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SENSORY PROPERTIES AND CONSUMER ACCEPTANCE OF NOVEL HIBISCUS (*Hibiscus sabdariffa)* BEVERAGES

Thesis submitted to *Universidade Católica Portuguesa* to attain the degree of PhD in Biotechnology, with specialization in Food Science and Engineering

Maria João Nepomuceno Pereira Monteiro Palermo de Faria

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Maria João Nepomuceno Pereira Monteiro Palermo de Faria

Supervisor: Prof. Maria Manuela Estevez Pintado
Co-supervisors: Dr. Keith Tomlins and Prof. Emídio Gomes

This thesis is dedicated to

The loving memory of my parents Maria Madalena e Joaquim Manuel Pereira Monteiro. Not a day goes by that I don't miss you.

My husband, Luís Carlos, for his almost infinite patience, love and support.

As bebidas à base de extratos de hibisco são ricas em ácidos orgânicos, compostos fenólicos e polissacáridos, substâncias com potenciais benefícios para a saúde, sendo tradicionalmente consumidas na Africa Ocidental, na Ásia e na parte sul da América do Norte. A sua divulgação na Europa e dos Estados Unidos tem sido lenta e dirigida sobretudo a nichos de mercado, no entanto, o crescente interesse dos consumidores europeus e norte-americanos em bebidas com ingredientes únicos ou exóticos, com potenciais benefícios para a saúde, criaram recentemente novas oportunidades de mercado para bebidas de hibisco de alta qualidade.

Entre 2010 e 2015, o projeto Europeu African Food Tradition rEvisited by Research (AFTER) investigou a produção, a comercialização e o consumo de bebidas de hibisco (*Hibiscus sabdariffa* var. *sabdariffa ruber*) no Senegal e na Europa, com o objetivo de otimizar a sua produção industrial, minimizando a degradação dos compostos fitoquímicos. Pouco se sabia sobre o perfil sensorial destes produtos, na sua forma tradicional ou melhorada e sobre como as mesmas eram avaliadas pelos consumidores, fatores essenciais para o sucesso destes produtos em particular fora dos seus mercados tradicionais. Esta dissertação teve como objetivo avaliar o impacto da otimização dos processos de produção industrial das bebidas de hibisco levadas a cabo pelo projeto AFTER (1) nas propriedades físico-químicas dos produtos resultantes com impacto na qualidade sensorial; (2) no seu perfil sensorial; (3) na perceção e aceitação por parte dos consumidores em mercados tradicionais (Senegal) e potenciais (França, Portugal e Reino Unido).

O uso de cálices de hibisco secos à sombra e triturados das cultivares Vimto e Koor (50:50) e de condições moderadas de tempo e temperatura de extração e pasteurização, deu origem a novas bebidas com uma cor mais intensa e mais vermelha, maior concentração em antocianas monoméricas e polifenois e consequentemente maior capacidade antioxidante do que as bebidas convencionais, maior equilíbrio entre a concentração de açúcares e de ácidos e maior intensidade aromática. O estudo efetuado com consumidores no Senegal (N=150), mostrou que as novas bebidas foram positivamente avaliadas e melhor apreciadas que bebidas convencionais, sem que a perceção do caracter tradicional tenha sido alterada. Três segmentos de consumidores com distintos padrões de aceitação das bebidas foram identificados. A avaliação

hedónica foi influenciada por preferências pessoais relativamente à cor, aroma e equilíbrio doçura-acidez, por características sociodemográficas e hábitos de consumo.

Vinte e duas bebidas de hibisco foram avaliadas por painéis de provadores no Senegal e em Portugal. Léxicos em Português, Inglês e Francês contendo 21 descritores definidos, e referenciados foram desenvolvidos. As características distintivas das bebidas de hibisco eram a cor vermelha, o aroma floral (hibisco) e frutado (aronia/arando, ginja e framboesa) e o sabor predominantemente ácido (infusões de hibisco e bebidas preparadas a partir de concentrados) ou doce (bebidas preparadas a partir de xaropes). Eram ainda percetíveis aromas de chá preto, passa de uva, mel e feno, assim como sabor amargo e adstringência. O vocabulário usado por profissionais não-treinados (N=30) e por consumidores foi estudado usando entrevistas semiestruturadas (N=75) e questões *Check-all-that-apply* (N=490) no Senegal, em França, Portugal e Reino Unido. Léxicos gráficos contendo 27 atributos sensoriais e 15 termos gerais foram desenvolvidos. Diferenças com origem na cultura e familiaridade com o produto foram observadas. A comparação dos perfis sensoriais obtidos com provadores treinados e consumidores permitiu uma melhor compreensão da linguagem destes últimos.

Estudos com consumidores em França, Portugal e Reino Unido (N=403) mostraram padrões similares de aceitação e perceção pelos consumidores. Diferenças importantes foram observadas relativamente ao Senegal. Na generalidade, os consumidores na Europa, gostaram menos das bebidas do que no Senegal, embora avaliações hedónicas médias positivas tenham sido observadas para todas as bebidas. No Senegal os consumidores avaliaram mais favoravelmente uma maior intensidade de cor e maior doçura e de forma mais depreciativa menor acidez. Na Europa e caracter floral e frutado das bebidas constituíram importantes fatores de aceitação das mesmas. Segmentos de consumidores foram identificados. A aceitação e perceção foram influenciadas pela familiaridade com o produto e com produtos com características de algum modo similares na Europa (caso dos sumos de frutos vermelhos); por características sociodemográficas; e por preferências individuais inatas ou culturalmente adquiridas. Foram observadas diferenças entre segmentos de consumidores na Europa relativamente ao perfil sensorial de uma bebida ideal, mas não no Senegal.

Abstract

Hibiscus beverages are rich in organic acids, phenolic compounds and polysaccharides, which have been demonstrated to be linked to important health benefits. They are traditionally consumed in West African countries, in Asia and in the Southern part of the North American continent. In Europe and United States of America, hibiscus beverages have slowly penetrated niche markets. Due to the rising number of health conscious consumers, along with the growing interest of North Americans and Europeans in beverages made from unique or exotic ingredients, new mainstream market opportunities for high quality hibiscus beverages have been recently created.

Between 2010 and 2015, the African Food Tradition rEvisited by Research (AFTER) project, investigated the production, trading and consumption of hibiscus beverages made from dried hibiscus (*Hibiscus sabdariffa* var. *sabdariffa ruber*) calyx extracts in Senegal and Europe. Key improvements were proposed to increase production process efficiency and minimize degradation of phytochemical compounds. The aims of this thesis encompassed the evaluation of the impact of the reengineering processes held under AFTER project scope (1) in the main chemical-sensory properties of hibiscus beverages; (2) on their sensory profile; (3) on consumer acceptance in traditional markets (Senegal) and potential markets (France, Portugal and United Kingdom).

The selection of a blend of shade dried ground hibiscus calyces (Vimto:Koor cultivars 50:50) and the use of mild water extraction and pasteurization conditions, led to the production of beverages with a more intense redder colour, higher monomeric anthocyanin, total phenolic content and higher antioxidant capacity than their conventional counterparts. Colour evaluation by trained panelists was mainly linked to colour density and to anthocyanin/polyphenol content, while flavour assessments were associated to titratable acidity and sugar-to-acid ratio. New beverages presented also a more intense aroma than conventional ones. Results of hedonic assessments held in Senegal (N=150), indicated that all improved beverages were liked by roughly three-quarters of consumers and were better accepted than a traditional one (baseline comparison). Three clusters of consumers with distinct overall liking patterns were identified. Acceptance was driven personal preferences regarding colour intensity, aromatic character, balance between sweetness and acidity but also by gender, age,

frequency of consumption and education level. Importantly, new beverages were still perceived by consumers as traditional products.

Given the scarcity of information about hibiscus beverages sensory profile, sensory lexicons were developed in French, Portuguese and English. Twenty-two samples, including freshly prepared and ready-to-drink infusions, syrups, concentrates and an instant tea, were evaluated by trained panelists, resulting in 21 defined and referenced descriptors, subsequently assembled in a sensory wheel. A distinctive red colour, a floral (hibiscus) and fruity aroma (aronia/cranberry, sour cherry and raspberry) and a predominant acid (in case of infusions and beverages prepared from concentrates) or sweet taste (for beverages prepared from syrups) were the main sensory attributes. Also moderate or low intensities of black tea, honey, raisin and hay odours and of bitter taste and astringency were perceived in all products. The vocabulary used by untrained panelists was also investigated in Europe and Senegal through professional meetings (N=30), consumer focus groups (N=75) and check-all-that-apply questions (N=490), resulting in graphical lexicons with 27 sensory and 15 overall impression terms. Training, food culture and product familiarity influenced results. Trained panelists used precise terms to describe the aromatic profile of hibiscus beverages (e.g., reminiscent of dried hibiscus flowers, with berry, raisin, hay and sweet notes), whereas untrained ones were more prolific in general expressions (e.g., strong in hibiscus in Senegal; floral and fruity in Europe).

Consumer studies in France, Portugal, United Kingdom (N=403), showed similar patterns of acceptance and perception for hibiscus beverages in European countries. Positive mean overall liking ratings were observed for all beverages. Major differences were observed relatively to Senegal where consumers favored higher intensities of red colour and sweetness. In Europe fruity and floral character constituted important drivers of liking. Consumer segments were identified. Perception and acceptance was influenced by consumers' sociodemographic characteristics and by innate and culturally acquired preferences. Besides exposure to product category, also exposure and liking of familiar products, with somewhat similar characteristics to hibiscus beverages (like red fruit beverages), was shown to influence liking in Europe. Differences across consumer segments in the profile of an ideal beverage were observed in Europe but not in Senegal.

This thesis is organized in 3 parts.

Part I includes **Chapter 1** which presents the framework of the research performed and the state of the art pertaining hibiscus beverages - raw materials, beverages production, physical-chemical composition, sensory evaluation and consumer acceptance. The sensory methodologies employed are also briefly reviewed. Finally the objectives of the thesis are presented.

Part II includes **Chapters 2, 3, 4** and **5**, reflecting the four studies performed. **Chapter 2** presents the investigation on chemical-sensory properties of three new hibiscus beverages developed under AFTER project scope (an under-vacuum concentrate, a diluted to taste syrup and a ready-to-drink infusion) vis-à-vis those of a conventionally manufactured infusion. Relationships between process parameters, physical-chemical composition, sensory characteristics and consumer preference are explored. In **Chapter 3** consumer acceptance of these hibiscus beverages in Senegal is explored. Consumer characteristics and impact of judgements of appropriateness of sensory attribute intensities on acceptance are addressed. **Chapter 4** presents the research leading to the cross-cultural development of trilingual sensory lexicons for trained and untrained panelists. Classical descriptive analysis was used to establish trained panelists lexicon. Vocabulary employed by untrained professionals and consumers was explored in semi-structured meetings and by using check-all-that-apply questions with consumer panels in Senegal, Portugal, France and United Kingdom. Research presented in **Chapter 5** explores and compares perception and liking of hibiscus beverages by consumers in Senegal, France, United Kingdom and Portugal. Impact of familiarity, exposure and innate or cultural acquired preferences are addressed. Consumer segmentation based on hedonic acceptance is explored and differences rooted on individual reactions to beverages, socio-demographic characteristics and consumption patterns evaluated.

In **Part III**, **Chapter 6** summarizes the main findings of this thesis and proposes avenues for future research.

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My colleagues and long-time friends at ESB and particularly in CINATE for their companionship and friendship.

Last, but not least, I wish to thank my family and friends for their love and support.

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$ABTS^{\bullet+}$	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical cation
AEAC	Ascorbic acid equivalent antioxidant activity
AFTER	European project African Food Tradition rEvisited by Research
ANOVA	Analysis of variance
ºBX	Brix degree
CA	Correspondence analysis
CATA	Check-all-that-apply
CINF	Conventional manufactured ready-to-drink infusion
ColourD	Colour density
CONC; Conc	Diluted-to-taste and sweetened-to-taste tea/beverage prepared from concentrate
CTi	Conventional manufactured ready-to-drink infusion
Cy-3-glu	Cyanidin-3-glucoside equivalents
Cy3G	Cyanidin-3-glucoside
Cy3S	Cyanidin-3-xylosylglucoside
Dy3G	Delphinidin-3-glucoside
Dy3S	Delphinidin -3-xylosylglucoside
DW	Dry weight
EU	Europe
FINF; FInf	Freshly brewed infusion
FP	Flash profile
FR	France
FRAP	Ferric reducing antioxidant power
GAE	Gallic acid equivalents
GPA	Generalised Procrustes Analysis
HSD	Honest significant difference
ID	Ideal beverage
INST	Instant tea/beverage
JAR	Just-about-right
LSD	Least significant difference
MFA	Multiple factor analysis
NINF	Ready-to-drink infusion manufactured using a new process
OL	Overall liking
PA	Penalty analysis
PT	Portugal

REi	Ready-to-drink infusion manufactured using a new process
REs	Dilute-to-taste syrup manufactured using a new process
RInf	Ready-to-drink infusion
RV	Regression vector
SN	Senegal
SYR; Syr	Dilute-to-taste tea/beverage prepared from syrup
TAC	Total antioxidant capacity
TMA	Total monomeric anthocyanins
TPhenolics	Total phenolics
TS	Too strong
TSS	Total soluble solids
TW	Too weak
US, USA	United States of America
UVc	Dilute and sweetened-to-taste beverage prepared from under vacuum concentrate
UK	United Kingdom
WP	Weight penalty analysis

CHAPTER 1

General introduction

Framework

Beverages with enhanced health benefits provided by plant-based ingredients are growing in popularity (Hernández-Carrión *et al.* (2015), Sun-Waterhouse, 2011, Lawless *et al.*, 2012), particularly when made from ingredients perceived as new or exotic by European and North American markets (Orjuela-Palacio *et al.*, 2014, Vidigal *et al.*, 2011). This trend is fueled by the food & beverage industry's current focus on the development of products with a health claim (as a strategy of differentiation), as well as by consumers' growing desire for more natural and healthier diets, on one hand, and new and exciting food and drink experiences, on the other (Menezes *et al.*, 2011). In recent years, for instance, North-American companies, like Starbucks' Teavana and Dunkin' Donuts, have been developing premium beverage lines that carry several ready-to-drink and instant beverages based on hibiscus extracts, as well as many herbal blends containing hibiscus.

The bioactivity of the organic acids, phenolic compounds and polysaccharides present in hibiscus calyces and hence their extracts has been demonstrated in different biological models to be linked to important pharmacological properties (Maganha *et al.*, 2010, Patel, 2014, Da-Costa-Rocha *et al.*, 2014). In view of the above, much research has been undertaken to uncover more effective ways of enhancing the amount and stability of such compounds in hibiscus extracts and extraction by-products, in countries where the production of hibiscus products is relevant, like Mexico, Nigeria and Senegal (Aurelio *et al.*, 2008, Awe *et al.*, 2013, Cid-Ortega and Guerrero-Beltrán, 2014, Cisse *et al.*, 2012, Ramírez-Rodrigues *et al.*, 2011a, Ramírez-Rodrigues *et al.*, 2011b, Camelo-Méndez *et al.*, 2013, Gonzalez-Palomares *et al.*, 2009, Salazar-González *et al.*, 2012, Sáyago-Ayerdi *et al.*, 2014, Sáyago-Ayerdi *et al.*, 2007, Ramírez-Rodrigues *et al.*, 2012, Pérez-Ramírez *et al.*, 2015). Yet, the sensory profiling and hedonic evaluation of both traditional and improved products has scarcely been undertaken (Bechoff *et al.*, 2014, Ramírez-Rodrigues et al., 2011a, Bolade *et al.*, 2009, Foline *et al.*, 2011), particularly outside domestic markets (Ramirez *et al.*, 2010, Ramírez-Rodrigues et al., 2012).

Between 2010 and 2015, the European project African Food Tradition rEvisited by Research (AFTER) (www.after-fp7.eu) investigated the production, trading and

consumption of hibiscus beverages in Senegal, with the aims of improving the safety, nutritional value and sensorial quality of the beverages. Firstly extant production processes were mapped and evaluated on the basis of known technological constraints and potential for further optimization (Cisse *et al.*, 2012). The chemical-sensory properties of commonly consumed beverages and their impact on consumer acceptance were furthermore investigated (Bechoff et al., 2014). This highlighted the importance of harmonizing the sensory profile of these beverages as part of the product re-engineering process, and re-assessing their acceptability amongst the Senegalese population. Based on the results, improvements were proposed which encompassed raw materials and production processes definition and standardization; optimization of efficiency of extraction and minimization of degradation of phytochemical compounds; optimization of production hygienic conditions and extension of shelf life (Boucher *et al.*, 2014). The implementation of a new manufacturing process enabling local production of hibiscus concentrates for the beverage industry was also suggested.

New processes of manufacturing hibiscus infusions, syrups and concentrates, incorporating the above-mentioned technological improvements, were developed and pilot tested in a second stage of the AFTER project. These were produced using dried decorticated hibiscus calyces 50:50 Vimto:Koor cultivars to equilibrate colour and acidity. To improve extraction efficiency a first step of grinding of calyces was introduced in the production process. This allowed decrease to one half the amount of hibiscus calyces required to produce the beverages and to decrease the aqueous extraction time from 2 hours to 30 min, at ambient temperature. The use of calyces dried at moderate temperatures (away from direct sunlight), of ambient temperature aqueous extraction conditions, of mild pasteurization temperature (85ºC), were set to minimize degradation of phytochemical compounds while contributing to minimize energy production cost. Chemical-sensory characteristics of AFTER developed products as well as consumer perception and acceptance on both in regional and international markets remained however undetermined.

State of the art

1. *Hibiscus sabdariffa*

The *Hibiscus sabdariffa* L. is a species in the genus *Hibiscus* of the Malvaceae family. Thought to be native to Africa (Sudan region) (Mc Clintock and El Tahir, 2004) or Asia (India to Malaysia) (Ismail *et al.*, 2008), this herbaceous plant is nowadays widely grown in the tropical and subtropical areas of Africa, Asia and North-America (Patel, 2014). Two varieties of *H. sabdariffa* are typically cultivated for their commercial value: the *altissima* Wester and the *sabdariffa* (Morton, 1987). While the former includes different tall-growing, unbranched types – cultivated primarily for their stem fibre -, the latter entails several shorter, bushy shrubs, grown mainly for their leaves, seeds and pigmented calyces (Mc Clintock and El Tahir, 2004, Plotto *et al.*, 2004).

H var. *sabdariffa* plants can be classified into four races: the *bhagalpuriensi*, with green, red-streaked, inedible calyces; the *intermedius* and the *albus*, both with yellow-green edible calyces and also yielding fiber; and the *ruber*, with deep crimson, or deep magenta, edible calyces and greater economic importance than the previous (Figures 1 and 2) (Morton, 1987). Indeed, over 100 cultivars or seed strains of the race *ruber* have been identified around the world (Plotto et al., 2004), being vernacularly known as *hibiscus*, *roselle*, *red sorrel* or *Florida cranberry* in English language (Plotto et al., 2004), *jamaica* in Spanish (Sáyago-Ayerdi et al., 2014), *karkadeh* in Arabic (Ali *et al.*, 2005), *hibiscus, l'oseille de Guinée* or *oiselle rouge* in French (Palé *et al.*, 2004), *asam susur* in Malay (Mohd-Esa *et al.*, 2010), *krachiap daeng* in Thai (Maganha et al., 2010).

Due to their intense red colour and unique flavor, the sepals of *H* var. *sabdariffa ruber* flowers, hereinafter referred to as *hibiscus calyces*, are one of the highest volume speciality botanical products in international trade (Plotto et al., 2004, Tsai *et al.*, 2002). Several cultivars and seed varieties are being extensively grown in China, Thailand, Malaysia, the Philippines, Mexico, Jamaica, West Indies, USA, Sudan, Senegal, Mali, Chad, Nigeria, Niger, Egypt and Tanzania (Mc Clintock and El Tahir, 2004, Wong *et al.*, 2002, Atta *et al.*, 2013, D'Heureux-Calix and Badrie, 2004, Patel, 2014, Chen *et al.*, 2005).

Figure 1. *Hibiscus* var. *sabdariffa ruber plant* (Photo by Roy Cui).

Figure 2. *Hibiscus* var. *sabdariffa ruber* fruit and calyx. Reprint from Cisse *et al.* (2009b)

The United States is the world's largest importer of hibiscus calyces, followed by Germany and Japan (Anonymous, 2016)

Dried, rather than fresh, hibiscus calyces are sold and consumed in most areas of the world, being currently employed in traditional cuisines and food companies to produce hot and cold infusions, fermented beverages, jams, confectionary, salads, side-dishes and desserts (Sáyago-Ayerdi et al., 2007, Mounigan and Badrie, 2007, Morton, 1987, Mohamed *et al.*, 2012, Bamishaiye *et al.*, 2011, Wong *et al.*, 2003, Ramírez-Rodrigues et al., 2011a, Cisse et al., 2009b). Their extracts in syrup, powder or concentrate form, are furthermore widely employed in the European and North-American food, beverage and pharma industries as natural coloring and flavoring ingredients (Cisse et al., 2009b, Cid-Ortega and Guerrero-Beltrán, 2015, Cisse et al., 2012). Dried hibiscus calyces are red with some background purple hues, depending on the cultivar (Figure 3).

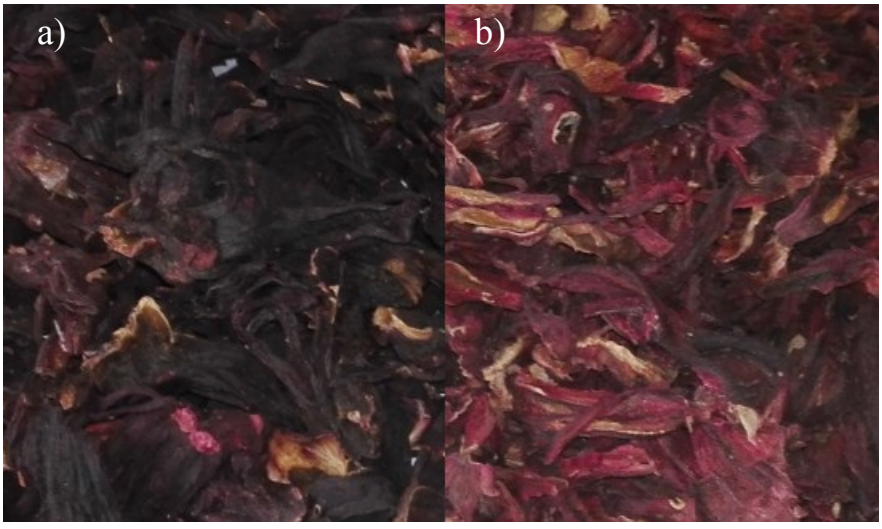

Figure 3. Dried calyces of *Hibiscus sabdariffa* var. *sabdariffa*: a) Vimto cultivar; b) Koor cultivar

They have a distinctive floral, berry-like aroma, a sour taste and an astringent, slightly mouth-drying flavor, similar to that of cranberry (Patel, 2014, Plotto et al., 2004, Abou-Arab *et al.*, 2011, Salinas-Moreno *et al.*, 2012). Their moisture content varies between 8% and 16%, but should not exceed 12% if they are to be internationally traded (Plotto

et al., 2004, Juliani *et al.*, 2009, Cisse et al., 2009b). The concentration in organic acids can attain 60% of dry weight (DW) and above (Khafaga and Koch, 1980a), the pH is around 2.5 and the average total soluble solid content 13.5 ºBx. Hibiscus calyces are very rich in anthocyanins and other phenolic compounds but their concentration depends markedly on the cultivar, growing conditions and maturity (Khafaga and Koch, 1980b, Juliani et al., 2009).

Four main cultivars of hibiscus are currently grown in Senegal for the production of calyces: the Sudanese Vimto, the local Koor, the Thai Thaïlandese and the Mexican CLT 92. The Thaïlandese and the CLT 92 have only been recently introduced in this country, being thus less extensively cultivated than the traditional Vimto and Koor (Cisse et al., 2009b). Vimto plants reputedly yield the best calyces and the biggest harvests, Koor plants are also widely cultivated in this region, but their production is relatively less developed and the yields considerably lower (Mc Clintock and El Tahir, 2004, Plotto et al., 2004). The first is rich in anthocyanins and other phenolic compounds – and has thus good coloring and antioxidant properties -, while the second has a high organic acid content and thus an intense sourness, a characteristic which is much appreciated by local consumers (Cisse *et al.*, 2009a).

Hibiscus calyces should be harvested soon after the flowers have dropped being gradually handpicked from the fields when they are still bright red, tender and fleshy. Typically within a day after harvesting, calyces are manually shelled to remove the seedpods and sepals are sun-dried to ensure their proper preservation during storage, transportation and trade (Cisse et al., 2009a). Post-harvest operations, particularly the calyces' drying process, play a very important role in guaranteeing end-product quality (Plotto et al., 2004, Mc Clintock and El Tahir, 2004, Khafaga and Koch, 1980c). In Senegal, shelled calyces are often spread over mats or plastic sheets placed on the ground, where they are left to dry under direct sunlight for 6 to 11 days, until the desired moisture content is attained (Cisse et al., 2009a). This is however a highly inefficient process that often results in substantial anthocyanin and organic acid degradation, with negative impact on the quality of dried calyces and their extracts (Khafaga and Koch, 1980c, Plotto et al., 2004).

2. Traditional hibiscus beverages

Cold beverages prepared from hibiscus extracts are commonly known by hibiscus juices in African countries, hibiscus beverages in Europe and hibiscus teas in North American countries. Hibiscus beverages have a translucent intense red appearance (Figure 4), distinctive aroma an acid taste usually counterbalanced by the addition of sugar (Aurelio et al., 2008, Bechoff et al., 2014).

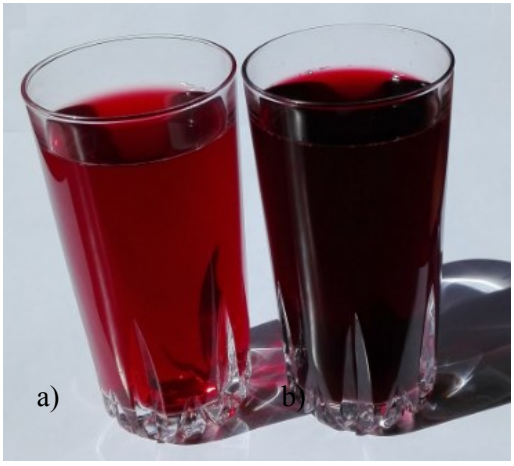

Figure 4. Beverages prepared prepared from a) Koor and b) Vimto hibiscus calyces

Due to their appealing colour, pleasantly refreshing taste and perceived nutritional value and health benefits, beverages prepared from water extracts of hibiscus calyces are consumed, either as refreshment or folk remedy, in countries as diverse as Mexico, Jamaica, Thailand, Philippines, Taiwan, Egypt and many others (Mohamed et al., 2012, Morton, 1987, Cisse et al., 2009a, Sáyago-Ayerdi et al., 2007, Cid-Ortega and Guerrero-Beltrán, 2015, Mc Clintock and El Tahir, 2004). Still, it is nowhere more widespread that in West Africa, where dried hibiscus calices are found in every market and variations of ready-made or bottled hibiscus infusions are commonly sold on the streets (Bolade et al., 2009, Cisse et al., 2009b, Plotto et al., 2004). Hibiscus beverages are known as *wanjo* in Gambia, *dabileni* in Mali, Ivory Coast and Burkina Faso, *soborodo, zobo and Isapa pupa* in Nigeria (Bamishaiye et al., 2011), *soboro* in Ghana and *jus de bissap* in Senegal (Bolade et al., 2009, Cisse et al., 2009b). In fact, drinking

cold hibiscus infusions is such an ubiquitous tradition in the latter, particularly during Ramadan, that the *jus de bissap* has become known as Senegal's national drink (Cisse et al., 2009b). In Europe and United States of America, hibiscus beverages have slowly penetrated niche markets targeted mainly the Hispanic, Caribbean, Asian and Africa diasporas.

Several different types of hibiscus beverages can be found in Africa (Bamishaiye et al., 2011, Cisse et al., 2009b, Mohamed et al., 2012). More often than not, the dried calices are sold directly to customers at local markets, who then use them to prepare infusions either for private consumption at home or for sale at the surrounding stalls and stores. Such infusions are usually named "traditional" in the literature (Bechoff et al., 2014, Cisse et al., 2009a). Nevertheless, both local small-scale and industrial manufacturers are also involved in the production of hibiscus beverages, namely bottled infusions and syrups (Cisse et al., 2009a). These so-called "commercial infusions" (Bechoff et al., 2014) are then sold in larger retail stores, hotels and restaurants in West Africa, mainly as a cheap alternative to imported soft drinks (Bolade et al., 2009).

Hibiscus beverages are typically prepared by soaking dried calyces in water and then straining them to obtain an infusion (Cisse et al., 2009b, Ramírez-Rodrigues et al., 2011b), with the notable exceptions of Malaysia and the West Indies, where fresh calyces are employed instead (Wong et al., 2003, Mounigan and Badrie, 2006). Sucrose and sometimes other flavoring ingredients are added. Calyx quality (cultivar, drying and storage processes), blending, extraction conditions (calyx particle size, calyx-to-water soaking ratio, extraction temperature and time) and flavoring of resulting infusions (with sucrose, fruit juices, pieces of fresh fruits, herbs and spices or artificial flavors) vary greatly according to available technology and tradition, leading to high variability in the chemical and sensory characteristics of end-products (Bolade et al., 2009, Fasoyiro *et al.*, 2005, Cid-Ortega and Guerrero-Beltrán, 2014, Ramirez et al., 2010). To produce syrups relatively higher ratios of calyx-to-water are used in the production of extracts and larger amounts of sucrose are added during or after stepping. Other flavouring ingredients are not normally added. Subsequent concentration may be performed usually by thermal processes (Cisse et al., 2009a, Cisse *et al.*, 2011a). Beverages are prepared by dilution with water and flavoring may be added to taste.

Homemade infusions are usually stored and consumed cold within days of their preparation. Manufactured products usually undergo pasteurization and packaging steps to extend shelf-life (Bechoff et al., 2014, Cisse et al., 2009a).

3. Physicochemical composition of hibiscus beverages

Hibiscus beverages are characterized by a low pH, most often between 2.1 and 2.8. For hibiscus beverages representative of the consumption of Senegal and Nigeria, pH was on average 2.6 (Bechoff et al., 2014, Babajide *et al.*, 2005, Foline et al., 2011), for Mexico 2.4 (Ramírez-Rodrigues et al., 2011b) and for Burkina-Faso between 2.1 and 2.4 (Diessana *et al.*, 2015).

Hibiscus calyces are rich in organic acids and have a high titratable acidity, although it may vary considerably with hibiscus cultivar, maturity at harvest, climatic and geographical conditions (Wong et al., 2002, Khafaga and Koch, 1980a, Galicia-Flores *et al.*, 2008). Hydroxycitric acid, hibiscus acid (a lactone of hydroxycitric acid) and its derivatives are the major acids present in calyces extracts. Citric, malic and tartaric acids are also present as major compounds, whereas oxalic and ascorbic acids are minor compounds (Da-Costa-Rocha et al., 2014). Hibiscus beverages acidity varies greatly. Titratable acidities of 1.6 to 2.2 gL^{-1} malic acid were reported in Nigeria (Bolade et al., 2009), of 3.7 to 4.0 gL^{-1} malic acid for Mexican infusions (Ramírez-Rodrigues et al., 2011b), of 9.1 to 10.0gL^{-1} malic acid for commercial Senegalese infusions and 1.9 gL^{-1} to 3.3 gL^{-1} malic acid for Senegalese commercial syrups (Bechoff et al., 2014). Ascorbic acid concentrations of 0.21 gKg^{-1} to 0.36 gKg^{-1} were reported for beverages prepared for three cultivars in Nigeria (Bamishaiye et al., 2011).

Hibiscus calyces are poor in sugars (El Afry *et al.*, 1980). Sugar content of hibiscus beverages depends thus mainly on the amount of sucrose added to equilibrate natural acidity, varying greatly (10 ºBx - 18 ºBx) according to local uses and consumer preferences (Bechoff et al., 2014, Bolade et al., 2009, Boucher et al., 2014). Calyces are also poor in starch (El Afry et al., 1980), but relatively rich in mucilage and pectins, contributing to the interesting soluble dietary fiber content of extracts (Da-Costa-Rocha et al., 2014). Indeed, the total dietary fiber content of the dried calyces of four Mexican

hibiscus cultivars (Criolla, China, Rosalis and Tecoanapa) was found to vary between 37% and 39% dry weight (DW), with 19% to 22% DW of it being soluble dietary fiber (Sáyago-Ayerdi et al., 2014). For hibiscus beverages a soluble dietary fiber content of 0.66 gL^{-1} was reported by Sáyago-Ayerdi et al. (2007).

Colour of hibiscus calyces varies considerably across cultivars, production areas and degree of calyx maturation at harvest (Babalola *et al.*, 2001, Khafaga and Koch, 1980b, Galicia-Flores et al., 2008, Salinas-Moreno et al., 2012). Is due to the presence of anthocyanins, which are pigmented water-soluble vacuole flavonoids (Castañeda-Ovando *et al.*, 2009), and depends of the interaction of these compounds with other constituents and physicochemical characteristics (Delgado-Vargas and Paredes-López, 2002, Du and Francis, 1973). Anthocyanins constitute the major group of phenolic compounds present in hibiscus calyces and their water extracts (Ramírez-Rodrigues et al., 2011b). Anthocyanins play an important role both in plant physiology, visual attraction for pollinators and seed dispersers, having been implicated in tolerance to stressors as drought, UV-B, and heavy metals, as well as resistance to herbivores and pathogens (Stintzing and Carle, 2004, Gould, 2004). Their content in hibiscus calyces was reported to vary between 0.2% and 2.5% DW (Cisse et al., 2009b, Du and Francis, 1973, Palé et al., 2004, Cisse *et al.*, 2009c, Salinas-Moreno et al., 2012), being thus seemingly higher than that of many fresh fruits and berries, such as strawberries, red plums, red raspberries or cranberries, but lower than blueberries or blackberries (Cisse et al., 2009c, Kalt *et al.*, 1999, Clifford, 2000). Purple, mauve delphinidin-3-xylosylglucoside (D3S) and magenta, crimson cyanidin-3-xylosylglucoside (Cy3S) are the predominant anthocyanins found in hibiscus calyces, hibiscus extracts and beverages; delphinidin-3-glucoside (D3G) and cyanidin-3 glucoside (Cy3G) are also found in smaller quantities (Fernández-Arroyo *et al.*, 2011, Rodríguez-Medina *et al.*, 2009, Delgado-Vargas and Paredes-López, 2002, Du and Francis, 1973). Galicia-Flores et al. (2008) analyzed hibiscus extracts prepared using calyces from China, Sudan and Mexico, finding significant differences in total anthocyanins concentrations, proportion of D3S and C3S and colour (colour lightness, hue and chroma). In Senegal, where hibiscus beverages are extremely popular, the Sudanese Vimto cultivar, which has a

very high average content of anthocyanins (1.7 g $100g^{-1}$ DW) is often used for its strong colour to blend with the local Koor variety (0.5 g $100g^{-1}$ DW) (Boucher et al., 2014).

