Chemical-sensory properties and consumer preference of hibiscus beverages produced by improved industrial processes

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Abstract
The need to increase sustainability and add value to traditional foods claiming health benefits led to the introduction of key improvements in the production of hibiscus beverages in Senegal. The physicochemical and sensory properties of three resulting products (an under-vacuum concentrate, a dilute-to-taste syrup and a ready-to-drink infusion) were assessed, vis-à-vis those of conventionally manufactured beverages, and their impact on local consumer preference determined (n = 146). New beverages had more intense, redder colour and higher monomeric anthocyanin content, total phenolic content and antioxidant capacity. Moreover, their colour evaluations by trained panellists were mainly linked to colour density and anthocyanin/polyphenol content, while flavour assessments were associated to titratable acidity and sugar-to-acid ratio. Consumer evaluations, in turn, were driven by the beverages’ red colour intensity, aroma strength and balance between sweetness and acidity. This explained why they overwhelmingly preferred the under-vacuum concentrate, regardless of their age, gender or frequency of hibiscus beverage consumption.

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1. Introduction

Hibiscus sabdariffa L. is an herbaceous plant of the genus Hibiscus of the Malvaceae family, widely grown in tropical and subtropical Africa, Asia and North-America (Mc Clintock & El Tahir, 2004). Due to their deep red colour, distinctive floral, berry-like aroma and unique acidic flavour, the dried sepals of H var. sabdariffa ruber flowers (hereinafter referred to as hibiscus calyces) are one of the highest volume specialty botanical products in international trade, being used worldwide in the production of foods, beverages, pharmaceuticals and cosmetics (Da-Costa-Rocha, Bonnlaender, Sievers, Pischel, & Heinrich, 2014; Plotto, Mazaud, Röttger, & Steffel, 2004).

The consumption of sweetened, hot or cold hibiscus infusions (either as refreshment or folk remedy) is very common in such diverse countries as Mexico, Thailand and Egypt (Mc Clintock & El Tahir, 2004; Plotto et al., 2004; Ramirez-Rodrigues, Balaban, Marshall, & Rouseff, 2011).

Still, it is nowhere more widespread that in West Africa, where dried hibiscus calices are found in every market and variations of homemade or manufactured infusions are commonly sold on the streets, mainly as a cheap alternative to imported soft drinks (Bolade, Oluwalana, & Ojo, 2009; Cisse, Dornier, Sakho, Ndiaye, et al., 2009). In Senegal, for instance, hibiscus beverages are commonly prepared from calyces of the Sudanese ‘Vimto’ and/or the native ‘Koor’ cultivars. The first are reputedly rich in anthocyanins and other phenolic compounds (and thus possess good colouring and antioxidant properties), while the latter have a high organic acid content, resulting in beverages with intense sourness, a sensory characteristic much appreciated by locals (Cisse, Dornier, Sakho, Ndiaye, et al., 2009).

Indoctrination, flavouing and thickening properties of hibiscus calyces have been shown to derive largely from their distinctive phenolic, organic acid and pectin composition (Fernández-Arroyo et al., 2011; Ramirez-Rodrigues, Plaza, Azeredo, Balaban, & Marshall, 2011; Sáyago-Ayerd, Velázquez-López, Montalvo-González, & Goñi, 2014). Moreover, the bioactivity of these compounds has been demonstrated in different biological models, being linked to important
pharmacological properties of extracts, namely nephro- and hepato-protective, renal/diuretic, anti-cholesterol, anti-hypertensive, anti-diabetic, hypo-lipidemic and anti-tumoral effects (Da-Costa-Rocha et al., 2014; Maganha et al., 2010).

The need to increase sustainability and add value to traditional foods that can potentially claim health benefits, in both domestic and foreign markets, has led to the implementation of several research projects in countries where the production of hibiscus is important, namely Nigeria, Mexico and Senegal (Cisse et al., 2012; Diessana, Parkouda, Cissé, Bréhima, & Dicko, 2015; Pérez-Ramírez, Castaño-Tastado, Ramírez-de León, Rocha-Guzmán, & Reynoso-Camacho, 2015; Ramírez-Rodrigues, Balaban, et al., 2011; Ramírez-Rodrigues, Plaza, et al., 2011). While these projects aimed primarily at enhancing the physicochemical and phytochemical composition of calyx extracts, the optimization of the sensory characteristics and consumer acceptance of hibiscus beverages has scarcely been undertaken, particularly in recent years (Babajide, Bodunde, & Salami, 2005; Bechoff et al., 2014; Mounigan & Badrie, 2006; Ramirez, Wysocki, Ramirez, Sims, & Badrie, 2012; Diessana, Parkouda, Cissé, Bréhima, & Dicko, 2015; Pérez-Ramírez-Rodrigues, Plaza, et al., 2011). The chemical-sensory properties of hibiscus beverages are largely determined by raw material quality and processing variables. Calix drying methods, in particular, play a very important role (Ramírez-Rodrigues, Balaban, et al., 2011; Ramírez-Rodrigues, Plaza, et al., 2011). In Africa, shellfore calyces are often spread over mats or plastic sheets placed on the ground, where they are left to dry under direct sunlight for 6–11 days, until a moisture content of 16% is attained (or under 12%, if they are intended for international trade) (Cisse, Dornier, Sakho, MarDiop, et al., 2009; McClintock & El Tahir, 2004). This is a highly inefficient process that often results in substantial anthocyanin and organic acid degradation, with subsequent negative impacts on the colour and flavour of beverages (Khaftaga & Koch, 1980b; Plotto et al., 2004).

Regarding processing variables, Ramírez-Rodrigues, Plaza, et al. (2011) compared the effects of cold (25 °C) and hot (90 °C) water extraction conditions on the physicochemical and phytochemical properties of extracts of whole, sun-dried, ‘Criollo’ hibiscus calyces. They observed that cold extraction led to less anthocyanin degradation and detrimental colour changes, but required much longer extraction times (15-fold), than hot extraction. The influence of other relevant extraction parameters, like particle size, calyx-to-water soaking ratio and time, on yield and anthocyanin concentration has also been investigated. Diessana et al. (2015) showed that the use of crushed (rather than whole) dried calyces and a 1:5 w/w calyx-to-water soaking ratio resulted in maximal anthocyanin extraction after 30 min at 30 °C. Meanwhile, studies of the thermal kinetic degradation of anthocyanins in water extracts of sun-dried hibiscus calyces uncovered that rate of degradation increases dramatically above 80 °C, revealing the importance of using mild (rather than harsh) pasteurisation conditions (Cisse, Vaillant, Acosta, Dhuque-Mayer, & Dornier, 2009).

