b_0$  are the values for fresh uncooked leaves and/ or 1st brew infusion from fresh leaves.

## 2.8. Statistical analyses

For cooked leaves the main and interaction effects of species and state of leaves used for sensory attributes and colour were determined using analysis of variance (ANOVA) by the Modelling data option (XLSTAT 2014 by AddinSoft™ SARL, Paris, France). Similarly, for leaf infusions the main and the interaction effects were determined for species, state of leaves used and infusion cycle on sensory attributes and colour. 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 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.

## 3. Results

### 3.1. Effects of species and state of leaves used for cooking on aroma and flavour of cooked leaves

Nine aroma (Table 2), and 13 flavour descriptors (nine aroma attributes perceived retronasally, two basic tastes and two mouthfeel) (Table 3) differed

Table 2. The mean ( $\pm$  standard deviation) intensities of aroma descriptors of cooked leaves from fresh and oven dried nettle and spinach

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

<sup>ab</sup> 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 3. The mean ( $\pm$  standard deviation) intensities of flavour descriptors of cooked leaves from fresh and oven dried nettle and spinach

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

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

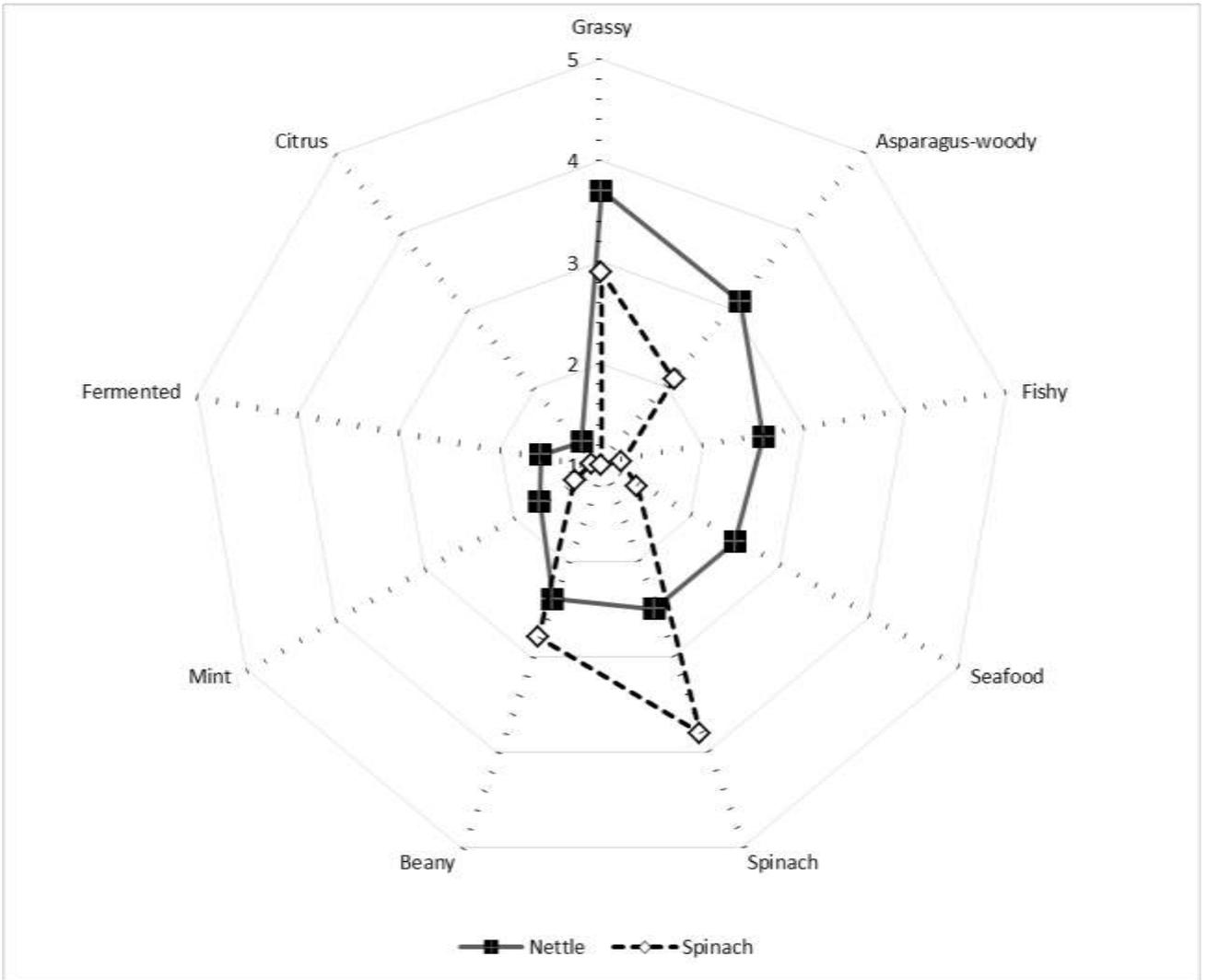
\*A five-point category scale (none=1, slight=2, moderate=3, strong=4 and extreme=5).



