Cold brewing of rooibos tea affects its sensory profile and physicochemical properties compared to regular hot, and boiled brewing

Cold brewing of rooibos tea affects its sensory profile and physicochemical properties compared to regular hot-, and boiled-brewing

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ABSTRACT

Cold-brewing of rooibos tea has gained popularity, in particular in Japan, one of its major markets, due to the convenience of preparation. The sensory profile of traditional ('fermented') rooibos, prepared by regular brewing (5 min infused in freshly-boiled water) and served hot, is well described. However, no sensory profiling of cold-brewed rooibos tea, rooibos tea served at ambient temperature or green rooibos tea has been performed to date. In this study, the sensory profile of both fermented and green rooibos prepared by cold brewing (8 h at ≤5 °C), regular brewing and boiling (5 min), and consumed at ambient temperature (21 °C), were investigated. 'Rooibos woody' and 'fynbos-floral' aroma notes of fermented rooibos were unaffected by brewing procedure, but its cold brew was sweeter and less astringent than the other brews. The sensory profile of green rooibos, dominated by a 'hay/dried grass' aroma, bitter taste and astringency, was affected to a greater extent by brewing procedure, but mostly, cold and regular brews had similar aroma attribute intensities. Higher levels of flavonoids may explain the trends for astringency. Fe and Al were present in the highest levels in boiled brews, although at levels too low to impact on health.

Keywords

Rooibos; cold brew; sensory profiling; flavonoids; aspalathin; Fe and Al content

1. Introduction

Sugar-sweetened beverages, including ready-to-drink teas, come at a price, not only in terms of 'sugar-tax', but also the socio-economic burden of non-communicable diseases. These beverages make a substantial contribution to sugar intake and have consequently been linked to the onset of obesity, diabetes, and heart diseases (Benade & Essop, 2017). In response, the beverage industry has reformulated beverages with lower sugar content and/or replaced sugar with non-nutritive sweeteners. A recent report predicted huge growth in the global sugar-free tea market by 2025 with the increasing consumer awareness towards healthy drinks and a growing acceptance of natural herbs identified as some of the market drivers (https://www.openpr.com/news/2015719/sugar-free-tea-market-to-see-huge-growth-by-2025-unilever).

Another market trend is the emergence of cold-brewed tea, which could boost the acceptance of sugar-free tea. Cold brewing delivers a beverage with less caffeine and subsequently a reduced bitter taste as demonstrated for green tea (Lin, Liu, & Mau, 2008; Lin et al., 2014). A caffeine-free herbal tea that has grown in popularity on the global market is rooibos (*Aspalathus linearis*) (Joubert & De Beer, 2011). The main product is 'fermented' (oxidized) rooibos with its natural, slightly sweet taste and mild astringency. The characteristic sweet-associated aroma notes, 'caramel' and 'honey', of hot-brewed fermented rooibos (Jolley, Van der Rijst, Joubert, & Muller, 2017) could even enhance the perception of sweetness through cross-modal aroma-taste interactions (Alcaire, Antúnez, Vidal, Giménez, & Ares, 2017). In contrast to fermented rooibos, green (unoxidized) rooibos is a relatively new product, developed to capitalize on the market opportunities for antioxidant-rich beverages. High levels of aspalathin, a potent

C-glucosyl dihydrochalcone antioxidant, can be found in the unoxidized plant material (Joubert & De Beer, 2011). Mounting evidence of aspalathin and green rooibos extracts in the prevention of the metabolic syndrome (Johnson et al., 2018; Muller et al., 2018) merits the development of green rooibos as a functional beverage. However, a ready-to-drink beverage containing aspalathin-enriched green rooibos extract as an ingredient was perceived as having an overt 'plant-like' character, disliked by consumers (Viljoen, Muller, De Beer, & Joubert, 2017). Studies on functional foods have demonstrated that consumers are not inclined to compromise on flavor and taste for health (Verbeke, 2006). Changing the brewing procedure of green rooibos may, therefore, be merited to improve flavor and taste. Brewing temperature and duration of green tea were found to affect flavor (Lee & Chambers, 2009) and the extraction of health-promoting substances (Pastoriza, Pérez-Burillo, & Rufián-Henares, 2017). Cold-brewing of fermented rooibos during summer has already gained acceptance in Japan, one of its main markets, due to convenience. Adding a rooibos tea bag to water and infusing it for a prolonged period at ambient temperature or in a refrigerator is a convenient alternative to steeping in hot water or boiling on a stove. The latter practice was traditionally used to prepare rooibos tea (Joubert, Gelderblom, Louw, & De Beer, 2008).

Against this background, the aim of this study was to determine the effect of brewing procedure on the sensory profile and composition of both fermented rooibos and green rooibos. The sensory profile, color, and turbidity of cold-brewed rooibos were therefore compared with that of regular- and boiled-brewed rooibos. Color and turbidity are important parameters for visual appeal. The chemical composition of the beverages was quantified in terms of their *Z*-2-(β-D-glucopyranosyloxy)-3-phenylpropenoic acid

67 (PPAG), flavonoid, Al, and Fe content. PPAG was previously demonstrated to elicit a
68 bitter taste (Joubert et al., 2013).
69