The EU FP7-funded, African Food Tradition rEvisited by Research (AFTER) project (www.after-fp7.eu) intended to improve the safety, nutritional value and sensory quality of hibiscus beverages made in Senegal. To that end, it developed and pilot-tested new manufacturing processes entailing several changes in raw materials (shade-drying of calyces), formulation (optimised cultivar blend and sucrose addition), extraction (grinding of calyces, optimised time, temperature and soaking ratio), concentration (under-vacuum evaporation) and pasteurisation (reduced temperature), relatively to traditional production processes (Bechoff et al., 2014; Boucher, Cisse, Dornier, Gabor, & Doucet, 2014). This paper evaluates the physicochemical properties of three beverages resulting from such changes, vis-à-vis those of conventionally manufactured ones, and determines their impact on the products’ sensory evaluation and local consumer preference.

2. Materials and methods

2.1. Beverage production

Four beverages were studied: an under-vacuum concentrate (UVC), a dilute-to-taste syrup (RES) and two ready-to-drink infusions (REI and CTI). Beverages were produced in Dakar using manually decorticated, shade-dried (moisture < 14%) hibiscus calyces from ‘Vimto’ and ‘Koor’ cultivars (50:50) grown in the Senegalese region of Kaolack, according to the process depicted in Fig. 1. Selection of cultivars was determined by their availability and popularity in Senegal, as well as the organoleptic qualities of the extracts made from their calyces (Cisse, Dornier, Sakho, MarDiop, et al., 2009). The proportion of each cultivar to be used in the blend was decided based on the results of previous studies investigating the chemical-sensory properties and consumer acceptance of hibiscus beverages made from different cultivars and blends (Babajide et al., 2005; Bechoff et al., 2014; Cisse, Vaillant, et al., 2009).

UVC, RES and REI were made in the pilot plant of Centre Sectoriel de Formation Professionnelle aux Métiers des Industries Agroalimentaires according to good hygiene and manufacturing practices. Their production resulted from incremental improvements of the traditional manufacturing processes of hibiscus beverages in Senegal, which did not introduce any safety or health concerns. Hibiscus calyces were manually ground and subsequently extracted for 30 min at ambient temperature (25–30 °C), with periodic stirring. Calyx-to-water soaking ratios of 1:5, 1:10 and 1:40 w/w were used to obtain the extracts for the production of UVC, RES and REI, respectively. Extracts were filtered with a stainless steel filter ( pore size ca. 1 mm) and a pocket filter ( pore size 25 μm). UVC was produced by evaporating the corresponding extract up to 62 °Bx at 0.4 bar, while RES and REI were obtained through the addition of sucrose to corresponding extracts up to 62 and 17 °Bx, respectively. All three beverages were pasteurised (75 °C for 30 min), rapidly cooled and adequately packaged. REI was then stored under refrigeration (<10 °C) for preservation, whereas UVC and RES were kept at ambient temperature (25–30 °C) due to its high Brix value.

CTI was produced by a local company using a conventional manufacturing process. Whole calyces were extracted (calyx-to-water soaking ratio of 1:20 w/w) for 120 min at ambient temperature (25–30 °C), with periodic stirring. The extract was filtered with a stainless steel filter ( pore size ca. 1 mm) and a pocket filter ( pore size 25 μm), and sweetened up to 15 °Bx. The resulting infusion was pasteurised (90 °C for 20 min), rapidly cooled, adequately packaged and stored under refrigeration (<10 °C).

2.2. Sample preparation

Samples of UVC were prepared by dilution with potable water (1:40 v/v) and sweetening with 130 g L−1 of commercial sucrose. Samples of RES were prepared by dilution with potable water (1:4 v/v). Samples of REI and CTI did not require any further preparation. All samples were coded with random three-digit numbers and stored at 6 °C for 24 h prior to testing.

2.3. Physicochemical analyses

All analyses were performed at least in duplicate. Reagents used were all analytical grade, except for HPLC eluents, which were HPLC grade.
2.3.1. Colour

Colour density and hue tint were determined with a Shimadzu UV-1800 spectrophotometer (Shimadzu, Tokyo, Japan), by measuring the absorbance at 420, 520 and 700 nm. Calculations were performed as described by Giusti and Wrolstad (2001), where colour density = \[(A_{420 \text{ nm}}/A_{700 \text{ nm}}) + (A_{520 \text{ nm}}/A_{700 \text{ nm}})] and hue tint = \[(A_{420 \text{ nm}}/A_{700 \text{ nm}})/(A_{520 \text{ nm}}/A_{700 \text{ nm}})], for a 10 mm optical path length.

2.3.2. Total soluble solids and sugars

Total soluble solids (TSS) (ºBx) were measured according to the European Standard EN 12143 at 20.0 ± 0.5 ºC, using an Atago 3T Abbe refractometer (Atago, Tokyo, Japan). Glucose, fructose and sucrose contents were determined according to method 463.1 of the Manuel Suisse des Denrées Alimentaires. A Beckman Coulter HPLC chromatograph (Brea, California, USA), fitted with a Jasco RI 1531 refractive index detector (Jasco Corporation, Tokyo, Japan) and an Altima NH2 (4.6 x 250 mm; 5 µm) column (Grace Davison, Columbia, Maryland, USA), was used. The eluent was a mix of acetonitrile and water (76:24 v/v) at a flow rate of 1.5 mL min⁻¹. Total sugars were calculated as the sum of glucose, fructose and sucrose.

2.3.3. pH and titratable acidity

Potentiometric determination of pH was performed at 20 ºC ± 2 ºC. Titratable acidity was determined by potentiometric titration of a 25 mL aliquot with NaOH 0.1 M. The endpoint was calculated using the first derivative.