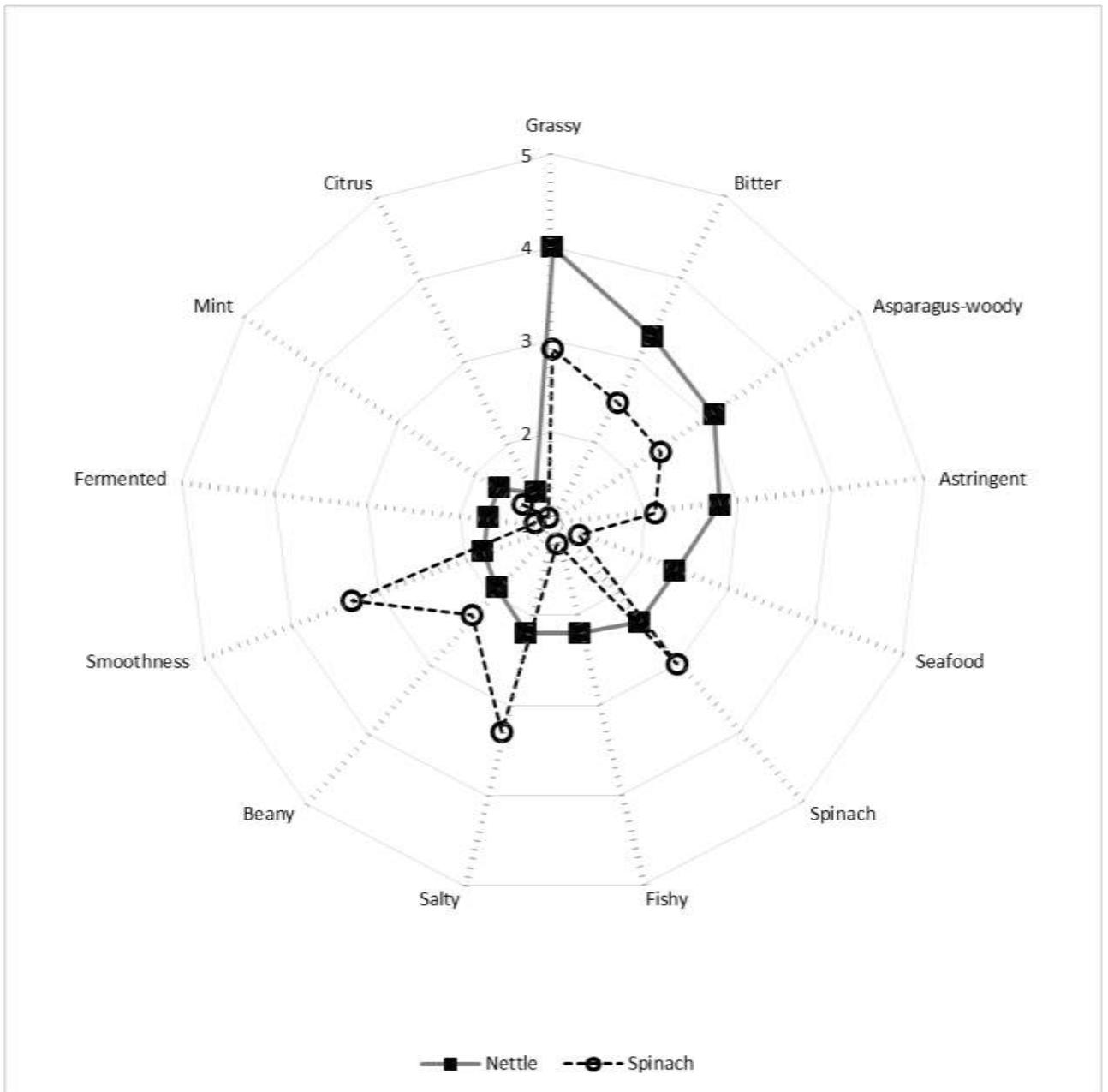
ODN) on the left from spinach (FrS and ODS) on the right while F2 differentiated cooked leaves prepared from leaves that were oven dried (ODN and ODS) at the bottom of the plot from those that were prepared from fresh leaves (FrN and FrS) at the top of the plot.

