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Impact of steam pasteurization on the sensory profile and phenolic composition of rooibos (*Aspalathus linearis*) herbal tea infusions

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ABSTRACT

The effect of steam pasteurization of fermented rooibos leaves and stems on the sensory characteristics and phenolic composition of infusions was determined. The extent to which this processing step changes the sensory profile and whether compositional changes influence taste and astringency of the beverage was determined. These were achieved by examining the changes in the concentrations of soluble solids (SS), total polyphenols (TP) and 14 individual non-volatile monomeric phenolic compounds, as well as the changes in 17 aroma, flavor, taste and mouthfeel attributes of rooibos infusions. Steam pasteurization significantly reduced the SS, TP and aspalathin contents, as well as the "total color" (area under the curve: 380 to 520 nm). Neither the intensities of the taste attributes, sweetness and bitterness, nor the levels of individual phenolic compounds changed significantly, except that of aspalathin which were significantly reduced. A small but significant decrease in the astringency of rooibos infusions was observed. The intensities of most of the aroma and flavor attributes decreased significantly as a result of steam pasteurization. "Green" and "caramel" notes exhibited the largest reductions in attribute intensity. The prominent "green" flavor of unpasteurized rooibos was frequently changed to a "hay-like" flavor after steam pasteurization.

1. Introduction

Leaves and fine stems of the endemic South African fynbos plant, *Aspalathus linearis*, are processed to produce rooibos, a herbal tea of increasing global popularity. It is mostly consumed in the "fermented" (oxidized) form (Joubert & De Beer, 2011) which has a characteristic rooibos flavor that can be described as a combination of honey, woody and herbal-floral notes with a slightly sweet taste and subtle astringency (Koch, Muller, Joubert, Van der Rijst, & Næs, 2012). It contains no caffeine (Joubert & De Beer, 2011), which could contribute to a bitter taste. Flavor plays a predominant role in the grading of rooibos. The characteristic sensory attributes and absence of negative attributes are associated with high quality tea. Attributes such as "green grass" and "hay-like" aroma notes which are undesirable in some products (Hongsoongnern & Chambers, 2008) could have a negative impact on the quality and grade of rooibos as these would signify under-fermentation.

Commercial processing of rooibos involves shredding of the shoots followed by wetting with water and bruising to initiate enzymatic and chemical oxidation before overnight fermentation at ambient temperature and sun-drying the next day (Joubert & Schulz, 2006). Before packaging the dried and sieved product is steam pasteurized at 96 °C for 60 s to ensure that the final product is microbiologically safe.

After the introduction of steam pasteurization in the 1980s, consumers noted a softening of the flavor and the prominent "medicinal aroma" of rooibos. This led to a more acceptable product for some consumers while others preferred the flavor of unpasteurized rooibos (A. Redelinghuys, Rooibos Ltd., Clanwilliam, pers. comm.). These changes in the sensory quality of rooibos infusions due to steam pasteurization have not yet been scientifically substantiated, nor have such changes been accurately described or quantified. Recently a rooibos sensory wheel was developed (Koch et al., 2012) summarizing the variation in sensory attributes of rooibos infusions. The development of the sensory wheel has provided a basis for subsequent studies on the sensory quality of rooibos, because it has supplied the necessary terminology with which changes in the sensory profile of rooibos could be described and quantified.

The objective of this study was thus to determine the effect of steam pasteurization of fermented rooibos leaves and fine stems on the sensory characteristics of the infusion and to profile the changes in aroma, flavor, taste and mouthfeel (astringency) attributes. Furthermore, the link between the changes in the sensory profile and the phenolic composition of rooibos infusions was examined to determine whether compositional changes in non-volatile compounds could be associated with changes in taste and mouthfeel attributes.

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2. Materials and methods

2.1. Chemicals

Chemicals required for HPLC analysis were 99.8% acetic acid (Fluka, Sigma-Aldrich, Steinheim, Germany), acetonitrile (LiChrosolv, gradient grade for liquid chromatography, Merck, Darmstadt, Germany) and ascorbic acid (Sigma-Aldrich). Enolic phenylpyruvic acid-2-*O*-glucoside (PPAG), isolated from green rooibos (purity > 95% by HPLC and LC–MS), was supplied by the Post-Harvest & Wine Technology Division of the Agricultural Research Council of South Africa (ARC Infruitec-Nietvoorbij, Stellenbosch, South Africa). Aspalathin and nothofagin (purity > 95% by HPLC and LC–MS) were supplied by the PROMEC Unit of the Medical Research Council of South Africa (Cape Town, South Africa). Iso-orientin, iso-vitexin, luteolin, chrysoeriol and hyperoside were obtained from Extrasynthese (Genay, France). Sigma-Aldrich provided quercetin and rutin, while Roth (Karlsruhe, Germany) supplied orientin, vitexin, quercetin-3-*O*-glucoside (isoquercitrin) and luteolin-7-*O*-glucoside.

Laboratory grade deionized water was purified using a Milli-Q 185 Academic Plus water purifier (Merck Millipore, Billerica, MA, USA) to obtain HPLC grade water. The reagents required for the quantification of the total polyphenol content were Folin–Ciocalteu's phenol reagent (Merck), anhydrous sodium carbonate (Saarchem, South Africa) and gallic acid (Sigma Aldrich).

