South African Journal of Botany 108 (2017) 8–14

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

A comparative study of the chemical composition of lucerne (*Medicago sativa* L.) and spinach beet (*Beta vulgaris* var. *cicla* L.)

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ARTICLE INFO

Article history:
Received 20 April 2016
Received in revised form 22 August 2016
Accepted 9 September 2016
Available online xxxx

Edited by M Moyo

Keywords:
Chemical composition
Lucerne
Spinach beet
Cultivar
Human consumption

ABSTRACT

The chemical composition of three lucerne (*Medicago sativa* L.) cultivars ('SA Standard', 'WL711' and 'WL525') were compared to spinach beet (*Beta vulgaris* var. *cicla* L.), a familiar leafy vegetable (as control), in order to establish its potential as an alternative green leafy vegetable for human consumption. The degrees Brix, macro- and micro-minerals, protein, amino acids, dry matter, moisture, ash, fat, fibre, carbohydrates and energy contents were determined. The protein content of the cooked 'SA Standard' and 'WL525' lucerne cultivars had significantly ($p < 0.001$) higher protein contents than cooked spinach beet. Cooked lucerne cultivar 'SA Standard' had a significantly ($p < 0.001$) higher energy content than cooked lucerne cultivar 'WL525' and spinach beet. Based on these findings, the chemical composition of lucerne compared well to the properties of spinach beet, making them desirable in terms of nutrition and could be used as a potential vegetable for human consumption.

1. Introduction

Lucerne is the most widely grown (over 32 million ha worldwide) forage crop in the world. Recently, with the increase in the demand for healthy, green food, consumers are paying more attention to the utilization of lucerne, because of its high nutritional contents (Lamsal et al., 2007; Colas et al., 2013), high digestibility and unique proportions of structural to non-structural components (Yari et al., 2012). In addition, lucerne is one of the plants producing the most protein per hectare (15–20% crude protein) (European Food Safety Authority, 2009).

South Africa (SA) has an estimated population of 50.6 million people (Statistics South Africa, 2011), of which 79% are Black Africans. The majority of SA households live in poverty, with a limited variety of foods (mainly staples) available in homes. In essence, most SA children consume a diet low in energy, and poor in protein quality and micronutrient density, while children from urban areas are increasingly overweight (Labadarios et al., 2001). What is striking is that Africa, as a continent, does not play a role or make a contribution towards protein supplementation in the world. The main issues affecting future growth trends in SA are thought to be population growth and food supply. Africa is the only continent where population growth rates (3.1%) have not yet started to decline (Steyn et al., 2001). Therefore, it is becoming a reality that SA consumers need more plant protein legumes, such as lucerne, that can play a major role in consumers' diets and help maintain health and prevent disease (e.g. malnutrition). The nutritional value of lucerne hay in SA has been researched mainly for animal grazing purposes (Scholtz et al., 2009). However, little information is available about the nutritional value of fresh lucerne leaves, purposive for human consumption.

Therefore, the objective was to investigate the chemical (degrees Brix, macro- and micro-minerals, protein, amino acids, dry matter [DM], moisture, ash, fat, fibre, carbohydrates and energy contents) composition of three lucerne cultivars and compare it to spinach beet (SB), a familiar leafy vegetable as control, to determine the nutritional value of lucerne cultivars.

2. Material and methods

2.1. Evaluation of soil

Before the establishment of lucerne cultivars, the soil of the trial site was analysed by NviroTek Laboratories Pty Ltd., Brits, SA, to determine if any deficiencies in soil pH, P, K, Ca and Mg levels needed to be corrected (Pioneer Brand Products Manual, 2011). Soil pH was measured in 1 N potassium chloride (KCl) extract at a 1:1 soil to solution ratio, using a Beckman Expandomatic pH meter. The soil available P was determined according to Bray No. 1 methods (Bray and Kurtz, 1945), using an auto analyser (International Institute of Tropical Agriculture, 1982).

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http://dx.doi.org/10.1016/j.sajb.2016.09.006
0254-6299/

Potassium, Ca and Mg, present in the soil sample, were extracted with neutral 1 M ammonium acetate, by shaking the soil-extract mixture (Motsara and Roy, 2008). The extraction was then measured by an inductively coupled plasma-mass spectrometry emission spectroscopy (ICP-MS) instrument (Ziadi and Sen Tran, 2007).

2.2. Establishment of cultivars

Three lucerne cultivars, 'SA Standard' ('SAS') (Code: 2010029), 'WL525' (Code: 1020249) and 'WL711' (Code: 1017152) and spinach beet (SB; *Beta vulgaris* var. *cicla* L.; control) were cultivated in Potchefstroom in the North-West Province, during January 2012, in the morning at a temperature of 25 °C, humidity of 64% and wind speed of 12 km/h. The pastures were irrigated weekly during the summer mornings (Pioneer Brand Products Manual, 2011). Lucerne requires 0.65–0.80 mm of water to produce 10 kg of lucerne. Potchefstroom is situated in the summer rainfall region of SA, with a long term average annual rainfall of 613 mm, occurring mainly between September and April. The high evaporation rates of the area imply a water deficit during the whole year (Aucamp, 2000).

These three cultivars were of high quality, certified seed and were purchased from K2 Agri Klein Karoo Seed Marketing Pty Ltd., in Potchefstroom in the North-West Province. These cultivars were chosen, as they provide better germination rates and establishment, better DM production, better aphid and disease resistance, and are weed-free (K2 Agri, 2011).