The spider plot (Figure 2) is a representation of aroma descriptors whereas Figure 3 represent the flavour 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 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 3.** Spider plot representation of flavour 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).

### 3.2. The effect of species, state of leaves used and infusion cycles on aroma and flavour of infusion

Two aroma (burnt and fishy) (Table 4) and three flavour descriptors (burnt, fishy and bitter taste) (Table 5) described differences between the species, whereas eight aroma and four flavour descriptors differentiated the infusions made from fresh and

Table 4. The mean ( $\pm$  standard deviation) intensities of aroma descriptors of first and second infusion from fresh and oven dried nettle and spinach leaf infusion

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

<sup>ab</sup> 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 5. The mean ( $\pm$  standard deviation) intensities of flavour descriptors of first and second infusion from fresh and oven dried nettle and spinach leaf infusions

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

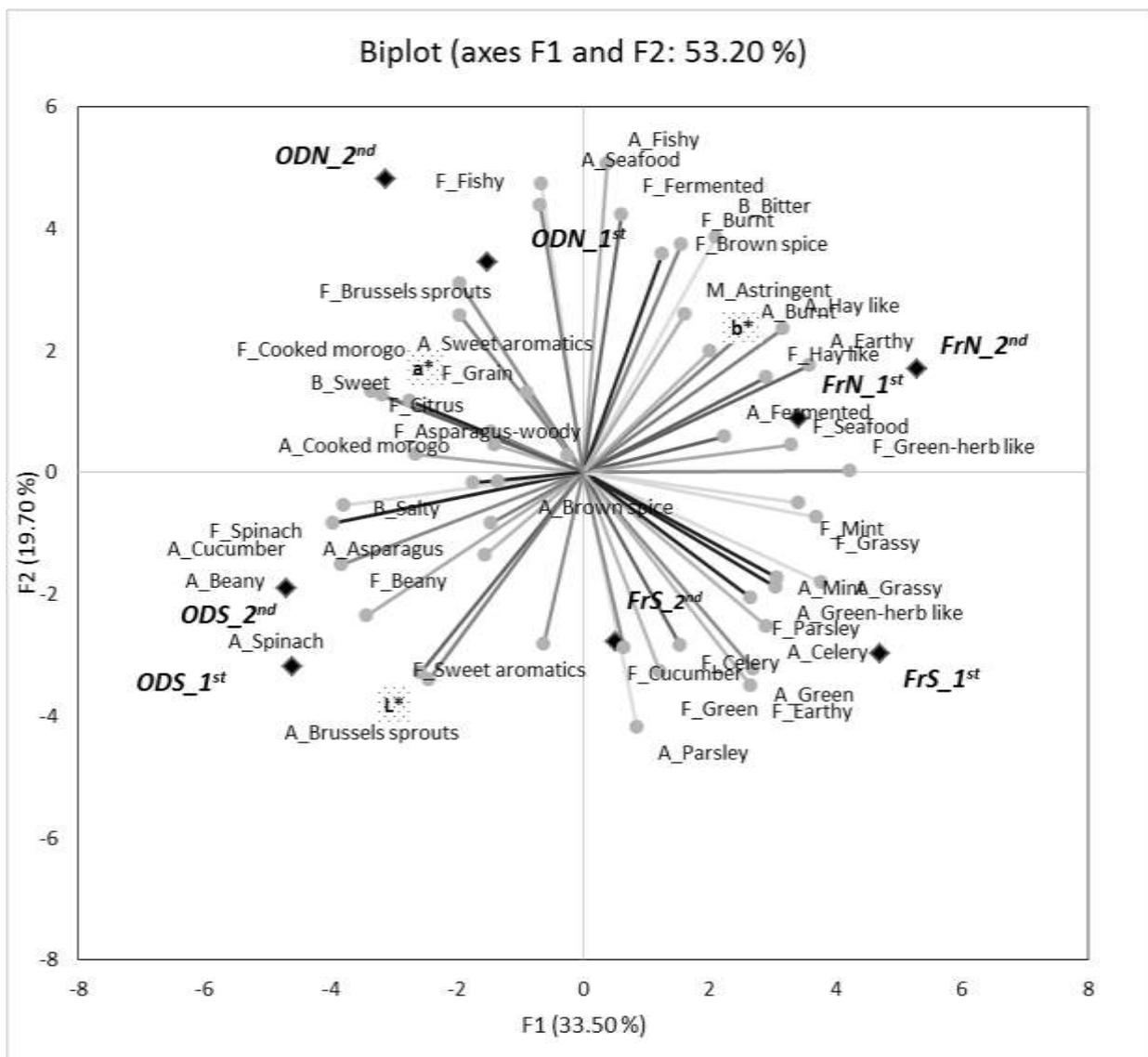
<sup>ab</sup> Means for a specific main effect (species, state of leaves used) within a row not sharing a superscript letter are significantly different ( $p < 0.05$ ).