70 **2. Experimental**

71 *2.1. Chemicals*

72 Reagent-grade chemicals were obtained from Sigma-Aldrich (St. Louis, MO,
73 USA) or Merck (Darmstadt, Germany). Ultrapure water for HPLC and metal analysis
74 was prepared by a two-stage water purification system (Elix and Milli-Q) (Merck).
75 Authentic standards (purity ≥ 95%) were purchased from Extrasynthese (Genay, France;
76 isoorientin, orientin, 7-β-D-glucopyranosyleriodictyol (miscanthoside), isovitexin and
77 hyperoside), Carl Roth (Karlsruhe, Germany; vitexin), Sigma-Aldrich (isoquercitrin,
78 ferulic acid), Transmit (Gieβen, Germany; rutin), Phytolab (Vestenbergsgreuth,
79 Germany; aspalathin (91% purity)) and SAMRC (Cape Town, South Africa;
80 nothofagin). PPAG was from our compound library (Plant Bioactives Group, ARC
81 Infruitec-Nietvoorbij). Stock solutions of the standards (ca. 1 g/L) were prepared in
82 DMSO and diluted with water as required. The diluted standard mixtures contained ca.
83 10 g/L ascorbic acid (Sigma-Aldrich) and were filtered through 0.45 μm hydrophilic
84 PVDF filters (Merck).
85 Working standard solutions for metal analysis were prepared by appropriate
86 dilution of inorganic atomic absorption standard solutions (1000 mg/L) of Fe (Carlo
87 Erba, Milan, Italy) and Al (Sigma-Aldrich) and stored in a refrigerator at ca. 4 °C
88 protected from light. Ultrapure nitric acid (670-690 g/kg) was purchased from Carlo
89 Erba.

90 *2.2. Rooibos samples*

Samples from production batches (n=12) of both fermented rooibos and green rooibos were sourced from Bokkeveld Rooibos (Nieuwoudtville, Northern Cape, South Africa) and Rooibos Ltd. (Clanwilliam, Western Cape, South Africa), respectively. Samples were mechanically sieved to remove dust (<0.42 mm) and coarse material (>1.68 mm) (Jolley et al., 2017) and then stored at 21 °C in sealed glass jars.

2.3. Preparation of infusions

A cold brew (C), regular brew (R), and boiled brew (B) were prepared from each batch of fermented and green rooibos by adding 12.5 g plant material to 1 L of distilled water (equivalent to 'cup-of-tea' strength) as outlined in Fig. 1. Cold brewing entailed adding water (21 °C) to the plant material in a wide-necked Schott bottle and steeping for 8 h in a refrigerator (0-5 °C). For regular brew, freshly-boiled water was added to the plant material in a glass jug followed by stirring for 5 s and steeping for 5 min (Koch, Muller, Joubert, Van der Rijst, & Næs, 2012). The boiled brew was prepared by adding boiling water to the plant material in a glass jug followed by microwave heating (1000 W) for 5 min with stirring every minute to prevent boiling over. All brews were strained through a fine-meshed strainer into 1 L Schott bottles directly after brewing and stored overnight in a refrigerator (12 h). The brews were equilibrated to 21 °C prior to sensory analysis.

2.4. Descriptive sensory analysis

Panel training and testing were performed separately for fermented and green rooibos. Eleven assessors, with extensive experience in DSA of fermented rooibos, were trained according to a basic protocol (Koch et al., 2012) to generate appropriate aroma, flavor, taste, and mouthfeel attributes for green and fermented rooibos (supplementary material, Table A.1). Attribute intensities were scored, using an

115 unstructured line scale (0 = not detected; 100 = high intensity), and the scores were
116 captured electronically (Compusense®, Guelph, Canada). Three batches of plant
117 material of each of the brew variants were analyzed per day in three consecutive
118 sessions. The sample order was randomized per assessor per session. The blind-coded
119 samples were served in black wine tasting glasses (ca. 70 mL per glass) covered with
120 plastic lids. Analyses were conducted in individual tasting booths under standard
121 lighting and controlled temperature (21 °C).

122 *2.5. Physicochemical analyses*

123 Turbidity, objective color, and soluble solids (SS) content of the brews were
124 determined in triplicate as described by Sishi, Muller, De Beer, Van der Rijst, & Joubert
125 (2019).

126 The concentration of the major flavonoids and PPAG in the brews was
127 determined in duplicate by high performance liquid chromatography with diode array
128 detection (HPLC-DAD) using an Agilent 1200 HPLC system (Agilent Technologies,
129 Santa Clara, CA, USA). All compounds, except aspalathin and ferulic acid, were
130 quantified using the validated method described by Walters, De Villiers, Joubert, & De
131 Beer (2017). Aspalathin and ferulic acid were quantified after separation on a Poroshell
132 120 EC-C_{18} column (50×3 mm, 2.7 µm particle size; Agilent Technologies) at 25 °C.
133 The mobile phases (0.1% aqueous formic acid (A) and acetonitrile (B)) were run at 0.43
134 mL/min using the following gradient: 0–5 min, 12.4–14.5% B; 5–5.5 min, 14.5–80% B;
135 5.5–6.5 min, 80% B; 6.5–7 min, 80–12.4% B; 7–11 min, 12.4% B. UV-Vis spectra were
136 recorded between 200 and 450 nm with selected wavelength monitoring at 288 nm for
137 aspalathin and 320 nm for ferulic acid and the compounds quantified, using eight-point
138 calibration curves. Peak identity was verified by performing the same separation on a

139 Waters Acquity UPLC coupled to a Synapt G2 quadrupole-time-of-flight (Q-TOF) MS
140 detector, equipped with an electrospray ionization (ESI) source (Waters, Milford, USA).
141 The mass spectrometer was operated in negative MS^E mode with a collision energy
142 ramp from 20–60 V. All other MS parameters were as described by Walters et al.
143 (2017).