Monomeric anthocyanins concentrations of 194, 238, 245 and 559 mgL^{-1}, expressed as D3S equivalents, were reported for aqueous extracts prepared from calyces of Koor, Thai, Guatemala and Vimto cultivars grown in Senegal (Cisse et al., 2009c). Also in Senegal, Bechoff et al. (2014) reported concentrations of 174 and 241 mgL^{-1} D3S for infusions prepared from Vimto cultivar, between 73 and 167 mgL^{-1} D3S for infusions of Koor and 141 mgL^{-1} D3S for infusions prepared from a blend of 50:50 Vimto and Koor cultivars. In the same study for diluted to taste syrups prepared from Vimto and Koor cultivars, concentrations of 60 and 80 mgL^{-1} D3S were found, respectively. For infusions of hibiscus Criollo cultivar grown in Mexico, Ramírez-Rodrigues et al. (2011b) reported concentration of monomeric anthocyanins from 128 mgL^{-1} to 146 mgL^{-1}, depending on extraction conditions.

Phenolic acids and flavonols are other main groups of phenolic compounds present in hibiscus calyces. Their content in hibiscus extracts also depends chiefly on the hibiscus cultivar and extraction conditions employed (Sáyago-Ayerdi et al., 2014, Ramírez-Rodrigues et al., 2011b, Pérez-Ramírez et al., 2015, Prenesti *et al.*, 2007). Chlorogenic acid and its isomers I and II, caffeic acid and derivatives of protocatechuic and gallic acids are the main phenolic acids (Pérez-Ramírez et al., 2015, Ramírez-Rodrigues et al., 2011a, Ramírez-Rodrigues et al., 2011b, Reyes-Luengas *et al.*, 2015). Quercetin and its glycosides, glycosides of myricetin, proanthocyanidins, catechin, epigallocatechin and epigallocatechin-galate (Fernández-Arroyo et al., 2011, Pérez-Ramírez et al., 2015, Sáyago-Ayerdi et al., 2007, Ramírez-Rodrigues et al., 2012, Reyes-Luengas et al., 2015) have been identified as the main flavonols. Total phenolic contents of 207, 266 and 340 mgL^{-1} expressed as galic acid equivalents (GAE) were reported for extracts prepared from Rosalis, Tecoanapa and China hibiscus cultivars, respectively (Sáyago-Ayerdi et al., 2014), between 143 and 480 mgL^{-1} GAE were found for Criollo infusions by Ramírez-Rodrigues et al. (2011b) and Sáyago-Ayerdi et al. (2014). For infusions from Koor and/or Vimto cultivars, concentrations between 597 and 695 mgL^{-1} GAE were reported by Bechoff et al. (2014), whereas for syrups concentrations were 218 and 296 mgL^{-1} GAE. The phenolic compounds present in aqueous hibiscus beverages and

extracts exhibit a wide range of interesting physiological properties, with their health benefits being mainly attributed to a high antioxidant activity (Prenesti et al., 2007). Anthocyanins (Tsai et al., 2002) and chlorogenic acid and its derivatives (Fernández-Arroyo et al., 2011) being identified as the major contributors. According to Tsai et al. (2002), hibiscus extracts have ca. 20% of the antioxidant capacity of green tea and 33% of that of black tea. Hibiscus extracts have been shown to exhibit interesting antibacterial, anti-oxidant, nephro- and hepato-protective, renal/diuretic effect, effects on lipid metabolism (anti-cholesterol), anti-diabetic and anti-hypertensive effects among others (Da-Costa-Rocha et al., 2014, Patel, 2014, Maganha et al., 2010). Phenolic acids (namely protocatechuic acid), organic acid (namely hydroxycitric acid and hibiscus acid), anthocyanins, as well as triterpene derivatives and phytosteroids are likely to contribute to the reported effects (Da-Costa-Rocha et al., 2014, Maganha et al., 2010)

4. Chemical sensory characteristics of hibiscus beverages

Hibiscus calyces reputedly have a sour taste and an astringent flavor similar to that of cranberries (Patel, 2014, Plotto et al., 2004, Aurelio et al., 2008), with derived beverages being described by trained panelists as having a prevailingly acid or sweet taste (Bechoff et al., 2014, Wong et al., 2003), a moderate mouth-drying astringency and a weak bitter taste (Mounigan and Badrie, 2006).

Colour is one of the principal attributes used to access quality and acceptability of hibiscus beverages (Bolade et al., 2009, Ramirez et al., 2010). As the preparation of beverages varies from one location the other, important variations in colour density, chroma, lightness and hue are found (Bolade et al., 2009, Galicia-Flores et al., 2008, Ramírez-Rodrigues et al., 2011b).

While sourness is associated to the rich organic acid composition of hibiscus calyces, and the correspondingly high titratable acidity of their extracts (Wong et al., 2002, Wong et al., 2003), mouth-drying astringency and bitterness are most likely linked to their phenolic composition (Mohd-Esa et al., 2010, Bechoff et al., 2014), as is often the case with plant-based foods and beverages (Ignat *et al.*, 2011, Hufnagel and Hofmann, 2008, Lawless et al., 2012, Laaksonen *et al.*, 2013). Whereas (free) anthocyanins

contribute essentially to colour (Wrolstad, 2004, Ignat et al., 2011) and do not seem to significantly impact other sensory dimensions (Vidal *et al.*, 2004), tannins, phenolic acids, flavonols and flavanols are known to be associated to the astringency and bitterness taste properties of phenol-rich beverages (Hufnagel and Hofmann, 2008, Laaksonen *et al.*, 2014, Soares *et al.*, 2017, Laaksonen et al., 2013). Bound hydroxycinnamic acids, in particular caffeoylquinic acids were determined to make up 38% of the total phenolic content of hibiscus extracts, while sugars of hydroxybenzoic acids, namely protocathecuic acid glucoside, totaled only 0.5%. Meanwhile, flavonol glucosides, including quercetin-3-rutinoside, accounted for about 10% (Ramírez-Rodrigues et al., 2011b). This type of phenolic derivative is known to be associated to velvety astringency perception in red wines (Hufnagel and Hofmann, 2008). High molecular weight polyphenols like tannins, however, have been detected in water extracts in negligible amounts only (Wong et al., 2003).

Volatiles present in hibiscus extracts contribute for its unique flavor and include aldehydes, alcohols, ketones, terpenes and acids (Ramírez-Rodrigues et al., 2011a). Aroma intensity and aroma profile of hibiscus infusions depends on balance of the various components present on the calyces naturally, induced by calyces' preservation method and extraction conditions (Chen *et al.*, 1998, Ramírez-Rodrigues et al., 2011a). Twenty-eight and twenty-five volatiles, were identified by Ramírez-Rodrigues et al. (2011a) in hot and cold Criollo hibiscus extracts, respectively, with aldehydes, followed by alcohols comprising the largest group of volatiles, whereas ketones, acids and terpenes were found in lower concentrations. By using Gas chromatography-olfactometry these authors found in hot and cold hibiscus extracts 17 and 16 active aromatic compounds. The most intense odorants were the ketone 1-octen-3-one (described as mushroom dirt and green), and the aldehyde nonanal (fruity, green) followed by geranylacetone (fruity like and apple sauce), eugenol (sweet spices) and 2(E)-Nonenal (cucumber, green, floral).

5. Process effects

The choice of hibiscus cultivar is greatly dependent on local availability and consumers preference, whereas product process derives mainly from customs and available

technology. Variable calyx-to-water soaking ratios are used for preparation of hibiscus beverages. In Nigeria mass ratios of dried calyx to water of 1:71 to 1:26 were reported (Bolade et al., 2009, Foline et al., 2011, Fasoyiro et al., 2005), in Senegal 1:20 to 1:30 for infusions and 1:4 to 1:8 for syrups (Cisse et al., 2012), in Mexico 1:20 to 1:40 (Ramírez-Rodrigues et al., 2012, Sáyago-Ayerdi et al., 2007). Time and temperature of extraction are also extremely variable. Long extraction times are commonly used for extraction at ambient temperatures, typically from 120 to 270 min, while for hot extraction, soaking time usually varies from 3 to 30 min at boiling temperature (Bolade et al., 2009, Bamishaiye et al., 2011, Bechoff et al., 2014, Foline et al., 2011, Suliman *et al.*, 2011, Cisse et al., 2012, Adinsi *et al.*, 2011).

Several authors investigated the impact of processing conditions in the composition of hibiscus extracts and beverages. Wong et al. (2003) compared the ascorbic acid and anthocyanins content, colour density and polymeric colour of hibiscus infusions extracted between 30 and 90ºC, from 30 to 300 min. Prenesti et al. (2007) compared the antioxidant power, total phenolics and absorbance of anthocyanin pigments of infusions prepared by decoction (3 min) and by soaking calyces in cold water (5 to 930 min). Ramírez-Rodrigues et al. (2011b) compared pH, titratable acidity, total solids, colour, anthocyanin content, total phenolics and antioxidant capacity of hibiscus infusions prepared using cold water (25ºC, 30 to 240 min) and hot water (90ºC, 2 to 16 min). Sindi *et al.* (2014) studied the effect of temperature (25ºC to boiling temperature) and extraction time (3 to10 min), on antioxidant capacity, anthocyanins and total phenolics content. Diessana et al. (2015) studied the effect of calyx particle size (whole and 250 µm), calyx-to-water ratio (1:25 to 1:5) on pH, titratable acidity, anthocyanins and total phenolics content, and antioxidant capacity of hibiscus extracts at 30ºC from 10 to 240 min. Cisse et al. (2012) studied the impact of calyx-to-water ratios (1:10 to 1:30), calyx particle size (whole and 150 µm), extraction temperature (25 to 90ºC) and time (0 to 600 min) on anthocyanins extraction yield and degradation. Aurelio et al. (2008) researched the degradation kinetics of anthocyanins from 60 to 100ºC up to 120 min. Cisse et al. (2011a) and Cisse *et al.* (2011b) studied extracts' concentration processes: osmotic evaporation, vacuum evaporation and pressure-driven ultrafiltration and nanofiltration membranes. Ramírez-Rodrigues et al. (2011a) compared the volatile

composition and aroma profile of hot (98ºC, 16 min) and cold water extracted infusions (22ºC, 240 min).

Anthocyanins content of hibiscus beverages increases proportionally with the amount of calyces used (Aurelio et al., 2008, Tsai et al., 2002). Extraction rate is strongly affected by calyces particle size with similar extraction yields being obtained in 10 min instead 120 min, at 25ºC, by reducing the size of the from 2 cm to 150 μm (Cisse et al., 2012). Diessana et al. (2015) showed that the use of crushed calyces (≤ 250 μm) and a 1:5 w/w calyx-to-water ratio resulted in maximal anthocyanin extraction 30ºC for 30 min.

In spite of the stabilizing effect of the low pH (Aurelio et al., 2008), higher extraction temperatures promote anthocyanins and other phenolic compounds extraction but anthocyanin's degradation also increases. In the study conducted by Ramírez-Rodrigues et al. (2011b) similar anthocyanins extraction yields were found for extraction at 25ºC during 240 min and 90ºC for 16 min. Wong et al. (2003) and Aurelio et al. (2008) observed that for mild temperatures anthocyanins concentration increases with an increase in temperature and soaking time, whereas for higher temperatures (>60ºC), anthocyanins degradation rate increases mainly during long soaking times. Prolonged extraction times and harsher thermal treatments may promote the polymerization of anthocyanins in plant-based beverages by processes of direct condensation and co-pigmentation (Wrolstad *et al.*, 2005). Polymeric anthocyanins are chemically more stable than free monomeric forms, but are known to contribute towards an undesirable change to a more yellowish hue in both fruit juices and hibiscus extracts (Wong et al., 2003, Cisse et al., 2009c, Rein and Heinonen, 2004), leading to higher colour hue tints and polymeric colour values. Studies on thermal kinetic degradation of anthocyanins showed that the rate of degradation increases dramatically over 80 ºC, revealing the importance of using mild (rather than harsh) pasteurization conditions (Cisse et al., 2009c).

Being directly related to beverages composition in anthocyanins, similar trends were reported for colour than the ones described for anthocyanins, namely in relation with extraction conditions (calyx-to-water soaking ratios, time and temperature) (Ramírez-Rodrigues et al., 2011b, Wong et al., 2003, Bolade et al., 2009). When comparing

colour densities and hue tints of infusions extracted at 25ºC and 90ºC, Ramírez-Rodrigues et al. (2011b) concluded that although cold extraction required much longer extraction times (15-fold increase) resulted in lower colour degradation. In the investigation held by (Wong et al., 2003), the increase of polymeric colour was observed mainly for temperatures above 60ºC.

Extraction conditions also affect polyphenols content and antioxidant activity of hibiscus extracts (Prenesti et al., 2007, Wong et al., 2003, Ramírez-Rodrigues et al., 2011b, Sindi et al., 2014). Prenesti et al. (2007) and Sindi et al. (2014) concluded that extraction temperature affected the total content in phenolic compounds, that were better extract with boiling water without damaging its antioxidant ability, resulting in higher antioxidant capacity. In the study conducted with F141 cultivar, Tsai et al. (2002) concluded that anthocyanins accounted for 51% of the total FRAP (ferric reducing ability of plasma) activity and 24% was due to other phenolic compounds. In the same study, it was shown, however, that the degradation of monomeric anthocyanins into polymerized phenolics during storage (from about 80% to 50% of total phenolics) only had a minor effect on overall antioxidant level that decreased only a few percent.

Calyx-to-water soaking ratio and time and temperature of extraction were shown to have a very limited impact on the pH of the beverages (Diessana et al., 2015, Ramírez-Rodrigues et al., 2011b). Along with calyx cultivar, calyx-to-water soaking ratio had, however, a main impact in hibiscus beverages' acidity. Galicia-Flores et al. (2008) compared the titratable acidity of extracts obtained from calyces from China, Sudan and Mexico, finding much lower concentrations for beverages prepared from Sudanese calyces than from Chinese and Mexican calyces. Diessana et al. (2015) found that by increasing the mass ratio calyx-to-water from 1/20 to 1/5 a corresponding 3.5 fold increase of titratable acidity was attained. Major organic acids present in the calyces are easier to extract than other constituents (namely anthocyanins and other polyphenols) and do not extensively degrade in aqueous solutions (Prenesti et al., 2007), consequently , time and temperature of extraction were shown to have a limited impact on the acidity and pH of the beverages, except when very short extraction periods were used (Ramírez-Rodrigues et al., 2011b, Diessana et al., 2015, Wong et al., 2003, Prenesti et al., 2007).

Extraction temperature was also shown to influence the total amount and composition in volatile compounds of hibiscus extracts. Ramírez-Rodrigues et al. (2011a) analyzed the total volatiles content of a cold extracted infusion (22 ºC, 240 min) having found only 60% of the content of hot extracted one (98ºC, 16min). In the same study, in both hot and cold extracts, aldehydes comprised the largest group of compounds followed by alcohols and ketones, with terpenes and acids being the less abundant, nevertheless aldehydes were present in much higher proportion in hot than in cold extracts.

6. Sensory evaluation and consumer acceptance studies of hibiscus beverages

Some sensory analysis of beverages has been previously conducted (Wong et al., 2003, Bechoff et al., 2014). In the study held by (Bechoff et al., 2014) in Senegal, eight hibiscus beverages (2 diluted to taste syrups, 3 ready-to-drink commercial infusions and 3 traditional infusions) prepared from Vimto and/or Koor cultivars were evaluated by a panel of 17 semi-trained panellists using 11 sensory attributes. Three attributes were used to describe appearance (red colour, clarity and concentration) and nine for odour (hibiscus and fermented) and flavour (acidic, sweet, hibiscus, bitter, irritant and fermented). Significant differences were found for all attributes. The main sensory attributes of hibiscus beverages were a characteristic red colour, a predominant hibiscus odour a prevailingly acid taste for infusions and sweet taste for syrups, with moderate (infusions) to low (syrups) bitter taste. Meanwhile, quality requirements for the export of hibiscus calyces to Europe and the US have also been established, taking into account their sensory characteristics as well as those of their aqueous extracts (Plotto et al., 2004). For extracts these were: clear appearance, deep red colour with some background purple hues (blue hues were considered undesirable), slight berry aroma and a well-balanced, tart and astringent flavor with some cranberry notes as well as a slight drying effect. Excessively tart, acidic and bitter taste as well as of-flavors and extraneous spice or botanical notes were considered undesirable.

Red colour, hibiscus odour and flavor, acid and sweet taste intensities are considered the key drivers of consumer acceptance of hibiscus beverages in West African markets (Bechoff et al., 2014, Foline et al., 2011, Bolade et al., 2009). Hedonic evaluations of

two ready-to-drink infusions and two diluted to taste syrups, manufactured according to Senegalese tradition were evaluated by 160 consumers in Senegal, yielding mean overall liking (OL) ratings of 6.2 and 5.2 out of 9 for infusions; and 5.9 and 5.6 out of 9 for syrups. Such differences in acceptance were statistically significant for infusions ($p < 0.01$), but not for syrups. Importantly, the existence of groups of consumers with highly distinct patterns of sensory preferences for hibiscus beverages was uncovered (Bechoff et al., 2014).

Ramirez et al. (2010) used consumer-based sensory optimization to develop a ready-to-drink hibiscus Criollo infusion for the US market, based on the traditional hibiscus Mexican beverage *Agua de Jamaica*. Extraction conditions (calyx-to-water ratio) and formulation (sugar-to-acid ratio) were experimentally varied and resulting products tested with 75 consumers in Florida, with the goal of identifying the parameters maximizing acceptance. A subsequent test with a similar consumer sample uncovered that an optimized infusion, with a calyx-to-water soaking ratio of 1:40 (w/v) and a sugar-to-acid ratio of 25 ºBx g^{-1} malic acid 100 mL^{-1}, was liked by 79% of participants and disliked only by 13% of them, which corresponded to a mean OL of 6.3 out of 9. The attribute with the highest mean OL was colour (7.4), with flavor and aroma being rated considerably lower (5.9 and 5.7, respectively). Mainly due to differences in the cultivar and formulation employed in its production, the optimized infusion displayed a brighter red hue, but much lower titratable acidity and colour density than its West African counterparts, being actually more comparable to diluted syrup in terms of sensory profile. It had, nonetheless, lower sugar content than any of the aforementioned products. In spite of the product improvement process ensued and due to a low level of familiarity with hibiscus beverages, Florida consumers mainly described the optimized hibiscus infusion as having an exotic, floral aroma and an unusual, tart taste, suggesting therefore the addition of sugar or fruit juices to enhance its palatability (Ramirez et al., 2010). Significant differences in preference for the levels of some attributes were nonetheless observed among consumers: those over 30 years old found flavor and tartness significantly more often too weak than their younger counterparts, while females liked the colour of this product significantly more than males. Ramírez-Rodrigues et al. (2012) subsequently investigated the effects of applying flash and cold

pasteurization on the sensory quality of an optimized infusion. The acceptance of resulting products was tested also with consumers in Florida, along that of a control (unpasteurized) infusion. Mean OL ratings reportedly varied between 5.0 (cold pasteurization) and 5.2 (control and flash pasteurization).

7. Sensory evaluation methods

Sensory evaluation is often described as a scientific method used to evoke, measure, analyze and interpret those responses to products perceived through the senses of sight, smell, touch, taste and hearing (Anonymous, 1975). It comprises a set of techniques which intend to accurately measure human responses to sensory properties of foods (and other products), while seek to minimize the potentially biasing effects of brand identity and other information (Lawless and Heymann, 2010). Sensory evaluation is traditionally divided into two categories of testing: objective and subjective. In objective testing, the sensory attributes of a product are evaluated by a selected or trained panel. In subjective testing, hedonic, acceptance and other reactions of consumers to the sensory properties of products are measured. Often, these two elements are combined to reveal insights in the way sensory properties drive consumer acceptance and emotional benefits. Furthermore, linking sensory properties to physical, chemical, formulation and/or process variables, enables the product to be designed to deliver optimum or appropriate consumer benefits and to relate sensory properties to specific ingredients or process variables (Kemp *et al.*, 2011).

Over the last half century a number of scales have been developed and utilized to measure hedonic responses in both basic psychophysical and applied research (Lim, 2011). However, the nine-point hedonic scale occupies a unique niche in terms of its applicability to the measurement of products acceptance and preference (Stone *et al.*, 2012) since its development by Jones *et al.* (1955) and Peryam and Pilgrim (1957). Being easy easily understood by naive consumers with minimal instruction, results have proven to be remarkably stable, with product differences (in liking) being reproducible within different groups of subjects (Stone et al., 2012). Its validity and reliability has been furthermore accessed with considerable success, as for instance Moskowitz and

Sidel (1971) and Warren *et al.* (1982) independently concluded that magnitude estimation was not superior. In recent years, interest in these comparisons have not demonstrated any evidence for the superiority of other proposed scales, with the nine-point hedonic scale being used on a worldwide basis (when translated) by researchers and professionals to access product likes and dislikes (Stone and Sidel, 2004, Stone et al., 2012).

How much do you like the sample 351?

- ☐ Like extremely
- ☐ Like very much
- ☐ Like moderately
- ☐ Like slightly
- ☐ Neither like nor dislike
- ☐ Dislike slightly
- ☐ Like moderately
- ☐ Dislike very much
- ☐ Dislike extremely

How would you rate the sweetness of sample 351?

- ☐ Too strong
- ☐ Just-about-right
- ☐ Too weak

Figure 5. Example of sample ballot with a 9 point hedonic scale and a 3 point JAR scale

Just-about-right (JAR) scales are bipolar scales used to measure the level of an attribute relative to participants ideal level, having a midpoint labelled just-about-right or just right. They are commonly used in consumer research to identify whether product attributes are perceived at levels that are too high, too low or just-about-right for the product. The emergence of these scales in sensory and market research is not well

documented. In the early 1970s, Moskowitz (1972) discussed JAR scales as an alternative to sensory intensity scales for identifying optimal formulations of food products. A decade later, the use of scales with the center "just right" was reported by McBride (1982) and Booth *et al.* (1983), among others. Today, JAR scales are a tool commonly used for guiding product development, being most often used in nonexperimental product optimization studies (Popper, 2014). Of particular interest has been the relationship between JAR scales and hedonic scales through penalty analysis, which accesses the impact of respondents finding an attribute of the product not just-about-right in lowering overall liking (Popper, 2014).

Classical or generic sensory descriptive analysis is the most powerful, sophisticated, and most extensively used tool in sensory science, being able to provide a complete description of the sensory characteristics of a food product (Heymann *et al.*, 2014, Varela and Ares, 2012, Lawless and Heymann, 2010). In this methodology, assessors (usually between 8 and 12) are trained in attribute recognition and scaling, they use a common and agreed sensory language, and products are scored on repeated trials to obtain a quantitative description. The high specialization of descriptive panels allows obtaining detailed, reliable and consistent results (Moussaoui & Varela, 2010). This gold standard technique (Lawless and Heymann, 2010) has, nevertheless, two major drawbacks: a) is very expensive and time-consuming, since vocabulary and associated panel training must be adapted to each type of product; b) trained assessors may describe products differently from consumer and/or take into account attributes that may be irrelevant for them (ten Kleij and Musters, 2003). This has led, in recent years, to an increasing interest in alternative methods to obtain products' characterization that can be used with semi trained assessors (i.e. trained in sensory recognition and characterization, but not in the specific category of products or in scaling) as well as in methods that gather sensory information directly from consumers. Extensive research has hence been conducted to developed and validate such methods, with remarkable success, since sensory maps similar to those resulting from classic descriptive analysis can be obtained. (Varela and Ares, 2012). Check-all-that-apply (CATA) questions and Flash profiling are among these novel methods.

Flash profiling (FP) was suggested by Dairou and Sieffermann (2002) for rapidly profile products according to their most salient sensory attributes (Varela and Ares, 2012) . FP usually uses six to twelve semi-trained panelists and combines individual vocabulary generation through free choice profiling with ranking classification of all product set for each attribute. Results are evaluated using Generalized Procrustes Analysis (Gower, 1975). Flash Profile has been applied to describe different foods, including jams (Dairou and Sieffermann, 2002), dairy products (Delarue and Sieffermann, 2004), commercial apple and pear purees (Tarea *et al.*, 2007) and fish nuggets (Albert *et al.*, 2011), among others. More recently, FP was been used with consumer panels to evaluate hot beverages (Moussaoui and Varela, 2010, Veinand *et al.*, 2011).

CATA questions are multiple choice questions, which are commonly used in marketing research in order to reduce response burden (Rasinsky *et al.*, 1994). The use of CATA to gather information about consumer's perception of sensory characteristics of food products was proposed by Adams *et al.* (2007), being increasingly applied in sensory and consumer science for getting consumer-based sensory characterizations (Ares *et al.*, 2017, Ares *et al.*, 2015). Consumers are presented with a list of terms (words or expressions) and are asked to indicate which appropriately describe their perception of the samples being evaluated. The terms may include sensory attributes, hedonic responses, emotional responses or other terms that consumers might associate with the samples (Meyners and Castura, 2014). The first step when analyzing data from CATA questions is to assess the existence of significant differences in elicitation frequencies for each CATA term across samples, usually by means of a Cochran Q test, followed by bi-dimensional representation of terms and samples using Correspondence Analysis or Multifactorial Analysis (Varela and Ares, 2012, Meyners and Castura, 2014)

Objectives of the thesis

Sensory characteristics are known to constitute the main bottleneck in the path to market success of beverages with enhanced phytochemical properties, as many of the plant-based ingredients with important health benefits often translate into products with low palatability (Hernández-Carrión et al., 2015, Jaeger *et al.*, 2009, Laaksonen et al., 2014, Lawless et al., 2012) and consumers are almost always unwilling to compromise on taste for health (Ares *et al.*, 2010, Sabbe *et al.*, 2009). Understanding sensory perception and the main chemical-sensory drivers of acceptance of such beverages, as well as developing new products that effectively meet consumer's expectations and preferences, are hence among the key challenges faced by the food & beverage industry today (Sun-Waterhouse, 2011). This is a critical step for the validation and/or further development of products resulting from AFTER research, as findings can contribute to understand the impact of implemented technological changes, to the identification of opportunities for further improvement and, importantly, to the assessment of new products market potential in Senegal and in European countries.

In view of the above the objectives of this thesis are to:

- evaluate the impact of the reengineering processes held under AFTER project scope in the chemical-sensory properties of hibiscus beverages;
- compare the perception and consumer preference of conventional and new beverages in traditional markets (Senegal);
- develop hibiscus beverages sensory lexicons that can be used by trained panelists, professionals and consumers;
- investigate individual and cultural effects on perception and preference of hibiscus beverages in new markets through cross-country studies in Europe (France, Portugal and United Kingdom).

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CHAPTER 2

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

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.

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 and 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 *et al.*, 2014, Plotto *et al.*, 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 (Ramírez-Rodrigues *et al.*, 2011a, Plotto et al., 2004, Mc Clintock and El Tahir, 2004). 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 *et al.*, 2009, Cisse *et al.*, 2009b). 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 have 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 *et al.*, 2009a). Indeed, the colouring, flavouring and thickening properties of hibiscus calyces have been shown to derive largely from their distinctive phenol, organic acid and pectin composition (Fernández-Arroyo *et al.*, 2011, Sáyago-Ayerdi *et al.*, 2014, Ramírez-Rodrigues *et al.*, 2011b). 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 (Diessana *et al.*, 2015, Cisse *et al.*, 2012, Pérez-Ramírez *et al.*, 2015, Ramírez-Rodrigues et al., 2011a, Ramírez-Rodrigues et al., 2011b). 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 *et al.*, 2005, Mounigan and Badrie, 2006, Ramirez *et al.*, 2010, Wong *et al.*, 2003, Bechoff *et al.*, 2014).

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 et al., 2011a, Ramírez-Rodrigues et al., 2011b). In Africa, shelled calyces are often spread over mats or plastic sheets placed on the ground, where they are left to dry under direct sunlight for 6 to 11 days, until a moisture content of 16% is attained (or under 12%, if they are intended for international trade) (Cisse et al., 2009a, Mc Clintock and 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 (Plotto et al., 2004, Khafaga and Koch, 1980b).

Regarding processing variables, Ramírez-Rodrigues et al. (2011b) compared the effects of cold (25 ºC) and hot (90 ºC) 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 30ºC. 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 ºC, revealing the importance of using mild (rather than harsh) pasteurisation conditions (Cisse *et al.*, 2009c).

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 (Boucher *et al.*, 2014, Bechoff et al., 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 Figure 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 et al., 2009a). 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 et al., 2009c). 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 ºC), 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 ºBx at 0.4 bar, while REs and REi were obtained through the addition of sucrose to corresponding extracts up to 62 and 17 ºBrix, respectively. All three beverages were pasteurized (75 ºC for 30 min), rapidly cooled and adequately packaged. REi was then stored under refrigeration (< 10 ºC) for preservation, whereas UVc and REs were kept at ambient temperature (25-30 ºC) 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 ºC), 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 ºBx. The resulting infusion was pasteurized (90 ºC for 20 min), rapidly cooled, adequately packaged and stored under refrigeration (< 10 ºC).

2.2 Sample preparation

Samples of UVc were prepared by dilution with potable water (1:40 v/v) and sweetening with 130 gL^{-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 ºC 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.

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 & Wrolstad (2001), where colour density = [(A420nm − A700nm) + (A520nm − A700nm)] and hue tint = (A420nm − A700nm)/(A520nm − A700nm), for a 10 mm optical path length.

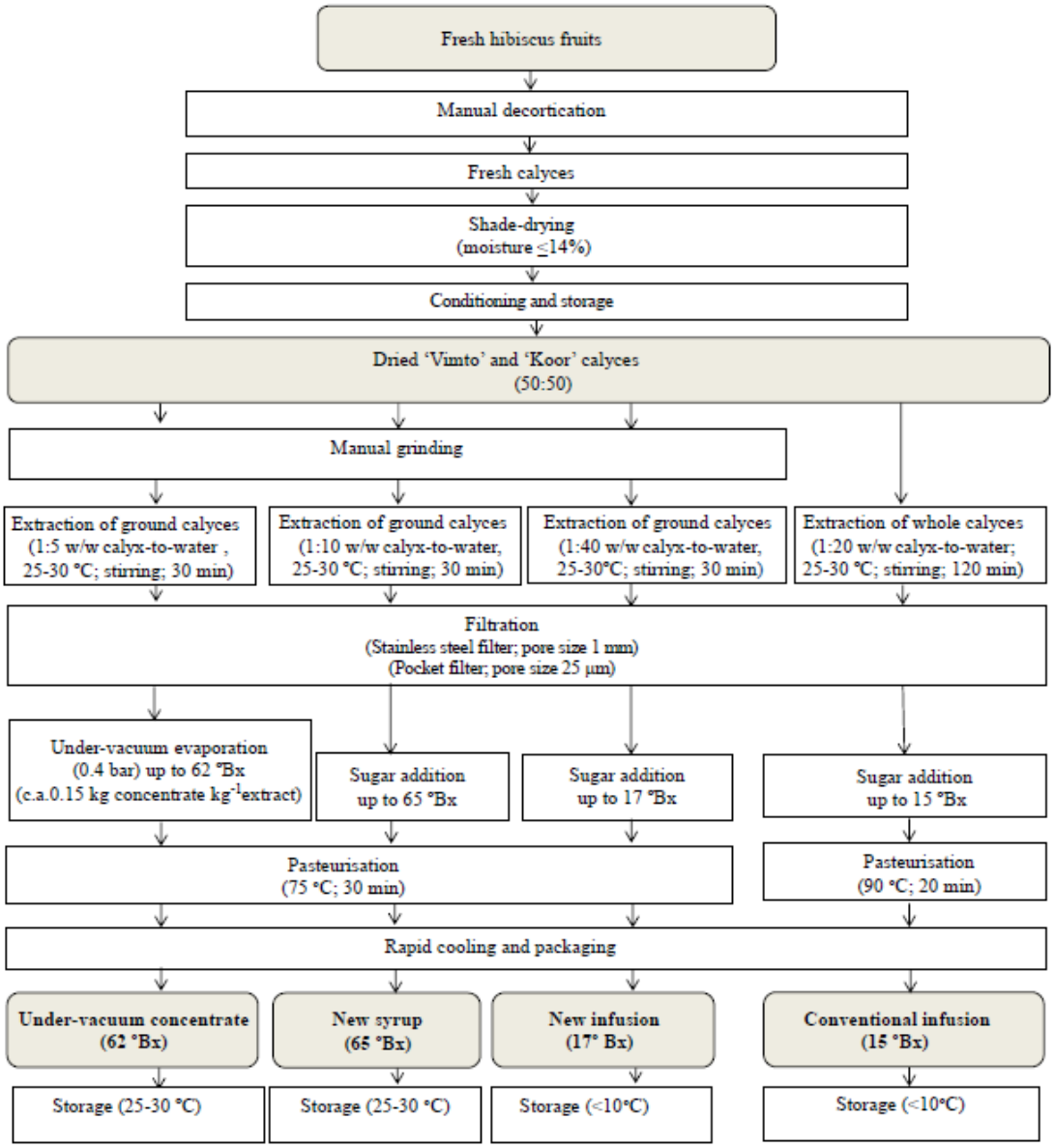

Figure 1. Processes of production of the four hibiscus beverages

Total soluble solids and sugars

Total soluble solids (TSS) (ºBx) were measured according to the European Standard EN 12143 at 20.0 ºC ± 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 (4.6x250 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 $mLmin^{-1}$. Total sugars were calculated as the sum of glucose, fructose and sucrose.

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.

Total monomeric anthocyanins, phenolic content and antioxidant capacity

The spectrophotometric pH differential method was used to investigate the anthocyanin content of the samples (Lee *et al.*, 2005). Degraded anthocyanins in the polymeric form, as well as non-enzymatic browning pigments, are hardly reversible 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 $Lcm^{-1}mol^{-1}$ at pH=1 and 520 nm. Concentrations were expressed as cyanidin 3-glucoside equivalents (Cy3glu) (MW=449 $gmol^{-1}$).

Total phenolic content was measured by 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 $ABTS^{\bullet+}$ 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 (mgL^{-1} AEAC).

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

2.4 Sensory evaluation

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.

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 and 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.

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* (*Parcelles 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.

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.

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 *et al.*, 2010).

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 Senegalese 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, education 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.

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 *et al.*, 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

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 et al., 2009c, Ramirez et al., 2010, Ramírez-Rodrigues et al., 2011b). 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, (Delgado-Vargas and Paredes-López, 2002). However, given that no significant differences in the pH of tested beverages were observed, such effects could not be investigated.

UVc had the highest colour density of the beverages tested, followed by REI, REs and finally CTi. New beverages also had lower hue values (i.e., were redder and less yellow/brown) than CTi. The changes introduced by AFTER in the traditional manufacturing process, particularly the introduction of a grinding step prior to extraction and milder pasteurisation conditions (Figure 1), appear therefore 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-Rodrigues et al. (2011b) 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 steps (Bechoff et al., 2014, Diessana et al., 2015, Khafaga and 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 ($\rho=0.957$, $p<0.01$) 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 (Rein and Heinonen, 2004, Cisse et al., 2009c). Prolonged extraction times and harsher thermal treatments may promote the polymerisation of anthocyanins by processes of direct condensation and co-pigmentation (Wrolstad *et al.*, 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 et al., 2009c, Rein and 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.