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

oven dried leaves. There were significant species x state of leaves interaction effects found for 'burnt' and 'fishy' aroma. Aroma and flavour of infusions were not significantly affected by the two infusion cycles. However, the interaction effects of species x infusion cycle was significant for 'cooked-morogo' aroma, and the interaction of species x state of the leaves x infusion cycle significantly influenced the perception of 'green-leafy' flavour.

The first two principal components (F1 and F2) explained 53 % of the variation in aroma, flavour, basic taste and mouthfeel of infusions (Figure 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) of the plot. F2 differentiated nettle leaves at the top of the plot (ODN and FrN) from spinach leaves at the bottom (ODS and FrS).

According to Figure 4, 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 notes 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.



**Figure 4.** Principal component loadings and scores of aroma (A), flavour (F) and mouthfeel (M) descriptors as well as colour values [L a\* b\*] of 1<sup>st</sup> and 2<sup>nd</sup> brews from fresh (Fr) and oven dried (OD) nettle and spinach leaves. N-nettle leaves, S-spinach leaves

### 3.3. Colour values

Tables 6 and 7 presents the results for the L, a\*, b\* and  $\Delta E$  colour values for leaves and infusions, respectively. In both uncooked and cooked leaves, higher L, a\* and b\* values were mostly observed in spinach compared to nettle leaves. Lightness (L values) 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\* values increased from fresh

Table 6. The mean ( $\pm$  standard deviation) colour values of uncooked and cooked leaves from fresh and oven dried nettle and spinach leaves