144 *2.6. Quantification of Fe and Al*

145 Samples were prepared and analyzed in triplicate in a clean room laboratory ISO
146 14644–1 Class 6, with areas at ISO Class 5 under laminar flow. All the laboratory
147 materials were acid-cleaned as described by Illuminati, Annibaldi, Truzzi, & Scarponi
148 (2014). An aliquot of each sample (5 mL) was put into Teflon vessels of a Microwave
149 Accelerated Reaction System, MARS-5, 1500 W (CEM, Mathews, NC, USA) and
150 digested (program reported in supplementary material, Table A.2) without any
151 pretreatment with 3 mL of HNO_3 (Annibaldi et al., 2019; Łozak, Sołtyk, Ostapczuk, &
152 Fijałek, 2002). An HP-500 control vessel containing the same matrix of samples was
153 used to control the temperature and pressure during the process. Water (2 mL) was used
154 to clean up the vessels at the end of the mineralization process and added to the
155 samples, to reach a final volume of 10 mL. The quantitative determinations of Al and Fe
156 were carried out using a graphite furnace atomic absorption spectrophotometer (DUO
157 240FS AA-GTA120 Graphite Tube Atomizer, Agilent, Santa Clara, CA, USA),
158 equipped with Zeeman background correction with argon (purity = 99.998%) as carrier
159 gas and multi-element hollow cathode lamps as light source. The instrumental
160 parameters used for each element are reported in the supplementary material (Table
161 A.3). The quantitative values were calculated from calibration curves generated using
162 diluted standard solutions (20–80 µg/L for Al and 10–50 µg/L for Fe).

For assessment of the accuracy of the data, Al and Fe were determined using a certified reference material (dogfish muscle DORM-2, NRCC; Ottawa, ON, Canada). Certified mean values and experimental mean values obtained from the analysis of DORM-2, expressed in mg/kg dry weight (d.w.), are as follows: Fe, Certified value = 142 ± 10, Analytical result found = 132 ± 1; Al, Certified value = 10.9 ± 1.7, Analytical result found = 10.2 ± 0.8. No statistically significant differences ($P \geq 0.05$, Student's T-test) between certified and measured values were detected, and results were in good agreement with the certified values, proving good accuracy of the analytical methodology.

2.7. Statistical procedures

Data obtained for fermented and green rooibos were analyzed separately. DSA data were subjected to various statistical techniques to confirm panel reliability (Næs, Brockhoff, & Tomic, 2010) and normality. Subsequent statistical analyses were conducted on means over assessors. DSA and composition data were subjected to analysis of variance (ANOVA) to test for treatment differences using SAS software (Version 9.2, SAS Institute Inc., Cary, USA). Fisher's least significant difference (LSD) was calculated (5% level) to compare treatment means. P values < 0.05 were considered significant.

Principal component analysis (PCA), using the correlation matrix, was performed, using XLStat (Version 2018, Addinsoft, Paris, France). Using the combined fermented and green rooibos data sets, the Pearson correlation coefficient (r) was calculated to determine the relationship between individual compound content and bitterness and astringency, respectively.

3. Results and discussion

Twelve batches of both fermented and green rooibos were subjected to three different brewing procedures, i.e. cold (C), regular (R), and boiled (B), to determine the impact on their sensory profiles. As the mean intensity scores for the aroma and flavor attributes followed similar trends with flavor attributes mostly perceived at lower intensities, the focus will fall on the aroma.

3.1 Sensory profile

The PCA bi-plot for fermented rooibos (Fig. 2a) shows that only 52.7% of the variance is explained. The regular- and boiled-brewed samples tend to associate on the left of PC1, while the cold-brewed samples were positioned on the right, indicating that the application of heat played a role in discriminating between treatments. On PC2, samples largely separated between the boiled-brewed samples at the top and the cold- and regular-brewed samples at the bottom. The intensities of the primary aroma attributes ('rooibos-woody', 'fynbos-floral' and 'honey') of regular-brewed fermented rooibos consumed hot (Jolley et al., 2017), as well as 'cooked apple', a secondary aroma attribute of fermented rooibos, were not significantly ($P \geq 0.05$) affected by treatment (Fig. 3a). The boiled-brewed samples associated with 'raisin' and 'caramel' (Fig. 2a), which were perceived at significantly higher ($P < 0.05$) intensities in these brews (Fig. 3a). The cold-brewed samples associated with 'tobacco' and sweet taste, but not with astringency (Fig. 2a). The cold-brewed samples were significantly sweeter and less astringent ($P < 0.05$) than the regular- and boiled-brewed samples (Fig. 3a), which could contribute to consumer acceptance of cold-brewed rooibos. In view of the need to reduce the sugar content of beverages to limit sugar intake, the inherent slightly sweeter taste of cold-brewed rooibos is beneficial. The 'tobacco' aroma note is generally

associated with the dry ‘fermented’ leaf product. Furthermore, ‘seaweed’, a negative aroma note, was not perceptible in the cold- and boiled-brewed samples, and just perceptible in the regular brews ($P<0.05$).

For green rooibos, PC1 and PC2 explained 77.6% of the variance and a clear distinction between the samples of the three brewing procedures is evident (Fig. 2b). On PC1, the boiled-brewed samples clustered on the right, whereas the cold- and regular-brewed samples formed separate clusters on the left. On PC2, the separation between samples was driven by brewing temperature with cold-brewed samples positioned at the top and both regular- and boiled-brewed samples at the bottom. The cold brews had significantly higher intensities ($P<0.05$) of the aroma notes ‘fresh apple’ and ‘tobacco’, as well as sweet taste, compared to those of the other brew types (Fig. 3b). In contrast, the boiled brews had significantly higher intensities of the attributes ‘oats porridge/cooked grains’, ‘caramel’, ‘stewed fruit’, bitter taste and astringency ($P<0.05$) than the other two treatments, whereas the negative attributes, ‘green grass’ and ‘putty’, were barely perceptible in the boiled brews ($P<0.05$) (Fig. 3b). The ‘seaweed’ aroma note was prominent in the regular brews (intensity >15), barely perceptible in the cold brews (<5), and practically not perceptible in the boiled brews. The trend is the same as for fermented rooibos, suggesting that the short heat exposure is required to form volatile compound(s) responsible for this negative aroma note, while a more severe application of heat resulted in sufficient loss of the formed volatile compound(s).