Table 1 – Physicochemical characteristics of hibiscus beverages.

Sample	Colour density (UA)	Hue tint	TSS (ºBx)	Total sugars (gL^{-1})	pH	TAcidity (mM NaOH)	TSS/ TAcidity (ºBx mM^{-1} NaOH)	TMA (mgL^{-1} Cy-3-glu)	TPhenolics (mgL^{-1} GAE)	TAC (mgL^{-1} AEAC)
UVc	17.1^{a}±0.9	0.38^{b}±0.08	13.4^{d}±0.1	127^{c}±3	2.52^{a}±0.10	100.0^{b}±7.1	0.134^{c}±0.009	234^{a}±3	677^{a}±9	557^{a}±18
REs	7.3^{c}±0.2	0.38^{b}±0.02	14.9^{c}±0.0	151^{b}±2	2.61^{a}±0.09	51.5^{c}±2.1	0.290^{a}±0.012	83^{c}±2	329^{c}±19	332^{b}±9
REi	12.3^{b}±0.0	$0.49^{a,b}$±0.01	17.4^{a}±0.1	167^{a}±3	2.56^{a}±0.01	142.5^{a}±0.7	0.122^{c}±0.001	112^{b}±3	587^{b}±2	409^{c}±14
CTi	4.9^{d}±0.0	0.58^{a}±0.00	15.8^{b}±0.1	149^{b}±2	2.45^{a}±0.01	104.0^{b}±0.0	0.151^{b}±0.001	44^{d}±1	344^{c}±4	292^{d}±13

Mean ± standard deviations. Different superscripts within a column indicate significant differences according to Tukey's HSD ($p<0.05$). TSS = Total soluble solids; TAcidity = Titratable acidity; TMA = Total monomeric anthocyanins; TPhenolics=Total phenolics; TAC = Total antioxidant capacity; UVc = under-vacuum concentrate; REs = dilute-to-taste syrup; REi = ready-to-drink infusion; CTi = conventionally manufactured, ready-to-drink infusion.

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 to 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 the 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 ºBx) (Bechoff et al., 2014). The same 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 º Brix (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 ºBx and resulting beverages 17 to 18 ºBx after dilution to taste (Bechoff et al., 2014, Cisse et al., 2009a). In view of this, one of the process improvements implemented by AFTER was the reduction of the TSS content of syrups to 65 ºBx, 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, climatic and harvest conditions (Khafaga and 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., 2009a, Diessana et al., 2015, Cisse et al., 2009c, Khafaga and Koch, 1980b, Cisse et al., 2012). 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 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 110L of REs, whereas the same amount yielded about 50L of REi and 25L 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 ºC, 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 ºC, 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 optimized 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 ºC) (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 (Diessana et al., 2015, Bechoff et al., 2014, Khafaga and 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 (Figure 1), have most likely results 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 (Ramirez et al., 2010, Bechoff et al., 2014, Bolade et al., 2009). 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 ºBx 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 ºBx mM^{-1}NaOH) (Bechoff et al., 2014), but fairly lower than that of the optimised infusion earlier described (0.18 ºBx 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 although the also lower addition of sucrose. Finally, the sugar-to-acid ratios of tested beverages were significantly correlated to their titratable acidity (ρ=-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.

Anthocyanins, polyphenols and antioxidant activity

The total monomeric anthocyanin content of tested beverages varied significantly between a minimum of 44 mgL^{-1} Cy-3-glu (for CTi) and a maximum of 234 mgL^{-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 methods and parameters (calyx-to-water ratio, stirring, temperature and time) and extract processing operations (e.g., evaporation or pasteurisation) (Khafaga and Koch, 1980b, Diessana et al., 2015, Ramírez-Rodrigues et al., 2011b, Wong et al., 2003, Cisse et al., 2012, Cisse et al., 2009c). Values of 106, 60 and 45 mgL^{-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 (Wrolstad et al., 2005). Since low water activity is one the factors known to promote anthocyanin stability in plant-based beverages during thermal treatment (Delgado-Vargas and 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 (Figure 1). Such effect was likely enhanced by differences thermal treatment: conventionally manufactured syrups were pasteurised at 105 ºC and infusions at 85 ºC (Bechoff et al., 2014), against 75 ºC 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 ºC), relatively to both new and conventional ready-to-drink infusions (for comparable treatment times). Cisse et al. (2009c) have showed that the rate of kinetic degradation of anthocyanins in hibiscus extracts increases dramatically over 80 ºC, while Pérez-Ramírez et al. (2015) observed a gradual growth of this rate up to 70 ºC, but a rather accelerated one after this temperature and up until 100 ºC. These findings seem to support our hypothesis. Anthocyanins concentrations of 146 and 421 mgL^{-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 25ºC) (Cisse et al., 2009c). Such high values, when compared to those observed for REi and CTi, are likely explained by the use of calyces from either single cultivar, by the much higher calyx-to-water ratio and a much longer extraction time, as well as the lack 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 mgL^{-1} GAE were previously reported for conventionally manufactured 'Vimto' and 'Koor' (50:50) infusion, 'Vimto' syrup and 'Koor' syrup, respectively (Bechoff et al., 2014). These values were lower than the ones observed in the present study for REs, higher for REi and similar for 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 with the corresponding conventionally manufactured beverage, it should be noted that although ½ of the calyx-to-water ratio was used the decrease of total phenolic content was of only about 15%. A longer extraction time might, nevertheless, contribute to an increase of the total phenolic content of new beverages. Ramírez-Rodrigues et al. (2011b) and Pérez-Ramírez et al. (2015) studied the optimization of extraction under different extraction conditions, showing that the total phenolic content of aqueous hibiscus extracted at 25ºC increased with extraction time up to 240 min. For CTi a very low total phenolics content was observed when compared with REi

and with the earlier reported conventional infusion. Again, a possible explanation may be linked to harsher pasteurization conditions used.

The main polyphenols in hibiscus beverages are anthocyanins, phenolic acids and flavonols (Sáyago-Ayerdi et al., 2014, Ramírez-Rodrigues et al., 2011b, Pérez-Ramírez et al., 2015).They 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 *et al.* (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 (ρ=0.874, $p<0.01$), between the monomeric anthocyanin and antioxidant activity, (ρ=0.978, $p<0.01$), which indirectly result in polyphenol content and antioxidant activity appearing significantly associated to colour density (ρ=0.974, $p<0.01$ and ρ=0.957, $p<0.01$, respectively).

3.2. Sensory evaluation

Descriptive analysis

Panellists generated a set of nine descriptors to discriminate between hibiscus beverages: one related to appearance (red colour), three to odour (hibiscus: 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 manufacture in Senegal (Bechoff et al., 2014). Ramírez-Rodrigues et al. (2011a) 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 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 and Badrie, 2006, Wong et al., 2003).

Figures 2 a-b depicts the results of the descriptive analysis. They show that there was good agreement between panellists 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. Panellists 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.

Expectedly, the correlation coefficients estimated showed that red colour intensity was significantly ($p<0.01$), positively associated to colour density ($\rho=0.932$) and monomeric anthocyanin content ($\rho=0.895$). This explains why UVc and REi was judged to have a higher red colour intensity than REs of CTi (Figure 2b). On the other hand, the intensities of some aromatic notes, namely hibiscus and honey odour, were strongly, negatively associated with hue tint ($\rho=-0.764$, $p<0.05$ and $\rho=-0.863$, $p<0.01$, 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 *et al.*, 1997).

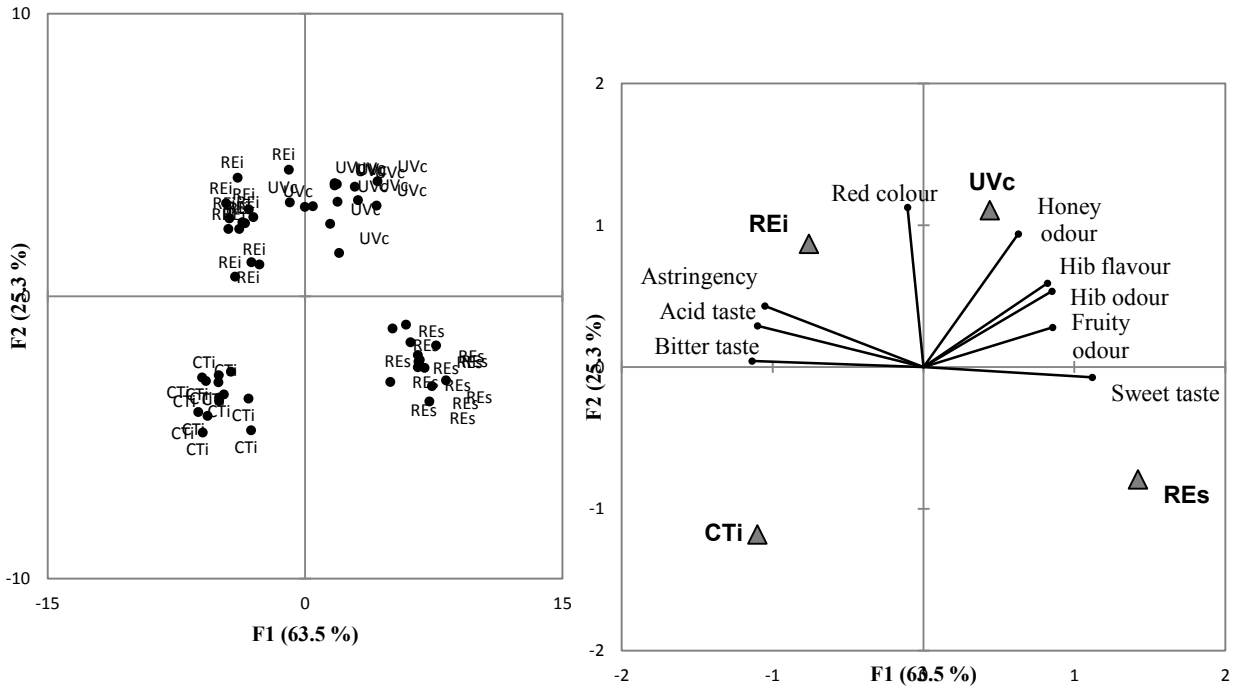

Figure 2 – Generalized Procrustes Analysis of Flash Profile data: (a) representation of panellists and samples; (b) representation of samples and sensory descriptors. UVc = under-vacuum concentrate; REs = dilute-to-taste syrup; REi =ready-to-drink infusion; CTi=conventionally manufactured, ready-to-drink infusion.

Strong, positive relationships were identified between acid taste intensity and titratable acidity (ρ=0.845, $p<0.01$), and between sweet taste intensity and sugar-to-acid ratio (ρ=0.819, $p<0.05$), as expected from the 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 that 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 (ρ=0.724, $p<0.05$), and between astringency and titratable acidity (ρ=0.934, $p<0.01$). Conversely, significant negative associations were found between these two sensory descriptors and sugar-to-acid ratio (ρ=-0.709, $p<0.05$ and ρ=-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 (Lesschaeve and Noble, 2005, Laaksonen *et al.*, 2014, Keast and Breslin, 2002). Similar enhancement and suppression effects were also observed in prior sensory evaluations of hibiscus beverages (Bechoff et al., 2014, Wong et al., 2003, Mounigan and Badrie, 2006). 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 and Breslin, 2002). Given that UVc had the most the strongest red colour and aromatic character of the beverages studies, 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, flavanols and tannins (Lesschaeve and Noble, 2005, Laaksonen et al., 2014), have been detected in in hibiscus extracts in negligible amounts only, or not at all. Conversely, anthocyanins, that are by far the most abundant flavonoid in hibiscus extracts (Ramírez-Rodrigues et al., 2011b, Fernández-Arroyo et al., 2011, Wong et al., 2003, Rodríguez-Medina *et al.*, 2009), were shown to have little to no direct impact on the flavour perception of plant-based beverages (Soares *et al.*, 2017).

Consumer study

Figure 3 presents the results of the preference and similarity questions included in the consumer questionnaire. About 55% of participants indicated UVc as most liked beverage and only 3% as 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 most liked ($p<0.05$) and not significant for least liked. Finally, no significant differences were found between REs and REi for either most or 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 smaller 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 (Figure 2).

Meanwhile, consumers over 30 years old found the flavour and tartness intensities of an optimized hibiscus infusion more often too weak than their younger counterparts, while female consumers liked its intense red colour more than males ones (Ramirez 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), THses results are gebnerally supportive of our findings.

The proportion 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 most often and beverages prepared from syrups least often (Table 2), which is line with consumption habits in Senegal (Cisse et al., 2009a).

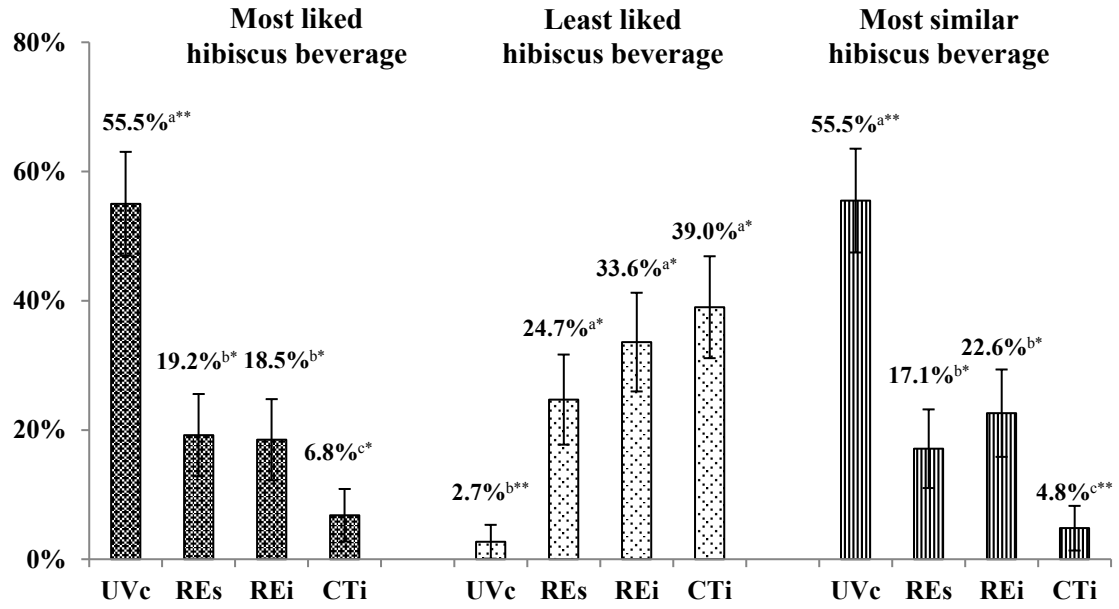

Figure 3 – Consumer preference and similarity judgments of hibiscus beverages (n=146). Error bars represent 95% confidence intervals. Different superscripts within each type of judgment indicate significant differences according to Pearson chi-square tests and Marascuilo pairwise comparisons (*p<0.05; **p<0.01). UVc = under-vacuum concentrate; REs = dilute-to-taste syrup; REi = ready-to-drink infusion; CTi = conventionally manufactured, ready-to-drink infusion.

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 (Figure 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.

Table 2 – Associations between preference/similarity judgments and participants' age, gender, education level and frequency of consumption of hibiscus beverages (overall and per type) (n=146).

Sample	Number of respondents	Number of male respondents	Age (mean ± std. dev.)	Education level of participants (mode)	Frequency of consuming hibiscus beverages - overall (mode)	Frequency of consuming hibiscus beverages - per type (mean of ranks)		
						Homemade infusions	Ready-to-drink infusions	Dilute-to-taste syrups
Most liked								
UVc	81	44	$30.6^{b} \pm 10.6$	higher education	several times/month	2.72^{a}	1.96^{b}	1.32^{c}
REs	28	17	$36.3^{a,b} \pm 16.6$	higher education	several times/month	2.63^{a}	2.11^{a}	1.26^{b}
REi	27	21	$35.4^{a,b} \pm 14.0$	higher education	several times/day	2.67^{a}	2.11^{a}	1.22^{b}
CTi	10	5	$43.2^{a} \pm 12.8$	secondary education	several times/week	2.85^{a}	$1.85^{a,b}$	1.30^{b}
Least liked								
UVc	4	3	$45.0^{a,b} \pm 25.9$	higher education	*multiple*	2.36^{a}	2.50^{a}	1.13^{a}
REs	35	24	$38.7^{a} \pm 13.5$	higher education	several times/month	2.79^{a}	1.96^{b}	1.26^{c}
REi	49	27	$30.5^{b} \pm 11.9$	secondary education	several times/week	2.77^{a}	1.88^{b}	1.36^{c}
CTi	58	33	$32.9^{a,b} \pm 11.9$	higher education	several times/month	2.65^{a}	2.11^{b}	1.26^{c}
Most similar to usual								
UVc	81	42	$31.0^{b} \pm 10.6$	higher education	several times/month	2.67^{a}	2.00^{b}	1.32^{c}
REs	25	14	$33.4^{a,b} \pm 14.2$	higher education	several times/month	2.72^{a}	1.96^{b}	1.32^{b}
REi	33	25	$38.5^{a} \pm 15.0$	higher education	several times/week	2.71^{a}	2.14^{a}	1.15^{b}
CTi	7	6	$43.0^{a} \pm 18.1$	secondary education	several times/week	3.00^{a}	1.57^{b}	1.43^{b}

The existence of significant differences ($p<0.05$) in the frequency distributions of age, education 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 differences of gender. Friedman tests were conducted to evaluate differences in rankings of frequency of consuming beverages per type. For age, different superscripts within a column indicate significant differences ($p<0.05$). For frequency of consuming hibiscus beverages - per type, different superscripts within a line indicate significant differences ($p<0.05$). No significant differences were found in proportion of male respondents, education level of participants or frequency of consuming hibiscus beverages - overall for Most liked, Least liked and Most similar to usual. UVc = under-vacuum concentrate; REs = dilute-to-taste syrup; REi = ready-to-drink infusion; CTi = conventionally manufactured, ready-to-drink infusion.

Figure 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 Figure 2, sweet and acid taste intensities (F1) have now more explanatory power than colour driven 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 are the dominant sensory factor driving the food preferences of consumers, which explains this inversion.

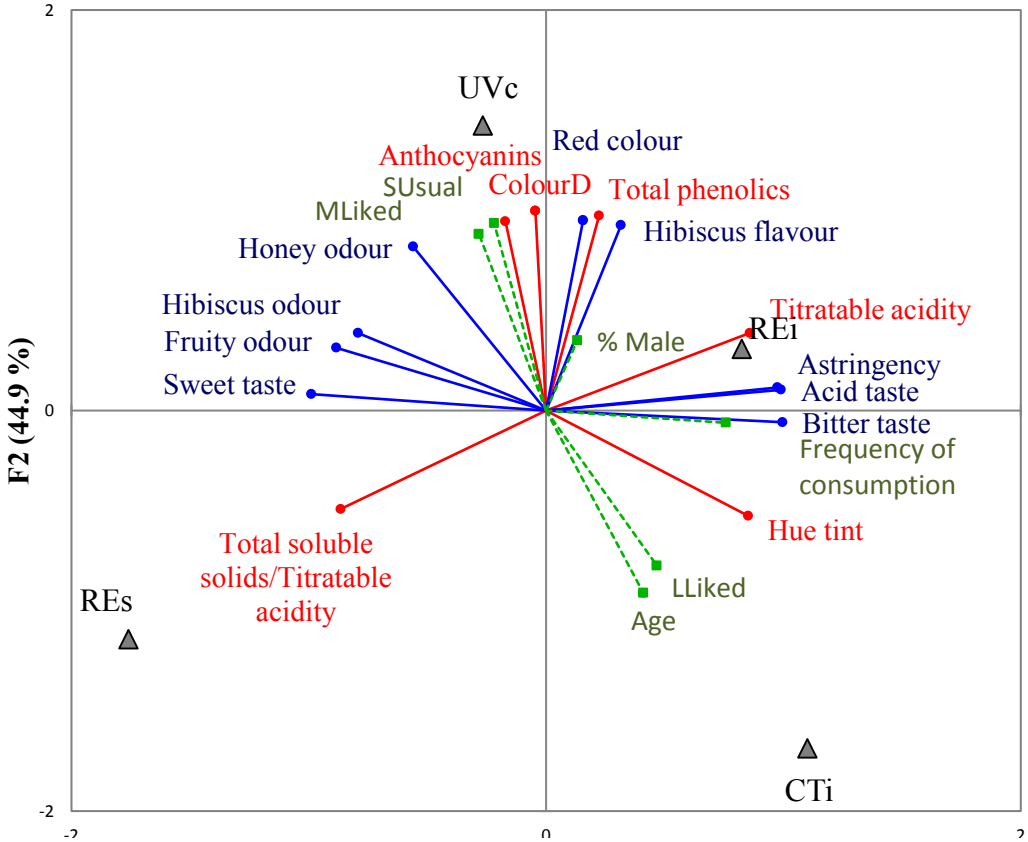

Figure 4 – MFA plot representing the relationships between the hibiscus beverages' physicochemical properties, Flash Profile descriptors and consumer evaluation data. MLiked = Most liked; LLiked = Least liked; SUsual = Most similar to the usually consumed beverage; UVc = under-vacuum concentrate; REs = dilute-to-taste syrup; REi = ready-to-drink infusion; CTi = conventionally manufactured, ready-to-drink infusion.

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 colour tone than improved beverages and a weak aromatic character, CTi seem to disfavoured by most of the 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 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 further 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 are nearly unknown to the majority of European consumers. Future research should explore their potential in these markets by identifying important drivers of liking and new hence new opportunities for product improvement and marketing.

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CHAPTER 3

Consumer acceptance of improved hibiscus beverages in Senegal

CONSUMER ACCEPTANCE OF IMPROVED HIBISCUS BEVERAGES

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Abstract

Much research is being undertaken to uncover effective ways of preserving, or even increasing, the health benefits of hibiscus beverages during processing. Nevertheless, acceptance of resulting products is seldom evaluated. Four hibiscus beverages were tested by consumers (n=150) in Senegal: three with improved chemical-sensory properties – an under-vacuum concentrate (UVc), a dilute-to-taste syrup (REs) and a ready-to-drink infusion (REi) -, and a conventionally manufactured, ready-to-drink infusion (CTi). Results of hedonic assessments indicate that all improved beverages were better accepted than CTi, being likely accepted by roughly three-quarters of consumers in Senegal. With an average overall liking of 7.1 (out of 9), the under-vacuum was the preferred beverage. Three clusters of consumers with distinct overall liking patterns of REs, REi and CTi were identified. Acceptance was driven personal preferences regarding colour intensity, aromatic character, balance between sweetness and acidity but also by gender, age, frequency of consumption and education level.

1. Introduction

The dried sepals of *Hibiscus sabdariffa* var. *sabdariffa ruber* flowers – hereinafter referred to as hibiscus calyces -, are one of the highest volume, specialty botanical products in international trade, mainly due to their intense red color and fruity acid (cranberry-like) flavor (Da-Costa-Rocha *et al.*, 2014). Hibiscus calyces are used in traditional cuisines and food companies worldwide to produce hot and cold beverages, jams, confectionary and desserts. Their extracts – in powder or concentrate form -, are furthermore widely employed in the European and North-American food, beverage and pharma industries as natural coloring and flavoring ingredients (Cid-Ortega and Guerrero-Beltrán, 2015, Cisse *et al.*, 2012a).

Due to their appealing red color, pleasantly refreshing taste and perceived health benefits, beverages prepared from the aqueous extracts of hibiscus calyces are consumed in many countries outside Europe and North America, like Thailand, Egypt, Mexico, Mali, Taiwan, Senegal, Jamaica, Nigeria, the Philippines or Sudan (Cid-Ortega and Guerrero-Beltrán, 2015, Da-Costa-Rocha et al., 2014). Their chemical-sensory properties are due to the rather exceptional organic acid, pectin and phenolic composition of hibiscus calyces, particularly in what respects anthocyanins (Fernández-Arroyo *et al.*, 2011, Sáyago-Ayerdi *et al.*, 2007, Cisse *et al.*, 2012b). Moreover, the bioactivity of such compounds in aqueous extracts has been demonstrated in different biological models and they have been shown to have important pharmacological effects (e.g., nephro- and hepato-protective, renal/diuretic, anti-cholesterol, anti-hypertensive, anti-diabetic, hypolipidemic and anti-tumoral) (Maganha *et al.*, 2010, Da-Costa-Rocha et al., 2014).

Hibiscus beverages are nowhere more widespread than in West Africa, particularly in Nigeria and Senegal, where homemade or manufactured ready-to-drink infusions and dilute-to-taste syrups are ubiquitous in homes, street vendors, retail stores and restaurants alike, serving as a cheap, local alternative to carbonated soft-drinks (Cisse *et al.*, 2009b, Bolade *et al.*, 2009). They are made by soaking sun-dried hibiscus calyces in water and straining them to obtain an extract. Calyces of Sudanese *Vimto* (dark red) and Senegalese *Koor* (bright red) cultivars are typically used. The former are very rich in anthocyanins and

other phenolic compounds – and have thus good coloring and antioxidant properties -, while the latter have a high organic acid content, resulting in beverages with intense sourness, a characteristic very much appreciated by local consumers (Cisse *et al.*, 2009a, Foline *et al.*, 2011).

Ready-to-drink infusions are prepared by adding sucrose and other flavoring ingredients to hibiscus extracts (e.g., fruit juices, fresh fruit, fresh herbs, spices or artificial flavorants). Making syrups, on the other hand, requires using relatively higher calyx-to-water soaking ratios and adding large amounts of sucrose after steeping, to obtain concentrated extracts; other flavoring ingredients are usually not added. Beverages can then be prepared by diluting the hibiscus syrup with water and flavoring it to taste just prior to consumption (Cisse et al., 2009a). Homemade infusions are typically stored and consumed cold within days of their preparation, whereas homemade syrups may last a few weeks under refrigeration. Manufactured products, however, undergo additional pasteurization and packaging steps, which extend infusions' shelf-life for weeks or even months, when kept under refrigeration, and syrups' up to one year at ambient temperature (Cisse et al., 2009a). Importantly, calyx quality (drying and storage processes), blending, extraction conditions (particle size, solid-to-water soaking ratio, temperature and time), flavoring formulations, heat treatment parameters and end-product storage conditions change greatly with local culinary customs and available technology, leading to high variability in the chemical and sensory characteristics of such beverages (Bolade et al., 2009, Cisse et al., 2009b, Cisse et al., 2012a, Cisse et al., 2009a, Cisse et al., 2012b).

The need to add value to local crops and traditional food products with high nutritional quality – and which can thus potentially claim important health benefits in both domestic and foreign markets -, has led to the implementation of several research projects in countries where the production of hibiscus products is relevant, like Mexico, Nigeria and Senegal. These focused mainly on developing more effective ways of preserving, or even increasing, the nutritional value of these products during manufacturing (Awe *et al.*, 2013, Ramírez-Rodrigues *et al.*, 2011a, Ramírez-Rodrigues *et al.*, 2012, Cid-Ortega and Guerrero-Beltrán, 2015, Pérez-Ramírez *et al.*, 2015, Cisse et al., 2012b, Cisse *et al.*, 2011a, Cisse *et al.*, 2011b). Understanding the sensory quality and consumer acceptance of foods with enhanced health benefits is known to be key to their market success (Sivakumar *et al.*,

2010, Arancibia *et al.*, 2013, Lawless *et al.*, 2012b). Yet, the sensory profiling and hedonic evaluation of hibiscus beverages, whether conventional or improved, have scarcely been undertaken (Ramirez *et al.*, 2010, Wong *et al.*, 2003, Mounigan and Badrie, 2006, Ramírez-Rodrigues et al., 2012, Ramírez-Rodrigues et al., 2011a).

Between 2010 and 2015, the African Food Tradition rEvisited by Research (AFTER) EU FP7-funded project (www.after-fp7.eu) investigated the production, trading and consumption of hibiscus beverages in Senegal, with the aim of improving the safety and nutritional value of these products while maintaining, or even enhancing, their sensory quality. Firstly, extant production processes were mapped and evaluated on the basis of known technological constraints and potential for further optimization (Cisse *et al.*, 2012). In order to identify key drivers of process improvement, the chemical-sensory properties of commonly consumed beverages and their impact on sensory acceptance were also studied (Bechoff *et al.*, 2014). Findings led to the development and pilot-testing of improved processes of manufacturing hibiscus beverages, and the subsequent investigation of the physicochemical and antioxidant properties of three new products – an under-vacuum concentrate, a dilute-to-taste syrup and a ready-to-drink infusion (Boucher *et al.*, 2014, Monteiro *et al.*, 2017).

Beverages with enhanced health benefits provided by plant-based ingredients are growing in popularity (Laaksonen *et al.*, 2014, Lawless *et al.*, 2012a, Sun-Waterhouse, 2011, Hernández-Carrión *et al.*, 2015), particularly when made from ingredients perceived as new or exotic by European and North American markets (Vidigal *et al.*, 2011, Orjuela-Palacio *et al.*, 2014, Bolling *et al.*, 2015). This trend is fueled by the food & beverage industry's current focus on the development of products with a health claim (as a strategy of differentiation), as well as by consumers' growing desire for more natural and healthier diets, on one hand, and new and exciting food and drink experiences, on the other (Sabbe *et al.*, 2009b). Sensory characteristics remain, nevertheless, the main bottleneck in the path to market success of such novel beverages, as many of the plant-based ingredients with important health benefits often translate into products with low palatability (Jaeger *et al.*, 2009, Laaksonen et al., 2014, Lawless et al., 2012a, Hernández-Carrión et al., 2015), and consumers are almost always unwilling to compromise on taste for health (Sabbe *et al.*, 2009a, Ares *et al.*, 2010).

The AFTER project led to the introduction of important changes in the manufacturing of hibiscus beverages in Senegal, with the goal of enhancing their health benefits and hedonic value in both domestic and foreign markets. Overall, resulting beverages were shown to have better physicochemical and antioxidant properties than those commonly consumed in this country (Boucher et al., 2014, Monteiro et al., 2017). Still, their market acceptance remains undetermined. The objectives of this paper are thus to assess the acceptance of such improved hibiscus beverages in Senegal and determine how this is affected by consumers' evaluations of the appropriateness of key sensory attributes and individual characteristics. Findings here reported also advance extant knowledge about the hedonic evaluation of hibiscus beverages and their sensory attributes by different consumer groups.

2. Materials and methods

2.1. Hibiscus Beverages

Samples of an under-vacuum concentrate (UVc), a dilute-to-taste syrup (REs) and two ready-to-drink infusions (REi and CTi) manufactured 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 -, were tested.

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. Calyces were manually ground and subsequently extracted for 30 min at ambient temperature (25 ºC), with periodic stirring. Extracts were filtered with a stainless steel filter (pore size ca. 1 mm) and a pocket filter (pore size 25 μm). UVc was obtained by evaporating the most concentrated extract (calyx-to-water soaking ratio of 1:5 w/w) up to 62 ºBx at 0.4 bar, while REs and REi were obtained by adding sugar to the remaining ones (up to 65 ºBx for a ratio of 1:10 w/w and 17 ºBx for 1:40 w/w, respectively). All products were pasteurized (75 ºC for 30 min), rapidly cooled

and adequately packaged. UVc and REi were kept under refrigeration (< 10 ºC), whereas REs was stored at ambient temperature (25 ºC).

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 ºC), 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 ºBx. The resulting infusion was pasteurized (90 ºC for 20 min), rapidly cooled, adequately packaged and stored under refrigeration (< 10 ºC).

Samples of UVc and REs were prepared for taste sessions by dilution with potable water (1:40 v/v) and sweetening with 130 gL^{-1} of commercial sucrose, or just dilution with potable water (1:4 v/v), respectively. Samples of REi and CTi did not require any preparation. All samples were stored at 6 ºC for 24 h prior to testing.

2.2. Consumer test

Consumer study was conducted in accordance with the Declaration of Helsinki and their protocol approved by the Ethics Committee of the EU AFTER project (FP7 245 - 025).

Consumers of hibiscus beverages (n=152) were non-probabilistically recruited at four different locations in Dakar – *Association Culturelle d'Aide a la Promotion Educative et Social* (*Parcelles Assainies*, suburbs; n=44), *Point E* (residential area; n=42), *Université Cheikh Anta Diop* (university campus; n=37) and *Centre Culturel Français* (city centre; n=29) -, according to their willingness and availability to participate. Taste sessions were conducted at the four recruiting sites under central location test conditions. Participants' age ranged between 18 and 73 years old (M = 34, SD = 13); 61% were male, 90% were Senegalese or long-term Senegal residents and 52% had a university diploma.

A paper-and-pencil questionnaire, written in French, was administered to participants, which started with questions about 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 these products, 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 the reasons why they consumed them, by choosing from a 7-item list of motives ("pleasure", "health", "energy", "thirst-quenching", "nutrition", "suitable price", "tradition").

Thirty milliliters of each sample were subsequently 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. Sample acceptance was measured by overall liking ratings (OL) provided on a 9-point hedonic scale. The appropriateness of the intensities of five sensory attributes - color, hibiscus odor, hibiscus taste, sweet taste and acid taste -, was evaluated by ratings provided on a 3-point, just-about-right scale ["too weak" (TW), "just-about-right" (JAR), "too strong" (TS)]. 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.

2.3. Statistical Analysis

XLSTAT software (Addinsoft SARL, France) was used to carry out the statistical analyses. The significance of statistical tests was evaluated at $p < 0.05$, unless stated otherwise. One participant rated all samples with the same OL and all corresponding sensory attributes with the same appropriateness value, while another rated UVc with an atypical OL (z-score >4). Their questionnaires were hence excluded from further analysis, yielding a final sample of 150 consumers.

One-way Analysis of Variance (ANOVA) was performed on OL, considering participants and samples as sources of variation. When overall significant differences in sample means were detected, Fisher's LSD (Least Significant Difference) multiple comparison tests were applied post-hoc. Pearson's chi-square tests with a Marascuilo procedure were used to test the significance of differences in the proportions of participants who disliked ($1 \leq OL \leq 4$), were indifferent to ($OL = 5$), or liked ($6 \leq OL \leq 9$) each sample.

The frequencies of TW, JAR and TS ratings for the five sensory attributes evaluated were determined for each sample, and the resulting proportions calculated. A Correspondence

Analysis (CA) was performed on the contingency table of proportions for all samples and attributes. A weighted penalty analysis (PA) was then conducted to relate attribute intensity ratings to OL for each sample and participant. Weighted penalties of 0.2 or smaller (in absolute value) were considered negligible (Popper, 2014).

A Hierarchical Cluster Analysis (HCA), using Euclidean distances and the Ward's Agglomeration method, was conducted to identify groups of participants with similar patterns of sample liking. One-way ANOVA was then performed on OL within clusters, considering participants and samples as sources of variation. When overall significant differences in sample means were detected, Fisher's LSD multiple comparison tests were applied post-hoc. In the case of clusters with less than 30 participants, non-parametric Kruskal-Wallis H tests and Dwass-Steel-Critchlow-Fligner multiple comparison analysis were used instead.