Species									p-values						
	Nettle				Spinach				Cooking x Cooking	Species x Species	State of leaves x State of leaves	Cooking x Species	Cooking x State of leaves	Species x State of leaves	Cooking x Species x State of leaves
	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 <sup>c</sup> (0.2)	53.1 <sup>a</sup> (0.2)	24.1 <sup>c</sup> (0.4)	23.5 <sup>c</sup> (0.4)	47.6 <sup>b</sup> (0.5)	53.0 <sup>a</sup> (0.1)	27.2 <sup>d</sup> (1.5)	24.4 <sup>e</sup> (0.6)	0.00	0.00	0.00	0.50	0.00	0.00	0.22
a*	-8.5 <sup>b</sup> (0.0)	-9.7 <sup>bc</sup> (0.1)	-5.8 <sup>a</sup> (0.2)	-8.6 <sup>b</sup> (0.6)	-12.0 <sup>de</sup> (0.2)	-10.5 <sup>cd</sup> (0.0)	-12.2 <sup>e</sup> (2.6)	-9.0 <sup>bc</sup> (0.7)	0.01	0.00	0.66	0.14	0.90	0.00	0.06
b*	9.0 <sup>c</sup> (0.1)	15.5 <sup>a</sup> (0.2)	2.6 <sup>e</sup> (1.0)	5.8 <sup>d</sup> (0.5)	12.8 <sup>b</sup> (0.2)	14.8 <sup>ab</sup> (0.1)	9.2 <sup>c</sup> (3.7)	6.2 <sup>d</sup> (0.2)	0.00	0.00	0.00	0.11	0.00	0.00	0.47
$\Delta E$		11.1	21.2	20.9		5.9	20.7	24.3							

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

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

Where  $\Delta L = L - L_0$ ,  $\Delta a = a - a_0$ ,  $\Delta b = b - b_0$ ; and where L, a, and b are the values for dried or cooked leaves;  $L_0$ ,  $a_0$ ,  $b_0$  are the values for fresh uncooked leaves.

Table 7. The mean ( $\pm$  standard deviation) colour values of first and second brew from fresh and oven dried nettle leaf and spinach infusion

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

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

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

Where  $\Delta L = L - L_0$ ,  $\Delta a = a - a_0$ ,  $\Delta b = b - b_0$ ; and where L, a, and b are the values for dried or fresh leaf infusions; L<sub>0</sub>, a<sub>0</sub>, b<sub>0</sub> are the values for fresh leaf first brew infusion.

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.

L value was higher for spinach compared to nettle infusions and also for uncooked versus cooked leaf infusions. Lightness values increased from the 1<sup>st</sup> brew to the 2<sup>nd</sup> brew in both leaf infusions from fresh and oven dried leaves. In contrast,  $a^*$  values increased from the 1<sup>st</sup> brew to the 2<sup>nd</sup> brew in both leaf infusions made from fresh and oven dried leaves. A significant interaction effect of infusion cycle x species x state of leaves was found for  $b^*$  values.

#### **4. Discussion**

Grassy, asparagus-woody, mint, citrus, fermented, seafood and fishy aromas and flavours typified cooked nettle leaves compared to spinach leaves. Nettle leaf infusion was more intense in fishy and burnt aromas and flavours as well as bitter taste 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 (parsley, bay leaf, spearmint, basil) (Diaz-Maroto et al., 2004) 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 leaf part of spinach was used.

When leaves were oven dried, all green-associated aromas and flavours were preserved in the cooked product, but more pronounced fermented aroma, bitter and salty tastes were noted. 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 compounds in the leaves.