2.3.4. Total monomeric anthocyanins, phenolic content and antioxidant capacity

The spectrophotometric pH differential method was used to investigate the anthocyanin content of the samples (Lee, Durst, & Wrolstad, 2005). Degraded anthocyanins in the polymeric form, as well as non-enzymatic browning pigments, are hardly reversible.

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**Fig. 1.** Processes of production of the four hibiscus beverages.
with pH. Sample absorbance is thus first measured at pH 1.0, enabling the determination of total anthocyanin content, and then at pH 4.5, allowing the quantification of the polymeric anthocyanins, as well as other browning pigments. Total monomeric anthocyanins (hereinafter referred as anthocyanins) are finally calculated from differences in absorbance. Calculations were performed using a molar extinction coefficient of ε = 26900 L cm⁻¹ mol⁻¹ at pH = 1 and 520 nm. Concentrations were expressed as cyanidin 3-glucoside equivalents (Cy3glu) (MW = 449 g mol⁻¹).

Total phenolic content was measured using the Folin-Ciocalteu reagent. Gallic acid was used as standard molecule for calibration and results were expressed as Gallic Acid Equivalents (GAE). To minimize interference from reducing molecules, calibration standards were prepared with concentrations of fructose and glucose similar to those of samples.

Total antioxidant capacity was assessed by determining ABTS⁺ scavenging activity (Re et al., 1999). Results were calculated as the percentage of inhibition of the chromophore by an aliquot of each sample, under controlled conditions. Ascorbic acid was used as calibration standard, with results being expressed as ascorbic acid equivalent antioxidant activity (mg L⁻¹ AEAC).

All spectrophotometric measurements were carried out using a Shimadzu UV-1800 spectrophotometer (Shimadzu, Tokyo, Japan).

2.4. Sensory evaluation

2.4.1. Ethics

The study was reviewed by the Ethics Committee of AFTER. Participants were informed about its general aim and procedures for handling personal data, and gave written informed consent prior to participation. All samples were produced and prepared according to good hygiene and manufacturing practices. The improved beverages tested resulted mainly from incremental changes in conventional manufacturing processes. No safety or health hazards were introduced by such changes.

2.4.2. Descriptive analysis

The Flash Profile method was used. This method combines two classic sensory evaluation techniques – Free Choice Profiling and Attribute Intensity Ranking –, to map products according to their most salient, non-hedonic sensory attributes (Delarue & Sieffermann, 2004). Sessions took place in the ISO 8589:2007 compliant sensory testing facilities of Escola Superior de Biotecnologia – Universidade Católica Portuguesa (ESB-UCP). Twelve experienced panellists participated: seven belonged to the sensory evaluation panel of ESB-UCP for over ten years, the other five had approximately one year of experience as panellists. The later were selected and trained in compliance with ISO 8586:2012, and their performance was evaluated according to ISO 11132:2012, prior to panel admission.

In order to generate a list of discriminant sample descriptors, panellists evaluated and compared the four samples during the first study session. Descriptors were then compiled, synonyms discarded and a final pooled list of descriptors made by the end of the session. In a second session, each panellist started by choosing the descriptors he/she deemed more suitable to discriminate among the four samples, whether or not these had been listed at the end of the first session. Then, he/she ranked the four samples according to the relative intensity of each selected descriptor. Responses were given on a horizontal, 10-cm long, line scale, anchored with verbal labels at the left (‘less intense’) and the right (‘more intense’) ends. Ties in rankings were allowed and panellists could re-taste the samples as often as they wanted. Water was supplied between tastings to clean the palate.

2.4.3. Consumer study

Consumers of hibiscus beverages (n = 152) were non-probabilistically recruited at four different locations in Dakar – Université Cheikh Anta Diop (university campus; n = 37), Centre Culturel Français (city centre; n = 29), Point E (residential area; n = 42) and Association Culturelle d’Aide à la Promotion Educative et Sociale (Parcels Assainies, suburbs; n = 44) -., according to their willingness and availability to participate in the study. Taste sessions were conducted at the four recruiting sites under central location test conditions.

A paper-and-pencil questionnaire, written in French, was administered to participants. It started with questions about their socio-demographic characteristics (gender, age and education level) as well as consumption of hibiscus beverages. Namely, participants were asked to indicate the frequency with which they consumed this product category, by choosing from four ordered classes (‘several times per year’, ‘several times per month’, ‘several times per week’ and ‘several times per day’), as well as specific types of beverages, by ranking four products (‘homemade infusions’, ‘ready-to-drink infusions packaged in a plastic pouch’, “bottled, ready-to-drink infusions” and “dilute-to-taste syrups”) from the most to the least consumed.

Thirty millilitres of each of the four samples were served in clear plastic glasses (identified by a random, 3-digit code) and presented to participants in a sequential monadic mode, according to a complete balanced experimental design. Water was supplied to clean the palate between tastings. Participants were then asked to indicate the sample they most liked, the one they least liked and the one they found most similar to the hibiscus beverage they usually consumed. Trained enumerators assisted participants in French, or in the local Wolof language, when required. No information about the samples was provided, except for safety and hygiene considerations related to their preparation.

2.5. Statistical analyses

XLSTAT software V. 2015 (Addinsoft, Paris) was used to carry out all statistical analyses. The significance of statistical tests was evaluated at p < 0.05, unless otherwise stated.

2.5.1. Physicochemical analyses

Analysis of Variance (ANOVA) was performed on the results of the physicochemical analyses. Mean sample values were calculated and significant differences between them tested post hoc, using Tukey’s Honest Significant Difference (HSD) tests. Physicochemical characteristics discriminating significantly between samples were included in subsequent statistical analyses. Pearson’s correlation coefficients were calculated to investigate relationships among variables.

2.5.2. Descriptive analysis

In order to obtain consensual product and attribute configurations for the hibiscus beverage samples and descriptors investigated, a Generalized Procrustes Analysis (GPA) was performed. GPA reduces scale effects by detecting and minimizing individual differences and allowing a comparison of the proximity between sensory descriptors (Næs, Brockhoff, & Tomic, 2010).

