

**ÇUKUROVA UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES**

MSc THESIS

Oscar ZANNOU

**CHARACTERIZATION OF AROMA AND AROMA-ACTIVE
COMPOUNDS OF ROSELLE (*Hibiscus sabdariffa* L.) TEAS
OBTAINED BY THREE DIFFERENT INFUSIONS**

DEPARTMENT OF FOOD ENGINEERING

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ABSTRACT

MSc THESIS

<p style="text-align: center;">CHARACTERIZATION OF AROMA AND AROMA-ACTIVE COMPOUNDS OF ROSELLE (<i>Hibiscus sabdariffa</i> L.) TEAS OBTAINED BY THREE DIFFERENT INFUSIONS</p>
--

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**ÇUKUROVA UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF FOOD ENGINEERING**

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In this study, the effects of two different traditional brewing methods used frequently in Benin (TI₁ (40 minutes/100°C) and TI₂ (24 hours/ ambient temperature)) and an alternative brewing method suggested by Ramirez-Rodrigues et al. (2011) (IR (16 minutes/ 98 °C)) on aroma, aroma-active compounds, total phenolic content and antioxidant activity of *Hibiscus sabdariffa* tea were investigated. Liquid-liquid extraction method was used to determine the aroma of the samples. The aroma-active compounds were investigated by GC-MS-Olfactometry technique by applying Aroma Extract Dilution Analysis (AEDA). A total of 38, 39 and 38 aroma compounds were identified by GC-MS in TI₁, TI₂ and IR, respectively. The highest amount of aroma compounds was found in TI₂ sample (176545 µg/kg). Furans were the most dominant volatiles in *Hibiscus sabdariffa* teas with the lead of furfural in general. Furfural was identified as the most dominant aroma-active compound (FD=2048-4096) responsible for caramel, bread odors in all samples. Another aroma-active compounds detected in roselle infusions with high intensities were 5-methyl-2-furfural (caramel), acetic acid (vinegary), 3-penten-2-ol (oily, herbal), nonanal (herbal, grassy), benzaldehyde (almond), eugenol (spicy) and 3-methyl-3-buten-2-one (fruity). The FD factors of these *Hibiscus sabdariffa* aroma-active compounds were relatively higher in TI₂. Sensory evaluation revealed that TI₂ is more preferred by panellists and displayed higher intensities of aroma, fruity, sweet and caramel attributes than TI₁ and IR. This is in agreement with results from the olfactometric analysis. The total phenolic contents of TI₁, TI₂ and IR determined by Folin-Ciocalteu method were 714, 358 and 761 mg/kg gallic acid equivalents, respectively. Antioxidant activity

of TI₁, TI₂ and IR investigated with DPPH assay were 714, 358 and 761 mM/kg Trolox equivalents respectively. This study has shown that sample preparation methods can significantly affect the total phenolic compounds, antioxidant activities, aroma and aroma-active profiles of *Hibiscus sabdariffa*. When compared to the tea samples, TI₂ (24 hours/ ambient temperature) tea was determined to be richer in aroma and aroma-active compounds and this infusion had greater sensory acceptability than other teas (TI₁ and IR).

Key Words: *Hibiscus sabdariffa*, Phenolic compounds, Antioxidant activity, Aroma, Aroma-active compounds, GC-Olfactometry.



ÖZ

YÜKSEK LİSANS TEZİ

ÜÇ FARKLI İNFÜZYON TEKNİĞİYLE ELDE EDİLEN HİBİSKUS
(*Hibiscus sabdariffa* L.) ÇAYLARININ AROMA VE AROMA-AKTİF
BİLEŞİKLERİNİN KARAKTERİZASYONU