The fermented and green rooibos samples did not originate from the same batches of fresh plant material. However, the sensory profile of green rooibos was consistently and vastly different to that of fermented rooibos, especially in that the vegetal (‘green grass’, ‘cooked vegetables’) and cereal-like (‘oats porridge/cooked grains’) aroma attributes

234 were present in moderate to high intensities in green rooibos brews, depending on the
235 brewing procedure. While these aroma notes seem integral to the sensory profile of
236 green rooibos, they are considered to be negative for fermented rooibos (Jolley et al.,
237 2017). The 'hay/dried grass' intensity was not affected by the brewing procedure. A
238 short steam treatment of the dry fermented rooibos leaves also did not affect the 'hay-
239 like' intensity of rooibos infusions (regular-brewed; consumed hot) (Koch, Muller, De
240 Beer, Næs, & Joubert, 2013). Fermented rooibos brews had a sweeter taste, while the
241 green rooibos brews were more astringent with a prominent bitter taste.

242 *3.2 SS content, color and turbidity*

243 The SS content and turbidity of the fermented rooibos brews were significantly
244 ($P<0.05$) affected by brewing procedure, increasing progressively from cold to regular
245 to boiled brew (Table 1). The CIELab color parameters, lightness (L*), and hue (h)
246 decreased and redness (a*) increased significantly ($P<0.05$) (Table 1) with the increase
247 in the soluble solids content of the brew. The yellowness parameter, b*, and
248 subsequently also chroma of the cold brews were significantly ($P<0.05$) lower than
249 those for the regular and boiled brews. Whilst the color parameters indicate differences
250 between the brews, the values are difficult to relate to the actual visual color in a cup or
251 glass due to dichroism, i.e. the hue or color depends on the degree of dilution or
252 container size (Francis & Clydesdale, 1975).

253 The different procedures used for the brewing of green rooibos had similar
254 effects to fermented rooibos on the soluble solids content, turbidity, L*, a* and hue of
255 the brews ($P<0.05$) (Table 2). For all these parameters, except a*, higher values were
256 obtained for the green rooibos brews than for the fermented rooibos brews (Tables 1 and

2). The lower a* values of green rooibos brews (1.0-14.3 vs 16.01-30.9) were to be expected as the plant material is not oxidized.

3.3 PPAG and flavonoid content

The PPAG concentration in the fermented rooibos brews was slightly increased ($P<0.05$) by boiling compared to cold and regular brewing (Table 1; representative chromatograms shown in Fig. A.1). The concentration of most of the flavonoids increased significantly ($P<0.05$) when heat was applied during the brewing of fermented rooibos, with the boiling procedure being most effective in extracting the compounds from the plant material (Table 1). The aspalathin, bioquercetin, hyperoside, and isoquercitrin concentration of the regular and boiled brews did not differ significantly ($P\geq0.05$).

For the green rooibos brews, most phenolic compounds showed a significantly ($P<0.05$) higher concentration in the boiled brew compared to the other brews (Table 2; representative chromatograms shown in Fig. A.2). Some exceptions were PPAG, bioquercetin, and rutin with the lowest ($P<0.05$) concentration in the regular brew. The PPAG concentration of the cold and boiled brews was not significantly ($P\geq0.05$) different, but the bioquercetin and rutin concentrations were significantly ($P<0.05$) higher in the boiled brew than in the cold brew. Our previous study on the same sample set provided more insight into the phenolic profile of the brews. A large number of compounds were putatively annotated (Damiani et al., 2019). Of 187 putatively annotated compounds, 32 and 35 polyphenols were considered as markers that discriminate between cold and boiled brews, and cold and regular brews, respectively.

The investigation of the association between the flavonoid content of rooibos infusions and the intensities of the taste modalities and astringency (when served hot) is

281 limited (Koch et al., 2013). The association between PPAG and sweet taste was notable,
282 however, when tested at 21 °C as a single compound, dissolved in water, it was bitter at
283 a concentration similar to that of regular-brewed rooibos. The PPAG concentration of
284 the brews analyzed for the present study (Tables 1 and 2) was much higher than its
285 detection threshold in water (0.4 mg/L) (Joubert et al., 2013). PPAG could, therefore, be
286 expected to contribute to the bitter taste. Considering that the green rooibos brews were
287 substantially more bitter than the fermented rooibos brews, while their respective PPAG
288 content did not differ to the same extent, other compounds or interaction with other
289 compounds (i.e. masking and/or enhancing effects) contributed to bitterness. A
290 moderate correlation was found between the content of the individual flavonoids and the
291 bitter intensity of the brews ($r \geq 0.5$; $P \leq 0.002$). The eriodictyol glucosides had the highest
292 correlation with bitter intensity ($r > 0.65$; $P < 0.0001$).

293 It is well-known that phenolic compounds contribute to the astringency of food,
294 wine, and tea (Bordenave, Hamaker, & Ferruzzi, 2014). Despite the green rooibos
295 brews having a substantially higher flavonoid content than the fermented rooibos brews,
296 the green rooibos brews were not substantially more astringent (Tables 1 and 2; Fig. 3).
297 A moderate, but significant correlation was found between the individual flavonoid
298 content of the brews and astringency ($r \geq 0.41$; $P < 0.01$). PPAG did not correlate
299 significantly with astringency ($r = 0.325$; $P = 0.053$).