2.5.3. Consumer study

Six questionnaires were either incomplete or contained invalid answers. Their data was thus excluded from further analysis, yielding 146 valid questionnaires. Participants’ age ranged between 18 and 73 years old (M = 34, SD = 13); 60% were male, 89% were Sénégalaise or long-term Senegal residents and 52% had a university diploma. 19% of participants consumed hibiscus beverages several times per year, 31% consumed hibiscus beverages several times per
month, 39% several times per week and 11% of participants consumed hibiscus beverages daily. Homemade infusions were the type of hibiscus beverage most often consumed by participants (75%), followed by ready-to-drink infusions packaged in a pouch or bottled, whereas beverages prepared from syrups were the least frequent consumed (85%). Preference and similarity judgements were tallied for each sample. Frequencies were compared using Pearson chi-square tests and Marascuilo multiple pairwise comparisons. The existence of significant differences in the frequency distributions of age, educational level and consumption of hibiscus beverages across judgments was tested with Kruskal-Wallis H tests and Steel-Dwass-Critchlow-Fligner analyses; Pearson chi-square tests were used to investigate significant differences of gender. Finally, Friedman tests were conducted to evaluate the significance of differences in rankings of types of hibiscus beverages according to frequency of consumption.

2.5.4. Relationships between physicochemical characteristics and sensory evaluation data

Multiple Factor Analysis (MFA), a statistical technique analysing sets of observations described by groups of variables, with direct application in sensory evaluation (Lassoued, Delarue, Launay, & Michon, 2008), was performed to explore the relationships between physicochemical properties, sensory descriptors and consumer data. This technique seeks the common structures present in all, or some of the groups of variables under study to then generate a perceptual map integrating both observations and group relationships. Physicochemical parameters, sensory descriptors, participants’ sociodemographic characteristics and frequencies of consumption of (types of) hibiscus beverages were defined as active variables; preference and similarity judgments were defined as supplementary variables.

3. Results and discussion

3.1. Physicochemical characteristics

3.1.1. pH, colour and anthocyanins

Significant differences between samples (p < 0.05) were found for all physicochemical characteristics, except pH (Table 1). Earlier studies reported the pH of aqueous hibiscus extracts to vary roughly between 2.2 and 2.6, with cultivar, post-harvest operations, calyx extraction conditions and formulation appearing to have little impact on this variable (Bechoff et al., 2014; Cisse, Vaillant, et al., 2009; Ramírez-Rodriguez, Plaza, et al., 2011; Ramírez et al., 2010). The range of values obtained is well in line with these findings. Due to its effect on colour and stability of anthocyanins, the pH is known to greatly influence the colour of plant-based beverages, such as berry juices and hibiscus extracts, is particularly the introduction of a grinding step prior to extraction and milder pasteurisation conditions (Fig. 1), appear to have decisively contributed to the improvement of the colour of new beverages. This occurred in spite of the shorter extraction time adopted and, in the case of REi, the lower calyx-to-water soaking ratio used. Ramírez-Rodriguez, Plaza, et al. (2011) reported lower colour density (1.04 UA) and hue tint (0.35) values for an unpasteurized infusion prepared from sun-dried, whole calyces of the ‘Criollo’ cultivar, when compared to REi (under similar extraction conditions). Such differences could be due to the use of sun-dried (rather than shade-dried) calyces from a single (different) cultivar and/or the absence of grinding and the absence of a pasteurisation step (Bechoff et al., 2014; Diessana et al., 2015; Khafaga & Koch, 1980b; Plotto et al., 2004). To the best of our knowledge, no assessments of colour density and hue tint of hibiscus syrups or concentrates have been previously reported.

A significant association (ρ = 0.957, p < 0.01) was found between the total monomeric anthocyanin content and the colour density of tested beverages. This was expected since the colour of plant-based beverages, such as berry juices and hibiscus extracts, is known to be driven by the amount of monomeric anthocyanins present (Cisse, Vaillant, et al., 2009; Rein & Heinonen, 2004). Prolonged extraction times and harsher thermal treatments may promote the polymerisation of anthocyanins by processes of direct condensation and co-pigmentation (Wrolstad, Durst, & Lee, 2005). Polymeric anthocyanins are chemically more stable than free monomeric forms, but may contribute to undesirable changes in hue, towards more yellowish/brownish tone, in both fruit juices and hibiscus extracts (Cisse, Vaillant, et al., 2009; Rein & Heinonen, 2004; Wong et al., 2003). Given that extraction time was longer and pasteurisation temperature higher for CTi than new beverages, this might explain why it had a higher hue tint value and was visibly more brick-yellow in tone than them.

3.1.2. TSS, total sugars, titratable acidity and sugar-to-acid ratio

Hibiscus calyces are naturally poor in sugars and other soluble solids, with maximum total sugar contents of 3–5% dry weight (Da-Costa-Rocha et al., 2014). The sweetness of derived beverages is thus mainly dependent on the amount of sucrose added during their processing and/or preparation for consumption. REi had the highest TSS and total sugar contents of all beverages tested while UVC had the lowest. Intermediate values were observed for both REs and CTi. The TSS content of CTi was well in line with that earlier reported for a ready-to-drink infusion conventionally manufactured in Senegal (15–16 °Bx) (Bechoff et al., 2014). The same