Oven drying cause losses or formation of volatiles as a result of oxidation and/or esterification reactions (Diaz-Maroto et al., 2002; King et al., 2006; Orphanides et al., 2013; Shilton, 2016). In stinging nettle leaves flavour volatiles could potentially be generated from fatty acids (e.g. linoleic acid and linolenic acids) (Farag et al., 2013; Guil-Guerreroa 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-Guerreroa 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 (Chen et al., 2004; Owuor, 2016). In fresh and cooked nettle leaves, polyunsaturated fatty acids are approximately two to three times more than saturated fatty acids (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 acids to trimethylamine and dimethylamine in marine fish and seafood (Hebard et al., 1982) 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 (Frag et al., 2013; Otles and Yalcin, 2012; Pinelli et al., 2008). Caffeine and saponins are also responsible for bitterness (Lee and Chambers, 2009) whereas epicatechin and epigallocatechin (Owuor, 2016; Wang et al., 2000) and tannins (Troszyńska 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, the flavours were perceived as being more intense than the aroma attributes of cooked leaves. This was probably due to more aromatic compounds being released during mastication of the leaves and perceived retronasally. Oral processing during mastication of food enhances flavour release from food (Neyraud et al., 2003; Neyraud et al., 2005). 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 action 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 for 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  $\Delta E$  value, showed variation in colour between the two infusion cycles and in cooked leaves as well. The high  $\Delta E$  between the brews could be due to the effect of dilution and duration of thermal processing. A high  $\Delta E$  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., 2002). The loss of green colour in green vegetables is due to degradation of chlorophyll a and b, and oxidation of carotenoids (Di Cesare et al., 2003; Kidmose et al., 2002). It is probable that the chlorophyll a and b (Alibas, 2007; Dey, 2013) and  $\beta$ -carotenoids (Guil-Guerreroa 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 (Baritoux 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; Alibas, 2007; Dey, 2013). For example, a higher change in L,  $a^*$ , and  $b^*$  values was observed in convection dried swiss chard leaves with  $\Delta E$  of 5.9-11.2 (Alibas, 2006) than freeze-dried samples  $\Delta E$  of 4.95 (Alibas, 2007).

## **5. 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 used to characterize the sensory profiles of nettle products produced 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 degree of bitterness and astringency. Similarly, the aroma and flavour of nettle leaf infusions are more burnt and fishy and the infusions tastes more bitter 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 sensory baseline data set could be utilized to describe, compare, and differentiate the characteristics of various nettle food products around the world. Further research should determine which sensory attributes of the products from the nettle plant drives consumer liking or disliking. This research could contribute to the understanding of the potential of stinging nettle for addressing food and nutrition security, and well-being of consumers.

### **Acknowledgments**

We appreciate and thank the personnel of the Crop Production and Soil Science Field Trial Section and the Experimental Farm Station of University of Pretoria that facilitated the field work. The financial support of University of Pretoria and the Organization for Women in Sciences for Developing Countries (OWSD) towards a PhD Scholarship for T T Shonte is acknowledged.

## Declaration of interests statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the manuscript.

## 6. References

- Alibas, I., 2006. Characteristics of chard leaves during microwave, convective, and combined microwave-convective drying. *Drying Technology* 24, 1425-1435.
- Alibas, I., 2007. Energy consumption and colour characteristics of nettle leaves during microwave, vacuum and convective drying. *Biosystems Engineering* 96 (4), 495-502.
- Baritau, O., Richard, H., Touche, J., Derbesy, M., 1992. Effects of drying and storage of herbs and spices on the essential oil, Part I. Basil, *Ocimum basilicum* L. *Flavour and Fragrance Journal* 7, 267-271.
- Bhat, R.B., Moskovitz, G., 2009. Herbal medicinal teas from South Africa. *International Journal of Experimental Botany* 78, 67-73.
- Chen, G., Hackett, R., Walker, D., Taylor, A., Lin, Z., Grierson, D., 2004. Identification of a Specific Isoform of Tomato Lipxygenase (TomloxC) Involved in the Generation of Fatty Acid-Derived Flavour Compounds. *Plant Physiology* 136, 2641-2651.
- Davidson, A., 2013. *The Oxford Companion to Food*. 2nd ed. United Kingdom: Oxford University Press.
- Dey, T., 2013. *Freeze drying of novel materials* [Thesis]. Jadavpur: Jadavpur University.
- Di Cesare, L.F., Forni, E., Viscardi, D., Nani, R.C., 2003. Changes in the chemical composition of basil caused by different drying procedures. *Journal of Agricultural*