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: Prof. Dr. Haşim KELEBEK

Bu çalışmada, *Hibiscus sabdariffa* çayının aroma, aroma-aktif maddeleri, toplam fenolik içeriği ve antioksidan aktivitesi üzerine Benin’de sıklıkla kullanılan iki farklı geleneksel demleme yönteminin (TI₁ (40 dakika/100°C) ve TI₂ (24 saat/oda koşullarında)) ve literatürde Ramirez-Rodrigues ve ark. (2011) tarafından önerilen alternatif bir çay demleme yönteminin (IR (16 dakika/98°C)) etkisi incelenmiştir. Örneklerin aroma maddelerinin belirlenmesinde sıvı-sıvı ekstraksiyon metodu kullanılmıştır. Çaylarda aroma-aktif bileşikler, Aroma Ekstrakt Seyreltme Analizi (AES) uygulanarak GC-MS-Olfaktometri tekniği ile araştırılmıştır. TI₁, TI₂ ve IR örneklerinde GC-MS yardımıyla sırasıyla 38, 39 ve 38 adet aroma bileşiği tespit edilmiştir. Oda koşullarında 24 saat ile hazırlanan çay örneklerinin aroma maddeleri miktarı açısından en zengin olduğu saptanmıştır (176545 µg/kg). Örneklerdeki aroma bileşiklerinin büyük bir kısmını furan bileşiklerinin oluşturduğu ve bu grupta furfural bileşiğinin yüksek miktarda bulunduğu belirlenmiştir. Aroma-aktif bileşikleri bakımından furfural (karamel) tüm örneklerde en baskın aroma-aktif bileşik olarak belirlenmiştir (SF=2048-4096). Diğer güçlü aroma-aktif bileşikler ise, 5-metil-2-furfural (karamel), asetik asit (sirke kokusu), 3-penten-2-ol (yağimsı, bitki), nonanal (bitki, çimenli), benzaldehit (badem), öjenol (baharat) ve 3-metil-3-büten-2-on (meyvemi) olarak belirlenmiştir. Aroma-aktif bileşiklerinin seyreltme faktörü TI₂ çay örneğinde daha yüksek çıkmıştır. Duyusal analize gelince, GC-MS-O sonuçları ile paralel olarak, TI₂ infüzyonu panelistler tarafından daha çok tercih edilmiştir. Örneklerin toplam fenolik içeriği Folin-Ciocalteu yöntemi ile belirlenmiş ve TI₁, TI₂ ve IR’nin toplam fenolik içeriği sırasıyla 2135; 1556 ve 2201 mg/L gallik asit eşdeğeri olarak

saptanmıştır. Hibiskus çayı örneklerinin antioksidan aktivitesi ise DPPH yöntemi kullanılarak belirlenmiştir. TI₁, TI₂ ve IR'in toplam antioksidan aktivitesi sırasıyla 714, 358 ve 761 mM Trolox eşdeğeri/kg olarak belirlenmiştir. Bu çalışma, çay hazırlama yöntemlerinin *Hibiscus sabdariffa*'nın toplam fenolik bileşikleri, antioksidan aktivitesi, aroma ve aroma-aktif profilini önemli ölçüde etkileyebileceğini göstermiştir. Çay örneklerinden, TI₂ (24 saat/oda koşullarında) infüzyonunun aroma ve aroma-aktif bileşikleri açısından daha zengin olduğu ve duyuşal açışından da diğler örneklere göre daha çok tercih edildiğı belirlenmiştir.

Anahtar kelimeleri: *Hibiscus sabdariffa*, Fenolik bileşikleri, Antioksidan aktivite, Aroma, Aroma-aktif bileşikler



EXTENDED SUMMARY

Roselle (*Hibiscus sabdariffa*) belongs to Malvaceae family and can be cultivated in tropical and subtropical areas of both hemispheres. Every parts of this roselle from roots to leaves are used as foodstuff or for medicinal purposes. This plant can thrive well in the warm dry climates. The production of *Hibiscus sabdariffa* is led by China followed up by India, Sudan, Uganda, Indonesia, Malaysia, and Mexico. Food and Agriculture Organization (FAO, 2010) reported that Sudan has the best roselle in terms of quality. It's important to note that Egypt is considered as one of the important suppliers of roselle (Frag et al., 2015).

Humans are greatly interested in foods which have good nutrition and medicinal properties. Roselles are used to produce several food products such as hibiscus tea, zobo, bissap, sour tea agua of Jamaica, karkade jams, juices, vines, vegetables, oil, jellied confectionaries, ice cream, chocolates, flavouring agents, puddings, cakes, syrup and incorporated in dairy products. Studies reported that *Hibiscus sabdariffa* calyces are rich in protein, fat, carbohydrates, fibre, vitamin C, β -carotene and minerals. Its seeds are potential source of nutrients essentially fatty oil, protein, carbohydrate, crude fibre, ash and minerals. The most important identified saturated fatty acids in the seed oil are palmitic and stearic acids and the main unsaturated fatty acids are linoleic and oleic acid (Nzikou et al., 2011). It has been revealed that *Hibiscus sabdariffa* extracts have bioactive properties that may play an important role in the prevention of chronic diseases such as hypertension, hepatic disease, cardiovascular disease, atherosclerosis and diabetes (Ali et al., 2005; Maganha et al., 2010; McKay et al., 2010; Prenesti et al., 2007; Chen et al., 2004 and Agoreyo et al., 2008). Extracts have been related to diminish high cholesterol and can also work as anticancer, antimutagenic and antiproliferative agents (Hainida et al., 2008). In addition, extracts of *Hibiscus sabdariffa* have antimicrobial properties and can increase reproductive function (Amin et al., 2008; Maganha et al., 2010). Furthermore, *Hibiscus sabdariffa* extract is used to stabilize

body temperature, to treat drunkenness, sore throats coughs as well as genital troubles, kidney disease and bladder stones (Leung and Foster, 1996; Morton, 1987; Neuwinger, 2000; Maganha et al.,2010). The decoction from the seeds can relieve pain in urination, indigestion and to enhance or induce lactation in cases of poor milk production, poor letdown and maternal mortality, to treat stomachic, liver and high pressure problems (Gaya et al., 2009; Morton, 1987).