<table>
<thead>
<tr>
<th>Sample</th>
<th>Colour density (UA)</th>
<th>Hue tint</th>
<th>Total soluble solids (°Bx)</th>
<th>Total sugars (g L⁻¹)</th>
<th>pH</th>
<th>Titratable acidity (mM NaOH)</th>
<th>Total soluble solids/ titratable acidity (°Bx mM⁻¹ NaOH)</th>
<th>Total monomeric anthocyanins (mg L⁻¹ Cy–3–glu)</th>
<th>Total phenolics (mg L⁻¹ GAE)</th>
<th>Total antioxidant capacity (mg L⁻¹ AEC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UVC</td>
<td>17.1±0.9</td>
<td>0.33±0.08</td>
<td>13.4±0.1</td>
<td>127.0±3</td>
<td>2.52±0.10</td>
<td>10.00.7±7.1</td>
<td>0.134±0.009</td>
<td>234±3</td>
<td>677±9</td>
<td>557±18</td>
</tr>
<tr>
<td>REs</td>
<td>7.3±0.2</td>
<td>0.39±0.02</td>
<td>14.9±0.0</td>
<td>151±3</td>
<td>2.61±0.09</td>
<td>151.5±2.1</td>
<td>0.290±0.012</td>
<td>83±2</td>
<td>329±19</td>
<td>332±6</td>
</tr>
<tr>
<td>REi</td>
<td>12.3±0.0</td>
<td>0.49±0.01</td>
<td>17.4±0.1</td>
<td>167±3</td>
<td>2.56±0.01</td>
<td>142.5±0.07</td>
<td>0.122±0.001</td>
<td>112±3</td>
<td>587±2</td>
<td>409±14</td>
</tr>
<tr>
<td>CTi</td>
<td>4.9±0.0</td>
<td>0.58±0.00</td>
<td>15.8±0.1</td>
<td>149±2</td>
<td>2.45±0.01</td>
<td>104.0±0.0</td>
<td>0.151±0.001</td>
<td>44±1</td>
<td>344±4</td>
<td>292±13</td>
</tr>
</tbody>
</table>

Mean ± standard deviations. Different superscripts within a column indicate significant differences according to Tukey’s HSD (p < 0.05).

UVC = under-vacuum concentrate; REs = dilute-to-taste syrup; REi = ready-to-drink infusion; CTi = conventionally manufactured, ready-to-drink infusion.
cannot be said about REi, however, which displayed a relatively high TSS and total sugar contents, as its formulation entailed the addition of greater amounts of sucrose, likely to compensate for the expected increase in acidity brought about by the introduction of a grinding step prior to extraction.

The optimization of formulations of hibiscus infusions in Nigeria and Mexico have reportedly yield beverages with TSS values between 11 and 13 °Bx (Bolade et al., 2009; Ramirez et al., 2010), being thus more in the range of that observed for UVC than REi or CTi. The manufacturing of hibiscus syrups requires adding larger amounts of sucrose than that of infusions or concentrates. Syrup formulations in Senegal typically reach 68 °Bx and resulting beverages 17–18 °Bx after dilution to taste (Bechoff et al., 2014; Cisse, Dornier, Sakho, MarDiop, et al., 2009). In view of this, one of the process improvements implemented by AFTER was the reduction of the TSS content of syrups to 62 °Bx, with REs therefore registering a lower TSS and total sugar content than its conventional counterparts.

The organic acid content of fresh hibiscus calyces can be as high as 60% DW, depending on cultivar, climate and harvest conditions (Khafaga & Koch, 1980a). Still, the titratable acidity of hibiscus extracts varies greatly with calyx quality (drying and storage processes), blend of cultivars used and extraction conditions (particle size, calyx-to-water ratio, temperature and time) (Bechoff et al., 2014; Cisse et al., 2012; Cisse, Dornier, Sakho, MarDiop, et al., 2009; Cisse, Vaillant, et al., 2009; Diessana et al., 2015; Khafaga & Koch, 1980b). REi had the highest titratable acidity of all the beverages tested while REs had the lowest. Intermediate, but quite similar values were observed for CTi and UVC. The low value of titratable acidity uncovered for REs was most likely due to the combination of the calyx-to-water ratio used in its extraction (1:10 w/w) and the dilution employed in its preparation prior to testing (1:4 v/v). Indeed, taking into account the amount of sucrose added during processing, it can be estimated that 1 kg of calyces yielded approximately 110 L of REs, whereas the same amount yielded about 50 L of REi and 25 L of CTi. A similar reasoning could explain why UVc had nearly the same titratable acidity as CTi, but a much lower one than REi, when also accounting for the yield of the evaporation step and the amount of sucrose added during preparation.

Bechoff et al. (2014) reported the titratable acidities of conventionally manufactured syrups made from sun-dried, whole calyces of the ‘Vimto’ and the ‘Koor’ cultivars in Senegal (1:4 w/w, 25 °C, 120 min) to be 28.1 and 49.8 mM NaOH, respectively, and that of a conventionally manufactured infusion made from a blend of similar ‘Vimto’ and ‘Koor’ (50:50) calyces (1:20 w/w; 25 °C, 120 min) to be 136.1 mM NaOH. Correspondingly, higher values of titratable acidity were obtained for REs and REi in the present study, despite the lower calyx-to-water ratios and extraction time employed. The same holds true when comparing REi to CTi, in spite of the aforementioned differences between their extraction yields. On the other hand, a titratable acidity of 58.2 mM NaOH was determined for an optimised infusion made of sun-dried, whole calyces from the ‘Criollo’ cultivar extracted for a longer time at a slightly lower temperature (1:40 w/w, 60 min, 22 °C) (Ramirez et al., 2010). This is only slightly higher than that obtained for the new syrup, but much lower than the values observed for more comparable beverages, particularly REi. Such differences are probably due to the use of sun-dried (rather than shade-dried) calyces from a single (different) cultivar, as well as the absence of a grinding step prior to extraction (Bechoff et al., 2014; Diessana et al., 2015; Khafaga & Koch, 1980b). Taken together, these findings seem to indicate that the production improvements envisaged by AFTER, namely the implementation of shade-drying methods, the use of a blend of calyces from local cultivars with complementary chemical-sensory properties (such as ‘Vimto’ and ‘Koor’) and the introduction of a grinding step prior to extraction (Fig. 1), have most likely resulted in beverages with relatively high organic acid content, as indicated by their high titratable acidities (with the possible exception of UVC).

Sensory quality and acceptability of hibiscus beverages are greatly affected by the balance between sweetness and acidity perceptions, which in turn is largely determined by the sugar-to-acid ratios prescribed by their formulations (Bechoff et al., 2014; Bolade et al., 2009; Ramirez et al., 2010). REs had the highest sugar-to-acid ratio of all beverages tested, and CTi the second highest, while UVC and REi had the lowest. These results are not surprising given the high sugar-to-acid ratios typically entailed by hibiscus syrup formulations. Indeed, values of 0.66 and 0.34 °Bx mM⁻¹ NaOH have been reported for syrups conventionally manufactured from calyces of the ‘Vimto’ and the ‘Koor’ cultivars, respectively (Bechoff et al., 2014). Due to its relatively high titratable acidity and lower total sugar content, however, a low sugar-to-acid ratio was obtained for REs. Among the remaining beverages, REi/UVc had the highest/lowest TSS and the highest/lowest titratable acidity, resulting in both having thus lower sugar-to-acid ratios than CTi, for which the greatest gap between total sugar content and titratable acidity existed.