The frequencies of TW, JAR and TS ratings for the five sensory attributes evaluated were determined for each sample and cluster, and the resulting proportions calculated. The existence of significant differences in sample OL, age, education level and frequency of consumption of hibiscus beverages between clusters was tested using non-parametric Kruskal-Wallis H tests and Dwass-Steel-Critchlow-Fligner analyses. The existence of significant differences in the frequency distributions of gender and motives to consume hibiscus beverages across clusters was tested using Pearson's chi-square tests with a Marascuilo procedure.

3. Results and discussion

3.1. Acceptance of Hibiscus Beverages

Mean OL was higher than 5.50 for every sample (Table 1), implying that participants positively appreciated all the beverages tested. Still, acceptance varied significantly across samples ($p < 0.001$). All improved beverages were liked "slightly" to "moderately" and much more appreciated than CTi ($p < 0.01$). Mean OL of 6.2, 5.9, 5.6 and 5.2 were previously reported for hibiscus beverages commonly consumed in Senegal – a *Vimto* and

Koor 50:50 infusion, a *Vimto* syrup, a *Koor* syrup and a *Koor* infusion, respectively. Differences in acceptance were significant between infusions ($p < 0.01$), but not syrups. Moreover, differences in acceptance between the *Vimto* and *Koor* infusion and the *Vimto* syrup were not significant (Bechoff et al., 2014). Such levels of acceptance are lower than those reported in the present study for improved beverages, except in the case of REi, which had the same mean OL as the *Vimto* and *Koor* infusion. This indicates that the hibiscus beverages developed by the AFTER project are likely to have good acceptability in Senegal.

Ramirez et al. (2010) used consumer-based sensory optimization to develop a ready-to-drink hibiscus (cv. *Criollo*) infusion for the US market. Calyx-to-water and sugar-to-acid ratios were experimentally varied and resulting infusions tested with consumers in Florida, with the goal of identifying the formulation that maximized acceptance. A later test with a similar consumer sample uncovered that an optimized infusion, with a calyx-to-water soaking ratio of 1:40 (w/v) and a sugar-to-acid ratio of 25 g sucrose 100 g^{-1} / g malic acid 100 mL^{-1}, was liked by 79% of consumers and disliked only by 13% of them, which corresponded to a mean OL of 6.28. More recently, Ramírez-Rodrigues et al. (2012) studied the effects of applying flash and cold pasteurization on the sensory quality of optimized hibiscus infusions. The acceptance of resulting products was also tested with consumers in Florida, along that of a control (unpasteurized) infusion. Mean OL varied between 5.01 (cold pasteurization) and 5.23 (control and flash pasteurization), but were not significantly different across infusions.

UVc was liked by nearly all participants, while REs was liked by the vast majority of them. In spite of having the lowest level of acceptance among improved beverages, REi was nonetheless disliked by only less than one fifth of participants (Table 1). The overall acceptability of these beverages compares thus favorably to that of the products optimized for the US market. Nevertheless, it should be noted that the consumption of hibiscus beverages is far less common and much more recent in the US than in Senegal (Cid-Ortega and Guerrero-Beltrán, 2015). This may have contributed to the differences observed, as familiarity is known to have a strong impact on food acceptance across cultures (Hong *et al.*, 2014, Lee *et al.*, 2010).

Table 1. Acceptance of hibiscus beverages (*N* =150).

Sample	Overall liking ratings	1 ≤ Overall liking ratings ≤ 4	Overall liking ratings = 5	6 ≤ Overall liking ratings ≤ 9
UVc	$7.07^{a} \pm 0.93$	$0\%^{a}$	$3\%^{a}$	$97\%^{a}$
REs	$6.65^{b} \pm 1.12$	$5\%^{b}$	$6\%^{a}$	$89\%^{a}$
REi	$6.19^{c} \pm 1.74$	$19\%^{c}$	$8\%^{a}$	$73\%^{b}$
CTi	$5.65^{d} \pm 1.59$	$27\%^{c}$	$11\%^{a}$	$62\%^{b}$

9-point hedonic scale, ranging from 1 "dislike extremely" to 9 "like extremely." Different superscripts within columns indicate significant differences ($p<0.05$). UVc = under-vacuum concentrate; REs = dilute-to-taste syrup; REi = improved ready-to-drink infusion; CTi = conventional ready-to-drink infusion.

Previous studies have identified color (intensity and red hue), odor (hibiscus odor and aroma) and taste (sweetness and acidity) as the key drivers of sensory quality and consumer acceptance of hibiscus beverages (Ramirez et al., 2010, Wong et al., 2003, Mounigan and Badrie, 2006, Bechoff et al., 2014, Bolade et al., 2009). In line with this, the relative levels of acceptance of the four beverages tested in this study reflect well their distinct chemical-sensory profiles. Physicochemical and sensory descriptive analyses of these products showed that UVc had the highest monomeric anthocyanin content and, consequently, the most intense red color of all the hibiscus beverages investigated so far (Boucher et al., 2014, Monteiro et al., 2017). The characteristic red hues of hibiscus products are highly appreciated by consumers, being typically the sensory attribute with the highest acceptability ("liked moderately") (D'Heureux-Calix and Badrie, 2004, Bolade et al., 2009). This occurs even in the case of beverages with rather low monomeric anthocyanin content and color intensity, and assessed by unfamiliar consumers (mean color liking 7.35, on a 9-point hedonic scale) (Ramirez et al., 2010, Ramírez-Rodrigues et al., 2012). Nevertheless, the impact of color assessments on overall product acceptance seems to be strongly moderated by taste and odor evaluations, as both enhancing and suppressing effects have been observed (Bechoff et al., 2014, D'Heureux-Calix and Badrie, 2004).

UVc presented a more favorable balance between sweetness and acidity intensities than REs (*i.e.*, not overly sweet), REi and CTi (*i.e.*, not overly tart) (Monteiro et al., 2017). A

similar conclusion can be drawn when comparing to the chemical-sensory properties of beverages commonly consumed in Senegal, which had either a much lower (in the case of infusions) or much higher (in the case of syrups) sugar-to-acid ratios and were hence evaluated as insufficiently or overly sweet, respectively (Bechoff et al., 2014). Moreover, UVc had the most intense odor (honey, hibiscus and fruity odors) and hibiscus aroma of the four samples tested, with these two sensory dimensions scoring the highest intensities across samples, after red color (Monteiro et al., 2017). By contrast, the hibiscus odor and aroma of beverages commonly consumed in Senegal have been described as weak (in the case of syrups) or moderate (in the case of infusions), with sensory evaluations being dominated by color and sweetness/acidity perceptions instead (Bechoff et al., 2014).

The olfactory qualities of UVc, paired with its exceptionally high red color intensity, help explain why it achieved a greater mean OL than beverages with higher sugar-to-acid ratios, such as REs, CTi and the infusions optimized for the US market. Sweetness intensity is known to rise with increasing intensities of congruent color or odors (e.g., honey, fruity) (Delwiche, 2004) Moreover, congruency between color (hue and lightness), aroma and taste perceptions was shown to have a positive impact on the acceptance of green tea samples among familiar (Korean) consumers, as it was associated to judgements of product typicality. On the other hand, the high green color and green aroma intensities of the most typical tea samples negatively affected their acceptance among unfamiliar (US) consumers, as they were primarily associated to unpleasant sensory dimensions, such as astringency or bitterness (Lee et al., 2010). A concurring finding was reported for the hibiscus infusion optimized for the US market, with local (unfamiliar) consumers mainly describing it has having an exotic, floral aroma and a unusual, tart taste (mean aroma liking 5.65 and mean flavor liking 5.93, on 9-point hedonic scales), and suggesting therefore the addition of sugar or fruit juices to enhance its palatability (Ramirez et al., 2010).

Beverages made from dilute-to-taste hibiscus syrups are usually characterized by high sweetness intensity, which is in turn strongly related to their acceptance (Bechoff et al., 2014). Moderate and high sweetness intensities are generally suppressive of acid taste (Keast and Breslin, 2002). Accordingly, REs had the highest sugar-to-acid ratio, the strongest sweetness and the weakest acidity intensities of the four samples tested (Boucher et al., 2014, Monteiro et al., 2017). This most likely explains why it was more positively

appreciated than REi and CTi. On the other hand, it had lower and much lower sugar-to-acid ratios than the conventionally manufactured *Koor* and *Vimto* syrups, respectively, which were correspondingly judged to have very weak acidity intensities and hence displayed an unfavorable balance between sweetness and acidity (*i.e.*, were overly sweet) (Bechoff et al., 2014).

Hibiscus beverages, including those made from dilute-to-taste syrups, are greatly appreciated in their domestic markets due to their pleasantly refreshing taste, *i.e.*, their intense sourness (Cisse et al., 2009a, Cid-Ortega and Guerrero-Beltrán, 2015, Foline et al., 2011). Formulations and/or modes of preparation that excessively enhance sweetness will overly suppress sourness (Keast and Breslin, 2002) and may thus decrease, rather than increase, the acceptance of these products, particularly (albeit not exclusively) among familiar consumers (Ramirez et al., 2010).

REs had a higher monomeric anthocyanin content, a more intense red color and a stronger aromatic profile than CTi and beverages commonly consumed in Senegal, except for the *Vimto* and *Koor* infusion (Monteiro et al., 2017, Boucher et al., 2014). These visual and olfactory qualities, paired with the aforementioned highly favorable balance between sweetness and acidity, no doubt explain why this improved syrup was better appreciated than conventionally manufactured beverages. They also contribute to clarify why formulation and process improvements that take into account not only taste, but also other key drivers of the sensory quality of hibiscus beverages, are more likely to succeed in terms of the consumer acceptance of end products. This appears to be the case of both UVc and REs when compared to the infusions optimized for the US market, for instance (Ramirez et al., 2010, Ramírez-Rodrigues et al., 2012).

Previous sensory evaluation of hibiscus infusions describes them as markedly sour, but only moderately astringent and weakly bitter beverages (Wong et al., 2003, Mounigan and Badrie, 2006, Bechoff et al., 2014). In line with this, REi had the lowest sweetness-to-acid ratio, the highest titratable acidity and the lowest pH of the four samples tested, being thus also the one deemed to have the highest acidity and astringency intensities. It was also judged to have lower sweetness and higher bitterness than UVc and REs, albeit not than CTi (Monteiro et al., 2017, Boucher et al., 2014). Both peripheral oral and central cognitive, mutual suppression effects of sweetness and bitterness have been uncovered,

with bitterness being therefore often used as a descriptor to express lack of sweetness and dislike (Lesschaeve and Noble, 2005, Keast and Breslin, 2002). These findings, combined with its higher acidity and astringency intensities, help understand to some extent why REi had lower acceptability than UVc and REs but was still more liked than CTi.

Low consumer acceptance of plant-based beverages with enhanced health benefits is often caused by acid and bitter taste evaluations, usually paired with astringency sensations (Jaeger et al., 2009, Lesschaeve and Noble, 2005, Lawless et al., 2012a, Laaksonen et al., 2014, Streit *et al.*, 2007). Both enhancement and suppression effects between acid and bitter tastes have been observed. Sourness tends to enhance bitterness when both their intensities are low, but it may actually suppress it at moderate and high intensities (Keast and Breslin, 2002).

Aqueous hibiscus extracts are rich in weak organic acids, namely hydroxycitric acid, hibiscus acid and derivatives (Ramírez-Rodrigues *et al.*, 2011b, Da-Costa-Rocha et al., 2014). Such acids are not only responsible for very intense sourness in plant-based beverages, but also for moderately intense, puckering astringency, particularly at the low pH values characteristic of hibiscus infusions (< 2.5) (Lesschaeve and Noble, 2005, Laaksonen *et al.*, 2013, Bechoff et al., 2014, Ramirez et al., 2010). Meanwhile, weak astringency and diminished bitter taste have been linked to the presence of phenolic (hydroxycinnamic) acids in *yerba mate* infusions, whereas flavonol glycosides are known to cause moderately intense, mouth drying astringency and weak bitterness in black tea, red wine and blackcurrant juice (Hufnagel and Hofmann, 2008, Scharbert *et al.*, 2004, Laaksonen et al., 2013, Streit et al., 2007). Both types of phenolic compounds have been detected in aqueous hibiscus extracts above taste threshold concentrations (Fernández-Arroyo et al., 2011, Ramírez-Rodrigues et al., 2011b). Nevertheless, phenolic compounds more commonly associated with very intense bitter taste and astringency sensations, such as hydroxybenzoic acids, flavanols and tannins (Lesschaeve and Noble, 2005, Laaksonen et al., 2013) have been detected in negligible amounts only, or not at all (Fernández-Arroyo et al., 2011, Ramírez-Rodrigues et al., 2011b, Wong et al., 2003, Rodríguez-Medina *et al.*, 2009).

REi had the highest monomeric anthocyanin content, the most intense red color and the strongest aromatic character after UVc, thus resembling more this sample in terms of visual and olfactory qualities than REs or CTi. The latter, in particular, had the lowest monomeric anthocyanin content, least intense red color and weaker aromatic profile of the four beverages tested (Monteiro et al., 2017, Boucher et al., 2014). This likely contributed to enhance perceptions of lack of sweetness and more intense bitterness comparatively to REi, resulting in lower overall acceptability (Delwiche, 2004, Keast and Breslin, 2002), in spite of CTi actually having higher sugar-to-acid ratios than either REi or UVc. Given that consumers in Senegal are generally highly familiar with hibiscus infusions, the relative lack of visual and olfactory qualities that are highly characteristic of these products in CTi may have led to judgements of atypicality, thereby further increasing dislike for this sample (Lee et al., 2010).

REi's physicochemical and sensory profile matched very closely those reported by Bechoff et al. (2014) for a 50:50 *Vimto* and *Koor* infusion commonly consumed in Senegal, being thus unsurprising that it reached a similar level of acceptability. However, its intense red color and highly aromatic character, which are sensory qualities positively appreciated by both familiar and unfamiliar consumers of hibiscus infusions, seem not have been sufficient to compensate for its intense sourness and astringency in terms of hedonic evaluation. The fact that the infusion previously developed for the US market by Ramirez et al. (2010) – which had a much more favorable balance between sweetness and acidity but a much less intense red color and aromatic character than REi -, was able to reach a higher level of acceptance among a sample of unfamiliar consumers further strengthens this hypothesis.

3.2. Appropriateness of Sensory Attribute Intensities and Impact on Acceptance

Most participants considered color JAR in UVc and CTi, but TW in REs and TS in REi (Figure 1a). Moreover, they found hibiscus odor JAR in all beverages except REs, where it was judged to be TW. Hibiscus taste was mainly deemed JAR in UVc, but TW in REs. There was relatively less consensus about the appropriateness of the intensity of this attribute in REi (TW = 11%; JAR = 39%; TS = 50%), and even less in CTi (TW = 26%;

JAR = 42%; TS = 32%). The majority of participants thought that sweet taste was JAR in UVc and REs, but TW in CTi. There was less agreement about the appropriateness of the intensity of this attribute in REi (TW = 48%; JAR = 43%; TS = 9%) than in other samples. Acid taste was mainly found JAR in UVc, but TS in CTi. There was relatively less agreement about the appropriateness of the intensity of this attribute in REi (TW = 8%; JAR = 41%; TS = 51%) and REs (TW = 50%; JAR = 47%; TS = 3%).

The CA map (Figure 1b) reinforces the idea that participants' assessments of the appropriateness of sensory attribute intensities were more discriminative of some samples and attributes than others. UVc was mainly found JAR in hibiscus, sweet and acid tastes, whereas RES was essentially deemed TS in sweet taste and TW in acid taste, hibiscus odor and color. This is line with descriptive analysis results (Monteiro et al., 2017), which showed that while the former had the most favorable aromatic character and balance between sweetness and acidity of all the beverages tested, the latter had the highest sweetness and the lowest acidity intensities, as well as the least intense color and odor among improved beverages. Conversely, REi was considered TS in color, hibiscus taste and hibiscus odor. This is in agreement with it having the most intense red color and aromatic character of all the beverages tested, except UVc, as well as the highest acidity and astringency intensities. Previous studies have yielded similar descriptive and hedonic evaluations of the sensory attributes of hibiscus infusions (Bechoff et al., 2014, Ramirez et al., 2010, Wong et al., 2003, Mounigan and Badrie, 2006).

Both REi and CTi were found mainly TS in acid taste and TW in sweet taste. Still, the proportion of JAR evaluations in sweet taste and acid taste was markedly higher in REi than CTi, since the latter was more often assessed TW in these attributes than the former. This is line with descriptive analysis results (Monteiro et al., 2017), showing that REi had the highest acidity and the second lowest sweetness intensities, while CTi had the lowest sweetness and the second highest acidity intensities, of the four beverages tested.

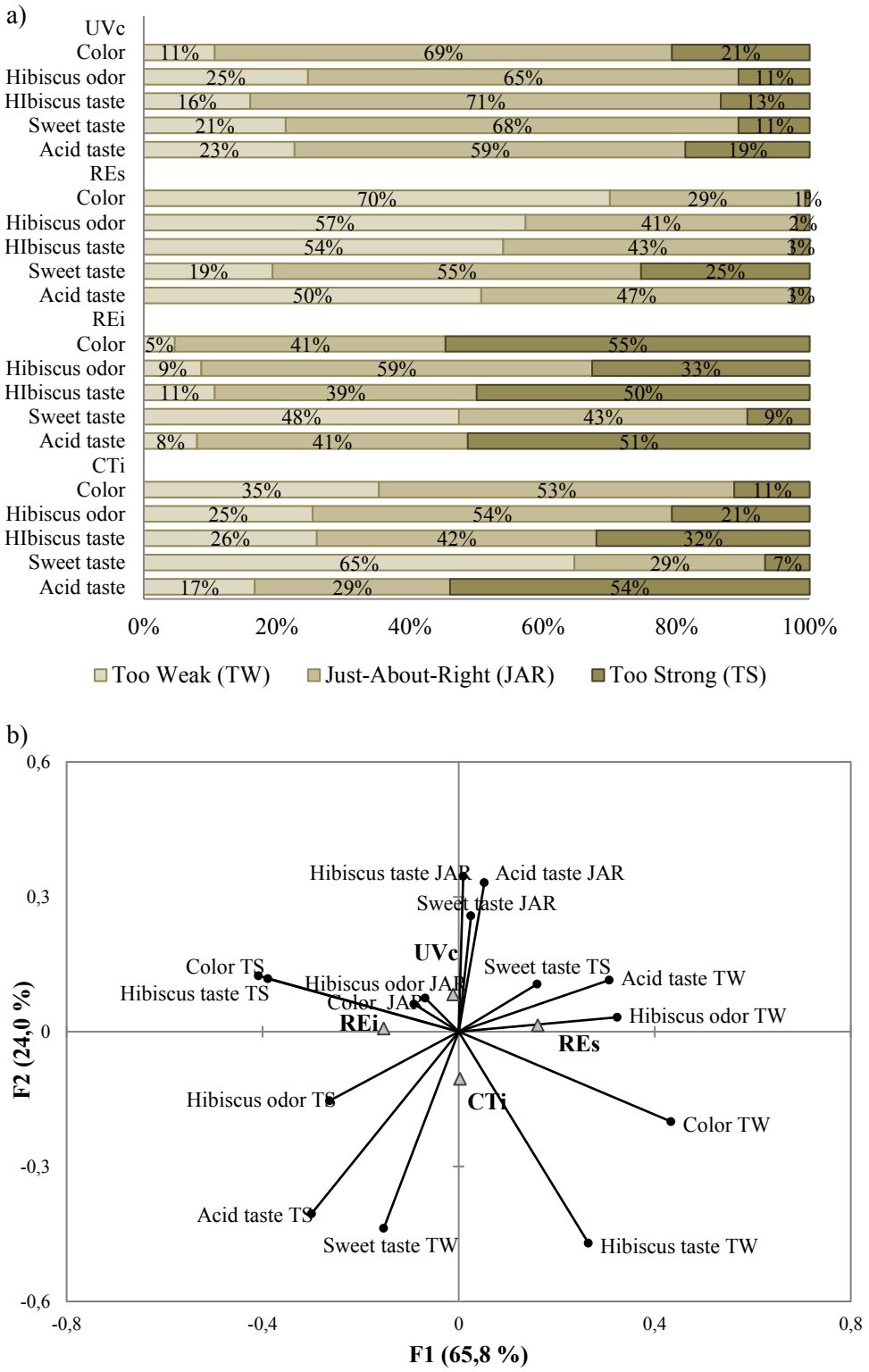

Figure 1. Consumer (N=159) evaluations of the appropriateness of the sensory attributes of hibiscus beverages on a 3-point just-about-right scale: a) distribution of ratings across samples and b) correspondence analysis map. UVc = under-vacuum concentrate; REs = dilute-to-taste syrup; REi = ready-to-drink infusion; CTi = conventionally manufactured, ready-to-drink infusion.

CTi was deemed to have the highest bitterness intensity of all samples (Monteiro et al., 2017), something which could explain its predominantly TW evaluations in sweet taste by consumers. Since bitterness was not among the attributes they were asked to evaluate, participants might have "dumped" their assessments of the appropriateness of its intensity on the ratings of the sensory quality most often associated to it, *i.e.*, lack of sweetness (Keast and Breslin, 2002). Such dumping effects would likely impact CTi' sweet taste evaluations the most. On the other hand, familiar consumers of hibiscus infusions expect them to be intensely sour, this being a sensory quality that positively contributes to their judgments of product typicality as well as hedonic evaluations (Bechoff et al., 2014, Foline et al., 2011). Given that CTi had lower acidity intensity than REi, a relatively higher proportion of participants might have thus rated it TW on this attribute.

JAR ratings predominated in the assessment of the appropriateness of color and hibiscus taste intensities in both UVc and CTi, as well as of hibiscus odor intensity in UVc, REi and CTi. Noticeably, these results do not reflect the fact that CTi was described as having a much less intense red color and aromatic profile than both UVc and REi (Monteiro et al., 2017). It is possible that some participants based their color intensity evaluations more on lightness and others on hue. Similarly, hibiscus odor assessments could have been more influenced by odor in some cases and aroma in others. Likewise, hibiscus taste evaluations are notoriously hard to disentangle from assessments of balance between sweetness and acidity in infusions (Ramirez et al., 2010). If this was the case, providing participants with an exact definition of the attributes to be evaluated could have improved their discriminative ability (Orjuela-Palacio et al., 2014).

The attributes with the highest TS weighted penalties were acid taste, hibiscus taste and hibiscus odor, essentially due to participants' assessments of these attributes in REi and CTi (Figure 2). Participants' TS ratings of color seem to have been also strongly detrimental of acceptance in the case of REi, but not CTi. Meanwhile, the highest TW penalties corresponded to sweet taste, equally due to assessments of REi and CTi, and hibiscus taste, due to assessments of REs and CTi. On the other hand, important impacts of TW ratings of color and hibiscus odor on acceptability were only observed for REs and CTi. These results indicate that a favorable balance between sweetness and acidity intensities is key to consumer acceptance of hibiscus beverages in Senegal, most likely also

affecting the hedonic evaluations of other taste characteristics, namely the strength of hibiscus taste. Color and odor judgements appear to have a relatively smaller impact on acceptability. Similar findings were reported by previous studies of consumers' hedonic evaluations of the sensory attributes of hibiscus beverages (Ramirez et al., 2010, D'Heureux-Calix and Badrie, 2004).

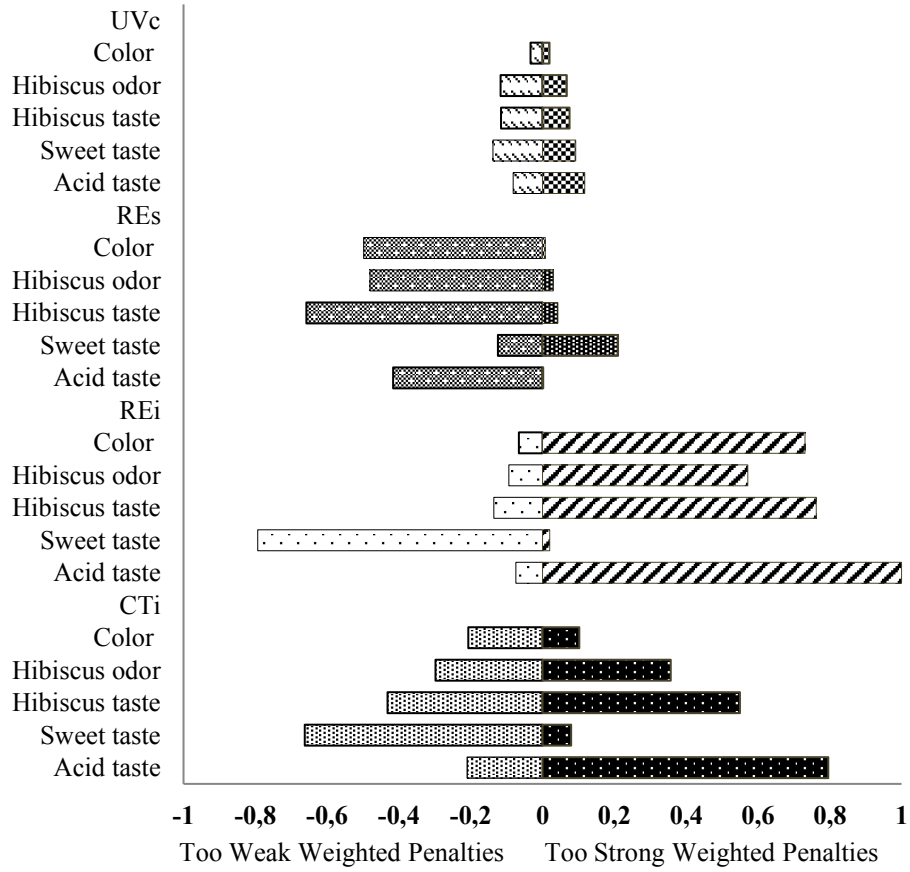

Figure 2. Weighted penalty analysis relating consumer (N=150) acceptance of hibiscus beverages to evaluations of the appropriateness of sensory attributes

No relevant weighted penalties (*i.e.*, ≥ |0.20|) were found for UVc on the attributes assessed (Figure 2). This result is well aligned with the chemical-sensory qualities earlier reported for this beverage (Sañudo *et al.*, 2015) and helps explain why it achieved an exceptionally high level of acceptance when compared to other improved or conventionally manufactured products. Important TW penalties were nonetheless

identified for REs in all attributes assessed, except for sweetness. Still, the somewhat unfavorable evaluation of the sensory attributes of this improved syrup, which reflects closely its chemical-sensory properties, did not prevent it from being much better accepted than the infusions tested, or the syrups commonly consumed in Senegal. This confirms the key role played by positive evaluations of sweetness intensity on the acceptance of diluted-to-taste hibiscus syrups (Bechoff et al., 2014).

Very high TS penalties were identified for REi in all attributes assessed except sweetness, which registered a very low TW penalty. These evaluations are in good agreement with the chemical-sensory qualities earlier reported for this infusion (Monteiro et al., 2017) and highly indicative of why it was the improved beverage least liked by participants. REi had the highest penalties (in absolute value) of all the beverages tested, for all attributes assessed (Figure 2). Still, it was found more acceptable than CTi, being also similarly or more positively appreciated than infusions commonly consumed in Senegal.

Low TW penalties were observed for CTi in all the attributes evaluated, although these were only marginally relevant in the case of color and acidity. This infusion also had fairly high TS penalties in hibiscus odor, hibiscus taste and acid taste. Like REi, CTi was judged to be overly tart and not sweet enough by most participants and this had thus an equally negative impact on acceptance. Hibiscus odor and taste intensities were also fairly detrimental of the overall appreciation of this beverage, although there was less agreement between participants as to why this was the case when compared to REi.

3.3. Consumer Characteristics and Impact on Acceptance

Three clusters of consumers with distinct overall liking patterns of hibiscus beverages were identified (Figure 3), with sample acceptance varying significantly ($p < 0.001$) in each of them. The largest cluster encompassed over half of the participants (56.0%) and comprised consumers who liked all four samples moderately, although still appreciating the improved beverages significantly more than CTi. UVc was significantly better accepted than REs, but not than REi. There were furthermore no significant differences in acceptance between the last two samples. Participants in this cluster were thus globally identified as Likers of Hibiscus Beverages (Cluster 1). The mid-sized cluster included roughly a quarter of the

participants (25.3%) and consisted of consumers who positively appreciated UVc and REs, but disliked REi slightly and neither liked nor disliked CTi. Significant differences in acceptance were observed across all samples, with differences between REi and all other samples, and between CTi and UVC/REs, being highly significant ($p < 0.01$). Participants in this cluster were hence collectively named Non-Likers of Hibiscus Infusions (Cluster 2).

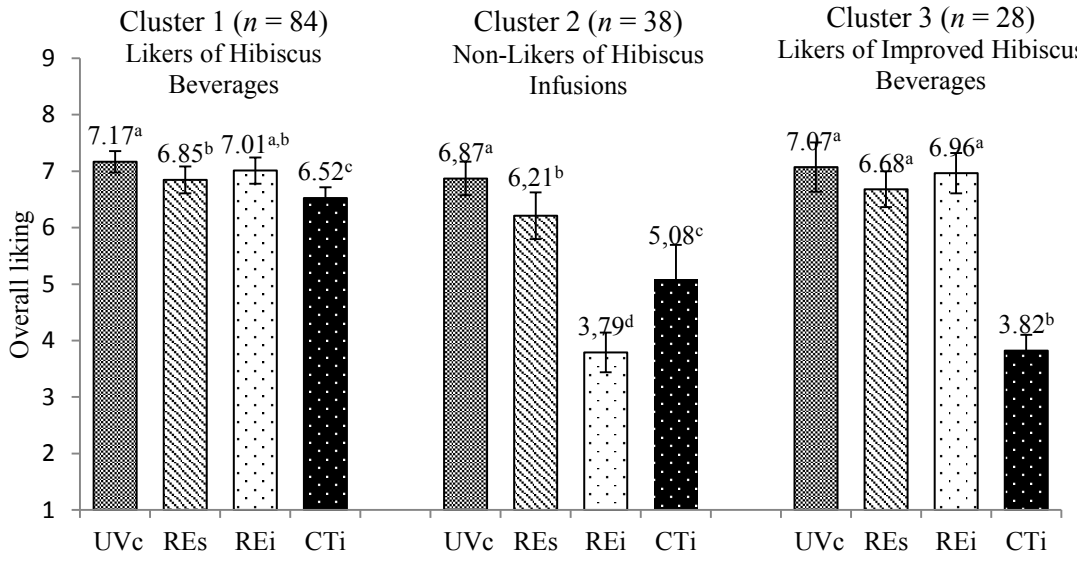

Figure 3. Hierarchical clusters of consumers (N=150) according to patterns of acceptance of hibiscus beverages

Finally, the smallest cluster covered a little less than one fifth of participants (18.7%) and comprised consumers that liked all improved beverages moderately, but disliked CTi slightly. There were no significant differences in acceptance between improved beverages and they were all significantly better accepted than CTi ($p < 0.01$). Participants in this cluster were therefore globally called Likers of Improved Hibiscus Beverages (Cluster 3). Moreover, there were no significant differences in acceptance of UVc, REs and REi between Clusters 1 and 3. These results indicate that all the hibiscus beverages developed by the AFTER project are likely to be well accepted (*i.e.*, liked moderately) by ca. 75% of consumers in Senegal. UVc, in particular, was equally well accepted by all clusters.

A comparison of the frequencies of TW, JAR and TS ratings per cluster, particularly in the cases of the samples showing the highest variation in acceptance across clusters (REi and

CTi, Figure 4), helps identify the key sensory drivers of acceptance of hibiscus beverages for different groups of consumers. Cluster 1 found REi JAR in all attributes except color, which was mainly deemed TS, whereas Cluster 2 found it predominantly TS in all attributes assessed except sweetness, which was considered mainly TW (Figure 4a). Cluster 3 found REi JAR in color and hibiscus odor (somewhat similarly to Cluster 1), but TS in hibiscus and acid tastes and TW in sweet taste (like Cluster 2, albeit less often). Overall, Cluster 2 presented thus by far the lowest frequencies of JAR ratings of the three clusters for the improved infusion. Accordingly, REi's acceptance was also significantly lower ($p < 0.001$) in Cluster 2 than in the remaining clusters.

Cluster 1 found CTi JAR in color, hibiscus odor and hibiscus taste, but TW on sweet taste, although much less often than the other two clusters (Figure 4b). CTi was meanwhile deemed JAR and TS in acid taste by nearly the same number of cluster members, which possibly explains why this beverage had a significantly lower acceptance than improved beverages among this group of consumers. Still, CTI achieved much higher frequencies of JAR ratings in sweet and acid tastes in this cluster than in the remaining ones, as well as a significantly higher mean OL. Cluster 2 found CTi mainly TS in hibiscus and acid taste (although slightly less often than REi), as well as predominantly TW in sweet taste (even more often than REi). The color and hibiscus odor of CTi were found mainly JAR, however, which is likely to explain why this beverage was still better accepted than REi by participants in this cluster. Cluster 3 considered CTi TW in sweet taste nearly as often as Cluster 2, but, opposite to it and Cluster 1, found this beverage predominantly TW in color, hibiscus odor and hibiscus taste. Consequently, Cluster 3 presented by far the lowest frequencies of JAR ratings of the three clusters for the conventional infusion. In line with this, CTi's acceptance was also significantly lower ($p < 0.001$) in Cluster 3 than in the remaining clusters.

Overall, it appears that the hedonic evaluations of Likers of Hibiscus Beverages were mostly determined by their judgements of the balance between sweetness and acidity of the samples. This was however not the case of participants in the other two clusters, whose acceptance of the tested beverages seemed to be strongly influenced by assessments of color, odor and aroma intensities as well.