Flavor of *Hibiscus sabdariffa* can originate from enzymes, fermentation, lipid oxidation, maillard reaction or processing operations and also reaction of amino acids and proteins with carbohydrates. Numerous aroma compounds have been discovered in roselle, however, the participation of each volatile compound of all aroma profile is different from each other. Aroma-active, also named potent odorant or key compound is that odorants which creates the olfactory percepts that affect memory, attention and behavior (Tan, 1998). Even though aroma-active compounds are in very low concentration around ppm and ppb, they can be perceived by human nose. Since these compounds are in trace amounts in the food, a suitable extraction method has to been chosen. Furthermore, the solvent choice is a determinant step during aroma extraction. After aroma isolation, the isolates are analysed depending on the objective. Thus, if the objective is to evaluate the amount of an aroma compound(s) in a food, gas chromatography (GC) may be enough or if the target is odorous compounds in a food, then GC/Olfactometry will be used. If the aim consist of identification of the aroma compounds in a food, this would require GC and mass spectrometry (or GC/MS /Olfactometry) (Reineccius, 2006).

In this work, *Hibiscus sabdariffa* cultivated and dried in University of Agriculture of Ketou's experimental field in 2016 were utilized. The phenolic compounds, antioxidant activity, aroma and aroma-active compounds analysis have been performed on this roselle.

Antioxidant activity was determined by DPPH method. Total antioxidants of TI₁, TI₂ and IR were 714, 358 and 761 mM/kg trolox equivalents. The total

phenolic content was determined by the Folin-Ciocalteu method and the total phenolic contents of TI₁, TI₂ and IR were 714, 358 and 761 mg/kg gallic acid equivalents, respectively. The results showed that sample preparation methods may influence on the total phenolic compounds and antioxidant activities of *Hibiscus sabdariffa*.

In tea, the aroma-active compounds were investigated by GC-MS-Olfactometry technique by applying Aroma Extract Dilution Analysis (AEDA). The liquid-liquid extraction method was used to determine the aroma of the samples. A total of 38, 39 and 38 aroma components were identified by GC-MS in TI₁, TI₂ and IR, respectively. The TI₁ extract had 134831.3 µg/kg, which includes furans (8), alcohols (8), ketones (5), volatile phenols (3), fatty acids (5), aldehydes (3), pyranone (1), pyrrole (1), lactones (2), terpene (1) and ester (1). The TI₂ extract had 176544.5 µg/kg, which includes furans (8), alcohols (9), fatty acids (5), ketones (5), ester (1), volatile phenols (3), aldehydes (3), pyranone (1), pyrrole (1), lactones (2), terpene (1) and ester (1). The IR extract had 89627 µg/kg, which includes alcohols (8), furans (8), fatty acids (5), ketones (5), aldehydes (3), lactones (2), terpene (1), pyranone (1), pyrrole (1) and ester (1). Furans is the most abundant group of aroma in all *Hibiscus sabdariffa* infusion in respect with concentration and the highest amount is found in TI₂, followed by TI₁ and IR. Olfactometric analysis revealed that furfural was the most strong aroma-active compounds in all *Hibiscus sabdariffa* extracts, providing caramel-bready odors. Another aroma-active compounds detected in roselle infusions with high intensities were 5-methyl-2-furfural (caramel), acetic acid (vinegary), 3-penten-2-ol (oily, herbal), nonanal (herbal, grassy), benzaldehyde (almond), eugenol (spicy) and 3-methyl-3-buten-2-one (fruity). The FD factor of these *Hibiscus sabdariffa* aroma-active compounds were relatively higher in TI₂ than others. Sensory evaluation revealed that TI₂ is more likeable and displays higher intensities of aroma, fruity, sweet and caramel attributes than TI₁ and IR. This confirms the results obtained from olfactometric analysis.