300 *3.4 Al and Fe content*

301 Al and Fe were present in significantly higher ($P < 0.05$) levels in the boiled
302 brews of both fermented and green rooibos (Tables 1 and 2), while no significant
303 differences ($P \geq 0.05$) between the cold and regular brews were found, except in the case

304 of the Fe content of fermented rooibos brews. In this case, the Fe content increased
305 significantly ($P<0.05$) from cold to regular to boiled brew.

306 The Fe and Al content values were similar to those previously reported for hot
307 water infusions of fermented rooibos (Joubert et al., 2008; Malik, Szakova, Drabek,
308 Balik, & Kokoska, 2008). Consumption of one cup of the brews would contribute
309 <0.2% of the nutrient reference value (NRV) for adults, i.e. 18 mg/day, according to
310 South African food labeling regulations. Chronic Al intake, on the other hand, has
311 negative health implications (Pérez-Granados & Vaquero, 2002). A tolerable weekly
312 intake (TWI) of Al is 1 mg/kg body weight/week (Aguilar et al., 2008), i.e. 10 mg/day
313 by a 70 kg adult. One cup of rooibos per day would contribute <0.3% of this intake.

314 *3.5 "To cold-brew or not to cold-brew"*

315 Boiling increased the flavonoid content of rooibos tea, irrespective of the type.
316 Similarly, in our previous study boiling also delivered brews with the highest
317 antioxidant capacities (ORAC and DPPH) (Damiani et al., 2019). However, it is not
318 clear whether these increases would translate into a specific health benefit. No
319 recommended daily intake values have been established for flavonoids, as this group of
320 phytochemicals differs vastly in terms of bioavailability and bioactivity, while the value
321 of antioxidant capacity in the assessment of health promotion is the subject of debate
322 (De Camargo et al., 2019; Williamson, Kay, & Crozier, 2018).

323 The flavor of beverage products is regarded as one of the main drivers of
324 consumer preference. The sensory profile associated with the respective rooibos brews
325 would be the deciding factor if convenience is not considered important. Cold brewing
326 of fermented rooibos delivered a similar primary and secondary sensory profile as
327 regular brewing, thus cold brewing offers no advantage or disadvantage in terms of its

328 sensory profile. The slightly higher intensities of ‘raisin’ and ‘caramel’ aroma notes of
329 boiled-brewed rooibos may be one of the reasons why rooibos was traditionally brewed
330 on a hot stove for extended periods. The ‘caramel’ note was also more prominent in
331 boiled-brewed green rooibos. The intensity of the ‘oats porridge/cooked grains’ note,
332 not present in fermented rooibos, was substantially increased with the boiling of green
333 rooibos. While the brewing procedure did not impact on astringency, boiling of green
334 rooibos increased the bitterness of the tea, linked to increased extraction of flavonoids.
335 To date, no study has been conducted to determine whether rooibos consumers are
336 willing to forfeit taste for health. Even the Fe and Al content of a cup of rooibos tea,
337 irrespective of type or brewing procedure, was found to be too low to impact,
338 respectively, positively and negatively on health and thus would not drive choice.

339 **4. Conclusions**

340 Cold brewing of both fermented rooibos and green rooibos did not offer many
341 advantages in terms of flavonoid content and sensory profile of the brew compared to
342 regular brewing. Notable was a less bitter and/or sweeter taste of the cold brew
343 compared to the boiled brew.

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Conflict of interest

The authors declare no conflict of interest.

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459 **Figure captions**

460 **Fig. 1.** Basic procedure used for the preparation of cold-, regular-, and boiled-brewed
461 fermented and green rooibos herbal tea.

462 **Fig. 2.** PCA bi-plot of the sensory profile of cold-, regular-, and boiled-brewed
463 fermented (a) and green (b) rooibos herbal tea with brewing procedure indicated by C,
464 R, and B, respectively. The numbers (1 to 12) refer to the individual samples,
465 representing independent replicates.

466 **Fig. 3.** ANOVA results of selected sensory attributes of cold-, regular-, and boiled-
467 brewed fermented (a) and green (b) rooibos herbal tea. Bars colored white, grey and
468 black represent cold-, regular-, and boiled-brewed rooibos, indicating mean values and
469 error bars (standard deviation).