2.2. Rooibos samples

Prior to pasteurization, 69 samples, representing different production batches of fermented rooibos of the 2009 harvest season, were randomly collected and graded by an expert industry grading panel (Koch et al., 2012). The samples comprised nine Grade A samples and 20 samples each of Grades B, C and D, with Grades A and D representing the highest and the lowest quality, respectively. The sample codes used in this study indicate the quality grade and sample number assigned to each batch (e.g. B13 = Grade B, Sample 13). A reference sample composed of six different Grade B rooibos samples was prepared. Grade B samples were chosen as this grade present good quality tea produced in the highest quantities. The sensory attributes of the reference sample were considered to be representative of the profile typically associated with rooibos, i.e. having a mixture of honey, woody and herbal-floral notes with a slight sweet taste and subtle astringency (Koch et al., 2012). It thus served as a "fixed" point during descriptive sensory analysis (DA), allowing panelists to calibrate their sensory perception at the start of each session.

2.3. Steam pasteurization of rooibos samples

A sub-sample of each unpasteurized rooibos sample (UPAS) was steam pasteurized for direct comparison. The finely cut, dried leaves and stems (50 g) were spread in a thin layer on stainless steel, 30-mesh trays which were placed in a pre-heated steam cabinet at ±96 °C for 60 s to simulate the industrial process. The steam pressure, generated with a THE 400 NM Electropac electrode boiler (John Thompson Boilers, Cape Town), was maintained at 2.76 N/m^2 at the inlet of the cabinet. In order to remove superficial moisture and reduce the moisture content of the pasteurized rooibos (PAS) below 10%, the trays were placed in a cross-flow dehydrator set to 40 °C for 10 min. The dried rooibos was then stored in air-tight, re-sealable plastic bags.

2.4. Preparation of tea infusions

Infusions of unpasteurized and pasteurized samples were prepared by adding freshly boiled distilled water (900 g) to 17.4 g rooibos and stirring for about 5 s, followed by a 5 min infusion period (Koch et al., 2012). Infusions were strained through fine-mesh stainless steel tea strainers into preheated stainless steel thermos flasks. Panelists were served ca. 100 mL of the infusions "as-is" in preheated porcelain mugs with plastic lids. The mugs were kept in water-baths at 65 °C throughout the sensory analysis session to keep the temperature of the infusion as constant as possible.

2.5. Descriptive analysis (DA)

2.5.1. Sensory panel

Nine female judges, who previously received extensive training in DA, as well as the sensory analysis of rooibos infusions (Koch et al., 2012; Lawless & Heymann, 2010), participated in the study. During 22 1-hour sessions the panel generated the aroma, flavor, taste and mouthfeel descriptors that were used to assemble the rooibos sensory wheel and lexicon as described by Koch et al. (2012). The current study is therefore based on the sensory terminology that was developed in the previous study.

2.5.2. Intensity rating

The panel was requested to rate the intensities of 17 aroma, flavor, taste and mouthfeel attributes for each of the samples as described by Koch et al. (2012). In each session the UPAS sample and its PAS counterpart were presented together with the reference standard so that the panelists could not only directly compare the two samples with one another, but also with the reference standard. The panelists were informed that they were to receive a number of sample pairs each consisting of an UPAS sample with its corresponding PAS sample. Samples were labeled with three-digit codes, while the reference sample was labeled as such so that it could be identified by the panelists. The presentation order of the different sample pairs, as well as the order of the UPAS and the PAS samples within each pair, was randomized. All 69 UPAS and PAS samples were analyzed in duplicate. The samples were tested during 40 sessions over a period of 8 weeks, with 2 sessions conducted per day during which 6 and 8 samples were analyzed, respectively.

2.6. Compositional analysis

2.6.1. Sample preparation

An aliquot (200 mL) of each infusion of UPAS and PAS rooibos prepared for sensory analysis was filtered through Whatman No. 4 filter paper, allowed to cool and the soluble solids (SS) content determined. The remaining part of the filtrate was transferred into 2 mL microfuge tubes which were stored in a freezer at −18 °C until required for further analyses.

2.6.2. Determination of soluble solids (SS) content

The SS contents of the infusions were determined gravimetrically by evaporating 20 mL aliquots of the filtrate to dryness on a steam bath in triplicate, followed by oven drying at 100 °C for 1.5 h. The moisture dishes were allowed to cool in a desiccator before re-weighing.

2.6.3. Determination of total polyphenol (TP) content

The TP contents of the rooibos infusions were determined according to the method developed by Singleton and Rossi (1965), scaled-down to 96-well microplate format as described by Arthur, Joubert, De Beer, Malherbe, and Witthuhn (2011). Gallic acid was used as calibration standard and results expressed as mg gallic acid equivalents (GAE)/L infusion.

2.6.4. Quantification of monomeric phenolic compounds with HPLC

Quantification of 14 monomeric phenolic compounds was achieved by high-performance liquid chromatography with diode-array detection (HPLC–DAD) using an Agilent 1200 system (Agilent, Santa Clara, CA, USA) comprising a quaternary pump, autosampler, in-line degasser,