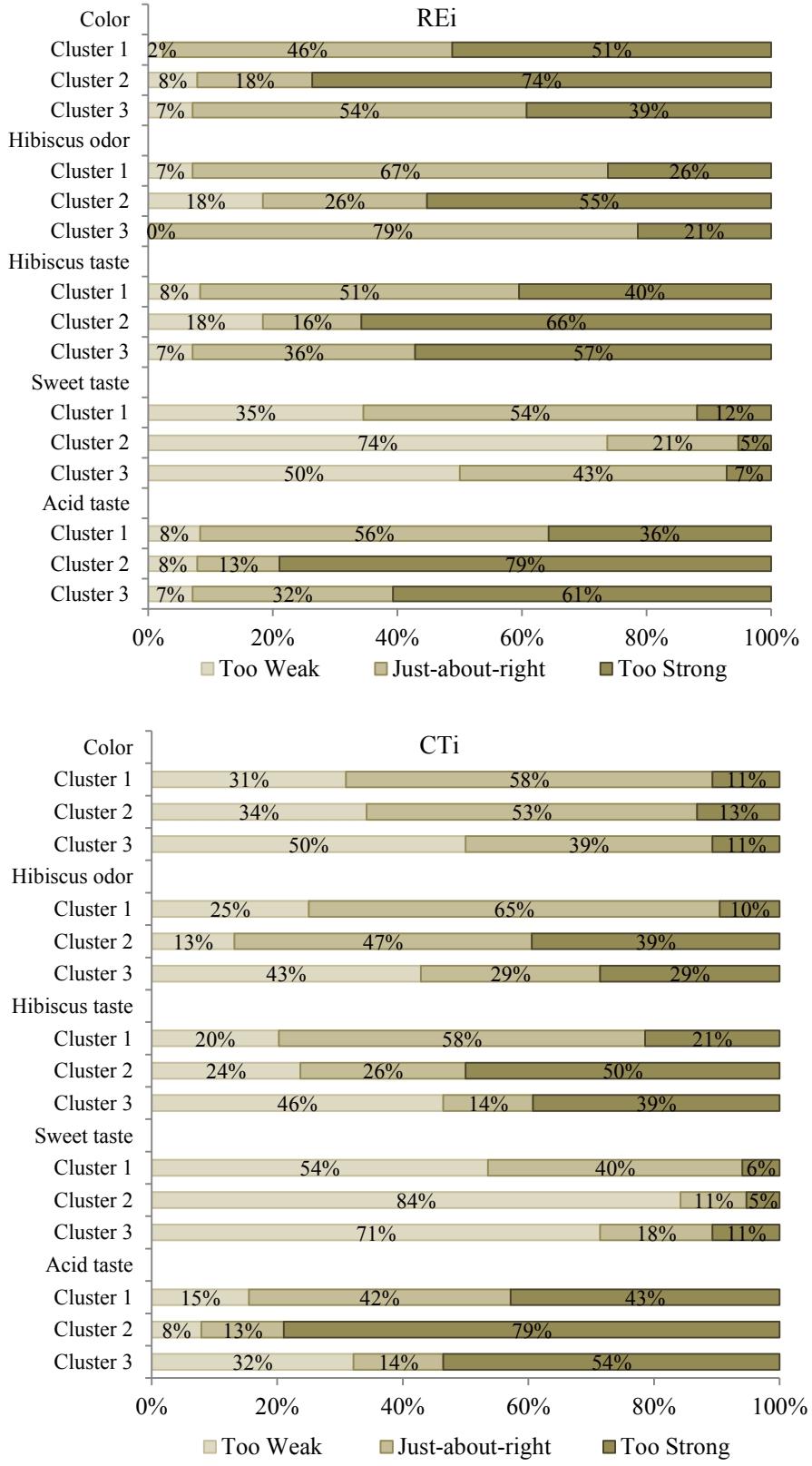

Figure 4. Consumer (*N*=150) evaluations of the appropriatness of sensory attributes of hibiscus beverages on a 3-point just-about-right scale: frequencies of ratings per cluster.

Non-Likers of Hibiscus Infusions, for instance, generally found both REi and CTi overly sour and lacking in sweetness. Yet, this seems to have affected acceptance more when color, aroma and odor were found too strong (as in REi), rather than too weak (as in CTi). Meanwhile, TW ratings of color, hibiscus odor and hibiscus taste appear to have determined the relative acceptance of infusions to a large extent in the case of Likers of Improved Hibiscus Beverages, irrespectively of their judgements of sweet and acid taste intensities.

HCA of the mean OL of hibiscus beverages commonly consumed in Senegal also identified three groups of consumers with distinct patterns of product acceptance (Bechoff et al., 2014). The largest one (42.5% of participants) was composed of consumers that liked syrups slightly, but not infusions (Syrup Likers). Conversely, the mid-sized group (36.2%) comprised those that liked infusions slightly or moderately, but not syrups (Infusion Likers). Finally, the smallest group (21.3%) encompassed consumers that liked both syrups and infusions moderately (Overall Likers). Similarly to the present study, different sensory drivers of beverage acceptance were identified at cluster level. Acid, bitter and sweet taste intensities were significantly associated to overall liking (in the expected directions) in the case of Syrup Likers, but not Infusion Likers. On the contrary, color (hue and lightness), hibiscus odor and hibiscus taste intensities were strongly related to hedonic evaluations in the case of Infusion Likers, but not Syrup Likers.

The socio-demographic profile and consumption patterns characterizing participants in each cluster (Table 2) provide further insights about the acceptance of hibiscus beverages by different groups of consumers in Senegal. Likers of Hibiscus Beverages were primarily men with an average age of 34 years, less than half of which had a university diploma. They were mainly frequent consumers of hibiscus beverages (mode = several times/week), products that they drank more for health reasons and less to quench thirst, comparatively to other clusters. Non-Likers of Hibiscus Infusions were chiefly women with an average age of 32 years, over 60% of which had a university diploma. They were mostly infrequent consumers of hibiscus beverages (mode = several times/month), products that they drank less for health reasons and more to quench thirst, comparatively to other clusters. Likers of Improved Hibiscus Beverages were mainly men with an average age of 35 years, slightly over half of which had a university diploma. Still, they included less participants with no

formal or only primary education than Likers of Hibiscus Beverages. This cluster included frequent and infrequent consumers of hibiscus beverages in equal proportion, who drank such products more for health reasons and less to quench thirst.

TABLE 2. Socio-demographic characteristics and hibiscus beverage consumption per cluster of consumers ($N = 150$).

	Male participants	Age	Education Level†	Frequency of hibiscus beverage consumption†	Motives of consumption	
					Health	Thirst-quenching
Likers of Hibiscus Beverages ($n = 84$)	68%[a]	34.4[a] ± 13.5	No formal education or primary education=14% Secondary education=38% Higher education=48%	several times/day = 14% several times/ week = 44% several times/month =26% several times/year =16%	69%[a]	54%[a,b]
Non-Likers of Hibiscus Infusions ($n = 38$)	39%[b]	32.4[a] ± 11.7	No formal education or primary education=5% Secondary education=34% Higher education=61%	several times/day = 5% several times/ week = 32% several times/month=37% several times/year =26%	42%[b]	71%[a]
Likers of Improved Hibiscus Beverages ($n = 28$)	68%[a,b]	34.6[a] ± 14.9	No formal education or primary education=7% Secondary education=39% Higher education=54%	several times/day = 7% several times/ week = 36% several times/month=36% several times/year =21%	54%[a,b]	46%[b]

Different superscripts within a column indicate significant differences ($p<0.05$). †The distribution of frequencies was significantly different ($p<0.05$) between Likers of Hibiscus Beverages and Non-Likers of Hibiscus Infusions. Most participants indicated "health" (59%), "pleasure" (57%) and "thirst-quenching (57%)" as motives to consume hibiscus beverages, followed by "tradition" (39%), "nutrition" (38%), "energy" (34%) and "suitable price" (23%). Significant differences ($p<0.05$) in frequency distributions between clusters were found solely for "health" and "thirst-quenching" motives, so only these are here reported.

Studies of the acceptance of plant-based beverages with enhanced health benefits show that the sourness, bitterness and astringency intensities that often characterized them are generally better tolerated by frequent/familiar than infrequent/unfamiliar consumers, particularly when such sensory qualities are linked to judgments of product typicality (Lee et al., 2010, Laaksonen et al., 2014). This may partially explain the significant differences

in acceptability observed between Likers of Hibiscus Beverages and Non-Likers of Hibiscus Infusions for both REi and CTi.

Tolerance to unpleasant sensory attributes of plant-based beverages appears to be also moderated by age and gender. For instance, older/male consumers liked sour, bitter and astringent blackcurrant juices significantly more than younger/female ones (Laaksonen et al., 2014). In line with this, acceptance of REi was found to be strongly associated to participants' gender, age and education level. Men liked this beverage significantly more than women (Mean OL = 6.60 and 5.54, respectively; $t=3.78$, $p<0.001$). Moreover, its acceptability increased significantly with age ($r=0.22$, $p<0.01$), while decreasing with education level ($r_s=0.17$, $p<0.05$). On the other hand, consumers over 30 years old found the flavor and tartness intensities of an optimized hibiscus infusion significantly more often TW than their younger counterparts, while female consumers liked the color of this product significantly more than male ones (Ramirez et al., 2010). This in line with the fact that that the optimized infusion had a very low monomeric anthocyanin content and color intensity, as well as a very high sugar-to-acid ratio, when compared to REi but not to CTi (Boucher et al., 2014, Monteiro et al., 2017, Ramírez-Rodrigues et al., 2012). Together with the results of the clusters' evaluations of the sensory attributes of infusions (Figure 4a,b), this is likely to explain, at least partially, why Non-Likers of Hibiscus Infusions liked REi significantly less than the other two clusters, while still liking CTI significantly more than Likers of Improved Hibiscus Beverages.

4. Conclusions

Understanding the sensory quality and consumer acceptance of foods with enhanced health benefits is key to their market success. Previous research on hibiscus beverages focused on the improvement of manufacturing processes, with the aim of preserving or enhancing the health benefits of these products. Yet, their sensory profiling and hedonic evaluation have seldom been investigated. This paper studied the acceptance of three improved hibiscus beverages recently developed by the AFTER project in Senegal and examined how this

was affected by evaluations of the appropriateness of key sensory attributes and consumer characteristics.

Results of hedonic assessments indicate that all three improved beverages are likely to be well accepted by roughly three-quarters of consumers in Senegal, particularly when compared to existing products. UVc achieved the highest OL, being equally well accepted across all the clusters of participants identified. Accordingly, no relevant weighted penalties were found for this product, suggesting that further sensory optimization will not be required. On the other hand, additional improvements of REs' formulation that result in products with more intense color, hibiscus character and acid taste (e.g., by using a higher calyx-to-water soaking ratio and/or adding less sucrose) could increase acceptability. Insight on how these improvements could be achieved without compromising too much on sweetness intensity – the main driver of hibiscus syrups' acceptability (Bechoff et al., 2014) -, is lacking and warrants future research.

The lack of a favorable balance between sweetness and acidity intensities was likewise responsible for the relatively low hedonic evaluations of both ready-to-drink infusions, particularly when complemented by a bland aromatic profile and weak color, as was the case of CTi. The latter finding does not generalize across all participants, however. Roughly a quarter of them – the Non-Likers of Hibiscus Infusions – indeed found both REi and CTi overly sour and lacking in sweetness. Yet, this seemed to have a higher impact on acceptance when color, aroma and odor were found too strong (as in REi), rather than too weak (as in CTi). The fact that this cluster entails younger, more highly educated and more female participants than the remaining ones, whose consumption of hibiscus beverages also relatively lower, is likely to explain this discrepancy to a great extent.

Intense sourness and/or lack of sweetness may originate perceptions of heightened astringency and bitterness (Lesschaeve and Noble, 2005, Keast and Breslin, 2002), which would have had additional detrimental effects on the acceptability of the ready-to-drink infusions tested. Future studies on the sensory improvement of these beverages should thus focus on reducing acidity intensity, preferably by means that do not involve increasing their caloric content (Pérez-Ramírez et al., 2015). Importantly, they should also seek to expand current knowledge about the sensory evaluation of sourness, astringency and bitterness descriptors in hibiscus beverages in general.

Overall, findings highlight the importance of focusing the development of new/improved hibiscus beverages on achieving a favorable balance between sweetness and acidity intensities, as this is key to increase their acceptance. Nonetheless, they also suggest that formulation and process improvements which take other relevant drivers of sensory quality (e.g., color and hibiscus aromatic character), into account should also be equated, as groups of consumers with highly distinct patterns of sensory preference were identified. Future studies should broaden the scope of this research by assessing consumer acceptance and sensory evaluation of hibiscus beverages in other country markets.

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CHAPTER 4

Cross-cultural development of hibiscus tea sensory lexicons for trained and untrained panellists

Beverages prepared from hibiscus extracts are commonly known by hibiscus teas in the United States of America, this designation was adopted in this paper.

CROSS-CULTURAL DEVELOPMENT OF HIBISCUS TEA SENSORY LEXICONS FOR TRAINED AND UNTRAINED PANELISTS

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Abstract

Given the growing interest in high quality hibiscus teas and the scarcity of information about their sensory profile, lexicons were developed in French, Portuguese and English. Twenty-two samples, including freshly prepared and ready-to-drink infusions, syrups, concentrates and an instant tea, were evaluated by trained panelists, resulting in 21 defined and referenced descriptors, subsequently assembled in a sensory wheel. The vocabulary used by untrained panelists was investigated in Senegal, Portugal, France and United Kingdom through professional meetings (n=30), consumer focus groups (n=75) and check-all-that-apply studies (n=490), resulting in graphical lexicons with 27 sensory and 15 overall impression terms. Training, food culture and product familiarity influenced results. Trained panelists used precise terms to describe the aromatic profile of hibiscus teas (e.g., ‘reminiscent of dried hibiscus flowers’, with ‘berry’, ‘raisin’, ‘hay’ and ‘sweet’ aromatic notes), whereas untrained ones were more prolific in general expressions (e.g., ‘strong in hibiscus’ in Senegal; ‘floral’ and ‘fruity’ in Europe).

1. Introduction

Tea is one of the most popular beverages in the world. The global tea beverage market was worth about $38.8 billion in 2013, being projected to reach $47.2 billion by 2020 thanks to the increasing popularity of green tea and associated health benefits (Anonymous, 2015). The United States is the fourth largest tea market in the world based on retail value, after China, Russia and Japan, and the second largest tea importer, after Russia, in spite of not being among the top-ten tea consuming countries (Bailey, 2015). This could be about to change, however. According to the Tea Association of the U.S.A. (Goggi, 2016) the total wholesale value of tea sold in this country grew from less than $2 billion in 1990 to over $11 billion in 2015, with it being the only western nation growing in both imports and consumption of this product.

Driven by a high demand for healthy and convenient beverages, Ready-To-Drink (RTD) offers currently represent about half of the United States tea market in sales, with premium brands experiencing annual growth rates of 5 to 7%. Meanwhile, as North Americans, particularly Millenials, increasingly revel in the discovery of new and differentiated teas flavors – such as those with ethnic or exotic origin, organic and/or fair trade certification, and craft blends -, specialty products enjoy growth rates of 8 to 10% and foodservice tea offers continue to rise in popularity (Bailey, 2015, Goggi, 2016).

Due to their deep red color, distinctive floral, berry-like aroma and slightly acidic taste, the dried sepals of calyces of *Hibiscus sabdariffa* var. *sabdariffa ruber* flowers (commonly known in the United States as hibiscus) 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 *et al.*, 2014, Plotto *et al.*, 2004). Importantly, hibiscus extracts constitute a rich dietary source of antioxidants, carrying a total polyphenol content similar to beverages like green tea, blood orange juice or grapefruit juice (Bechoff *et al.*, 2014, Pérez-Jiménez *et al.*, 2010). They have also been demonstrated to possess important nephro- and hepato-protective, renal/diuretic, anti-cholesterol, anti-hypertensive, anti-diabetic, hypolipidemic and anti-tumoral effects (Da-Costa-Rocha *et al*., 2014, Patel, 2014, Pérez-Ramírez *et al.*, 2015).

The United States is the world's largest importer of hibiscus calyces, followed by Germany and Japan (Anonymous, 2016), with RTD beverages based on their extracts being produced and distributed in this country since 2005 (Anonymous, 2006). At onset, these products targeted mainly the Hispanic, Caribbean, Asian and Africa diasporas, since the consumption of hot or cold hibiscus teas (either as refreshment or folk remedy) is highly popular in countries like Mexico, Jamaica, Thailand, Senegal, Nigeria or Egypt (Da-Costa-Rocha *et al*., 2014, Plotto *et al*., 2004, Pérez-Ramírez *et al*., 2015). Other hibiscus beverages have slowly penetrated additional Western niche markets. Dilute-to-taste extracts (in liquid or powder form) targeting the African diaspora in Europe, herbal teas and infusions aiming at the health-conscious, all-natural, ethnic food consumer, and specialty flavored syrups for foodservice professionals and end-customers seeking novel, exotic and exciting drink mixers or baking ingredients, are the main examples (Bennett *et al.*, 2014, Ramirez *et al.*, 2010). Bulk hibiscus concentrates have also been commonly employed in the European and North American food, beverage and pharma industries as natural coloring and flavoring ingredients for several decades (Cid-Ortega and Guerrero-Beltrán, 2015). More recently, however, the rising number of health conscious consumers, along with the growing interest of North Americans and Europeans in teas made from unique or exotic ingredients, are creating new, mainstream market opportunities for high quality hibiscus beverages (Bailey, 2015). Since 2011, Starbucks' Teavana, Argo Tea and Dunkin' Donuts, for instance, have been developing premium tea lines for both foodservice and retail outlets that carry several RTD and instant beverages based on hibiscus extracts, as well as many herbal blends containing hibiscus. In spite of these developments, research on the descriptive sensory attributes of hibiscus teas remains scarce (Bechoff *et al.*, 2014, Wong *et al.*, 2003). Minimum quality requirements for the export of hibiscus calyces to Europe and the United States that take into account the desirable sensory characteristics of their extracts have been established (Plotto *et al*., 2004). Nevertheless, due to their limited number and scope, as well as lack of empirical validation, such requirements are of very limited use for the sensory characterization of hibiscus teas.

Sensory lexicons provide accurate and reproducible results for descriptive sensory analysis (Cherdchu *et al.*, 2013) and constitute a tool for communication within panels and across diverse audiences(Hayakawa *et al.*, 2010, Lawless and Civille, 2013). Its application to describe and compare the quality of hibiscus teas should, therefore, lead

to a better understanding of the quality of existing products and suggest avenues for the development of new ones. Moreover, it should facilitate communication between the actors involved in research, production and regional/international trading, particularly in what steers the breeding of new hibiscus cultivars of high commercial value.

When developing lexicons for cross-cultural application, the involvement of researchers from all relevant cultures is desirable (Lawless and Civille, 2013). Furthermore, comparison of results between different cultures/countries/languages can provide additional lexicon validation (Cherdchu *et al*., 2013, Chambers *et al.*, 2012, Chung and Chung, 2007). On the other hand, food producing and trading companies do not always have the possibility to train or get access to trained panelists. The sensory vocabulary used by untrained professionals, or even by consumers, may also provide relevant product descriptions for marketing research, sales and customer support (Hayakawa et al., 2010, Kim and Lee, 2016). Moreover, its reliability can be assessed by comparison against sensory lexicons developed by trained panellists (Ares *et al.* 2015; Blanchet *et al.* 2012; Worch *et al.* 2010).

The main aims of this paper are to establish cross-culturally valid sensory lexicons for hibiscus tea in French, Portuguese and English, and to assemble them in simple and convenient formats for different actors in the hibiscus value chain. Additionally, it explores the impact of training, food culture and product familiarity on the generation and use of sensory vocabulary, and advances extant knowledge on the consumer profiling of plant-based beverages with health benefits, in both traditional and new markets.

2. Materials and methods

2.1. Ethical Approval

All sensory descriptive and consumer evaluation studies were conducted in accordance with the Declaration of Helsinki and their protocol approved by the Ethics Committee of the EU AFTER project (FP7 245 - 025).

2.2. Samples and Preparation

Twenty-two hibiscus teas were evaluated: eight freshly brewed infusions, five ready-to-drink infusions, six diluted-to-taste syrups, two diluted-to-taste concentrates and one prepared instant drink (Table 1). Samples were selected to represent the most important product market segments and ensure sufficient diversity in sensory characteristics. Moreover, sampling focused on teas containing hibiscus as the sole flavoring ingredient, in order to more truthfully reflect the sensory profile of this product.

2.3. Lexicon Development

Table 2 presents an overview of the stages of development of the hibiscus tea sensory lexicons for trained and untrained panelists. The strategy employed to develop and validate the lexicon for trained panelists entailed sequentially testing sets of samples of different hibiscus teas and assessing whether the descriptors proposed by panelists differentiated and described adequately their sensory profiles. This was done throughout Stages 1 to 3. Relatively to Stage 1, Stages 2 and 3 introduced different tea samples and a descriptive analysis performed by a trained, rather than a semi-trained panel (albeit highly familiar with the product). Moreover, the samples tested in Stage 3 included not only traditional hibiscus teas (like in Stages 1 and 2), but also some new beverages, with the aim of expanding and updating the lexicon vis-à-vis the range of available products.

The strategy pursued for the development and validation of a lexicon for untrained panelists also encompassed three stages. Stage 1 entailed a series of meetings with untrained professionals of processing and distributing companies, researchers and academics, all familiar with hibiscus teas, with the aim of compiling related sensory/hedonic/other vocabulary. The vocabulary used by consumers to characterize hibiscus teas was explored during a series of focus groups and validated through CATA-based sensory profiling studies, as well as comparison to trained panelists' lexicons.

Table 1. Hibiscus tea samples

Sample	Ingredients	Process	Origin	Preparation
Freshly brewed infusions				
FInf1	Whole hibiscus calyces (Vimto cultivar), sugar, water.	Extraction: calyx-to-water mass ratio 1:20, 25ºC, 120 min. Filtration, sugar addition 130 gL^{-1}.	Calyces: 2011 harvest (Kaolack region, Senegal), purchased locally (Latmingue, Senegal) Infusion: Made by a street vendor in Dakar, Senegal	None
FInf2	Whole hibiscus calyces (Koor cultivar), sugar, water.			
FInf3	Whole hibiscus calyces (Koor cultivar), sugar, water.	Extraction: calyx-to-water mass ratio 1:25, 98-100ºC, 20 min. Filtration, sugar addition 130 gL^{-1}.		
FInf4	Whole hibiscus calyces (Vimto cultivar), sugar, water.	Extraction: calyx-to-water mass ratio 1:20, 18-20ºC, 120 min. Filtration, sugar addition 130 gL^{-1}. Pasteurization (85ºC, 20 min).	Calyces: 2012 harvest (Senegal), supplied by Racines SA (Montpellier, France) Infusion: Made on site in sensory evaluation laboratories (Porto, Portugal; Montpellier, France; Chatam, Kent, United Kingdom)	
FInf5	Whole hibiscus calyces (Koor cultivar), sugar, water.			
FInf6	Whole hibiscus calyces (Vimto cultivar), sugar, water.	Extraction: calyx-to-water mass ratio 1:30, 98-100ºC, 60 min. Filtration, sugar addition 130 gL^{-1}.		
FInf7	Whole hibiscus calyces (Koor cultivar), sugar, water.			
FInf8	Ground hibiscus calyces [Vimto and Koor cultivars (50:50)], sugar, water.	Extraction: calyx-to-water mass ratio 1:33, addition of boiling water (98-100ºC), left to stand 20 min. Filtration, sugar addition 130 gL^{-1}.	Calyces: 2013 harvest from QABCOO (Kaolack, Senegal), purchased locally (Latmingue, Senegal) Infusion: Made on site in sensory evaluation laboratories (Porto, Portugal; Montpellier, France; Chatam, Kent, United Kingdom)	

Ready-to-drink infusions				
RInf1	Whole hibiscus calyces (Vimto cultivar), sugar, water.	Extraction: calyx-to-water mass ratio 1:20, 25ºC, 120 min. Sugar addition 130 gL^{-1}. Pasteurization (85ºC, 20 min).	Calyces: 2011 harvest (Kaolack, Senegal), purchased locally (Latmingue, Senegal) Infusion: Processed at Société Esteval Agro Alimentaire (Dakar, Senegal)	None
RInf2	Whole hibiscus calyces (Koor cultivar), sugar, water.			
RInf3	Whole hibiscus calyces [Vimto and Koor cultivars (50:50)], sugar, water.			
RInf4	Whole hibiscus calyces [Vimto and Koor cultivars (50:50)], sugar, water.	Extraction: calyx-to-water mass ratio 1:20, 25-30ºC, 120 min. Sugar addition up to 15ºBx. Pasteurization (90ºC, 20 min).	Calyces: 2013 harvest from QABCOO (Kaolack, Senegal), purchased locally (Latmingue, Senegal) Infusion: Processed at Société Esteval Agro Alimentaire (Dakar, Senegal)	
RInf5	Ground hibiscus calyces [Vimto and Koor cultivars (50:50)], sugar, water.	Extraction: calyx-to-water mass ratio 1:40, 25-30ºC, 30 min. Sugar addition up to 17ºBx. Pasteurization (75ºC, 30 min).	Calyces: 2013 harvest from QABCOO (Kaolack, Senegal), purchased locally (Latmingue, Senegal) Infusion: Processed at CSFPIAA (Dakar, Senegal)	
Syrups				
Syr1	Whole hibiscus calyces (Vimto cultivar), sugar, water.	Extraction: calyx-to-water mass ratio 1:4, 25ºC, 120 min. Pasteurization (up to 105ºC, cooled down immediately). 1.3kgL^{-1} sugar addition.	Calyces: 2011 harvest (Kaolack, Senegal), purchased locally (Latmingue, Senegal) Syrup: Processed at Société Esteval Agro Alimentaire (Dakar, Senegal)	Dilution with water 1:4 (v/v)
Syr2	Whole hibiscus calyces (Koor cultivar), sugar, water.			
Syr3	Whole hibiscus calyces [Vimto and Koor cultivars (50:50)], sugar, water.			
Syr4	Whole hibiscus calyces [Vimto and Koor cultivars (50:50)], sugar, water.	Extraction: calyx-to-water mass ratio 1:5, 25-30ºC, 120 min. Sugar addition up to 68ºBx. Pasteurization (90ºC, 20 min).	Calyces: 2012 harvest (Thiès, Senegal) Syrup: Produced and distributed by Société Esteval Agro Alimentaire (Dakar, Senegal)	

Syr5	Whole hibiscus calyces [Vimto and Koor cultivars (50:50)], sugar, water.	Extraction: calyx-to-water mass ratio 1:5, 25-30ºC, 120 min. Sugar addition up to 68ºBx. Pasteurization (90ºC, 20 min).	Calyces: 2013 harvest from QABCOO (Kaolack, Senegal), purchased locally (Latmingue, Senegal) Syrup: Processed at Société Esteval Agro Alimentaire (Dakar, Senegal)	
Syr6	Ground hibiscus calyces [Vimto and Koor cultivars (50:50)], sugar, water.	Extraction: calyx-to-water mass ratio 1:10, 25-30ºC, 30 min. Sugar addition up to 65ºBx. Pasteurization (75ºC, 30 min).	Calyces: 2013 harvest from QABCOO (Kaolack, Senegal), purchased locally (Latmingue, Senegal) Syrup: Processed at CSFPIAA (Dakar, Senegal)	
Concentrates				
Conc1	Whole hibiscus calyces [Vimto and Koor cultivars (50:50)], sugar, water.	Extraction: calyx-to-water mass ratio 1:5, 25-30ºC, 240 min. Concentration by osmotic evaporation at 0.15 bar up to 60ºBx.	Calyces: 2013 harvest from QABCOO (Kaolack, Senegal), purchased locally (Latmingue, Senegal) Concentrate: Processed at CIRAD (Montpellier, France).	Dilution with water 1:40 (v/v), sugar addition 130 gL^{-1}
Conc2	Ground hibiscus calyces [Vimto and Koor cultivars (50:50)], sugar, water.	Extraction: calyx-to-water mass ratio 1:5, 25-30ºC, 30 min. Concentration under-vacuum at 0.40 bar up to 62ºBx. Pasteurization (75ºC, 30 min).	Calyces: 2013 harvest from QABCOO (Kaolack, Senegal), purchased locally (Latmingue, Senegal) Concentrate: Processed at CSFPIAA (Dakar, Senegal)	
Instant tea				
Inst	Hibiscus (3%), dextrose, maltodextrin, citric acid, wild rose extract and aromas.	Spray-dried and granulated extract	Calyces: unknown Instant tea: Distributed by Racines SA (Montpellier, France)	Dilution with water 1:10 (m/v)

QABCOO: Quality Biological Agriculture Cooperative (Certified Organic and Fair Trade Production)

CSFPIAA: Centre Sectoriel de Formation Professionnelle aux Métiers des Industries Agroalimentaires

CIRAD: Centre de Coopération Internationale en Recherche Agronomique pour le Développement

Table2. Overview of the stages of development of the hibiscus tea sensory lexicons

Stage	Method	Sample	Panel	Goal
Trained panelists				
1	Descriptive analysis	FInf 1-3; RInf1-3; Syr 1-3	17 Senegalese semi-trained panelists	Initial lexicon development by panelists highly familiar with hibiscus tea
2	Descriptive analysis	FInf 4-7; Syr 4; Inst	7 Portuguese trained panelists	Lexicon development
3	Descriptive analysis	FInf 8; RInf4-5; Syr 5-6; Conc 1-2	13 Portuguese trained panelists	Lexicon expansion
4	Compilation and assembly of lexicons: translation of stage 3 descriptors to French and English; linguistic equivalence validation using ISO 5492:2008 and assembly of descriptors	Not applicable	12 senior panelists and native Portuguese, French and English speakers	Completion of lexicons in French, Portuguese and English, and assembly of a sensory wheel in English
Lexicon development for untrained panelists				
1	Professional meetings: vocabulary generation and reduction	Assorted teas and other products made with hibiscus calyces	30 staff and board members of food processors and/or distributors, researcher and academics from Senegal, Ghana, Cameroon, Portugal, France and United Kingdom	Vocabulary development by professionals familiar with hibiscus tea

2	Focus groups: vocabulary generation	FInf 4,6,8; RInf 4-5; Syr 4-6; Conc2; Inst	75 consumers recruited in Senegal, Portugal, France and United Kingdom	Vocabulary development by consumers across countries with different levels of hibiscus tea consumption
	Translation of focus group terms in French and English to Portuguese, and back-translation. Linguistic validation using ISO 5492:2008. Term reduction.	Not applicable	12 senior panelists and native Portuguese, French and English speakers	
3	Consumer profiling using check-all-that-apply questions	FInf4,6; RInf 4-5; Syr 4,6; Conc2; Inst	490 consumers recruited in Portugal, France, United Kingdom and Senegal	Comparison of consumer vocabulary: 1) across countries with different levels of hibiscus tea consumption; 2) with lexicon for trained panelists
4	Compilation, reduction and assembly of vocabulary: translation of terms from stage 1 to Portuguese, English and/or French and linguistic validation using ISO 5492:2008; reduction of terms from stages 2 and 3; assembly of terms	Not applicable	12 senior panelists and native Portuguese, French and English speakers	Assembly of graphical lexicons in French, Portuguese and English

Trained Panelists

Stage 1. Descriptive analysis of hibiscus tea by a semi-trained sensory panel

Participants. Seventeen Senegalese subjects with no relevant experience in sensory descriptive analysis (university technical staff and students, technical staff of food companies) were selected, based on their interest on the product and availability to participate. Subjects were pre-screened for high frequency of hibiscus tea consumption.

Procedure. Panel sessions were held in French (Senegal's official language) at the Cheikh Anta Diop University of Dakar, under the guidance of an experienced panel leader. Tastings were conducted in an air-conditioned room (22-25ºC) with controlled lighting. Nine hibiscus teas (FInf1-3, RInf1-3 and Syr1-3) were evaluated. Sensory terms were generated during initial orientation sessions. Eleven descriptors were subsequently developed by consensus and the corresponding definitions established (Bechoff *et al.* 2014). After a period of training in attribute recognition and intensity scoring, panelists evaluated the nine teas. Approximately 50 ml of the samples were served in clear plastic cups coded with three digit numbers. Samples were tested in triplicate and presented in randomized order. Mineral water was used to clean the palate.

Stage 2. Descriptive analysis of hibiscus tea by a trained sensory panel.

Participants. Seven Portuguese senior panelists belonging to the sensory evaluation panel of Escola Superior de Biotecnologia - Universidade Católica Portuguesa (ESB-UCP) in Oporto, with over ten years of experience in sensory descriptive evaluation and attribute intensity rating, were selected. Panelists had neither prior experience in hibiscus tea evaluation nor were they familiar with hibiscus products.

Procedure. Sessions were held in Portuguese at the ISO 8589:2007 compliant sensory evaluation laboratory of ESB-UCP. Six hibiscus teas (FInf4-7, Syr4 and Inst) were evaluated. Sensory terms were generated during initial orientation sessions. These included the discussion of a list of pre-existing descriptors broadly characterizing different types of hibiscus teas (Bechoff *et al.* 2014; Plotto *et al.* 2004; Wong *et al.* 2003), which were previously translated from French and English to Portuguese by the authors. Sensory terms and references were refined by consensus in subsequent

sessions. To this end, modalities odor (orthonasal) and aroma/flavor (retronasal) were defined according to ISO 5492:2008. To increase expertise with berry odors and flavors, reference samples of berry fruits and juices were provided. This led to the elimination of some related terms exhibiting low quoting frequency, intensity rating or discriminatory power (ISO 11035:1994). During the following sessions, definitions were proposed and discussed, reference standards established and tested, and the sequence of evaluation of each attribute defined. As a result, a score sheet was developed to rate attribute intensity using an unstructured-anchored line scale (0=none, 10=reference standard). Twelve 90-minute sessions were required to reach this phase. Panelists were then trained and their performance assessed according to ISO 11132:2012. Finally, they were requested to evaluate the samples in order to validate the developed lexicon (Chambers *et al.* 2016; Lawless and Civille 2013). Tastings were held in tasting booths under white light (6500 K) and controlled temperature (20-22 ºC). Samples were labelled with a three-digit code and presented to panelists in random order. Each sample was tasted in triplicate, with a maximum of four samples being evaluated per session. Water was supplied to clean the palate between tastings.

Stage 3. Descriptive analysis of hibiscus tea by a trained sensory panel to expand the lexicon.

Participants. Eight Portuguese senior panelists belonging to the sensory evaluation panel of ESB-UCP (including the seven involved in Stage 2), plus five panelists with no relevant experience in sensory descriptive analysis, were selected. The latter were selected according to ISO 8586:2012 (that is, based on the assessment of their health status, ability to discriminate and match basic tastes and odors, attribute intensity perception and descriptive ability), out of a population of 26 volunteers (university technical staff and doctoral students), and trained in descriptive sensory analysis during four 90-minute sessions. Two additional training sessions were conducted, where new panelists were introduced to the tasting of hibiscus tea using the lexicon developed in Stage 2.

Procedure. Sessions were held in Portuguese at the ISO 8589:2007 compliant sensory evaluation laboratory of ESB-UCP. Seven hibiscus teas (FInf8, RInf14-5, Syr5-6, and Conc1-2) were evaluated. Sensory terms were generated during initial orientation sessions, which included the discussion of the lexicon developed in Stage 2. Remaining

sessions were carried out using the procedure described in Stage 2. Overall, twelve 90-minute sessions were conducted.

Stage 4. Final compilation and assembly of the lexicons for trained panelists.

Procedure. The set of descriptors obtained at the end of Stage 3 was translated into English and French. A final lexicon for trained panelists was assembled and linguistic equivalence subsequently validated by 12 native speakers and senior sensory panelists using ISO 5492:2008 as reference. Lastly, descriptors in English were used to create a two-tiered sensory wheel (Koch *et al.* 2012), with the inner tier comprising terms that grouped together the terms in the outer tier (Noble *et al.* 1984; Theron *et al.* 2014).

Untrained Panelists

Stage 1. Meetings to investigate the hibiscus tea vocabulary of untrained professionals.