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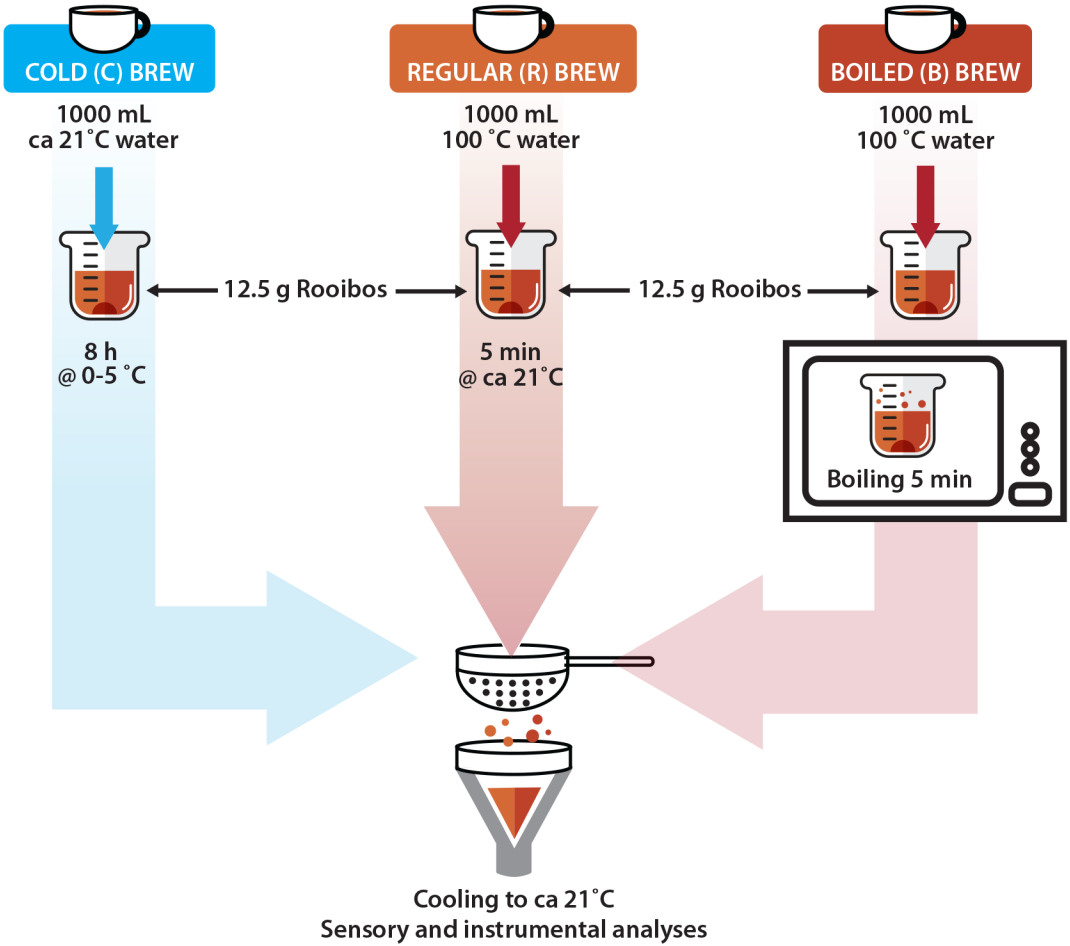

COLD (C) BREW
1000 mL
ca 21˚C water
REGULAR (R) BREW
1000 mL
100 ˚C water
BOILED (B) BREW
1000 mL
100 ˚C water
12.5 g Rooibos
12.5 g Rooibos
8 h
@ 0-5 ˚C
5 min
@ ca 21˚C
Boiling 5 min
Cooling to ca 21˚C
Sensory and instrumental analyses

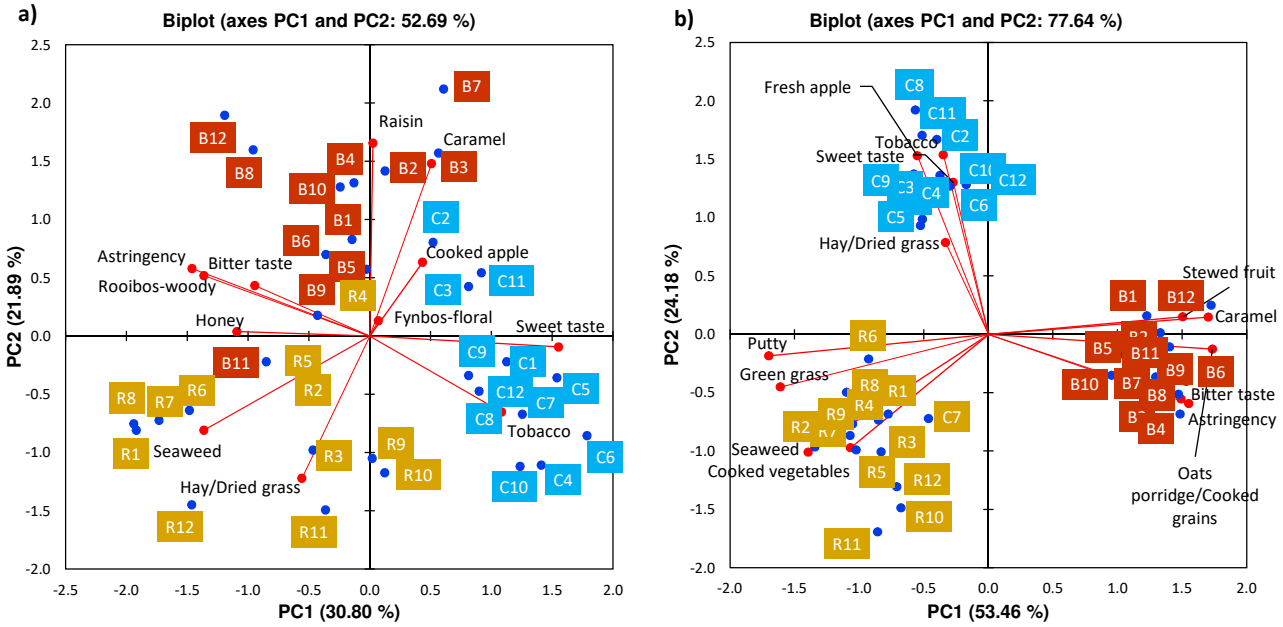

a)
Biplot (axes PC1 and PC2: 52.69 %)
PC1 (30.80 %)
PC2 (21.89 %)
Raisin
Caramel
Cooked apple
Astringency
Bitter taste
Rooibos-woody
Honey
Fynbos-floral
Sweet taste
Tobacco
Seaweed
Hay/Dried grass
b)
Biplot (axes PC1 and PC2: 77.64 %)
PC1 (53.46 %)
PC2 (24.18 %)
Fresh apple
Tobacco
Sweet taste
Hay/Dried grass
Stewed fruit
Caramel
Putty
Green grass
Bitter taste
Astringency
Seaweed
Cooked vegetables
Oats porridge/Cooked grains