The sugar-to-acid ratios of UVC and REi were only slightly higher than that of a comparable, conventionally manufactured infusion (0.11 °Bx mM⁻¹ NaOH) (Bechoff et al., 2014), but fairly lower than that of the optimised infusion earlier described (0.18 °Bx mM⁻¹ NaOH) (Ramirez et al., 2010). The ratio obtained for CTi was in fact closer to this value. Differences between new beverages and the optimised infusion derive from its lower acidity, in spite of the equally lower addition of sucrose. Finally, the sugar-to-acid ratios of tested beverages were significantly correlated to their titratable acidity (p = −0.899, p < 0.01), but not to their total sugar content. This was likely related to the fact that the first varied much more across the tested beverages than the latter.

3.1.3. Anthocyanins, polyphenols and antioxidant activity

The total monomeric anthocyanin content of tested beverages varied significantly between a minimum of 44 mg L⁻¹ Cy-3-glu (for CTi) and a maximum of 234 mg L⁻¹ Cy-3-glu (for UVC) (Table 1). The anthocyanin content of hibiscus extracts is known to vary widely with raw material characteristics (namely cultivar, drying method, blend and calyx particle size), extraction parameters (calyx-to-water ratio, stirring, temperature and time) and extract processing operations (e.g., evaporation or pasteurisation) (Cisse et al., 2012; Cisse, Vaillant, et al., 2009; Diessana et al., 2015; Khafaga & Koch, 1980b; Ramirez-Rodrigues, Plaza, et al., 2011; Wong et al., 2003). Values of 106, 60 and 45 mg L⁻¹ Cy-3-glu were previously reported for a conventionally manufactured ‘Vimto’ and ‘Koor’ (50:50) infusion, ‘Vimto’ syrup and ‘Koor’ syrup, respectively (Bechoff et al., 2014). These values were correspondingly much lower than those obtained for UVC and REs, but only slightly lower than for REi.

Anthocyanins are labile compounds in solution and may hence undergo a number of degradative reactions in beverages, progressively condensing with other phenolic compounds into polymeric pigments. This process is accelerated by high temperatures, such as those occurring during pasteurisation (Wiolstad et al., 2005). Since low water activity is one of the factors known to promote anthocyanin stability in plant-based beverages during thermal treatment (Delgado-Vargas & Paredes-López, 2002), it is possible that the high anthocyanin content of UVC (relatively to both new and conventionally manufactured beverages) resulted mainly from it being evaporated under-vacuum immediately after extraction, as implemented by AFTER. A similar reasoning may tentatively explain why the anthocyanin content of REi seemed to improve little when compared to the increase achieved by REs, particularly in relation to ‘Vimto’-only syrups (Bechoff et al., 2014). This could be
due to the concentration of the extract entailed by the addition of a large concentration of sucrose in the production of syrups (but not infusions), prior to pasteurisation (Fig. 1). Such effect was likely enhanced by differences thermal treatment: conventionally manufactured syrups were pasteurised at 105 °C and infusions at 85 °C (Bechoff et al., 2014), against 75 °C in the case of REs and REi, for comparable treatment times. Still, it is important to highlight that such increases in the anthocyanin content of both beverages (albeit small in the case of REi) were achieved in spite of the relatively low calyx-to-water ratios and extraction times introduced by AFTER in their production process.

The markedly low anthocyanin content of CTi may also be partially attributed to the high temperature at which it was pasteurised (90 °C), relatively to both new and conventional ready-to-drink infusions (for comparable treatment times). Cisse, Vaillant, et al. (2009) showed that the rate of kinetic degradation of anthocyanins in hibiscus extracts increases dramatically over 80 °C, while Pérez-Ramírez et al. (2015) observed a gradual growth of this rate up to 70 °C, but a rather accelerated one after this temperature and up until 100 °C. These findings seem to support our hypothesis. Anthocyanin concentrations of 146 and 421 mg L\(^{-1}\) Cy-3-glu were previously reported for unpasteurised, aqueous extracts prepared from sun-dried, whole calyces of the ‘Koor’ and the ‘Vimto’ cultivars, respectively, using a calyx-to-water ratio of 1:10 w/w (600 min at 25 °C) (Cisse, Vaillant, et al., 2009). Such high values, when compared to those observed for REi and CTi, are likely explained by the use of calyces from single cultivars, the much higher calyx-to-water ratio and much longer extraction time employed, as well as the absence of pasteurisation.

Total phenolic content was highest in UVc and second highest in REi, with REs and CTi presenting much lower, similar values. Values of 695, 296 and 218 mg L\(^{-1}\) GAЕ were previously reported for a conventionally manufactured ‘Vimto’ and ‘Koor’ (50:50) infusion, a ‘Vimto’ syrup and a ‘Koor’ syrup, respectively (Bechoff et al., 2014). Such values were lower than those observed in the present study in the case of REs, higher compared to REi and similar relatively to UVC. As observed for anthocyanins, the use of shade-dried ground calyces, low water activity and mild thermal processing conditions has likely led to good extraction/preservation of phenolic compounds in case of REs and UVC. When comparing REi to the corresponding conventionally manufactured beverage, it should be noted that while the production of the former used ½ of the calyx-to-water ratio employed to make the latter, its total phenolic content was still only about 15% smaller. A longer extraction time may, nevertheless, contribute to an increase of the total phenolic content in new beverages. Ramírez-Rodrigues, Plaza, et al. (2011) and Pérez-Ramírez et al. (2015) studied the optimization of extraction conditions, showing that the total phenolic content of aqueous hibiscus extracted at 25 °C increased with extraction time up to 240 min. For CTi, a very low total phenolic content was observed when compared to REi and earlier reported conventional infusions. Again, a possible explanation for this may reside in the harsher pasteurisation conditions used in conventional production methods.