Participants. Thirty subjects from Senegal, Ghana, Cameroon, Portugal, France and United Kingdom (staff and board members of food processors and/or distributors, researchers and academics) attended. They all tasted hibiscus teas in the course of their professional activities and were familiar with the product, but had no formal training in sensory evaluation.

Procedure. Eight semi-structured meetings of about 90 minutes were held in Ghana, Senegal, Portugal, France and United Kingdom between 2011 and 2013. They were conducted in French or English by experienced moderators and involved an average of eight subjects. The agenda included sensory-driven product optimization (hibiscus cultivar selection and breeding, improvement of harvest and post-harvest operations, tea processing methods), improvement of marketing and promotional activities, and identification of (potential) drivers of consumer acceptance in different countries. Half of the meetings entailed tastings of assorted teas and other products made with hibiscus calyces. Tea attributes (sensory, hedonic and others) were discussed in meetings conducted in 2011-12, along with the adequacy of the different terms used to describe them. As a result, a list of 60 terms characterizing this product was obtained. Subsequent meetings focused on term reduction by considering their relevance and

appropriateness, with purely hedonic expressions, synonyms and overlapping vocabulary being discarded by consensus.

Stage 2. Focus groups to investigate the hibiscus tea vocabulary of consumers.

Participants. Seventy-five subjects from Senegal, Portugal, France and United Kingdom (52% female; 19-65 years old) were selected. They were pre-screened for high frequency of hibiscus tea consumption in Senegal and regular consumption of fruit beverages or cold teas in the other countries.

Procedure. Nine focus groups of about 90 minutes were held in Oporto, Montpellier, Chatham and Dakar between 2012 and 2014. They were conducted in the local official language by experienced moderators and involved an average of eight subjects. These were asked to taste *ad libitum* 4 hibiscus teas with different sensory profiles, selected out of a set of 10 previously evaluated samples (Finf4,6,8; Rinf4-5; Syr4-6; Conc2; Inst), and to describe their attributes (sensory, hedonic and others). Senegalese subjects were requested to provide additional terms based on prior consumption experiences. Vocabulary describing hibiscus teas was compiled across all focus groups. Terms in French and English were translated to Portuguese by native speakers and senior sensory panelists and back-translated by bilingual individuals to check for linguistic equivalence, using the ISO 5492:2008 as reference. An overall list of 52 terms was obtained, which was subsequently reduced by the senior panelists. Reduction criteria were similar to those applied in Stage 1, but took the differences observed between the terms generated in Europe and Senegal into account.

Stage 3. Consumer profiling of hibiscus tea using check-all-that-apply (CATA) questions.

Participants. Four hundred and ninety subjects were non-probabilistically recruited in Oporto (N=100), Montpellier (N=120), Chatam (n=120) and Dakar (N=150). Subjects in France, Portugal and United Kingdom (52% female; 18-72 years old) were pre-screened for regular consumption of fruit beverages or cold teas. Subjects in Senegal (39% female; 18-73 years) were pre-screened for regular consumption of hibiscus tea.

Procedure. Based on the reduced list of terms obtained from European consumers, a trilingual CATA questionnaire comprising 28 terms – clear, red color, pink color, floral,

acid, bitter, sweet, astringent, fruity, red fruit, syrup like, tisane like, instant drink like, natural, artificial, watery, simple, strong, fresh, full bodied, viscous and fluid, herbaceous, honey, diluted and concentrated, smooth, rough -, was designed. Likewise, a paper-and pencil CATA questionnaire comprising eighteen terms – light red, dark red, odorless, tasteless, clear, acid, bitter, sweet, astringent, syrup like, natural, artificial, watery, refreshing, sharp odor, light in hibiscus, strong in hibiscus and appealing – was created in French from the terms generated by consumers in Senegal. All questionnaires were pre-trialed at the corresponding test location, upon which the order of terms was randomized within subjects and across samples and countries in Europe, and within subjects and across samples in Senegal. In the latter, trained enumerators assisted subjects in French or the local *Wolof* language when required.

Tasting sessions were conducted at recruiting sites under central location test conditions. Seven samples (Finf4,6,8; Syr4,6; Conc2; Inst) were evaluated in Europe and four (Rinf4-5; Syr6; Conc2) in Senegal. Samples were presented in Europe as non-alcoholic beverages, without further information, whereas in Senegal they were identified as *Bissap* juice (the common local designation for hibiscus tea). Thirty milliliters of each sample (labelled with a three-digit code) were served to subjects in random order in clear plastic glasses. Water was supplied to clean the palate between tastings.

Stage 4. Compilation and reduction of the hibiscus tea vocabulary of untrained panelists.

Procedure. The final set of terms obtained from the meetings with untrained professionals were translated to Portuguese, English and/or French. Native speakers and senior sensory panelists validated the translations using the ISO 5492:2008 as reference. These terms were then integrated with those retained from consumer focus groups and CATA questionnaires by senior panelists, and finally assembled in graphical lexicons according to sensory modality (appearance, odor and flavor, taste and mouthfeel) and overall impression (Hayakawa *et al.* 2010).

2.4. Statistical Analysis

XLSTAT software V. 2015 (Addinsoft, Paris) was used to carry out all statistical analyses. Assessment of trained panels' performance in Stages 2 and 3 was conducted according to ISO 11132:2012 using analysis of variance (ANOVA). Significant differences between the intensities of descriptive sensory attributes, evaluated by semi-trained (Stage 1) and trained panelists (Stages 2 and 3), were tested using ANOVA with Fisher post-hoc tests or Welch's ANOVA with Games-Howell post-hoc tests (in case of unequal variances). Principal components analysis (PCA) was used to investigate relations between descriptive sensory attributes and samples in Stage 3, whereas Pearson's correlation tests were used to investigate relationships among attributes in Stages 1, 2 and 3. The significance of these statistical tests was evaluated at $p<0.05$, unless otherwise stated.

The frequency of use of each CATA term to describe samples was tallied across consumers in each country. Multiple Factor Analysis (MFA) was then performed to explore relationships across countries and with intensities of descriptive sensory attributes evaluated by trained panelists (Stages 2 and 3) (Abdi *et al.* 2013; Escofier and Pagès 2008). To analyze similarities between sample configurations obtained from consumers across European countries, and between consumers and trained panelists in Europe and Senegal, regression vectors (RV) coefficients were used (Ares *et al.* 2015; Robert and Escoufier 1976) and their significance evaluated at $p<0.01$. The RV coefficient is a multivariate generalization of the Pearson correlation coefficient that measures the relationship between two sets of variables defined for the same individuals (Josse *et al*., 2008, Abdi, 2007). High RV values (close to 1) are regarded as indicators of good agreement between sample configurations (Ares et al. 2015; Lelièvre et al. 2008). Spearman correlation tests were finally conducted to investigate relationships between the frequencies of elicitation of CATA terms, and between these and the intensities of descriptive sensory attributes.

3. Results and discussion

3.1. Lexicon Development for Trained Panelists

Stage 1. Eleven sensory descriptors were obtained: three for appearance (red color, clarity and concentration), two for both odor and flavor (hibiscus and fermented) and four for taste/mouthfeel (sweet, acidic, bitter and irritant). Descriptors fermented and irritant were related to deviations in the quality of some samples (Tomlins *et al.* 2012). According to Lawless and Civille (2013), the set of samples used to develop a sensory lexicon should provide a fair representation of the whole product category, including products with defects. Hence, these descriptors, along with previously reported off odors and flavors resulting from the contamination of hibiscus calyces with extraneous botanical materials and spices (Plotto *et al.* 2004), were retained. Descriptive analysis results yielded significant differences across samples for all attributes, with the exception of fermented odor. This could be due to panelists' difficulty to discriminate low intensity odors (Bechoff *et al.* 2014). Concentration and red color were very strongly correlated (r=0.99) and were thus considered to assess essentially the same appearance attribute. Consequently, only red color was retained.

Stage 2. Sixty-one terms were initially elicited by panelists: 6 for appearance, 29 for odor and 26 for flavor, taste and mouthfeel. After reduction, fifteen were retained (Table 3), including new descriptors for visual and mouthfeel viscosity, astringency, odor and flavor (berry fruit: raspberry and sour cherry; dried fruit: raisin; sweet associated aromatics honey and cold black tea; and herbaceous: hay). Evaluations of viscosity appeared to be strongly affected by hibiscus calyx blend, being used by panelists only when describing samples made from a single cultivar. Previous studies have uncovered that blends of similar proportions of Koor and Vimto calyces lead to extracts with better sensory quality, particularly in terms of appearance and taste/mouthfeel (Babajide *et al.* 2005; Bechoff *et al.* 2014; Cisse, Vaillant, *et al.* 2009). Regarding odor and flavor, raspberry was considered the most suitable term to designate the berry fruit odor of the instant tea, whereas sour cherry was considered more appropriate to describe the remaining samples. The rather different selection of odor and flavor descriptors by Portuguese panelists, when compared to Senegalese ones, is likely to derive chiefly from cultural differences rooted on attribute familiarity (Chung and Chung 2007; Hersleth *et al.* 2013; Tu *et al.* 2010).

Table 3. Sensory Characteristics of Hibiscus teas

Sample	Appearance		Odor						Flavor and mouthfeel						
	Red Color	Viscosity	Hibiscus	Raspberry	Hay	Honey	Cold Black Tea	Raisin	Hibiscus	Sour Cherry	Acid	Sweet	Bitter	Astringency	Viscosity
FInf4	6.0^{b}±0.8	4.6^{b}±0.4	7.5^{a}±0.3	3.7^{b}±1.0	4.1^{a}±0.8	2.6^{b}±0.5	3.4^{b}±0.5	3.8^{a}±0.5	7.2^{a}±0.4	1.9^{b}±0.5	7.2^{a}±0.5	5.8^{c}±0.8	3.0^{a}±0.3	3.1^{b}±0.4	2.5^{a}±0.4
FInf5	3.9^{d}±0.4	2.3^{d}±0.7	7.4^{a}±0.4	2.9^{b}±0.8	4.6^{a}±0.4	2.1^{b}±0.2	$3.8^{a,b}$±0.4	3.5^{a}±0.5	7.0^{a}±0.1	1.9^{b}±0.5	6.8^{a}±0.3	6.5^{c}±0.6	2.7^{b}±0.1	$3.1^{a,b}$±0.7	2.3^{a}±0.5
FInf6	7.2^{a}±0.3	5.4^{a}±0.6	6.7^{b}±0.5	3.5^{b}±0.7	4.2^{a}±0.6	2.4^{b}±0.4	$3.8^{a,b}$±0.3	$3.1^{a,b}$±0.6	6.5^{b}±0.4	1.9^{b}±0.4	6.9^{a}±0.4	6.1^{c}±0.5	$2.9^{a,b}$±0.4	$3.5^{a,b}$ ±0.7	2.0^{a}±0.9
FInf7	4.8^{c}±0.7	3.2^{c}±0.5	7.8^{a}±0.6	3.5^{b}±0.8	4.7^{a}±0.6	2.6^{b}±0.7	4.0^{a}±0.2	3.7^{a}±0.1	7.4^{a}±0.3	1.9^{b}±0.5	7.1^{a}±0.3	6.6^{c}±0.5	$2.8^{a,b}$±0.1	3.9^{a}±0.6	2.4^{a}±0.7
Syr4	$4.2^{c,d}$±1.3	$2.7^{c,d}$±0.7	3.6^{c}±0.7	3.2^{b}±0.5	1.4^{b}±0.3	3.4^{a}±0.6	1.4^{a}±0.2	2.6^{b}±0.6	3.5^{c}±0.5	3.8^{a}±0.9	2.4^{b}±0.3	10.5^{a}±1.6	0.4^{c}±0.1	1.1^{c}±0.2	$1.9^{a,b}$±0.7
Inst	2.5^{e}±0.6	2.3^{d}±0.3	2.1^{d}±0.2	6.4^{a}±0.3	0.7^{c}±0.3	1.0^{c}±0.5	0.6^{d}±0.2	0.9^{c}±0.7	2.0^{d}±0.2	2.3^{b}±0.7	2.2^{b}±0.6	8.5^{b}±0.3	0.4^{c}±0.3	0.8^{c}±0.3	1.0^{b}±0.4

Mean ± standard deviation. Different superscripts within a column indicate significant differences ($p<0.05$).

Significant differences across samples were found for all descriptors (Table 3). The impact of calyx cultivar and extraction conditions on red color intensity was noticeable. Higher values of this attribute were obtained for samples prepared from Vimto calyces, compared to Koor, as well as for those extracted with boiling water rather than at ambient temperature. Calyces from the Vimto cultivar are known for their exceptionally rich content in anthocyanins, which are responsible for their very intense red color (Cisse *et al.* 2009). Meanwhile, the use of hot water leads to high extraction rates of anthocyanins and hence more deeply colored extracts (Cisse *et al.* 2012; Ramírez-Rodrigues *et al.* 2011; Wong *et al.* 2003). Red color intensity was also higher for infusions than for the syrup and instant tea. This was likely due to the higher proportion of calyces used in the production of infusions as well as the dilutions employed in the preparation of syrup and instant tea samples (Table 1). Similarly, hibiscus odor and flavor intensities, acid taste, bitter taste and astringency were generally higher for infusions than for the syrup or the instant tea. Conversely, sweetness was considerably lower, as the result of formulation. Likewise, an earlier study described the odor and flavor of hibiscus infusions as strong and sour, whereas those of syrups were predominantly characterized as sweet (Bechoff *et al.* 2014). Finally, raspberry odor was much more intense in the instant tea sample than in other samples, likely due to formulation, while hay, honey, cold black tea and raisin odors were much less intense.

Heightened perceptions of bitterness and astringency usually co-occur with strong acid taste in polyphenol-rich juices and teas (Bett-Garber and Lea 2013; Koch *et al.* 2012; Koppel and Chambers IV 2010), while moderate and high sweetness intensities are known to be generally suppressive of acid and bitter tastes (Keast and Breslin 2002). In line with this, strong positive associations were found between the acid taste, bitter taste and astringency of hibiscus teas ($p<0.01$), whereas negative associations where uncovered between these attributes and sweet taste.

Stage 3. As a result of initial orientation sessions, descriptors visual and mouthfeel viscosity, as well as berry fruit odors raspberry and sour-cherry were deemed to inadequately describe samples, since all teas evaluated at this stage were made from both Vimto and Koor calyces and did not include any instant drinks. A new term to describe the

berry fruit odor of tested samples (aronia/cranberry) was hence added to the lexicon, resulting in a grand total of twelve descriptors.

Significant differences across samples were found for all attributes, except raisin odor. The widest variations were observed in descriptors red color, hibiscus odor, hibiscus flavor, acid taste and sweet taste. Narrower variations and low to moderate intensities of honey, hay, cold black tea and aronia/cranberry odors, bitter taste and astringency were observed. Generally higher intensities of red color, hibiscus odor and flavor, and aronia/cranberry odor were observed for teas prepared from ground calyces than from whole ones, and for those manufactured using mild (rather than harsh) pasteurization conditions. Diessana *et al.* (2015) showed that the use of crushed calyces resulted in hibiscus extracts with a more intense color, while Cisse *et al.* (2009) uncovered that the rate of anthocyanin degradation in such products increased dramatically over 80 ºC, underscoring the importance of using mild pasteurization conditions to retain their characteristic and highly appreciated red hue. Present results indicate that besides improving color, such processing conditions are also more likely to effectively extract and preserve the highly fragrant, floral and berry-like aromatics of hibiscus calyces.

Figure 1 depicts the projection of hibiscus tea samples and descriptors in the first two principal components (PC) extracted, which together explained 85.3% of variance. Basic tastes and astringent mouthfeel, along with honey, cold black tea and raisin odors, were mainly correlated with PC1, while red color, aronia/cranberry and hay odor, as well as hibiscus odor and flavor were mainly correlated with PC2. A good discrimination between samples was observed. FInf8 was deemed to have the strongest aromatic character of all beverages tested, while displaying strong red color, moderate to strong sweetness and acidity, and weak to moderate bitterness and astringency. Syr5 and Syr6 were judged to have the strongest sweetness and the weakest acidity, bitterness and astringency of all beverages tested, while having moderate red color and aromatic character. Conversely, RInf4 and RInf5 exhibited the strongest acidity, bitterness and astringency, and the weakest sweetness. In particular, RInf5 exhibited strong red color and moderate to strong hibiscus odor and flavor, while RInf4 was deemed to have the weakest red color and aromatic character of all samples. Finally, Conc1 and Conc2 exhibited a sensory profile similar to RInf5, although displaying relatively weaker acidity, bitterness and astringency,

stronger sweetness and a more intense aromatic character. Differences in basic tastes and mouthfeel between beverages were most likely associated to the different proportions of calyces and sugar used in their production, as well as to the dilutions employed in the preparation of concentrates and syrups (Table 1).

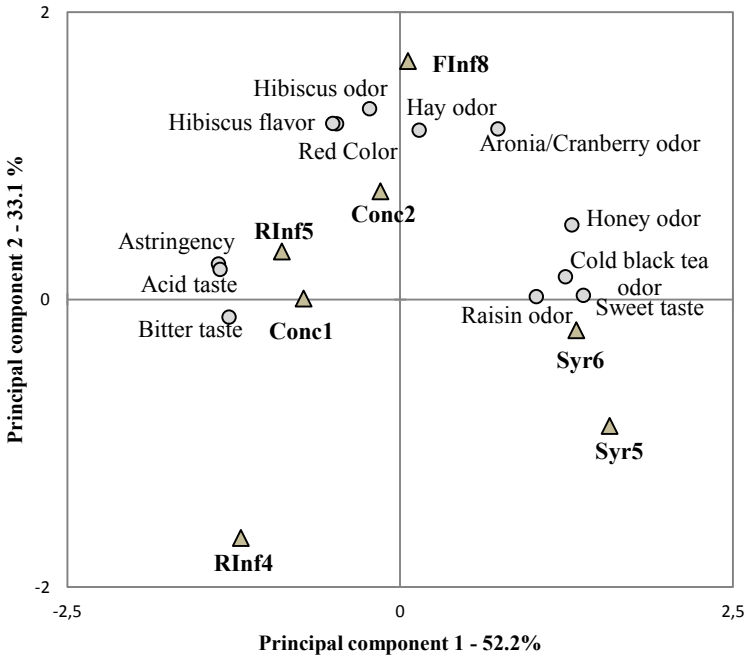

Figure 1. Principal component analysis of Hibiscus tea evaluations by trained panelists.

Stage 4. The results obtained from the performance of Stages 1to 3 led to the development of a set of 21 defined and referenced descriptors, enabling trained panelists to describe and discriminate well the sensory attributes of a broad range of hibiscus teas. Differences in the level of sensory training and familiarity with hibiscus tea and other beverages between Senegalese and Portuguese panelists, language specificities and the wide diversity of samples used in the different stages of lexicon development led to discrepancies in the definition and interpretation of some descriptors, namely red color, irritant and hibiscus flavor. It was concluded that Senegalese panelists used red color to describe both the intensity and the hue of the samples' color; therefore, both attributes were defined and referenced in the final lexicon. Additionally, it was observed that their evaluations of the intensity of hibiscus flavor were associated to their perceptions of the balance between the

sweetness and acidity of tea samples (Bechoff *et al*. 2014), while Portuguese panelists used this term exclusively to refer to the intensity of the aroma characteristic of dried hibiscus flowers. It was established that this was more likely related to their different level of training in sensory analysis, rather than to any effects of product familiarity (or lack thereof). Therefore, the descriptor hibiscus flavor was included in the lexicon only as part of the odor and flavor modalities. Lastly, the descriptor irritant, which was mentioned by Senegalese panelists only, was deemed to be related mainly to quality of the samples evaluated in Stage 1. Given the relatively low level of training of these panelists, this attribute was initially defined as a sensation in the tongue and later redefined by trained panelists to include sharp sensations in both the buccal and nasal mucous membranes, and included as such in the final lexicon.

An English version of this lexicon was subsequently developed (Table 4), with corresponding descriptors being conveniently assembled by sensory modalities in a two-tiered sensory wheel (Figure 2). To simplify the display and use of this wheel, all odor and flavor descriptors were assembled under a single sensory modality, with those referring specifically to off-odors/flavors being grouped together in a separate descriptor class (extraneous).

3.2. Lexicon Development for Untrained Panelists.

Stage 1. Twenty-five sensory terms (seventeen of which were also present in the trained panelists' lexicon) and five overall impression terms were obtained from the meetings with untrained professionals. These were: red color, wine color, clarity (clear), fluid/viscous appearance, hibiscus, floral, floral-sweet, red fruit, berry, cranberry, pomegranate, pungent, medicinal, fermented, clean/unclean, sweet, acid, bitter, astringent (mouth drying), smooth/harsh, fluid/viscous in the mouth, body (full bodied), watery, diluted/concentrated and syrup like.

Stage 2. Twenty-eight sensory terms were obtained from the focus groups with consumers, along with 15 overall impression terms. Most sensory terms used to describe appearance, taste and mouthfeel were similar across countries, as well as to those generated by untrained professionals. To describe odor and flavor, however, consumers in Europe used

terms floral, fruity, red fruits, honey and herbaceous, whereas those in Senegal employed the term hibiscus, along with odorless, tasteless, spoiled, irritant and sharp odor, as they were also asked to describe past consumption experiences.

Figure 2. Hibiscus tea sensory wheel

Table 4. Sensory lexicon describing visual, odor, flavour, taste and mouthfeel characteristics of hibiscus teas

Modalities and descriptors		Definition	Reference standard
Appearance			
Color (coleur; cor)	Intensity (intensité; intensidade)	Related to lighter (less intense) or darker (more intense) color.	50 ml reference hibiscus tea
	Red hue (teinte; matiz)	Related to hue value (Munsell color system); varies between red, purple and blue	50 ml reference hibiscus tea
Clarity (limpidité; limpidez)	Transparency/translucency (transparence/translucidité, transparência/translucência)	Related to the ability of seeing through the sample	100 NTU Turbidity standard
Viscosity (viscosité; viscosidade)	Viscosity (viscosité; viscosidade)	Related to resistance to flow. Evaluated by observing the displacement of the sample by swirling the tasting glass, to have the beverage wetting its inner surface. Varies from fluid to viscous.	Hibiscus syrup (undiluted) (Société Esteval Agro Alimentaire, Dakar, Senegal)
Odor and Flavor			
Floral (floral; floral)	Hibiscus (hibiscus; hibisco)	Fragrant aromatics with sweet, sour, berry and candy like notes, characteristic of the reminiscent odor of dry hibiscus flowers	Odor:10g shade-dried, coarsely ground, Vimto and Koor (50:50) hibiscus calyx blend (Racines SA, France) Flavor: reference hibiscus tea
Fruit (berry) [fruit (baie); frutado (frutos silvestres)]	Aronia/Cranberry (aronia/canneberge; aronia/arando)	Sweet and sharp berry aromatics found on a blend of aronia and cranberry juices	50 ml aronia and cranberry juice (50:50) blend (ProSain SAS, France)
	Raspberry (framboise; framboesa)	Sweet and sour aromatics commonly associated to raspberries	50g crushed raspberries (fresh or frozen)

	Sour cherry (griotte; ginja)	Slightly sweet and sour aromatics commonly associated to sour cherries	50g crushed sour cherries (fresh or frozen)
Fruit (dry) [fruit (sec); fruto seco]	Raisin (raisin sec ; uva passa)	Sweet aromatics with molasses notes, commonly associated to raisins	50g raisins (Ferbar, Lda. Portugal)
Sweet associated (sucré; doce)	Honey (miel; mel)	Sweet aromatics associated to bee honey	50g rosemary honey (Euromel, Lda., Portugal)
	Cold black tea (thé noir froid; chá preto frio)	Sweet and sharp aromatics associated to a cold, not freshly brewed, black tea infusion	50 ml Assan tea
Herbaceous (herbacée; herbaceo)	Hay (foin, feno)	Aromatics of air-dried hay	5g air-dried hay (local store, Oporto, Portugal)
Extraneous			
Extraneous (étranger, estranho)	Botanical* (botanical, botanico)	Aromatics associated to contamination of hibiscus calyces with extraneous botanical material	10g dry or 50g fresh plant material
	Spice * (épices, especiarias)	Aromatics associated to contamination of hibiscus calyces with spices	10g of ground commercial spice mix (cinnamon, cumin, cardamom, black pepper, fennel, turmeric, cilantro) (local store, Oporto, Portugal)
	Fermented (fermenté, fermentado)	Pungent, alcoholic and/or yeasty and/or sour odor or flavor indicating the occurrence of fermentation and consequent quality degradation	25 ml of diluted sauerkraut juice (1:2) (Beutelsbacher Fruchtsaftkelterei, GmbH)
	Irritant (irritant, irritante)	That produces a foreign and sharp sensation on the buccal and nasal mucous membranes	25 ml of diluted white wine vinegar (1:2) (Comtemp, Portugal)
Taste and mouthfeel			
Basic taste	Acid	Basic taste of substances such as malic acid	1.50 gL^{-1} malic acid solution

(saveur élémentaire, sabor básico)	(acide, ácido)		
	Sweet (sucrée, doce)	Basic taste of substances such as sucrose	100 gL^{-1} sucrose solution
	Bitter (amer, amargo)	Basic taste of substances such as caffeine or quinine	0.75 gL^{-1} caffeine solution
Mouthfeel (sensation en bouche, sensação tátil bucal)	Astringent (astringent, adstringente)	Drying and/or puckering of the mucosal surface of the mouth produced by hydroxycitric acid, hibiscus acid and derivatives and/or flavonol glicosides, respectively	50 ml reference hibiscus tea
	Viscous (visqueux, viscoso)	Related to resistance to flow. Evaluated by displacing the sample in the mouth. Varies from fluid to viscous.	Hibiscus syrup (undiluted) (Société Esteval Agro Alimentaire, Dakar, Senegal)

*Adapted from Plotto et al. (2004)

Reference hibiscus tea: 4 g of a shade-dried, ground (particle size: 1mm - 2.8 mm) Vimto and Koor (50:50) hibiscus calyx blend, extracted with 100g of water at 95-100ºC. Left to stand for 30 min with periodic agitation and then filtered (1 mm sieve). Sweetened with 130 gL^{-1} commercial sucrose.
Assan tea: 1 g of Assam tea (Lypton Yellow Label) extracted with 100 g of water at 95 ºC for 5 min. Left to stand overnight at ambient temperature.

To convey overall impression, consumers in Europe used terms simple, strong, diluted/concentrated, tisane like and instant drink like, whereas those in Senegal employed appealing and light/strong in hibiscus. Terms natural/artificial, watery, fresh/refreshing and syrup like were employed by both groups of consumers.

Individual experiences, along with noticeable cultural differences in the recognition of hibiscus aromatics, different levels of familiarity with red fruit stimuli and varying knowledge about hibiscus tea have likely contributed to these results. Similar effects have been observed for soy yogurts evaluated by French and Vietnamese panelists (Tu *et al.*, 2010) and descriptions of perilla oil by North American, Chinese and Korean panels (Yang *et al.*, 2012). In these studies, different descriptors that related to individual experiences, consumption habits and cultural background were used to characterize and evaluate samples.

Stage 3. Figure 3 depicts the first two dimensions of MFA plot of sensory descriptors and consumer CATA terms in A) Portugal, France and United Kingdom (82.0% of variance explained), and B) Senegal (92.5% of variance explained). CATA terms were generally well represented, with the exceptions of floral and tisane like in Europe. Moreover, while debriefing consumers in Senegal, it was noticeable that terms odorless and tasteless were interpreted by many of them as meaning without spoiled odor/flavor. Such terms were therefore not considered in subsequent statistical analyses.

The visual similarity between sensory spaces obtained using consumers across European countries was confirmed pairwise by the highly significant RV coefficients observed, with their values varying between 0.971 (Portuguese vs UK consumers) and 0.981 (French vs UK consumers). Such values indicate that there was very high agreement among the sample configurations obtained for each group of consumers (Ares *et al.* 2015; Lelièvre *et al.* 2008). RV values greater than 0.95 are also highly indicative of the attainment of stable and reliable consumer data configurations (Blancher *et al.* 2012). High cross-cultural similarities in consumer sensory perceptions have been earlier highlighted, notwithstanding important differences in the description of such perceptions (Andani *et al*. 2001). Likewise,

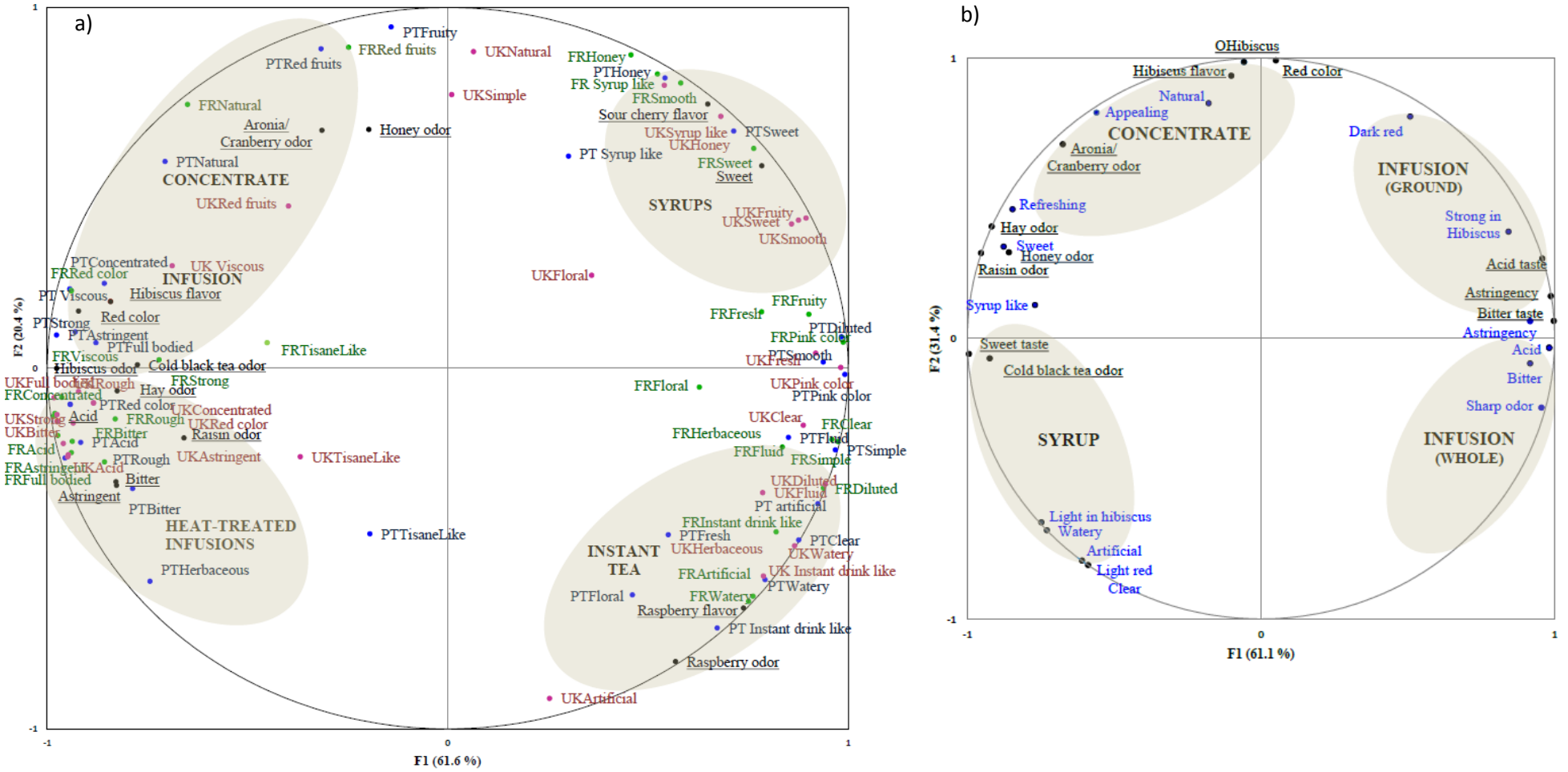

Figure 3. Multiple factor analysis plot of sensory descriptor and consumer CATA terms in a) Portugal (PT), France (FR) and United Kingdom (UK); b) Senegal

important differences across countries were observed in the present study for the sensory term herbaceous, and overall impression/hedonic terms simple and fresh [Figure 3 A)]. Herbaceous was associated to terms bitter, astringent and tisane like in Portugal, fluid, clear and simple in France, and watery and instant drink like in UK. Meanwhile, simple was related to terms natural and clear for consumers in UK, and diluted and fluid in both France and Portugal. Finally, fresh appeared related to fruity in France, pink and diluted in the United Kingdom, and watery in Portugal.

Highly significant ($p<.001$) RV coefficients were also observed for pairwise comparisons of the sensory spaces defined by trained panelists' evaluations and consumers' elicitation of CATA terms in Europe, with their values varying between 0.954 (trained panelist vs UK consumers) and 0.983 (trained panelist vs French consumers). Such figures indicate that there was very high agreement between the sample configurations obtained from each consumer group and trained panelists, as they are all well above both earlier reported and recommended threshold (RV = 0.70) values (Ares *et al.* 2015; Lelièvre *et al.* 2008). Indeed, a generally good agreement in terms of the perception of basic tastes and mouthfeel attributes between trained panelists and consumers was observed [Figure 3 A)]. Regarding odor attributes, descriptor aronia/cranberry odor was somewhat associated to the CATA term red fruit ($p<0.10$), but not to the term fruity. Meanwhile, the close positioning of the CATA term honey to panelists' evaluations of sweet taste indicates that it was most likely used by consumers to describe the level of sweetness of samples. On the other hand, no significant associations between trained panelists evaluations hay odor and honey odor attributes, and CATA terms herbaceous and honey, respectively, could be observed. Likewise, no significant associations between evaluations of descriptors hibiscus odor and hibiscus flavor, and the CATA term floral were uncovered. The latter may be attributable to the low familiarity of most European consumers with hibiscus flowers.

All hibiscus teas evaluated were clear, as diminished clarity (cloudy or milky appearance) is usually related with microbiological deviations and/or deficient filtration of extracts. However, untrained panelists often inadequately use the term 'clear' as a synonym of light in color. This much was noticed while debriefing consumers, being also confirmed

by the significant negative correlations observed between the terms clear and red color. Meanwhile, CATA terms full bodied, strong and concentrated were significantly correlated to each other ($p<0.05$), as well as with panelists' evaluations of red color, hibiscus odor and flavor and acid taste. In particular, correlations between strong and evalations of all four attributes were remarkably high ($p<0.01$).

Regarding overall impression/hedonic attributes, the CATA term natural was positioned close to both the term red fruit by consumers and to aronia/cranberry odor. Unsurprisingly, significant negative correlations between opposing terms were found: diluted/concentrated ($p<0.01$), fluid/viscous ($p<0.01$) and natural/artificial ($p<0.05$). Finally, the term syrup like was positively associated to sweet taste and sour cherry flavor ($p<0.05$).