- and Food Chemistry 51, 3575-3581.
- Diaz-Maroto, M.C., Pérez-Coello, M.S., Cabezudo, M.D., 2002. Effect of drying method on the volatilities in bay leaf (*Laurus nobilis* L.). Journal of Agricultural and Food Chemistry 50, 4520-4524.
- Diaz-Maroto, M.C., Palomo, E.S., Castro, L., Vinas, M.A.G., Perez-Coello, M.S., 2004. Changes produced in the aroma compounds and structural integrity of basil (*Ocimum basilicum* L.) during drying. Journal of the Science of Food and Agriculture 84, 2070-2076.
- Einstein, M.A., 1991. Descriptive techniques and their hybridization. In: Lawless HT, Klein B P, Editors. Sensory science theory and applications in foods. New York: Marcel Dekker; pp. 317-338.
- Farag, M.A., Weigend, M., Luebert, F., Brokamp, G., Wessjohann, L.A., 2013. Phytochemical, phylogenetic, and anti-inflammatory evaluation of 43 *Urtica* accessions (stinging nettle) based on UPLC-Q-TOF-MS metabolomic profiles. Phytochemical 96, 170-183.
- Francisco, M., Velasco, P., Romero, A., Vázquez, L., Cartea, M.E., 2009. Sensory quality of turnip greens and turnip tops grown in north western Spain. European Food Research and Technology 230, 281-290.
- Giada, M.de-L.R., 2013. Food phenolic compounds: Main classes, sources and their antioxidant power. In: Morales-González JA, editor. Oxidative stress and chronic degenerative diseases - a role for antioxidants". Rijeka, Croatia, InTech. pp. 87-112.
- Guil-Guerreroa, J.L., Reboloso-Fuentes, M.M., Torija-Isasa, M.E., 2003. Fatty acids and carotenoids from stinging nettle (*Urtica dioica* L.). Journal of Food Composition and Analysis 16, 111-119.
- Hebard, C.E., Flick, G.J., Martin, R.E., 1982. Chemistry and biochemistry of marine

- food products. United States: Avi Publishing Company.
- Hicks, M.B., Hsieh, Y.-H.P., Bell, L.N., 1996. Tea preparation and its influence on methyl xanthine concentration. *Food Research International* 29, 325-330.
- Hongsoongnern, P., Chambers IV, E., 2008. A lexicon for green odour or flavour and characteristics of chemicals associated with green. *Journal of Sensory Studies* 23, 205-221.
- Hughes, R.E., Ellery, P., Harry, T., Jenkins, V., Jones, E., 1980. The dietary potential of the common nettle. *Journal of Science of Food and Agriculture* 31, 1279-1286.
- Humphrey, S.P., Williamson, R.T., 2001. A review of saliva: Normal composition, flow, and function. *Journal of Prosthetic Dentistry* 85, 162-169.
- International Food Policy Research Institute (IFPRI), 2010. 2009–2010 Global Food Policy Report: Sustainable solutions for ending hunger and poverty. Washington DC, USA: International Food Policy Research Institute.
- Kara, D., 2009. Evaluation of trace metal concentrations in some herbs and herbal teas by principal component analysis. *Food Chemistry*, 114, 347-354.
- Kavalali, G.M., 2004. *Urtica: Therapeutic and nutritional aspects of stinging nettles*. New York: Taylor and Francis Ltd; pp.15-90.
- Khatiwada, B.P., Choulagain, B., Osti, S., 2011. Nepal Tragedy of the underutilized crops and people with changing climate: a case from Chepang tribal communities of Nepal. World Association for Sustainable Development (WASD). *World Sustainable Development Outlook*. pp. 327-345.
- Kidmose, U., Edelenbos, M., Nørbæk, R., Christensen, L.P., 2002. Colour stability in vegetables. In: MacDougall DB, Editor. *Colour in food Improving quality*. Canada: Woodhead Publishing Limited and CRC Press LLC; pp. 179-232.
- King, B.M., Arents, P., Duineveld, C.A.A., Meyners, M., Schroff, S.I., Soekhai, S.T.,