The main polyphenols in hibiscus beverages are anthocyanins, phenolic acids and flavonols (Pérez-Ramírez et al., 2015; Ramírez-Rodrigues, Plaza, et al., 2011; Sáyago-Ayerdi et al., 2014). These exhibit a wide range of interesting physiological properties, with their health benefits being mainly attributed to a high antioxidant activity (Sáyago-Ayerdi et al., 2014). According to Tsai, McIntosh, Pearce, Camden, and Jordan (2002), hibiscus extracts have ca. 20% of the antioxidant capacity of green tea and 33% of that of black tea. In the present study, total antioxidant capacity varied significantly between samples, with UVC presenting the highest value and CTi the lowest. This was expected, given that anthocyanins were reported to be a major source of antioxidant capacity in hibiscus extracts, accounting for up to about half of it (Tsai et al., 2002). This is also reflected in the significant correlations found between the monomeric anthocyanin content and polyphenolics content \((p = 0.874, p < 0.01)\), between the monomeric anthocyanin and antioxidant activity, \((p = 0.978, p < 0.01)\), which indirectly result in polyphenol content and antioxidant activity appearing significantly associated to colour density \((p = 0.974, p < 0.01\) and \(p = 0.957, p < 0.01\), respectively).

### 3.2. Sensory evaluation

#### 3.2.1. Descriptive analysis

Panelists generated a set of nine descriptors to discriminate between hibiscus beverages: one related to appearance (red colour), three to odour (hibiscus: calyx-like; fruity: cranberry- and aronia-like; sweet: honey-like), four to flavour (retro-nasal aromatics: hibiscus calyx-like: taste: sweet, acid and bitter) and one to mouthfeel (astringency). Comparable colour and taste descriptors, as well as one odour descriptor (hibiscus calyx-like), have been reported to describe the sensory profile of hibiscus beverages conventionally manufactured in Senegal (Bechoff et al., 2014). Ramírez-Rodrigues, Balaban, et al. (2011) studied the volatile composition of extracts of sun-dried, hibiscus calyces and identified several compounds responsible for their aroma (e.g., 1-octene-3-one, (E)/(E)2-nonanal, octanal, geranylacetone, hexanal). Hibiscus aroma was hence described as complex combination of earthy, green, floral and fruity notes, rather than a unique, idiosyncratic odour. This is in line with the number and type of odour descriptors identified in the present study. Finally, previous sensory evaluations of hibiscus beverages have also describe them as weakly bitter, weak to moderately astringent, sharp or irritant (Bechoff et al., 2014; Mounigan & Badrie, 2006; Wong et al., 2003).

Fig. 2a–b depicts the results of the descriptive analysis. They show that there was good agreement between panelists and that a good discrimination between samples could be achieved with the selected descriptors. Importantly, two main components, one related to odour and flavour (F1) and another to colour (F2), were extracted, which together explained about 88.8% of the variance in the data. Panelists characterized UVC as having the most intense red colour, nasal and retro-nasal aroma of all beverages, while displaying relatively moderate sweet and acid taste intensities. REi displayed the most intense red colour and strongest aromatic character after UVC, but was also judged to be the most tart, bitter and astringent sample of all. REs, on the other hand, was deemed to be the sweetest one, with the lowest acidity, astringency and bitterness, but also to have the weakest red colour. Finally, CTi was perceived to have a weak red colour as well, but to be simultaneously more sour, astringent and bitter, rather than sweet.

The Pearson correlation coefficients estimated showed that red colour intensity was significantly \((p < 0.01)\), positively associated to colour density \((p = 0.932)\) and monomeric anthocyanin content \((p = 0.895)\), as expected from the results discussed in 3.1.1. This explains why UVC and REi were judged to have a higher red colour intensity than REs or CTi (Fig. 2b). On the other hand, the intensities of some aromatic notes, namely hibiscus and honey odour, were strongly, negatively associated with hue tint \((p = -0.764, p < 0.05\) and \(p = -0.863, p < 0.01\), respectively). They were thus weaker in the more brick-yellow beverages, namely REi and CTi, and stronger in the redder ones (i.e., UVC and REs). Accordingly, panellist evaluated the latter to have a stronger aromatic character than the former. This indicates that loss of aroma and colour degradation (i.e., browning) may be closely associated in hibiscus beverages, as they are, for instance, in wine, which has many of the same volatiles in its composition (Ferreira, Escudero, Fernández, & Cacho, 1997).
Strong, positive relationships were identified between acid taste intensity and titratable acidity ($p = 0.845, p < 0.01$), and between sweet taste intensity and sugar-to-acid ratio ($p = 0.819, p < 0.05$), as expected from the results discussed in 3.1.2. In view of the sugar-to-acid ratios obtained, it is straightforward to understand why panellists deemed REi/REs to have predominantly an intense acid/sweet taste and UVC/CTi to be well/poorly balanced in terms of these taste attributes. These findings are in good agreement with those earlier reported for conventionally manufactured beverages, which described the taste of infusions as mainly sour, and of syrups as predominantly sweet, with such evaluations being positively correlated to their titratable acidity and TSS, respectively (Bechoff et al., 2014).

Significant, positive associations were found between bitter taste and titratable acidity ($p = 0.724, p < 0.05$), and between astringency and titratable acidity ($p = 0.934, p < 0.01$). Conversely, significant negative associations were found between these two sensory descriptors and sugar-to-acid ratio ($p = -0.709, p < 0.05$) and $p = -0.872, p < 0.01$, respectively). This likely explains why REi was deemed the most astringent and bitter beverage and REs the least. Moderate to weak bitter taste and astringency sensations often co-occur with perceptions of moderate to strong sourness in plant-based beverages, while moderate and high sweetness intensities are known to be generally suppressive of acid and bitter tastes (Keast & Breslin, 2002; Laaksonen et al., 2014; Lesschaeve & Noble, 2005). Similar enhancement and suppression effects were also observed in prior sensory evaluations of hibiscus beverages (Bechoff et al., 2014; Moungian & Badrie, 2006; Wong et al., 2003). The rationale behind the evaluations of UVC and CTi for these descriptors is less straightforward, however, as proximate, lower values of titratable acidity were found for these beverages and their sugar-to-acid ratios were about half of that of REi.