A highly significant RV coefficient (0.927) was equally obtained for pairwise comparisons of the sensory spaces defined by trained panelists' evaluations and consumers' elicitation of CATA terms in Senegal. The level of agreement between sample configurations of trained and untrained panelists was nonetheless lower in this country than in Europe, albeit still higher than 90%. It has been hypothesized that product familiarity may moderate associations between the sensory descriptions and evaluations of consumers from different countries, as well as between these and those of trained panelists (Andani *et al*. 2001; Ishii and O'Mahony 1990). On the other hand, it has been recently suggested that focal sample similarity and complexity may also produce important interaction effects (Ares *et al.* 2015), as consumers generally have a harder time discriminating among similar samples than trained panelists, as well as describing more complex ones (Ares and Varela 2017). The relatively high familiarity of consumers in Senegal with hibiscus teas and the fact that they evaluated less and more similar samples, in terms of their chemical-sensory characteristics, than consumers in Europe may therefore help explain the results obtained. Significant correlations ($p<0.01$) between trained panelists' evaluations and consumers' elicitations for acid taste, sweet taste, bitter taste and astringent attributes were nonetheless found. Meanwhile, elicitations of CATA term strong in hibiscus were significantly correlated to trained panelists evaluations' of acid taste ($p<0.01$), but not of hibiscus odor or flavor, as it would be expected. A possible reason for this may be the traditional use of hibiscus calyces from the Koor cultivar in the preparation of hibiscus teas in Senegal,

yielding infusions with a pronounced sourness, a sensory characteristic that is highly appreciated by local consumers (Bechoff *et al.* 2014).

Similarly to Europe, it was noticeable that many Senegalese consumers used the term clear to refer to lighter shades of red color, with CATA terms clear and light red being significantly correlated to each other ($p<0.05$). Regarding overall impression/hedonic terms, artificial was significantly associated ($p<0.01$) to light red, light in hibiscus and watery. Meanwhile, the term appealing was significantly associated ($p<0.01$) to panelists' evaluations of red color, hibiscus odor and hibiscus flavor. Likewise, natural was significantly correlated to panelists' evaluations of aronia/cranberry odor ($p<0.01$). Lastly, syrup like was closely related to panelists' evaluations of sweet associated aromatics raisin ($p<0.10$) and cold black tea ($p<0.10$).

Stage 4. The results of the CATA study led to the elimination of terms odorless, tasteless and honey from the untrained panelist lexicon. Although the results of CATA studies indicate that consumers are likely to misuse terms herbaceous and clear, these were nevertheless retained since they were considered be relevant by untrained professionals. Terms harsh and rough were considered to describe astringency in a similar way and hence only harsh was retained.

Figure 4 presents a graphical compilation of the vocabulary used by trained and untrained panelists. It comprises a grand total of 56 terms, including 41 sensory terms (16 of which were present in the sensory lexicon developed by trained and untrained panelists) and 15 overall impression terms. Trained panelists used precise terms to describe the aromatic profile of hibiscus teas (e.g., reminiscent of dried hibiscus flowers, with berry, raisin, hay and sweet notes), whereas untrained ones were more prolific in general expressions (e.g., strong in hibiscus in Senegal; floral and fruity in Europe). Previous studies comparing the sensory vocabulary generated by untrained and trained panelists uncovered similar trends in the way trained and untrained panelists described and assessed the odor and flavor (Chollet and Valentin 2001; Hayakawa *et al.* 2010).

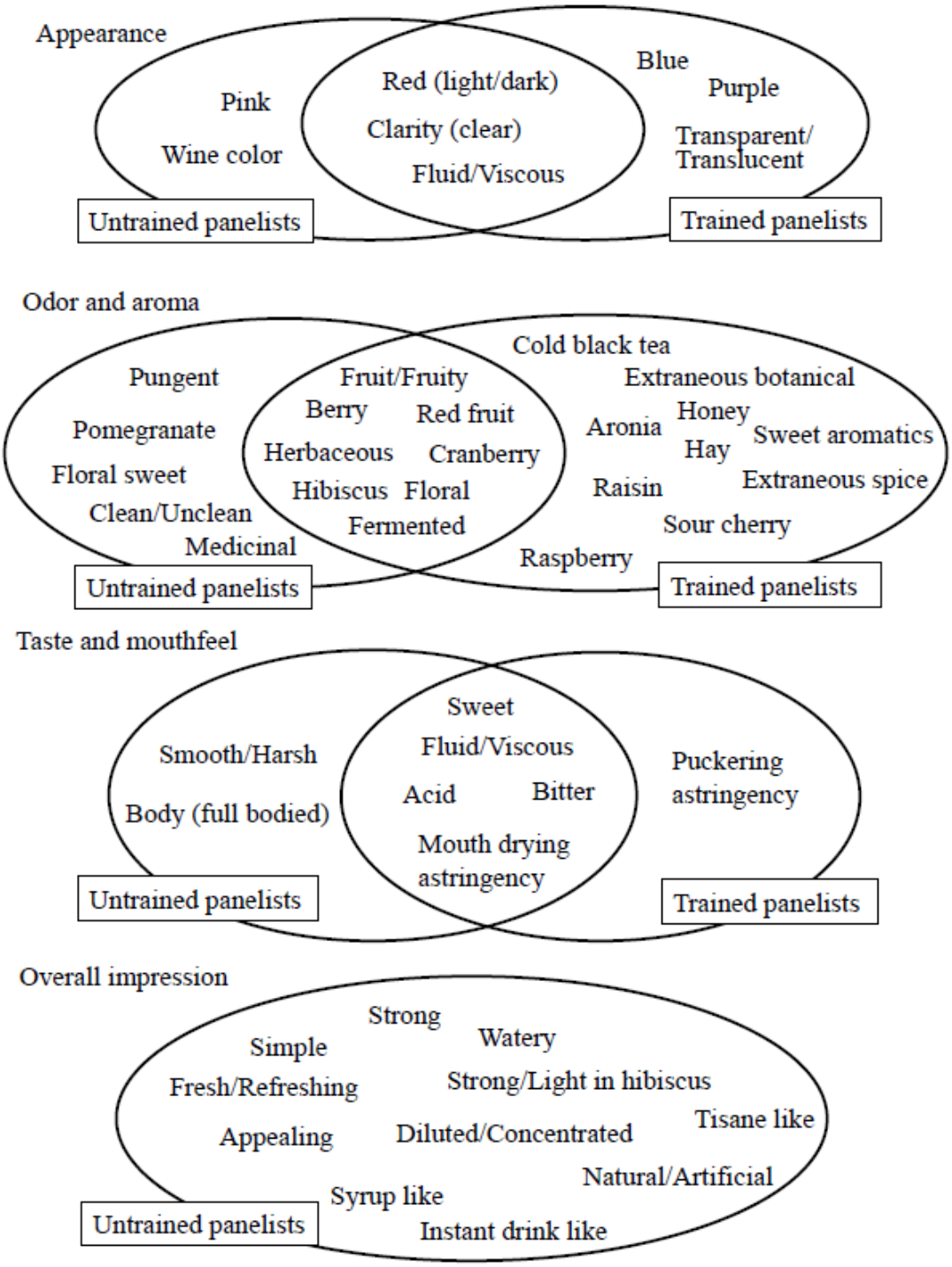

Figure 4. Hibiscus tea vocabulary of trained and untrained panellists

4. Conclusions

The vocabulary used by trained panelists, untrained professionals and consumers to describe and characterize hibiscus teas was investigated and compiled for the first time. Resulting lexicons adequately captured the main attributes known to drive the sensory quality and consumer acceptance of hibiscus teas: red color intensity, aroma character and strength, and balance between sweetness and acidity (Bechoff *et al*. 2014; Monteiro *et al.* 2017; Ramirez *et al.* 2010; Wong *et al.* 2003). Moreover, the methodology employed to this end successfully uncovered and accommodated differences rooted on subjects' sensory and professional expertise, culture and product familiarity. This brings important contributions to the sensory optimization of hibiscus tea production and the marketing of hibiscus products in international markets, as the vital role played by cross-culturally developed and validated, multilingual and easy-to-use lexicons encompassing the vocabulary of trained panelists, untrained professionals and consumers in such activities is increasingly acknowledged (Andani *et al.* 2001; Hayakawa *et al.* 2010; Kim and Lee 2016).

Notable, the comparison of consumers' sensory profiling of hibiscus teas using CATA question to the results of descriptive analysis by trained panelists yielded stable and reliable data configurations (Ares *et al.* 2015; Blanchet *et al.* 2012), providing important insights on the use and interpretation of sensory and overall impression/hedonic terms by consumers in different countries (Andani *et al*. 2001). Overall, the results presented in this paper suggest that deriving sensory vocabulary from panels of untrained professionals and consumers can yield representations of products' attributes in the minds of end-customers that are at least as valid and actionable as those resulting from trained panelists' lexicons and evaluations (Ares and Varela 2017; Hayakawa *et al.* 2010).

From a practical viewpoint, future studies carried out to validate or adapt hibiscus tea lexicons to other regions and cultures are recommended. Furthermore, blends of hibiscus with other flavoring, plant based ingredients are being increasingly used both in traditional and new beverage markets, constituting a worthy, but yet rather unexploited area of sensory research.

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CHAPTER 5

Perception and preference of hibiscus beverages: a cross-country study in Senegal, France, Portugal and United Kingdom

PERCEPTION AND PREFERENCE OF HIBISCUS BEVERAGES: A CROSS-COUNTRY STUDY IN SENEGAL, FRANCE, PORTUGAL AND UNITED KINGDOM

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Abstract

This study sought to compare acceptance and perception of hibiscus beverages by familiar consumers in Senegal and mostly unfamiliar consumers in France, United Kingdom and Portugal. Closer patterns of liking and product perception of hibiscus beverages were observed among consumers in European countries than in Senegal. Liking of hibiscus beverages appeared to mostly depend on familiarity, exposure and innate or cultural acquired preferences. In Senegal consumers favored higher intensities of red color and sweetness whereas in Europe fruity and floral character constituted important drivers of liking. Consumer segments were found, with differences rooted on individual reactions to the color and flavor of beverages, socio-demographic characteristics and consumption patterns of hibiscus and red fruit beverages (in Europe) being uncovered. Findings suggest that exotic hibiscus beverages might be more likely to be consumed in new markets if they share some similar attributes to existing products. Importantly, concomitant use of JAR and CATA questions was shown to provide valuable insights to understanding perception and liking of hibiscus beverages across cultures, countries and consumer segments.

1. Introduction

Understanding consumer preferences across countries, regions and continents is increasingly relevant to food companies acting in the global market (Astrom *et al.*, 2006). Differences in culture may reflect different customs, dietary experiences and product familiarity which have been reported to influence chemosensory perception and preference as well as expectations in relation to foods (Goldman, 2006, Prescott *et al.*, 2002, Prescott and Bell, 1995).

The segmentation of international markets according to distinct patterns of sensory preferences within a category of food products has long been advised (Moskowitz and Krieger, 1998) and is becoming increasingly relevant (Ares, *in press*). Recently, Koppel *et al.* (2014) when studying the acceptance of six pomegranate juices varying in flavor profile in four countries, found that individual consumer variations were greater than country-specific ones and that taste specific sensitivity and prior exposure were important in acceptability. These findings suggest that sensory preference segmentation, rather than country-market, may be a valuable approach for the international expansion of such beverages.

Plant based beverages which can claim health benefits are growing in popularity (Hernández-Carrión *et al.* (2015), Sun-Waterhouse, 2011, Lawless *et al.*, 2012a), particularly when made from exotic or new ingredients (Orjuela-Palacio *et al.*, 2014, Vidigal *et al.*, 2011). Still, understanding their sensory quality and consumer acceptance is key to market success (Lawless *et al.*, 2012b, Arancibia *et al.*, 2013) and remarkably few studies have examined how consumers' evaluations of such products are transferred from domestic to international markets (Jang *et al.*, 2016, Neely *et al.*, 2010a).

Hibiscus sabdariffa var. *sabdariffa ruber* flowers (hereinafter referred as hibiscus) are rich in polyphenols, organic acids and pectins which have been demonstrated to possess nephro- and hepato-protective, renal/diuretic, anti-hypertensive, anti-diabetic, hypolipidemic and anti-tumoral properties (Monteiro *et al.*, 2017a, Pérez-Jiménez *et al.*, 2010, Da-Costa-Rocha *et al.*, 2014, Patel, 2014). They are traditionally used in many countries in Africa, Asia and in the southern part of the North American continent (Cid-

Ortega and Guerrero-Beltrán, 2015, Da-Costa-Rocha et al., 2014) in the preparation of beverages, jams, confectionary and other food products. In the United States of America, Starbucks' Teavana, Argo Tea and Dunkin' Donuts, have recently developed premium lines of beverages based on hibiscus extracts, as well as herbal blends containing hibiscus (Bailey, 2015). In Europe, hibiscus products have slowly penetrated niche markets, with extracts and syrups targeting the African diaspora and consumers seeking novel and exotic drink mixes, and herbal mixtures aiming the health-conscious, all-natural, ethnic food consumers, being the main examples.

This paper aims to investigate and compare liking and perception of hibiscus beverages in traditional local market (Senegal) and in non-traditional international markets (France, Portugal and United Kingdom). The following questions are addressed: are there differences in perception and acceptance of different types of hibiscus beverages across countries? Are there consumer segments with different patterns of liking? If yes, are those differences driven by culture, familiarity and/or consumers individual specific characteristics?

2. Materials and methods

2.1. Ethical Approval

All sensory descriptive and consumer evaluation studies were conducted in accordance with the Declaration of Helsinki and their protocol approved by the Ethics Committee of the EU AFTER project (FP7 245 - 025).

2.2. Samples

Five hibiscus beverages were evaluated: a diluted-to-taste beverage prepared from syrup (SYR), a diluted-to-taste and sweetened-to-taste beverage prepared from a concentrate (CONC), a freshly brewed infusion (FINF) and two ready-to-drink infusions, one manufactured using a conventional process (CINF) and another manufactured using a new process (NINF). All beverages were prepared from a blend of dried hibiscus calyces of

Koor and Vimto cultivars (50:50 w/w). They were obtained by water extraction of whole (CINF) or ground (FINF, NINF, SYR, CONC) calyces, filtration, addition of sugar (FINF, CINF, NINF, SYR), concentration (CONC) and pasteurization (CINF, NINF, SYR, CONC). Process conditions and preparation prior to testing (if required) are presented in Table 1. All samples were stored at 6 ºC at least for 6 hours prior to testing and served cold.

Table 1. Hibiscus beverages

Sample	Process	Origin	Preparation prior to testing
Diluted-to-taste syrup (SYR)	Calyx-to-water ratio 1:10 (w/w) Water extraction: 25-30ºC, 30 min Sugar addition: up to 65ºBx Pasteurization: 75ºC, 30 min	CSFPIAA (Dakar, Senegal)	Dilution with water 1:4 (v/v)
Diluted-to-taste and sweetened-to-taste concentrate (CONC)	Calyx-to-water ratio 1:5 (w/w). Water extraction: 25-30ºC, 30 min. Concentration: 0.40 bar up to 62ºBx Pasteurization: 75ºC, 30 min.	CSFPIAA (Dakar, Senegal)	Dilution with water 1:40 (v/v), sugar addition 130 gL^{-1}
Freshly brewed infusion (FINF)	Calyx-to-water ratio 1:33 (w/w) Water extraction: addition of boiling water (98-100ºC), 20 min Sugar addition: 130 gL^{-1}	Infusion made on site	None
Conventional ready-to-drink infusion (CINF)	Calyx-to-water ratio 1:20 (w/w) Water extraction: 25-30ºC, 120 min Sugar addition: up to 15ºBx Pasteurization: 90ºC, 20 min	Société Esteval Agro Alimentaire, Dakar, Senegal.	None
New ready-to-drink infusion (NINF)	Calyx-to-water ratio 1:40 (w/w) Water extraction: 25-30ºC, 30 min Sugar addition: up to 17ºBx Pasteurization: 75ºC, 30 min	CSFPIAA (Dakar, Senegal)	None

CSFPIAA: Centre Sectoriel de Formation Professionnelle aux Métiers des Industries Agroalimentaires

2.3.Descriptive sensory analysis

Participants: Eight senior panelists (over ten years of experience in sensory evaluation), with previous qualification (ISO 11132:2012) in descriptive analysis of hibiscus beverages, participated in the study.

Procedure: The procedure and lexicon used were the same as those used by Monteiro *et al.* (2017b). Attributes color, hibiscus odor, aronia/cranberry, raisin, honey and cold black tea odor, hibiscus flavor, sweet, acid and bitter taste and astringency were evaluated. Intensities were rated using unstructured-anchored line scales (0 to 10).

2.4. Consumer studies

Consumer studies were held in Senegal, France, Portugal and United Kingdom. These locations enabled the study preference and perception in a traditional market with consumers familiar with the product (Senegal) vs. mostly unfamiliar consumers (Europe) (Cisse et al., 2009, Bennett et al., 2014). The selection of countries in Europe was determined by their long-term relationship with African countries together with availability of collaborators.

Focus group meetings to investigate hibiscus beverages perception by consumers

Participants: Semi-structured meetings (n=42; 55% men; 18-65 years old) were held in Europe and Senegal. Participants were pre-screened for high frequency of consumption of hibiscus beverages in Senegal and regular consumption of fruit juices or cold tisanes in the European countries.

Procedure: Subjects were asked to taste *ad libitum* the hibiscus beverages and to describe their attributes and impressions (sensory, hedonic and others). Vocabulary used by consumers to describe the beverages was compiled and compared across countries. To obtain reduced lists of attributes, appropriateness and relevance was assessed in collaboration with trained panellists.

Consumer testing in Senegal, France, Portugal and United Kingdom

Participants: Subjects were non-probabilistically recruited in Senegal (SN) (Dakar, N=150), France (FR) (Montpellier, N=142), Portugal (PT) (Porto, N=134) and United Kingdom (UK) (Chatham, N=127), based on willingness and availability to participate. All participants in the Senegalese study consumed hibiscus beverages, with 81% consuming them at least several times per month; their age ranged between 18 and 73 years old (M = 33.9, SD = 13.3) and 39% were female. In Europe, the majority (56%) of consumers had never consumed hibiscus products or were not sure to have ever consumed them; 41% of participants were infrequent consumers (less than several times a year) and 3% consumed these products several times per year or more; all participants consumed fruit juices or cold tisanes and 96% consumed red fruit juices. Participants' age ranged between 18 and 78 years old (M = 31.3, SD = 12.7) and 53% were female.

Procedure: Based on the results of focus group meetings with European and with Senegalese consumers, two questionnaires were used: a trilingual online questionnaire was employed in Europe (Qualtrics, LLC.) and a paper-and pencil version in French was employed in Senegal. Common questions and terms were translated from Portuguese to French and English and then back-translated by bilingual individuals to check linguistic equivalence (Ares, *in press*). Questions on socio-demographic information and on consumption of hibiscus were asked to participants in Senegal and Europe. In Europe, participants were also asked about the consumption of fruit juices, red fruit juices and cold tisanes. Consumer acceptance was evaluated by overall liking (OL) ratings provided on a 9-point hedonic scale. The intensity of color, sweet and acid taste of the product, relative to the participant ideal level, was measured using a 3-point, just-about-right (JAR) scale. Consumer sensory profiles were obtained for each sample, plus for an ideal beverage (ID) in Europe, employing check-all-that-apply (CATA) questions (Adams *et al.*, 2007, Ares *et al.*, 2010). The presentation order of JAR scales was fixed whereas the order of terms in CATA questions was randomized within subjects and across samples and countries in Europe, and within subjects and across samples in Senegal (Ares *et al.*, 2015b, Ares, *in press*). All questionnaires were pre-trialled at each test location.

Tasting sessions were conducted at recruiting sites under central location test conditions. Samples were presented in Europe as non-alcoholic beverages, whereas in Senegal they were identified as *Bissap* juice (the common local designation for hibiscus beverages). The two ready-to-drink infusions, syrup and concentrate beverages were evaluated in Senegal. In Europe, where ready-to-drink hibiscus infusions are not commercially available, but dry hibiscus calyces can be acquired, the fresh brewed infusion was evaluated along with the syrup and concentrate. Thirty millilitres of each sample (identified by a 3-digit code) were served to participants in clear plastic glasses following a balanced order. Water was supplied to clean the palate between tastings.

2.5. Data analysis

XLSTAT software (Addinsoft SARL, France) was used to carry out the statistical analyses. The significance of statistical tests was evaluated at $p<0.05$, unless stated otherwise.

To test the hypotheses of relevant country-specific differences among European countries, OL ratings were compared across countries and samples using two-way ANOVA. Since no significant interaction was found, one-way Welch's ANOVA Games-Howell post-hoc test was subsequently performed. To investigate the existence of within-country, preference-based segments, OL ratings of European and Senegalese consumers were clustered using K-means clustering. To control for relevant cultural differences in the perception and use of hedonic scales, clustering across European countries was conducted on mean-centered data (Hofstede, 1984). Within-cluster mean sample ratings were calculated, and significant differences between them tested using ANOVA with Fishers post hoc test or Welch's ANOVA with Games-Howell post hoc test (in case of dissimilar variances). Spearman or Pearson's correlation coefficients were calculated to investigate relationships among OL ratings, consumption patterns and sociodemographic variables.

Differences in gender across (European) countries and across clusters were tested with a Pearson chi-square test with Marascuilo procedure; differences in consumption frequencies of beverages were tested using Kruskal-Wallis test and multiple pairwise comparisons using Steel-Dwass-Critchlow-Fligner procedure; differences in age were tested with

ANOVA with Fisher's post-hoc analysis or Welch's ANOVA with Games-Howell post-hoc analysis.

Frequencies of too weak (TW), just-about-right (JAR) and too strong (TS) ratings were evaluated per attribute, sample and country and across clusters, and the resulting proportions calculated. To analyze similarity between sample configurations across European countries, regression vector (RV) coefficients (Robert and Escoufier, 1976) were calculated. Weighted penalty analysis (WP) was conducted to relate attribute intensity ratings to OL ratings for each sample and participant (Popper, 2014).

To analyze CATA question results, frequencies of use for each descriptor were determined by counting the number of consumers using that term to describe each sample. Cochran's Q-test, followed by the computation of multiple pair-wise comparisons with Marascuilo procedure, was employed to assess significant differences in elicitation frequencies between samples (Meyners and Castura, 2014, Meyners *et al.*, 2013). Similarity between samples configurations across European countries were assessed using RV coefficients. To obtain a bi-dimensional representation of CATA results across samples, and across samples per cluster, a correspondence analysis (CA) was performed (Meyners and Castura, 2014). Penalty-lift analysis (Williams, 2011) was finally used to relate consumers' choices of descriptors for each sample to the corresponding OL ratings.

3. Results

3.1. Descriptive sensory analysis

The widest variations across samples were observed for the intensities of the attributes red color, hibiscus odor and flavor, acid and sweet taste (Table 2). Narrow variations and low intensities of honey, raisin and cold black tea odor, bitter taste and astringency were perceived in all beverages. Intense red color, hibiscus odor and flavor, moderate aronia/cranberry odor were the main features of CONC. SYR had the strongest sweetness, the weakest acidity, bitterness and astringency and a moderate color intensity and aromatic profile (hibiscus odor and flavor and aronia/cranberry odor). FINF and NINF

Table 2– Sensory characteristics of hibiscus beverages

Sample	Red Color	Hibiscus odor	Aronia/ Cranberry odor	Hay odor	Honey odor	Cold Black Tea odor	Raisin odor	Hibiscus flavor	Acid taste	Sweet teste	Bitter taste	Astringency
SYR	$5.5^{b}\pm0.3$	$3.9^{bc}\pm0.6$	$3.1^{b}\pm0.5$	$1.9^{b}\pm0.5$	$2.2^{a}\pm0.2$	$1.7^{a}\pm0.3$	$1.4^{a}\pm0.5$	$5.2^{cd}\pm0.5$	$2.9^{c}\pm0.3$	$9.2^{a}\pm0.3$	$0.3^{c}\pm0.2$	$0.4^{b}\pm0.1$
CONC	$9.3^{a}\pm0.2$	$6.5^{a}\pm0.7$	$3.1^{b}\pm0.2$	$1.6^{b}\pm0.5$	$1.8^{b}\pm0.2$	$1.6^{a}\pm0.2$	$1.2^{a}\pm0.0$	$7.5^{b}\pm0.3$	$8.7^{b}\pm0.6$	$6.0^{c}\pm0.2$	$1.5^{b}\pm0.3$	$1.0^{a}\pm0.2$
FINF	$8.8^{a}\pm0.7$	$7.4^{a}\pm0.8$	$3.7^{a}\pm0.2$	$2.7^{a}\pm0.3$	$1.7^{b}\pm0.3$	$1.7^{a}\pm0.4$	$1.1^{a}\pm0.1$	$8.2^{a}\pm0.2$	$8.4^{b}\pm0.8$	$7.0^{b}\pm0.4$	$1.2^{b}\pm0.2$	$1.1^{a}\pm0.5$
NINF	$9.1^{a}\pm0.2$	$6.4^{a}\pm0.7$	$2.9^{b}\pm0.2$	$1.6^{b}\pm0.3$	$1.0^{c}\pm0.1$	$1.6^{a}\pm0.2$	$1.2^{a}\pm0.0$	$6.1^{c}\pm0.3$	$11.9^{a}\pm0.8$	$4.2^{d}\pm0.2$	$2.5^{a}\pm0.5$	$1.5^{a}\pm0.3$
CINF	$3.2^{c}\pm0.1$	$2.9^{c}\pm0.3$	$1.8^{c}\pm0.1$	$1.3^{b}\pm0.1$	$0.5^{d}\pm0.1$	$1.5^{a}\pm0.2$	$0.9^{a}\pm0.4$	$4.8^{d}\pm0.2$	$10.5^{a}\pm0.7$	$3.6^{d}\pm0.4$	$2.6^{a}\pm0.4$	$1.4^{a}\pm0.3$

Mean ± standard deviation. Values with different superscripts within a column indicate significant differences ($p<0.05$).

infusions presented intense red color, strong aromatic character and intense acidity whereas CINF had the weakest red color intensity and aromatic character.

3.2. Consumer studies

Focus group meetings

A reduced list of 24 hedonic, sensory and overall impression terms describing hibiscus beverages resulted from the focus group meetings held in Europe: light red, dark red, red fruits, fruity, floral, acid, bitter, sweet, astringent, syrup like, balanced flavor, natural, artificial, watery, tisane, simple, strong taste, invigorating, refreshing, healthy antioxidant, different/unknown, high calorie and new. In Senegal 22 terms were obtained, 14 of which were common to Europe: light red, dark red, acid, bitter, sweet, astringent, syrup, balanced flavor, natural, artificial, watery, invigorating, refreshing and healthy. The eight uncommon terms were: clear, sharp odor, light in hibiscus, strong in hibiscus, good taste, appealing, nutritional and traditional. These terms were used in CATA questionnaires. In Europe, prior to tasting most participants in focus group sessions misidentified hibiscus beverages as red wine containing beverages and after tasting as fruit beverages containing red fruits. It was hence decided to present samples to participants in European consumer tests as non-alcoholic beverages without specifying origin or ingredients.

Overall liking of hibiscus beverages

In Senegal, hibiscus beverages were slightly (CINF M = 5.7, SD = 1.6; NINF M = 6.2, SD = 1.7) to moderately liked (CONC M = 7.1, SD = 0.9; SYR M = 6.7, SD = 1.1) with significant differences in OL being found for all beverages. Frequencies of consumption of hibiscus beverages were correlated with OL of SYR and CONC ($p<0.01$). Significant relationships between age and gender with OL of NINF were observed, with men and older participants liking the NINF more ($p<0.01$) than women and younger participants.

Similar patterns of liking of hibiscus beverages were observed for the three European countries with no significant sample*country interactions being found. Unlike Senegal,

SYR was the most liked beverage (M=6.5, SD=1.6) preferred to CONC (M=6.0, SD = 1.9). FINF (M = 5.6, SD= 2.1) was the least liked beverage. Significant positive correlations were found between age and OL of CONC and FINF. Significant positive correlations were also found between the frequency of consumption of hibiscus products and red fruit juices with OL of FINF ($p<0.01$) and between the frequency of consumption of red fruit juices and OL of SYR. No relationships were observed between the consumption of fruit juices and cold tisanes and liking of hibiscus beverages.

Clustering revealed three consumer segments with different patterns of liking of hibiscus beverages in Senegal (SN1 to SN3) (Table 3) and four segments in Europe (EU1 to EU4) (Table 4). In Senegal and Europe, the larger clusters (SN1, 50% participants; EU1, 43% participants) were comprised by subjects who liked all hibiscus beverages and on average liked them more than subjects in the remaining clusters, whereas the smaller ones (SN3, 23% participants; EU4 13% participants) were constituted by subjects that liked hibiscus beverages less than in remaining clusters. In Senegal consumers in all clusters liked CONC and SYR but not the infusions, whereas in Europe only SYR was liked by consumers in all clusters. Some socio-demographic differences were observed across clusters: SN1 was constituted by larger proportion of males than SN3, consumers in EU1 were older than in EU4 and this last cluster was constituted by a much lower proportion of French consumers than the remaining clusters. No significant correlations between OL ratings and consumption of hibiscus beverages were found for SN1 to SN3. In Europe, frequency of consumption of hibiscus products was positively correlated with OL of CONC ($p<0.01$) and FINF (<0.01) in EU1 and with FINF in EU3; frequency of consumption of red fruit juices was positively correlated with OL of FINF in EU1. No significant differences and no relationships were found between the consumption of fruit juices and cold tisanes and liking of hibiscus beverages across clusters in Europe (data not shown).

Table 3. Clusters socio-demographic characteristics, consumption patterns and hibiscus beverages liking in Senegal

Senegal	SN1 (N=75) Hibiscus beverages likers	SN2 (N=40) Conventional infusion dislikers	SN3 (N=35) Infusions non-likers
Gender (% Female)	31% ^{B}	35% ^{B}	63% ^{A}
Age (mean ±st. dev.)	34.7 ± 13.5	34.2 ± 13.9	32.2 ± 11.8
Hibiscus beverages consumption:	SN1^{A}	SN2^{A}	SN3^{A}
At least several times a month	83%	80%	77%
Several times a year	17%	20%	23%
Hibiscus beverages overall liking:	SN1^{A}	SN2^{B}	SN3^{C}
SYR	6.8^{b} ± 1.1	6.6^{ab} ± 0.8	6.4^{a} ± 1.4
CONC	7.2^{a} ± 0.9	7.0^{a} ± 1.0	6.9^{a} ± 0.9
NINF	7.2^{a} ± 1.0	6.6^{ab} ± 1.0	3.7^{c} ± 1.0
CINF	6.7^{b} ± 0.8	4.0^{c} ± 0.8	5.4^{b} ± 1.8

Different uppercase case letter/superscripts indicate significant differences at $p<0.05$ across clusters
Different lower case superscripts indicate significant differences at $p<0.05$ within clusters

Table 4. Clusters socio-demographic characteristics, consumption patterns and hibiscus beverages liking in Europe

	EU1 (N=176) Hibiscus beverages likers	EU2 (N=93) Infusion dislikers	EU3 (N=82) Concentrate dislikers	EU4 (N=52) Hibiscus beverages non-likers
Gender (% female)	54% ^{A}	51% ^{A}	46% ^{A}	64% ^{A}
Age (mean ±st. dev)	32.6^{A} ± 12.5	32.0^{A} ± 12.1	30.8^{AB} ± 12.7	26.8^{B} ± 13.0
France	34% ^{A}	34% ^{A}	43% ^{A}	11% ^{B}
Portugal	33% ^{A}	35% ^{A}	19% ^{A}	46% ^{A}
United Kingdom	33% ^{A}	30% ^{A}	20% ^{A}	42% ^{A}
Hibiscus products consumption:	EU1^{A}	EU2^{B}	EU3^{AB}	EU4^{AB}
At least several times a year	4%	0%	6%	0%
Less than several times a year	45%	33%	42%	43%
Never consumed/ Do not know	55%	67%	51%	57%
Red fruit juices consumption:	EU1^{A}	EU2^{A}	EU3^{A}	EU4^{A}
At least several times a month	64%	56%	68%	54%
Several times a year	35%	41%	30%	31%
Less than several times a year	1%	3%	1%	15%
Hibiscus beverages overall liking:	EU1^{A}	EU2^{B}	EU3^{B}	EU4^{C}
SYR	6.5^{b} ± 1.5	6.6^{a} ± 1.5	6.7^{a} ± 1.6	5.5^{a} ± 1.9
CONC	7.3^{a} ± 0.8	6.8^{a} ± 0.9	4.3^{b} ± 1.2	2.7^{b} ± 1.9
FINF	7.1^{a} ± 0.9	3.5^{b} ± 1.1	6.7^{a} ± 0.8	2.4^{b} ± 1.1

Different uppercase case letter/superscripts indicate significant differences at $p<0.05$ across clusters

Different lower case superscripts indicate significant differences at $p<0.05$ within clusters

Attribute intensity ratings using JAR scales

Figure 1 depicts consumer evaluations of color, acid and sweet taste intensities as TW, JAR and TS intensities in Senegal for all consumers [1a)] and per cluster [1b)]. For CONC, the local preferred beverage, color intensity, sweet and acid taste were judged just-about-right by most consumers (59% to 69% of consumers). Although SYR presented a strong sweetness it was predominantly judged just-about-right for sweet taste (55% of consumers), conversely its moderate color and weak acidity were considered too weak by the majority of respondents (70% and 51%, respectively). The strong acidity of NINF and CINF infusions and the strong color of NINF were predominantly perceived as too strong (51% to 55% of respondents) whereas their moderate sweetness was perceived as too weak (47% and 65% of respondents, respectively). Similar attribute intensity ratings were observed across clusters for CONC and SYR (data not shown), whereas for infusions significant differences were observed. For NINF color and acid taste evaluations by consumers in SN3 were significant deviated towards too strong ratings relatively to the other clusters whereas sweet taste was deviated towards too weak ratings. For CINF sweetness and acid taste evaluations of consumers in SN3 were also deviated towards too weak and too strong ratings, respectively, relatively to SN1.

In European countries, paired comparisons across countries of intensity ratings of color, sweet and acid taste using JAR scales by means of RV coefficients, yielded values between 0.919 and 0.989 ($p<0.001$). Such high values have been previously regarded as indicators of good agreement between sample configurations (Lelièvre *et al.*, 2008, Ares *et al.*, 2015a). Analysis of pooled data from the three countries was hence performed (Figure 2). The moderate red color intensity and weak acidity of SYR were considered just-about-right by most participants in Europe (76% and 65% of consumers, respectively) whereas its strong sweetness was predominantly considered too strong (52% of consumers). Similarly to what was observed in Senegal, comparison of ratings across clusters in Europe showed similar evaluations for SYR (data not shown). For CONC and FINF, however, differences in perception across clusters were readily apparent. Analysis showed that evaluations of color and acid taste in clusters EU3 and EU4 were deviated towards too strong ratings relatively to EU1 and EU2 ($p<0.05$); for FINF sweetness of EU3 was deviated towards too weak appreciations relatively to all other clusters ($p<0.01$).