2006. Orthonasal and retronasal perception of some green leaf volatiles used in beverage flavours. *Journal of Agriculture and Food Chemistry* 54, 2664-2670.
- Lee, J., Chambers, D.H., 2007. A lexicon for flavour descriptive analysis of green tea. *Journal of Sensory Studies* 22, 256-272.
- Lee, J., Chambers, D.H., 2009. Sensory descriptive evaluation: brewing methods affect flavour of green tea. *Asian Journal of Food and Agro-products* 2(04), 427-439.
- Lee, J., 2009. Green tea: Flavour characteristics of a wide range of teas including brewing, processing, and storage variations and consumer acceptance of teas in three countries [PhD Thesis] Kansas USA: Kansas State University.
- Lee, J., Chambers, D., Chambers IV, E., 2013. Sensory and instrumental flavour changes in green tea brewed multiple times. *Foods* 2, 554-571.
- Leon, K., Mery, D., Pedreschi, F., Leon, J., 2006. Colour measurements in  $L^* a^* b^*$  units from RGB digital images. *Food Research International* 39, 1085.
- Masanetz, C., Guth, H., Grosch, W., 1998. Fishy and hay-like off-flavours of dry spinach. *European Food Research and Technology* 206, 108.
- Nencu, I., Viorica, I., Diana-Carolina, I., Valeria, R., 2013. Preliminary research regarding the therapeutic uses of *Urtica dioica* L. The dynamics of accumulation of total phenolic compounds and ascorbic acid. *FARMACIA* 61(2), 276-283.
- Neyraud, E., Dransfield, E., 2004. Relating ionisation of calcium chloride in saliva to bitterness perception. *Physiology and Behaviour* 81, 505-510.
- Neyraud, E., Peyron, M.A., Vieira, C., Dransfield, E., 2005. Influence of bitter taste on mastication pattern. *Journal of Dental Research* 84, 250-254.
- Neyraud, E., Prinz, J., Dransfield, E., 2003. NaCl and sugar release, salivation and taste during mastication of salted chewing gum. *Physiology and Behaviour* 79, 731-737.
- Orphanides, A., Goulas, V., Gekas, V., 2013. Effect of drying method on the phenolic

- content and antioxidant capacity of spearmint. *Czech Journal of Food Science* 31(5), 509-513.
- Otles, S., Yalcin, B., 2012. Phenolic compounds analyses of root, stalk, and leaves of nettle. *The Scientific World Journal* 2012, 1-12.
- Owuor, P.O., 2016. Tea: Processing, chemistry, analysis and tasting. In: Caballero B, Finglas PM, Toldra F, editors. *Encyclopaedia of Food and Health*. Oxford: Elsevier Science Ltd; pp.324-332.
- Ozcan, M.M., Unver, A., Arslan, T.U.D., 2008. Mineral content of some herbs and herbal teas by infusion and decoction. *Food Chemistry* 106, 1120-1127.
- Pinelli, P., Francesca, I., Pamela, V., Laura, B., Silvia, B., Annalisa, R., 2008. Extraction and HPLC analysis of phenolic compounds in leaves, stalks, and textile fibers of *Urtica dioica* L. *Journal of Agriculture and Food Chemistry* 56, 9127-9132.
- Roberts, M., 2012. *Healing foods*. Queenswood, South Africa: Briza Publications; pp. 235.
- Rutto, L.K., Xu, Y., Ramirez, E., Brandt, M., 2013. Mineral properties and dietary value of raw and processed nettle (*Urtica dioica* L.). *International Journal of Food Sciences* 2013, 1-9.
- Shilton, N., 2016. Drying: Chemical Changes. In: Caballero B, Finglas PM, Toldra F, editors. *Encyclopaedia of Food and Health*. Oxford: Elsevier Science Ltd; pp.1947-1950.
- Talavera-Bianchil, M., Chambers IV, E., Chambers, D.H., 2010. Lexicon to describe flavour of fresh leafy vegetables. *Journal of Sensory Studies* 25, 163-183.
- Troszyńska, A., Grzegorz, L., Hanna, K.G., 2003. Evaluation of astringency of preparations with different degree of tannin polymerisation. *Polish Journal of Food and Nutrition Sciences* 12(53), 84-86.

Upton, R., 2013. Nettles leaf (*Urtica dioica* L.): Extraordinary vegetable medicine. Journal of herbal medicine 3, 9-38.

Wang, L.F., Kim, D.M., Lee, C.Y., 2000. Effects of heat processing and storage on flavanols and sensory qualities of green tea beverage. Journal of Agriculture and Food Chemistry 48, 4227-4232.