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Steam treatment of green *Cyclopia longifolia* - Delivering herbal tea infusions with a high bioactive content and improved aroma

L. Alexander [a,b], D. de Beer [a,b], M. Muller [b], M. van der Rijst [c], E. Joubert [a,b,*]

[a] Plant Bioactives Group, Post-Harvest and Agro-Processing Technologies, Agricultural Research Council (ARC), Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch 7599, South Africa
[b] Department of Food Science, Stellenbosch University, Private Bag X1, Matieland, Stellenbosch 7602, South Africa
[c] Biometry Unit, Agricultural Research Council (ARC), Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch 7599, South Africa

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ABSTRACT

Steam treatment of fresh, shredded plant material (0, 30, 60, 90 and 120 s) and dried, shredded green plant material (0, 1, 2, 3 and 4 min) of *Cyclopia longifolia* was investigated to reduce negative aroma notes, such as "green grass" and "hay/dried grass", but without compromising the green leaf colour and phenolic content. Steam treatment not only decreased the vegetal aroma intensities, but also increased sweet, fruity intensities. The dried plant material was less susceptible to the impact of steam than the fresh plant material, and steam duration had little effect. Steam treatment had little impact on leaf colour, although the total chlorophyll content was decreased. The content of only two polyphenols, namely tetrahydroxyxanthone-di-*O*,*C*-hexoside isomer A and scolymoside, were slightly but significantly ($P < 0.05$) reduced by steam treatment of the fresh plant material. Infusions prepared from a large number of batches of green *C. longifolia* ($n = 50$) confirmed high levels of polyphenols at "cup-of-tea" strength, in particular the xanthones, mangiferin and isomangiferin, and the benzophenones, 3-β-D-glucopyranosyliriflophenone and 3-β-D-glucopyranosyl-4-β-D-glucopyranosyloxyiriflophenone. It could thus make a substantial contribution to the dietary intake of these types of polyphenols.

1. Introduction

Epidemiological data linking dietary polyphenols to prevention of diabetes and metabolic syndrome in target populations (Xiao and Högger, 2014) justify the identification of foods and beverages rich in certain bioactive compounds. Phenolic profiling of several "fermented" *Cyclopia* herbal teas revealed that *C. longifolia* and *C. subternata* contain relatively high levels of scolymoside (Schulze et al., 2015), a flavone that suppresses high-glucose-induced vascular inflammation (Ku and Bae, 2016) and enhances glucose uptake by muscle cells *in vitro* (Schulze et al., 2016). Distinctive differences in the phenolic composition of infusions of *C. subternata* and *C. longifolia* relate to substantially higher levels of mangiferin, a xanthone, and 3-β-D-glucopyranosyliriflophenone, a benzophenone, in the latter species (Schulze et al., 2015). Mangiferin is well-known for its anti-diabetic effects (reviewed by Vyas et al., 2012), while 3-β-D-glucopyranosyliriflophenone showed promise as an anti-diabetic agent by reducing fasting blood glucose levels of streptozotocin-induced diabetic rats (Pranakhon et al., 2015) and inhibiting mammalian α-glucosidase (Beelders et al., 2014).

Whilst "fermentation" of *Cyclopia* plant material is essential to develop the characteristic floral, fruity, woody, plant-like and sweet aromas associated with honeybush herbal tea (Erasmus et al., 2016), this high-temperature oxidation processing step has a detrimental effect on the phenolic content of the plant material. De Beer and Joubert (2010) showed that hot water extracts of several "unfermented" (green) *Cyclopia* species contained substantially more scolymoside than the extracts prepared from fermented plant material. Investigation of the thermal stability of the major xanthones and benzophenones present in *C. genistoides* (Beelders et al., 2015) showed that their thermal degradation followed first order reaction kinetics. Furthermore, the temperature-dependence of the respective degradation reaction rate constants of the compounds complied with the Arrhenius equation (Beelders et al., 2017). Green honeybush herbal tea thus provides a healthier alternative to herbal tea prepared from fermented plant material, due to its higher levels of bioactive phenolic compounds. Regardless, consumer acceptability of green honeybush herbal tea is low in comparison to the fermented product due to an overpowering vegetal aroma. We recently demonstrated that the vegetal aroma of green *C. maculata* infusions could be reduced by steam treatment of plant material, either pre- or post-drying. Not only were the vegetal aroma attribute intensities reduced, but those of fruity aroma attributes

* Corresponding author at: Plant Bioactives Group, Post-Harvest and Agro-Processing Technologies, Agricultural Research Council (ARC), Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch 7599, South Africa.
E-mail address: joubertL@arc.agric.za (E. Joubert).

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were more prominent after steam treatment (Alexander et al., 2017). Another positive outcome of this study was that steam treatment had little effect on the phenolic content of the plant material. These findings served as impetus for the present study on *C. longifolia*, investigating the effect of steam treatment on quality parameters such as the aroma of the infusion, as well as the phenolic content and colour of the leaf product. Similar to the investigation of green *C. maculata*, two steam treatment strategies, *i.e.* application of steam to fresh, shredded plant material prior to drying or to the dried herbal tea product, were followed.

In addition, hot water infusions of a large number of batches of green *C. longifolia* were analysed to provide content values for the major phenolic compounds of a typical "cup-of-tea" serving. This data serve to complement that previously compiled for fermented *C. longifolia* (Schulze et al., 2015), thus providing further context for consumption of green *C. longifolia* in terms of bioactive content.