Figure 2

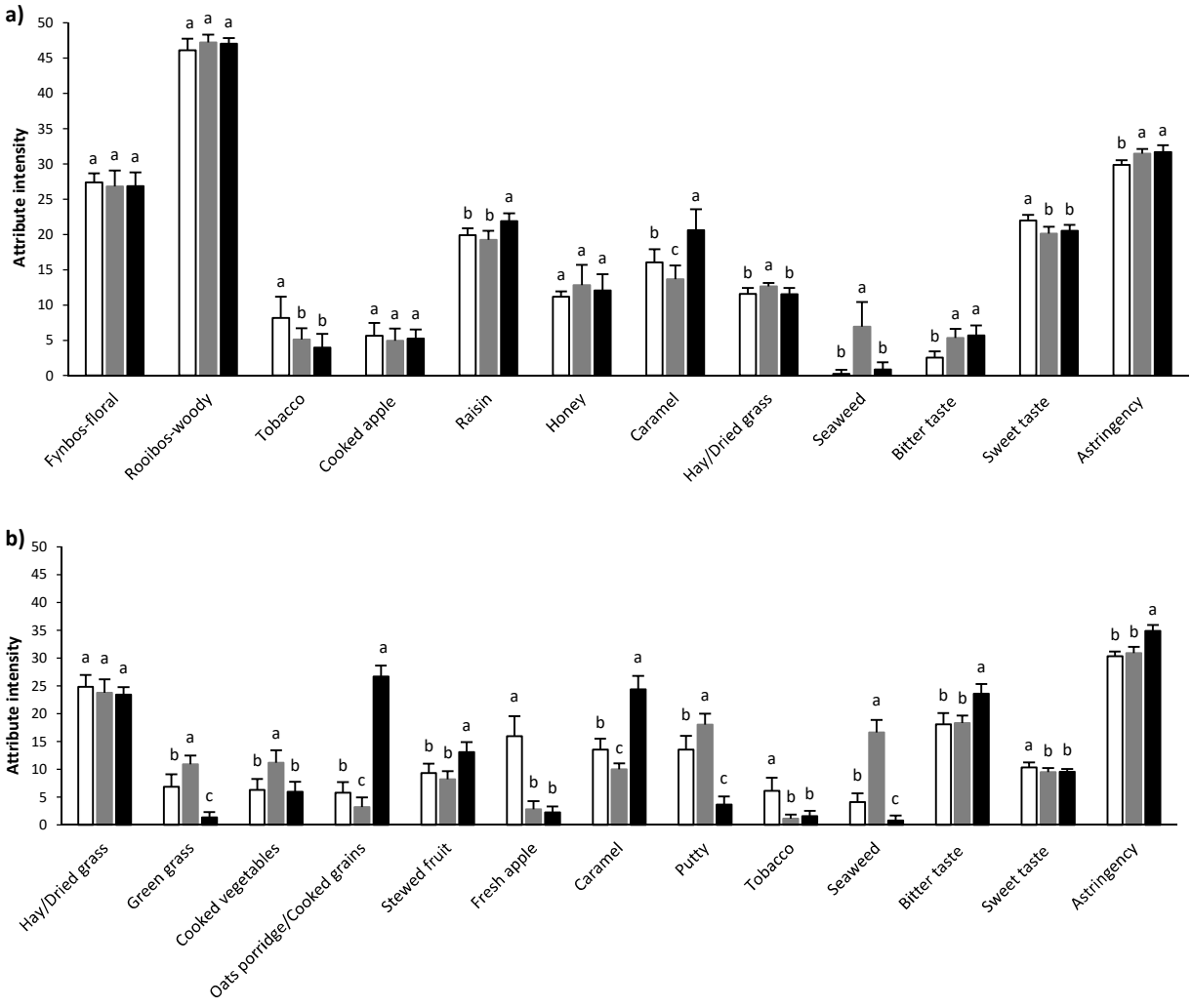

a)
Attribute intensity
Fynbos-floral
Rooibos-woody
Tobacco
Cooked apple
Raisin
Honey
Caramel
Hay/Dried grass
Seaweed
Bitter taste
Sweet taste
Astringency
b)
Attribute intensity
Hay/Dried grass
Green grass
Cooked vegetables
Oats porridge/Cooked grains
Stewed fruit
Fresh apple
Caramel
Putty
Tobacco
Seaweed
Bitter taste
Sweet taste
Astringency

Figure 3

Table 1

Colour, turbidity (NTU), soluble solids content (SS, g/L) and chemical composition (mg/L, except Fe and Al, µg/L) of cold brew, regular brew and boiled brew fermented rooibos.