A well-drained, sandy clay loam paddock was chosen and sprayed out with a rake to eliminate all weeds (including grasses) (Mbatani, 2000). The seedbed was made even with no compaction layers. For lucerne, seed was sown at 12–18 kg/ha and planted no deeper than 25 mm. For SB, seed was sown at 7–9 kg/ha and planted no deeper than 20 mm (SADAFF, 2014).

2.3. Sampling procedure

All the samples were harvested in the morning, when the dew or moisture was high, to limit leaf loss (Dickinson et al., 2010). The lucerne leaves were sampled during the mid-vegetative (stem length 15–30 cm, no buds, flowers or seed pods) to late vegetative stage (stem length greater than 30 cm, no buds, flowers or seed pods). Lucerne leaves contain 70% of the protein and 90% of the vitamins and minerals, found in the lucerne crown. Therefore, lucerne quality is highest when harvesting takes place at an early stage of development, when the leaves have a high percentage of the total DM yield (Pioneer Brand Products Manual, 2011).

Proper handling of the lucerne samples was ensured by hand picking clean samples (the upper 150 mm or 1/3 of the plant) and placing it in clean brown paper bags. Samples should not be placed in plastic bags or sealed containers as this promotes mould growth (Pioneer Brand Products Manual, 2011). Samples damaged by insects or climatic conditions were avoided and plant tissues were taken from the same relative position of the plant.

The SB leaves were harvested when the leaves were 10–12.5 cm long, by twisting the outer leaves sideways. Only leaves that were free of insect injury, worms, moulds, decay or other serious injury that affects its appearance, were picked. As with the lucerne samples, SB samples were placed in clean brown paper bags (SADAFF, 2014). All samples were cooled at 4 °C and evaluated within 24–48 h. All chemical analyses of the cultivars and SB were performed in triplicate.

2.4. Preparation of cooked samples

The leaves were cooked in filtered water at 96 °C for 10 min, and then cooled and stored at 4 °C, in order to prevent deterioration and to perform further chemical analysis (Mepba et al., 2007). One percent of sodium chloride (NaCl) was added to the cooking water, as a 1% addition of NaCl reduced the loss of mineral constituents in cooked spinach (Kimura and Itokawa, 1990).

2.5. Degrees Brix

The degrees Brix was analysed according to the Association of Official Agricultural Chemists (AOAC) 932.12 method (AOAC, 2000) using a PAL Digital Pocket Refractometer (Atago, Japan).

2.6. Macro- and micro-minerals of lucerne

The macro- and micro-minerals were analysed according to the wet digestion method for plant analysis (Food and Agriculture Organization of the United Nations, 2008). The samples were brought into solution through digestion with acids that dissolve the solid plant parts and convert the plant nutrients to a liquid for analysis (known as wet digestion). A mixture of nitric acid (HNO_3), sulphuric acid (H_2SO_4) and perchloric acid ($HClO_4$), in the ratio of 9:4:1, was used for tri-acid digestion. For di-acid digestion, two acids, HNO_3 and $HClO_4$ (9:4), were used. While di-acid digestion was used for determination of Ca, Mg, sulphur (S), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu), tri-acid digestion was used for P and K analysis (Motsara and Roy, 2008).

For mineral determination, 1 g of plant sample was ground with an electric stainless steel mill and placed in a 100 mL volumetric flask. Ten millilitres of acid mixture was added and the contents were mixed by swirling. The flask was placed on a hotplate in a fume hood and heated, starting at 80–90 °C, where after the temperature was raised to ≈150–200 °C. Heating continued past the production of red nitrogen dioxide (NO_2) fumes ceased, until the volume was reduced to 3–4 mL and became colourless (Motsara and Roy, 2008). After cooling the contents, the volume was made up to 50 mL with distilled water and filtered through Whatman No. 1 filter paper (Ahmad et al., 2013). The filtrate was then measured by an inductively coupled plasma-mass spectrometry emission spectroscopy (ICP-MS) instrument (Aucamp, 2000).

2.7. Average mineral cooking losses

The cooking losses of the macro- and micro-minerals were calculated on a wet weight basis for each replicate. The replicate results were averaged for the sample being analysed. The average value obtained from subtracting the specific mineral concentration, before and after cooking, was considered a loss if positive, or an apparent gain if marked with a positive symbol (Kawashima and Valente Soares, 2003).

2.8. Sample preparation for dry matter, moisture, ash, fat, fibre, carbohydrates, energy, protein and amino acids contents

All samples were freeze-dried and milled to obtain a homogeneous sample. The total DM, moisture, ash, protein, amino acids (AAs), fat, fibre, carbohydrates and energy content were determined in triplicate. All the results were calculated, using the freeze-dried values and the final results were expressed on a wet weight basis.

Analytical Standard Method (ASM) 013 was used to determine the percentage DM. Moisture was determined by the ASM 013 method. The percentage ash was determined according to ASM 048. The fat content was determined according to ASM 044 by means of the Soxtec method (AOAC, 2000). Fibre content was determined according to ASM 059. The carbohydrate content was analysed using ASM 075. The energy content (dry material) was determined according to ASM 074. The Kjeldahl method (ASM 056), which measures total organic nitrogen (N), was used to determine the protein content of raw and cooked leaves (AOAC, 2000).

The AA content was determined according to ASM 021 (AOAC, 2000). Seventeen AAs were analysed by High Performance Liquid Chromatography (HPLC), with a limit of quantification of 0.006 g 100/g