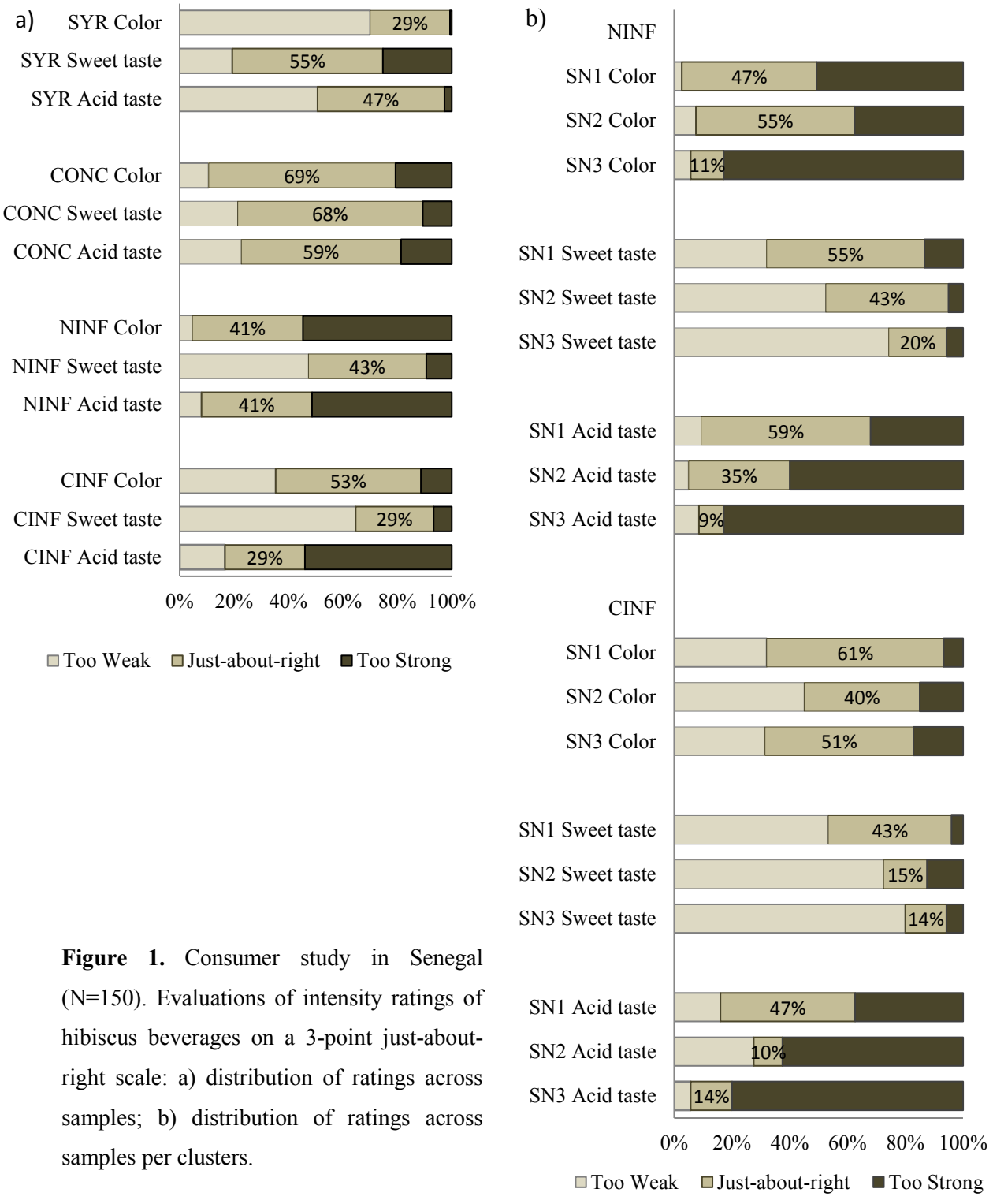

Figure 1. Consumer study in Senegal (N=150). Evaluations of intensity ratings of hibiscus beverages on a 3-point just-about-right scale: a) distribution of ratings across samples; b) distribution of ratings across samples per clusters.

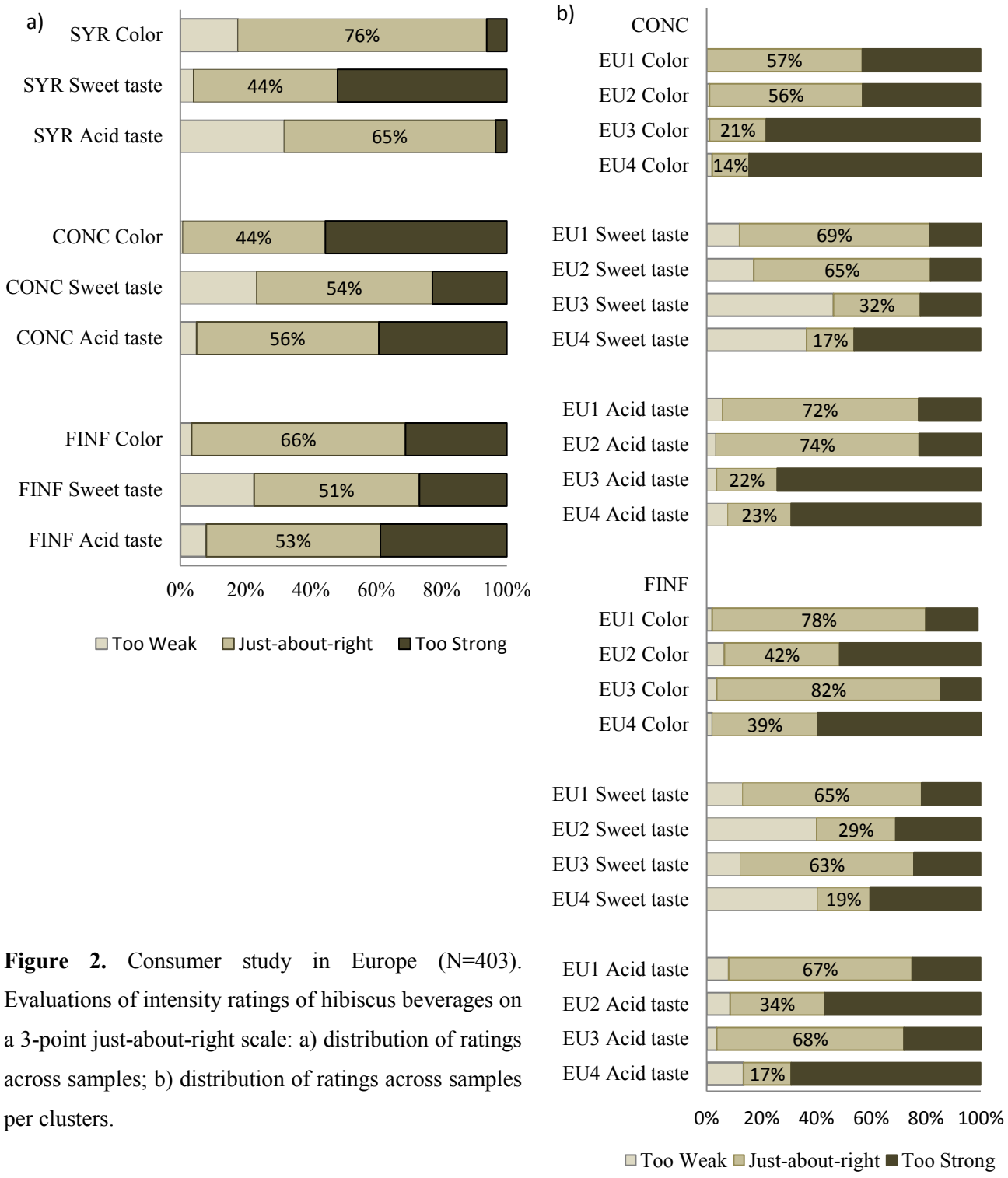

Figure 2. Consumer study in Europe (N=403). Evaluations of intensity ratings of hibiscus beverages on a 3-point just-about-right scale: a) distribution of ratings across samples; b) distribution of ratings across samples per clusters.

Consumer sensory profiles using CATA questions

In Senegal, consumers used between two and 14 CATA terms to describe each beverage, with an average of six terms. The most frequently selected term was natural (47%), refreshing (38%) and healthy (36%) and the less frequently selected terms were artificial (8%) sharp odor and astringent (7%). No significant differences in elicitation frequencies across samples and across clusters were found for terms syrup like, artificial and traditional, which were hence not considered for further analysis. The first two dimensions of the CA bi-plot accounting for 89.0% of the variability representing variables and samples in the Senegalese study are depicted in Figure 3a). Unsurprisingly, most hedonic and positive related terms were mainly associated by consumers to the most liked beverage CONC (balanced flavor, good taste, appealing, natural, nutritional, healthy, refreshing and invigorating). Light in hibiscus, light red, watery and sweet were the main terms used to describe SYR. For NINF main terms were dark red, strong in hibiscus, invigorating and acid and for CINF these were light red, bitter and acid. In line with JAR results, analysis of CATA data at cluster level showed that CONC and SYR were perceived in a rather similar way by consumers across clusters, but not NINF and CINF. Consumers in cluster SN1 described NINF in a similar way of CONC whereas consumers in SN3 described it chiefly as acid, bitter, astringent and with sharp odor, consumers in SN2 were in between these two. For CINF a significantly higher frequency of elicitation of bitter term was found for consumers in SN2 than in SN1, whereas light red/acid were less/more elicited by consumers of SN3 than SN1, respectively.

Expectedly, positive penalty lifts were observed for terms balanced flavor, good taste, appealing, invigorating, healthy, nutritional and refreshing [Figure 3b)] and also for sweet taste. For bitter, acid, and astringent, negative penalty lifts were observed for all clusters. Differences across clusters were observed for terms light red, watery and dark red with opposed signs significant penalty lifts being observed in SN1 and SN3.

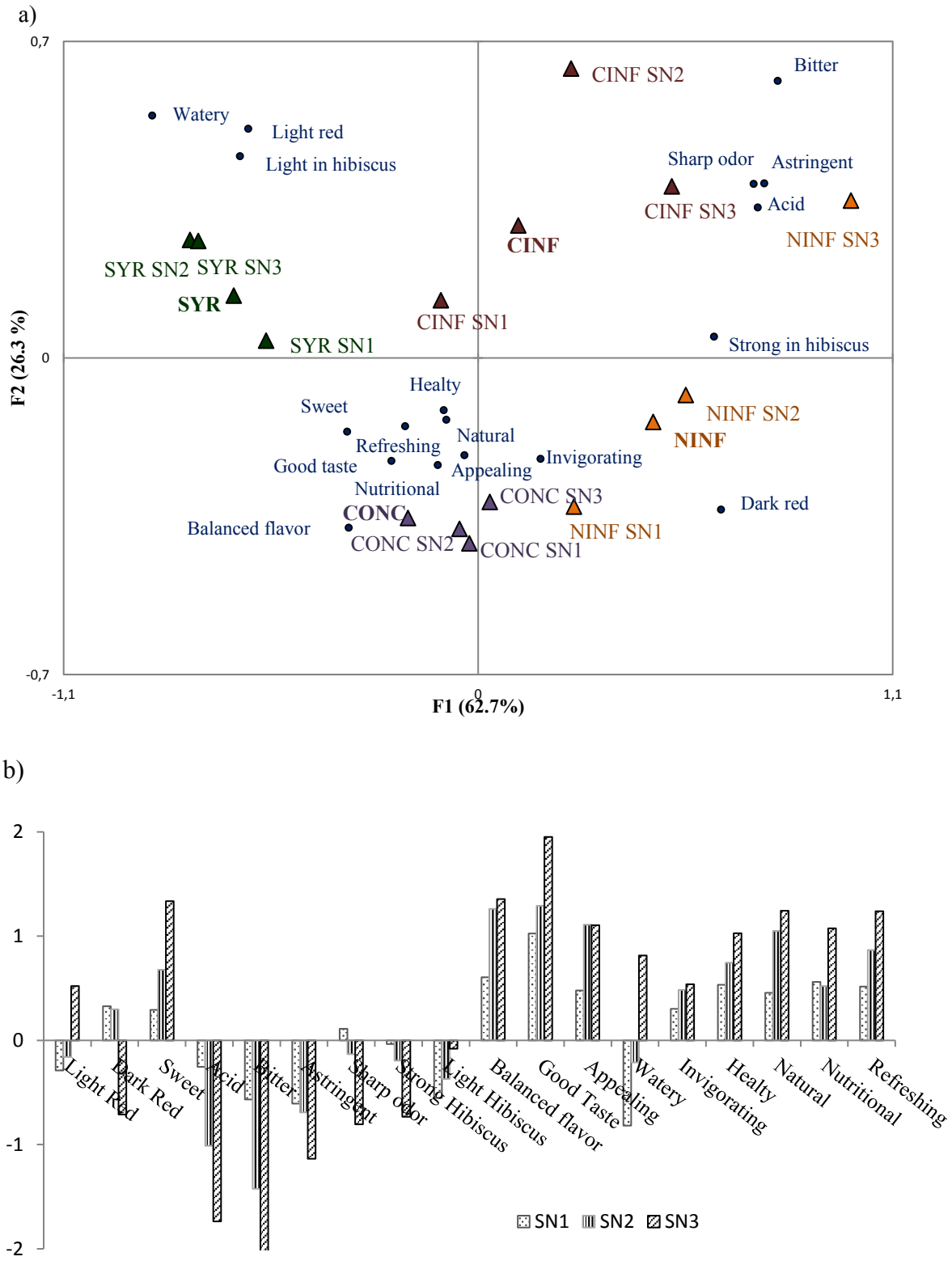

Figure 3. Consumer study in Senegal (N=150). a) Correspondence analysis plot of CATA terms and sample configurations obtained from evaluations of all consumers and for each cluster; b) penalty lift analysis relating consumer acceptance to elicitation frequencies of CATA terms per cluster.

In Europe CATA questions were used to describe the tested beverages plus an ideal beverage. Participants elicited between one and 15 CATA terms to describe each beverage, with an average of five terms. The less frequently selected terms were tisane, new and different/unknown (5% to 14%) whereas red fruit (50%) and fruity (44%) the most elicited terms. These results confirmed the initial impression that participants in general did not recognize hibiscus beverages as tisanes or extracts of a flower seldom used in Europe, misidentifying the samples as (red) fruit juices. Terms new and red fruit were non-discriminating in all countries for all samples including the ideal beverage. Terms balanced flavor, artificial, healthy, natural, refreshing, invigorating, antioxidant and simple were non-discriminating for the evaluated beverages but significant higher frequencies of elicitation were used to describe the ideal beverage.

In line with attribute intensity evaluations using JAR scales, paired comparisons of CATA counts across countries yielded also very high RV coefficients (between 0.959 and 0.997, $p<0.01$), indicating the high degree of similarity of the sensory spaces. Comparison of elicitation frequencies among samples across clusters uncovered significant differences for all terms, except new. Since this term was also not discriminant among samples at country level, it was not considered for further analysis. The representation of pooled results for the three countries and per cluster in the first two dimensions of the correspondence analysis of the CATA counts, explaining 87.5% of variability, is presented in Figure 4a). Fruity, sweet, watery, syrup, high calorie and light red were SYR main sensory descriptors. Also in line with JAR results, analysis at cluster level showed that this beverage was perceived in a similar way by consumers across clusters. Similar profiles were observed for CONC for subjects in EU1 and EU2 and for FINF for clusters EU1 and EU3, these beverages being described as fruity, refreshing, antioxidant, red fruit and dark red. Similar descriptive profiles were also observed for CONC for clusters EU3 and EU4 and FINF for clusters EU2 and EU4, these consumers described the beverages as having a strong, bitter and acid taste, astringent mouthfeel and different/unknown character. The ideal beverage, ID, was mainly described using non-sensory and hedonic terms (balanced flavor, natural, refreshing, healthy, simple and antioxidant) however significant differences in elicitation frequencies across clusters were uncovered.

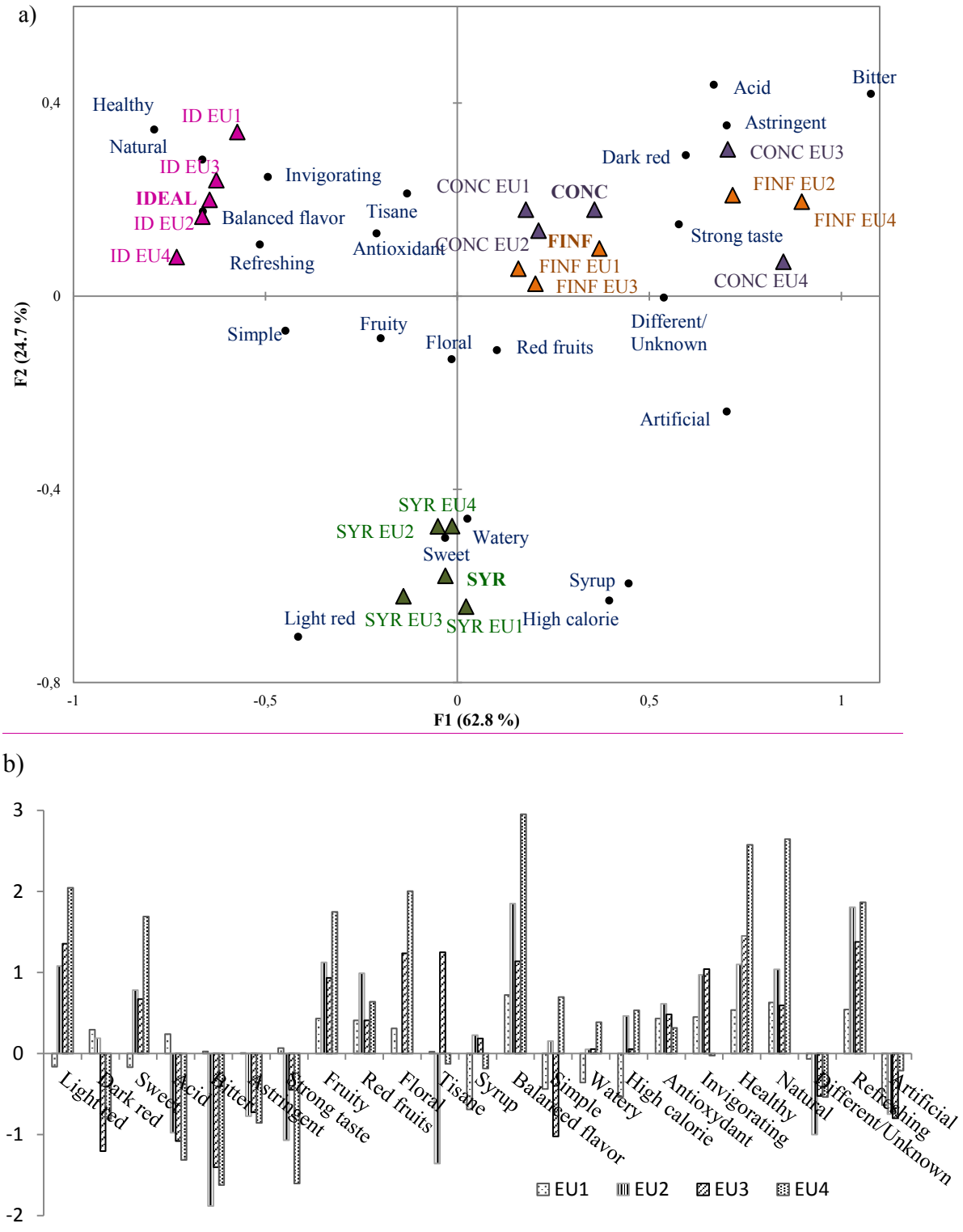

Figure 4. Consumer study in Europe (N=403). a) Correspondence analysis plot of CATA terms and sample configurations obtained from evaluations of all consumers and for each cluster; b) penalty lift

analysis relating consumer acceptance to elicitation frequencies of CATA terms per consumer cluster.

Consumers in EU1 described ID less often as sweet and light red than consumers in EU2 to EU4 and more often as acid, dark red and with strong taste. The penalty lift analysis for referred terms reflects these differences in perception across clusters: significant positive penalty lifts were found for terms sweet and light red for clusters EU2 to EU4, but not for EU1, whereas significant negative penalty lifts were found for dark red (EU3 and EU4), acid and strong taste (EU2 to EU4) but not for EU1 [Figure 4b)]. Expectedly, positive penalty lifts were observed for terms balanced flavor, antioxidant, invigorating, healthy, natural, refreshing fruity, red fruit and floral for all consumers whereas for artificial and different/unknown negative penalty lifts were observed. Term high calorie was associated with a significant negative penalty lift only for EU1.

4. Discussion

Focus group meetings showed that vocabulary used by consumers to describe hibiscus beverages was closer across European countries than in Senegal, mainly with respect to odor, aroma and overall impression terms. Consumer's individual experiences, familiarity with hibiscus products (by Senegalese), and with red fruit stimuli (European) have likely contributed to these differences. Tu *et al.* (2010) for soy yogurts evaluated by French and Vietnamese consumers and Yang *et al.* (2012) for perilla oil evaluated by American, Chinese and Korean panels, also reported that subjects used different descriptors that related to their cultural background, individual experiences and consumption habits to characterize the samples.

Familiarity and repeated exposure are known to be generally positive factors for food acceptance (Hong *et al.*, 2014, Borgogno *et al.*, 2015, Chung *et al.*, 2012, Koppel et al., 2014) whereas limited or a complete lack of prior experience with a food may result in poor consumer acceptability (Verbeke and Vackier, 2004). On the present study, besides the generally higher OL ratings of hibiscus beverages observed in Senegal than in European countries, a positive significant relationship between consumption of hibiscus products and

OL of FINF was uncovered in Europe. Moreover, high significant correlations between consumption of red fruit juices and OL of FINF and SYR were found indicating that exposure and (liking) of familiar products with somewhat similar sensory characteristics may constitute a tendency to like unfamiliar products which share similar attributes. Similar findings were earlier reported by Tuorila *et al.* (1998) who concluded that acceptance of unfamiliar foods by consumers was largely determined by how it related to familiar foods that were part of their diet.

Age seemed to have a positive impact on liking of NINF in Senegal and FINF and CONC in Europe, a possible explanation may reside on the lower aversion to high acidity with increasing age (Zandstra and de Graaf, 1998).

There is little evidence of cross-cultural differences in the sensory perception and hedonic evaluation of foods when products have similar levels of familiarity in the countries being compared (Jaeger *et al.*, 1998). This seems to be the case even when culturally novel foods are being assessed as long as consumers from different countries share similar levels of familiarity with the product category and with its general flavor profile (Neely *et al.*, 2010b, Neely et al., 2010a). This much can explain the closeness between OL mean ratings among European countries; still the much lower proportion of French consumers observed in EU4 may constitute a hint of cultural related hedonic differences among European consumers.

Comparison of ratings of color, sweetness and acidity using JAR scales showed a rather different perception of the appropriateness of these attributes by consumers in Europe and Senegal, as results indicate that generally Senegalese consumers favoured higher intensities of red color and sweetness than European consumers who favoured less weak acidities. Past studies in Senegal, Nigeria and US (Florida) also showed contrasting preferences to West African consumers who may be familiar with a product while US consumers were not. African consumers expected and preferred hibiscus beverages with a dark red color, strong aromatic profile and with high acidity and sweetness (but neither overly tart, in the case of infusions, nor overly sweet, in the case of syrups) attributes that positively contribute to their judgements of product typicality and quality (Bolade *et al.*, 2009, Foline *et al.*, 2011, Bechoff *et al.*, 2014). Largely unfamiliar US consumers seemed to appreciate more hibiscus

beverages with a brighter red color, but considerably blander in terms of hibiscus aroma and acid taste (Ramirez *et al.*, 2010).

Comparison of OL ratings and JAR evaluations of CINF and NINF in Senegal may indicate the high detrimental impact of the weak color of CINF on liking. Congruency between color and taste perceptions have been shown to have a positive impact on the acceptance among consumers as it was associated to judgements of product typicality (Delwiche, 2004), contributing to the observed difference between the two infusions.

Besides prior exposure or product familiarity, results showed that also individual variations were very important in acceptability and perception of hibiscus beverages both in Europe and Senegal. Three consumer segments with similar patterns of liking in Senegal and four segments in Europe were hence uncovered. Innate responses to some tastes, like sweet, acid and bitter as well as sensitivity (known to vary greatly for astringency and bitter taste), influence consumer liking (Koppel et al., 2014, Dinnella *et al.*, 2011, Dinehart *et al.*, 2006). This much can help explain differences in liking across clusters which were well reflected in the appropriateness ratings using JAR scales and on penalty lift analysis on CATA sensory terms. These show, for instance, that consumers in clusters EU1 and SN1 were more prone to accept and favour higher intensities of color, acidity, bitterness and astringency and lower levels of sweetness of hibiscus beverages than those in other clusters. Nevertheless, comparison of relative positioning of CATA term sweet in Senegal and Europe indicate that culturally acquired preferences have likely influenced consumer liking and perception of sweetness, as in the first this term was positioned close to good taste and balanced taste whereas in the last was located near to high calorie and watery terms.

JAR scales are a well-established approach to identifying the optimal intensity of sensory attributes (Popper, 2014). More recently, CATA questions have been demonstrated to provide information on the main deviations from the ideal and recommendations for product improvement (Ares *et al.*, 2017). In Senegal, for CONC, the high hedonic ratings observed for all clusters, the close proximity of CONC relatively to positive and hedonic related CATA terms, taken together with JAR evaluations, show no drivers for further improvement for this beverage in the local market. Therefore it should be adequate to consider this beverage has having an optimal or even an ideal sensory profile for Senegalese consumers.

Deviations from ideal for the remaining beverages in this market can therefore be assessed also by comparison of their sensory characteristics either evaluated by consumers or by trained panellists with CONC. In Europe, consumers' self-reported ideal beverage (ID) was chiefly described using non-sensory terms which cannot provide clear nor objective directions for product improvement and were unspecific of the type of beverage tested. This result can partially derive of asking respondents to describe the ideal characteristics of an unfamiliar product. Surprisingly, SYR, although being the preferred hibiscus beverage liked by subjects in all clusters, presented a very distinct sensory profile when compared with ID. A much closer proximity to ID was found between CONC profile described by subjects in clusters EU1 and EU2, and for FINF described by subjects in clusters EU1 and EU3, than for SYR. These results indicate that although all clusters described the theoretical ideal hibiscus beverage in a rather similar way, the sensory characteristics of such beverage is likely to differ across clusters.

5. Conclusions

Liking and perception of hibiscus beverages in Senegal, France, United Kingdom and Portugal was investigated. Rather similar patterns of acceptance and perception for hibiscus beverages were observed among consumers in Europe, but important differences were observed relatively to Senegal. The origin of such differences appeared to mostly depend on familiarity to hibiscus beverages and culturally acquired preferences. Both in Europe and Senegal, however, individual preferences of color intensity, sweet and acid taste as well as sensitivity to bitter and astringency seemed to greatly contribute to consumer segmentation. Besides familiarity to the product category, liking and/or exposure to products with somewhat similar characteristics (like red fruit juices) seemed to impact hibiscus beverages acceptance in Europe. Findings suggest that exotic hibiscus beverages might be more likely to be consumed in non-traditional/new markets if they share some similar attributes or perceptions to existing products. Ideal beverage profiling by consumers could not provide *de per se* clear and objective drivers of product improvement in Europe, but concomitant use of JAR and CATA questions was shown to provide valuable insights to understanding

perception and liking of hibiscus beverages across cultures, countries and consumer segments.

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CHAPTER 6

Conclusions and suggestions for future research

1. Conclusions

This thesis has brought new insights about the production and commercialization of hibiscus products, introduced new sensory methodologies for the analysis of hibiscus beverages and explored cross cultural preferences for unfamiliar foods, all of which are topics relatively under researched.

Studies conducted assessed the relationships between ingredients and process variables with the main physicochemical and sensory properties of novel hibiscus beverages and corresponding production processes developed by the AFTER project. Product and process improvements were in this way investigated. It was concluded that the use of a blend of shade-dried, ground hibiscus calyces of Vimto and Koor cultivars and of mild extraction and pasteurization conditions led to the production of beverages with higher monomeric anthocyanin, total phenolic content and antioxidant capacity then their traditional counterparts. Furthermore, the use of an under-vacuum concentration process led to a new product with excellent chemical-sensory properties.

Furthermore, it was shown that all novel products were well accepted by consumers in Senegal (where hibiscus beverages are the national drink), being actually preferred to the conventional ones. Achieving a favourable balance between sweetness and acidity intensities turned out to be crucial to guarantee consumer acceptance. Consumer segments with highly distinct patterns of liking of hibiscus syrups and ready-to-drink infusions were identified and distinctive preferences relatively to colour intensity and aromatic character were uncovered. Such findings are relevant to the development of new products and for sustainable market growth of local markets.

Acceptance and consumer perception of traditional and novel hibiscus beverages were subsequently explored outside Senegal, namely in France, United Kingdom and Portugal. Results of overall liking ratings, just-about right scaling and check-all-that apply questions were compared to those obtained in Senegal. This showed that product acceptance was significantly more driven by degree of familiarity with the product, personal preferences and socio-demographic factors, than by cross-cultural differences among European consumers. Namely, generally similar patterns of acceptance and perception for the beverages were observed among mostly unfamiliar consumers in Europe. These consumers often misidentified hibiscus beverages as red fruit beverages

and, in general, liked them less than consumers in Senegal. They also favoured beverages with milder sensory characteristics (weaker colour, sweetness and acidity), with fruity and floral aromatic characteristics constituting the main determinants of acceptance. These insights are important for the development and marketing of new hibiscus beverages outside traditional markets.

Prior to this dissertation, there was a lack of standardised approaches for identifying the sensory characteristics of hibiscus products in general, and beverages in particular. In view of this, sensory lexicons based on a series of studies with trained and untrained panellists across multiple countries were developed and applied for the first time.

Firstly, trilingual sensory lexicons for trained panellists were defined, referenced and assembled in a sensory wheel. Variations between beverage types (infusions, syrups, concentrates, instant drinks) were uncovered and relationships with raw materials and production process variables described. This provides practical support for future product optimization processes, aiding in hibiscus cultivar selection and breeding, harvest and post-harvest operations and beverage processing methods. Food producing and trading companies do not always have the possibility to train or get access to trained panellists. In such cases, the use of untrained professionals and consumers may provide relevant product descriptions for marketing research, sales and customer support. In view of this, a new multistage method – involving semi-structured meetings with untrained professional, focus groups with consumers and consumer sensory profiling of hibiscus beverages using check-all-that-apply questions -, was employed to subsequently develop graphical lexicons of hibiscus beverages for untrained panellists.

Taken together, these studies and the new methodology developed enabled to uncover and accommodate differences in product evaluation rooted on subjects' sensory and professional expertise, culture and product familiarity. Their outputs will further contribute decisively to facilitate the communication between the actors involved in the development, production and marketing of hibiscus extracts and products thereof across Africa, Europe and North/South America.

2. Suggestions for future research

The high failure rates of new products in the marketplace continues to be a major concern in the food industry (Næs et al., 2015). Past behaviour, habit and hedonic appreciation are the usual predictors of food choice behaviour (Köster, 2009). Nevertheless, perception and liking of new products may evolve with repeated exposure and increased familiarity (Dalenberg et al., 2014, Monteiro et al., 2016). In spite of this, the overwhelming majority of new food products are tested only prior, and not after, market launch for consumer liking and preference, most often on the basis of a single exposure test performed under blind testing conditions. The dynamic evolution in consumers' responses during exposure to a product over time questions thus the predictive value of single hedonic measurements for future liking and choice, being particularly relevant when launching new food products (Soerensen et al., 2015).

Research addressing the effects of repeated exposure to new products is still relatively scarce and its findings are often contradictory. Orjuela-Palacio et al. (2014), for instance, found that repeated exposure to new yerba mate/black currant beverage increased liking. Sulmont-Rossé et al. (2008) also studied the effect of repeated exposure on the liking of drinks, in this instance flavoured drinks, but concluded that positive effects were dependent on the arousal potential of products, with significant positive effects of exposure being observed only for fruit drinks with high arousal potential. Soerensen et al. (2015), on the other hand, found no relationship between pre-post exposure acceptance of chocolate products and arousal or complexity ratings, as well as no significant differences between pre- and post-exposure liking of products. In addition, Kinnear and de Kock (2011) found that exposure to sports drinks could actually lead to a decrease, rather than an increase in liking.

Whenever we are presented with a food product, we rapidly create an opinion (or a prediction) about the flavour, the oral-somatosensory and auditory properties of what we see and/or smell (Small, 2012, Spence and Piqueras-Fiszman, 2014) and make a judgment about how much we are going to like the experience (hedonic expectancy) (Cardello and Sawyer, 1992, Woods et al., 2011). In the case of new or unfamiliar products, this is based primarily on any previous experiences that we may have had with that food or foods perceived to be similar. As such, everything we perceive about the

product prior to consumption can set up powerful expectations in our mind about what we are about to experience (Spence and Piqueras-Fiszman, 2014).

Furthermore, consumers intuitively develop cross-modal associations between appearance, flavour, aroma and taste characteristics of foods. Such associations will be perceived as congruent if there is a high degree of fit among sensory characteristics of the product, or incongruent otherwise. Perception of sensory congruency is shaped by culture and product familiarity, as well as by individual characteristics, like age, gender, food neophobia or variety seeking traits (Piqueras-Fiszman and Spence, 2014, Spence et al., 2010, Spence and Piqueras-Fiszman, 2014). Congruency between appearance, odour and taste impressions have been shown to a major impact on food perception and acceptance (Delwiche, 2004, Wansink *et al.*, 2005, Yeomans *et al.*, 2008, Piqueras-Fiszman and Spence, 2015, Wan *et al.*, 2014, Spence et al., 2010), as generally it leads to experiences that the consumer finds preferable (Lindstrom, 2005).

Still, congruency is highly dependent on product information and consumer inferences and expectations as well. The need to try new foods, and the shifts in accepted fashions for them, occurs in all societies, particularly in those were food is plentiful. This trait may be driven by a basic human desire for sensory stimulation, novelty and change to counteract product boredom. In recent years, intentional use of sensory incongruity has gain some adepts among food developers in search for novelty, as reactions to such products will likely include attentional capture, surprise and memorability (Spence and Piqueras-Fiszman, 2014), all ofwhich can play an important role in product choice.

Hibiscus beverages configure a rare situation where an unfamiliar product is misidentified by many European consumers as a familiar red fruit juice, and related stimuli and evaluation context can be easily manipulated. Indeed, hibiscus beverages, red fruit juices and their blends can be easily manipulated to achieve different levels of complexity, as well as sensory and information (in)congruency. This provides exceptional experimental conditions to study evaluation and choice of new food products, both prior and aftermarket launch, that is, with exposure over time. Moreover, the application of traditional sensory evaluation tests combined with longitudinal experiments and the use of novel sensory and psychology research techniques would enable a more in-depth study of consumers' food evaluation and choice processes.

Overall, insights originating from this kind of research should greatly help European food companies to better understand new product adoption processes and minimize current marketplace failure rates. Importantly, they could also help better understand how to overcome current barriers to the acceptance of non-European foods, particularly those from African or Asian tradition with potential health benefits. This may be the case of Baobab (*Adansonia digitata)* beverages and Ziziphus (*Ziziphus mauritiana)* products, which have also been the object of study and improvement by the AFTER research project.

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