Parameter	Cold brew	Regular brew	Boiled brew	LSD
L*	81 ± 2 a	72 ± 2 b	64 ± 3 c	1
a*	16 ± 3 c	26 ± 2 b	31 ± 2 a	1
b*	93 ± 5 b	97 ± 3 a	95 ± 3 a	2
C	94 ± 5 b	101 ± 3 a	100 ± 3 a	2
h	80 ± 1 a	75 ± 1 b	72 ± 1 c	1
Turbidity	5 ± 1 c	22 ± 7 b	42 ± 14 a	6
Soluble solids	1.0 ± 0.1 c	1.5 ± 0.2 b	1.9 ± 0.2 a	0.1
SE6G[a]	7.3 ± 0.7 c	10.8 ± 1.0 b	14.0 ± 1.4 a	0.5
RE6G[b]	6.9 ± 0.8 c	10.5 ± 1.0 b	13.8 ± 1.5 a	0.5
SE8G[c]	2.0 ± 0.3 c	3.0 ± 0.4 b	4.3 ± 0.7 a	0.2
RE8G[d]	2.5 ± 0.2 c	3.4 ± 0.3 b	4.7 ± 0.5 a	0.2
Aspalathin	3 ± 2 b	5 ± 4 a	5 ± 3 a	1
PPAG[e]	9 ± 1 b	8 ± 1 b	10 ± 2 a	0.4
Nothofagin	0.3 ± 0.2 c	0.6 ± 0.3 b	0.9 ± 0.4 a	0.1
Isoorientin	4.4 ± 0.6 c	11.3 ± 1.4 b	15.9 ± 1.3 a	0.6
Orientin	8 ± 1 c	18 ± 2 b	25 ± 2 a	1
Ferulic acid	1.2 ± 0.4 c	1.3 ± 0.3 b	1.6 ± 0.4 a	0.1
Bioquercetin	5 ± 3 b	9 ± 4 a	10 ± 3 a	1
Vitexin	1.3 ± 0.1 c	3.1 ± 0.3 b	4.1 ± 0.3 a	0.2
Hyperoside	0.9 ± 0.9 b	2.4 ± 1.8 a	2.4 ± 0.9 a	0.5
Rutin	0.8 ± 0.6 c	1.5 ± 1.0 b	2.1 ± 1.4 a	0.5
Isovitexin	1.3 ± 0.1 c	3.2 ± 0.3 b	4.4 ± 0.3 a	0.2
Isoquercitrin	0.3 ± 0.4 b	1.8 ± 1.4 a	1.9 ± 1.0 a	0.6
Luteoleoside	0.02 ± 0.04 c	0.51 ± 0.18 b	0.94 ± 0.22 a	0.12
Sum of flavonoids	45	85	111	-
Fe	105 ± 13 c	119 ± 14 b	142 ± 5 a	7
Al	42 ± 22 b	57 ± 21 b	108 ± 35 a	20

[a] (*S*)-6-β-D-glucopyranosyleriodictyol.
[b] (*R*)-6-β-D-glucopyranosyleriodictyol.
[c] (*S*)-8-β-D-glucopyranosyleriodictyol.
[d] (*R*)-8-β-D-glucopyranosyleriodictyol.
[e] *Z*-2-(β-D-glucopyranosyloxy)-3-phenylpropenoic acid.

Table 2

Colour, turbidity (NTU), soluble solids content (SS, g/L) and chemical composition (mg/L, except Fe and Al, µg/L) of cold brew, regular brew and boiled brew green rooibos.

Parameter	Cold brew	Regular brew	Boiled brew	LSD
L*	93 ± 1 a	88 ± 3 b	80 ± 2 c	1
a*	1 ± 1 c	6 ± 2 b	14 ± 2 a	1
b*	40 ± 7 b	35 ± 6 c	46 ± 5 a	3
C	40 ± 7 b	35 ± 7 c	49 ± 5 a	3
h	89 ± 1 a	80 ± 2 b	73 ± 1 c	1
Turbidity	9 ± 3 c	33 ± 10 b	60 ± 9 a	5
Soluble solids	1.9 ± 0.2 b	1.7 ± 0.4 b	2.5 ± 0.4 a	0.2
Aspalathin	189 ± 27 b	176 ± 48 b	267 ± 40 a	17
PPAG[a]	13 ± 2 a	9 ± 2 b	13 ± 1 a	1
Nothofagin	19 ± 4 b	18 ± 6 b	28 ± 6 a	2
Isoorientin	13 ± 2 b	13 ± 3 b	21 ± 3 a	1
Orientin	19 ± 2 b	19 ± 4 b	30 ± 4 a	2
Bioquercetin	18 ± 5 b	14 ± 5 c	21 ± 6 a	1
Vitexin	3.0 ± 0.4 b	2.8 ± 0.7 b	4.5 ± 0.6 a	0.3
Hyperoside	5 ± 2 b	5 ± 2 b	7 ± 3 a	1
Rutin	8 ± 2 b	6 ± 2 c	10 ± 2 a	1
Isovitexin	3.6 ± 0.4 b	3.7 ± 0.9 b	5.7 ± 0.8 a	0.3
Isoquercitrin	8 ± 3 b	8 ± 4 b	13 ± 5 a	1
Luteoleoside	0.7 ± 0.3 c	1.1 ± 0.4 b	1.9 ± 0.6 a	0.2
Sum of flavonoids	286	267	409	-
Fe	21± 8 b	21 ± 7 b	53 ± 14 a	6
Al	82 ± 42 b	81 ± 38 b	149 ± 45 a	32

[a] *Z*-2-(β-D-glucopyranosyloxy)-3-phenylpropenoic acid.

FERMENTED ROOIBOS

GREEN ROOIBOS

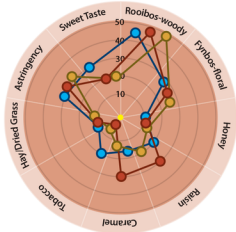
COLD (C) BREW

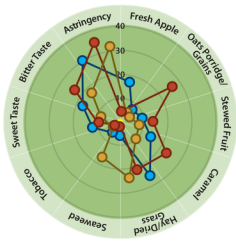
REGULAR (R) BREW

BOILED (B) BREW

PREPARATION PROCEDURE

AROMA PROFILE





C

R

B