Visual and olfactory sensations may affect reported taste and mouthfeel evaluations, even in the case of trained sensory panels. Perceived sweetness intensity, in particular, has been shown to rise with increasing intensities of congruent colour or odours (e.g., honey, fruity) (Delwiche, 2004). On the other hand, perceived lack of sweetness is known to enhance bitter taste and astringency perceptions (Keast & Breslin, 2002). Given that UVC had the strongest red colour and aromatic character of the beverages studied, while CTi had the weakest, such enhancement and suppression effects could explain why the first was deemed sweeter, less astringent and bitter than the second. This hypothesis is further supported by the fact that no significant associations were found between sweet, bitter and astringency intensities and the total phenolic content of beverages. Indeed, the phenolic compounds most commonly associated with intense bitter taste and astringency sensations in plant-based beverages, such as hydroxybenzoic acids, flavonols and tannins (Laaksonen et al., 2014; Lesschaeve & Noble, 2005), have been detected in hibiscus extracts in negligible amounts only, or not at all. Conversely, anthocyanins, that are by far the most abundant flavonoid in hibiscus extracts (Fernández-Arroyo et al., 2011; Ramírez-Rodrigues, Plaza, et al., 2011; Rodríguez-Medina et al., 2009; Wong et al., 2003), were shown to have little or no direct impact on the flavour perception of plant-based beverages (Soares, Brandão, Mateus, & De Freitas, 2017).

3.2.2. Consumer study

Fig. 3 presents the results of the preference and similarity questions included in the consumer questionnaire. About 55% of participants indicated UVC as the most liked beverage and only 3% as the least liked. Conversely, only 7% stated that CTi was the most liked beverage and 39% the least liked. Differences in these proportions were moreover highly significant ($p < 0.01$), as well as those observed between UVC and REs/REi. Differences between the latter and CTi were only moderately significantly for the most liked ($p < 0.05$) and not significant for the least liked. Finally, no significant differences were found between REs and REi for either the most or the least liked beverage.

No significant associations were found between participants’ hibiscus beverage preferences and gender, education level or frequency of consumption at category level (Table 2). Still, the majority of participants selecting REi as most liked beverage were male (77%). Significant differences were, nonetheless, found for participants’ age, with those declaring CTi the most liked beverage being, on average, more than a decade older than the ones referring UVC. Tolerance to high intensities of unpleasant sensory attributes in plant-based beverages, like sourness, bitterness and astringency, is reportedly higher in older and male consumers than in younger and female ones (Laaksonen et al., 2014). This could explain our results given the differences in the taste evaluations of these two beverages (Fig. 2). Meanwhile, consumers over 30 years old found the flavours and tartness intensities of an optimised hibiscus...
infusion more often than their younger counterparts, while female consumers liked its intense red colour more than males ones (Ramírez et al., 2010). Since this beverage had a much lower monomeric anthocyanin content and colour density, as well as a much high sugar-to-acid ratio than UVc (but not CTi), these results are generally supportive of our findings.

The proportions of participants choosing UVc, REs, REi or CTi as the hibiscus beverage most similar to the one they usually consumed were nearly the same as the corresponding values observed for the most liked beverage. The vast majority of participants declared to drink freshly brewed, homemade infusions the most often and beverages prepared from syrups the least often (Table 2), which is line with consumption habits in Senegal (Cisse, Dornier, Sakho, MarDiop, et al., 2009). The sensory properties of UVc, namely its intense red colour, strong aromatic character and well-balanced sweetness and acidity, should resemble more closely those of homemade infusions when compared to the remaining beverages, particularly CTi (Fig. 2). This might explain why the former was perceived to be most similar to the beverage participants usually consumed, while the latter was viewed as the least similar.

Fig. 4 depicts the relevant relationships obtained between physicochemical variables, sensory evaluations and consumer data (95.4% of variance explained). It is interesting to notice that, compared to Fig. 2, sweet and acid taste intensities (F1) have now more explanatory power than colour evaluations (F2). The characteristic red hues of hibiscus products are highly appreciated by consumers, being typically the sensory attribute with the highest acceptability (Bolade et al., 2009; Ramirez et al., 2010). Still, taste judgements remain the dominant sensory factor driving the food preferences of consumers, which explains this inversion.
UVC’s exceptionally high anthocyanin content and resulting deep red colour, its aromatic quality, both in odour and flavour dimensions, and its balanced sweetness and acidity, are noticeably related to consumers’ preference and similarity judgements. Meanwhile, with very different physicochemical characteristics and almost opposite sensory profiles, REi and REs presented nevertheless similar preference results. Still, male and more regular consumers of hibiscus beverages seemed to appreciate more REi than women and more infrequent ones, most likely due to its strong red colour, acidity, astringency and bitterness. They were also relatively less appreciative of REs, mainly due to its high sweet-to-acid ratio. With a more brick-yellow tone than improved beverages and a weaker aromatic character, CTi seemed to be disfavoured by most participants, particularly younger ones.

4. Conclusion

Findings here reported contribute to validate the introduction of important changes in the production of hibiscus beverages by AFTER, not only in terms of the improvements achieved in most of the key chemical-sensory characteristics of resulting products, but also in the high level of preference these seem to be able to achieve among consumers in Senegal. Excellent results were obtained particularly for UVC, which was overwhelmingly preferred by the majority of participants. Still, REi and REs appeared to be better accepted than conventionally manufactured alternatives, particularly when taking into account the individual characteristics of consumers. In line with this, the existence of distinct segments of hibiscus beverages consumers in Senegal according to hedonic preferences should be investigated in future studies, not only to identify the main sensory drivers of such preferences, but also relevant differences in the socio-demographic profile and consumption habits of segments. Importantly, hibiscus beverages produced according to West African tradition are virtually unknown to most Europeans. Future research should explore their potential in European markets by identifying important drivers of liking and new hence new opportunities for product improvement and commercialisation.

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