2. Materials and methods

2.1. Chemicals

High-performance liquid chromatography (HPLC) grade acetonitrile was purchased from Sigma-Aldrich (St Louis, USA). Other chemicals and solvents were analytical grade, sourced from Sigma-Aldrich or Merck Millipore (Darmstadt, Germany). Authentic phenolic reference standards (>95% purity) were sourced from Sigma-Aldrich (mangiferin, hesperidin and 3-β-D-glucopyranosyliriflophenone), Phytolab (Vestenbergsreuth, Germany: vicenin-2 and eriocitrin) and Chemos GmbH (Regenstauf, Germany: isomangiferin). 3-β-D-Glucopyranosyl-4-β-D-glucopyranosyloxyiriflophenone, 3-β-D-glucopyranosylmaclurin and scolymoside were isolated from *Cyclopia* (Beelders et al., 2014; Schulze et al., 2016). Stock solutions of the phenolic standards were prepared in DMSO at *ca.* 1 mg/L and aliquots kept frozen (−20 °C) until analysis.

2.2. Plant material

Five batches (5–6 kg per batch) of *C. longifolia* shoots were harvested for each of the two experiments. The plant material for experiment 1 and 2 was harvested from an experimental plantation on the farm, Toekomst (Bredasdorp, Western Cape Province, South Africa; GPS coordinates −34.24052, 20.47272) during May and August 2014, respectively. Prior to shredding, extra thick stems (>5 mm) were removed whereafter the trimmed shoots were shredded into small pieces (2–3 mm) using a mechanised fodder cutter. The shredded material of a batch was thoroughly mixed to improve homogeneity before steam treatment.

Additional batches of *C. longifolia* plant material were harvested during January to March 2017 from experimental plantations at Nietvoorbij (n = 18; GPS coordinates −33.90619, 18.87031) and Toekomst (n = 22) for phenolic content analysis. The plant material was shredded, dried in a laboratory cross-flow drying tunnel at 40 °C for 6 h to a moisture content of *ca.* 8%. The dried plant material was sieved (30 s at 90 rpm) to obtain the "tea bag" fraction (>0.42 mm; <1.68 mm), using a SMC Mini-sifter (JM Quality Services, Cape Town, South Africa).

2.3. Experimental layout for steam treatments

Two experiments were conducted on shredded *C. longifolia* plant material to evaluate the effects of steam treatment, as described by Alexander et al. (2017). Both experiments were implemented as randomised block designs with each of five steam treatments (exposure times) replicated at random on five independent batches (blocks) of plant material.

Fig. 1 depicts a schematic outline of the processing steps for one batch of plant material per experiment. The same procedure was followed for the other batches. For experiment 1, entailing steam treatment of the fresh, shredded plant material, the "homogenised" plant material of each batch was sub-divided into five treatment samples (T1–T5). The control sample (*ca.* 600 g) was dried without delay as described in Section 2.2. Steam treatment was effected by placing the shredded plant material, thinly spread across 8 drying trays (20 × 30 cm, 0.595 mm mesh stainless steel sieve; *ca.* 300 g per tray) in a pre-heated steam cabinet (±96 °C; inlet steam pressure 2.76 N/m^2). At each predefined time point (t = 30, 60, 90, and 120 s) two trays were removed and placed without delay in the drying tunnel. The dried plant material of two trays, allocated per treatment, was pooled and sieved to obtain the "tea bag" fraction.

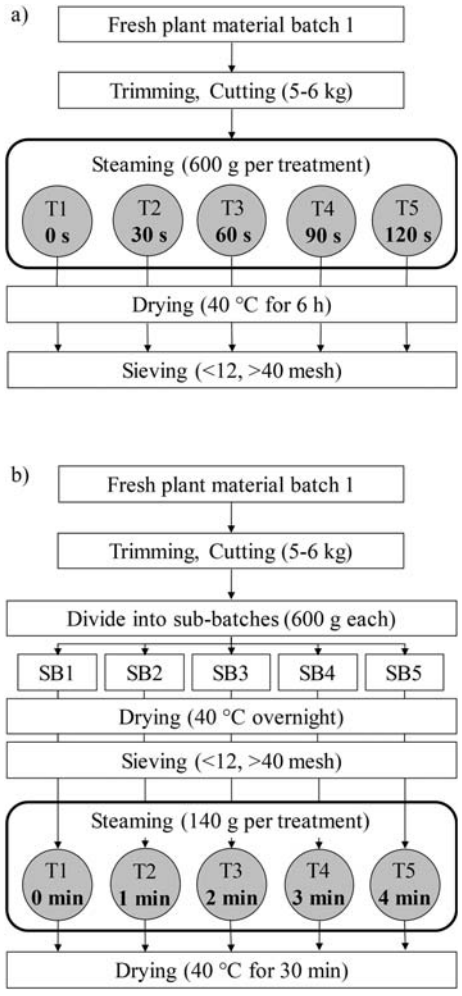

Fig. 1. Schematic outline of the processing steps to evaluate the effects of steam treatment on (a) fresh, shredded (experiment 1) and (b) dried, shredded (experiment 2) green *Cyclopia longifolia* plant material, respectively. The procedure outlined for one batch of plant material was replicated on a further four batches of plant material (n = 5).

Steam treatment of the dried plant material (experiment 2) required that each batch of shredded, "homogenised" material be divided into five sub-batches (SB1–SB5), which were dried and sieved to obtain the respective "tea bag" fractions. One sub-batch (SB1) was retained as control (t = 0), while sub-batches SB2–SB5 were steam-treated (1, 2, 3 or 4 min) as described for experiment 1 and dried (40 °C/30 min) to *ca.* 8% moisture content.

2.4. Analysis of plant material and infusions

2.4.1. Individual phenolic compound content

Finely-milled plant material, prepared with a Retsch MM301 ball mill (Retsch GmbH, Haan, Germany), was extracted with aqueous