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SYMBOLS AND ABBREVIATIONS

M	: Meter
cm	: Centimeter
mm	: Millimeter
g	: Gram
Kg	: Kilogram
Mg	: Milligram
GC	: Gas Chromatography
MS	: Mass Spectrometry
GC-MS-O	: Gas Chromatography-Mass Spectrometry- Olfactometry
AEDA	: Aroma Extract Dilution Analysis
°C	: Degree Celcius
SPME	: Solid-Phase Micro Extraction
GAE	: Gallic Acid Equivalent
TE	: Trolox Equivalent
DPPH	: 2,2-Diphenyl-1-picrylhydrazyl
TI ₁	: Traditional Infusion Obtained at 100°C/40 minutes
TI ₂	: Traditional Infusion Obtained at Ambient Temperature/ 24 hours
IR	: Infusion Obtained with 98°C/16 minutes (Ramires-Rodriguez et al., 2011)



1. INTRODUCTION

Hibiscus sabdariffa is the scientific name of roselle, hibiscus, Jamaica sorrel, red sorrel, bissap and karkadeh. *Hibiscus sabdariffa* can be cultivated in tropical and subtropical areas of hemispheres. Its native repartition is not known however it may be originated from India, Saudi Arabia or Africa (Ismail et al., 2008; Murdock, 1959; Changa et al., 2005; Lin et al. 2007).

Hibiscus sabdariffa belongs to Malvaceae family and delineated as a yearly, bushy plant with a size of up to 2.5 m (Mohamed et al., 2007). The calyx is red, consisting of 5 valves, each containing 3 to 4 kidney-shaped light-brown seeds. The flowers are reputed to be source of the anthocyanins and utilized to make teas like hibicus tea, zobo, bissap, sour tea agua of Jamaica and karkade (Mohamed et al., 2007, Ali, et al., 2005; McKay et al., 2010). The calyces taste sour and are available in the market as jams, juices, vines and incorporated in dairy products.



Figure 1.1. *Hibiscus sabdariffa*: (a) stem; (b) flowers ; (c) fruit and calyces (Cisse et al., 2009)

Food and Agriculture Organization (FAO, 2010) reported that Sudan has the best roselle in terms of quality. It's important to note that Egypt is considered as one of the important suppliers of roselle (Farag et al., 2015). From the genus

hibiscus, over 500 species worldwide were listed (Cid-Ortega & J. A. Guerrero-Beltrán, 2015). There are two types of *Hibiscus sabdariffa* with commercial potent. The first one is *Hibiscus sabdariffa* var. *altissima* Wester, cultivated for his fiber and the second is *Hibiscus sabdariffa* var. *sabdariffa*. It has been declared in the existent literature that some parts of *Hibiscus sabdariffa* var. *sabdariffa* have acid properties close to cranberry taste (Morton 1974).

Roselle flowers provide fruits that contain up to 5 g of seeds and the calyces are usually separated from those seeds before the drying process (Wong et al., 2002).

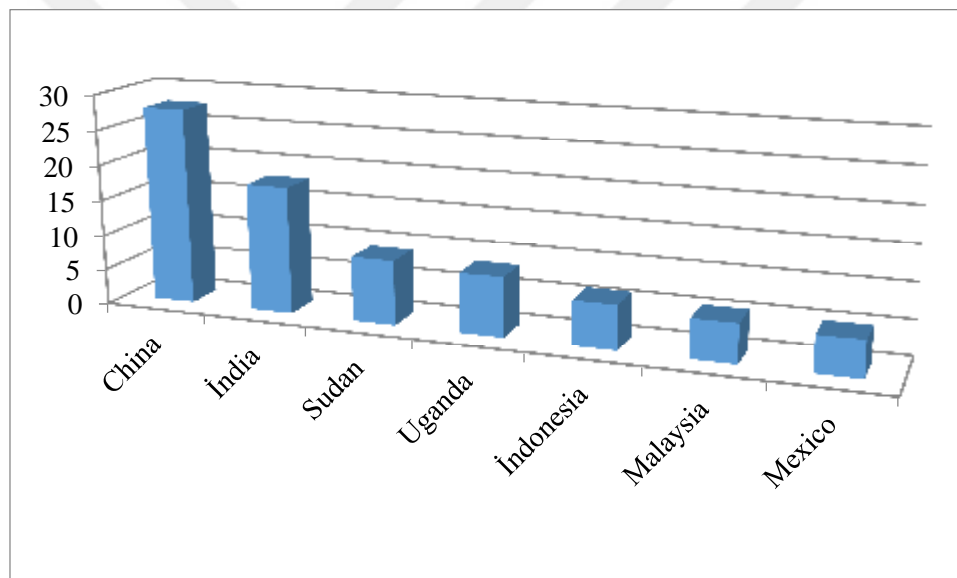


Figure 1.2. Roselle's worldwide production (%) (SAGARPA-CONACyT 2010)

The production of *Hibiscus sabdariffa* is led by China followed up by India, Sudan, Uganda, Indonesia, Malaysia, and Mexico as seen in Figure 1.2. The Roselle plant thrives good in warm and dry climates.

Nowadays, humans are greatly interested in foods which have good nutrition and medicinal properties. The nutritional composition of *Hibiscus sabdariffa* is

influenced by genetic, environmental, ecological, harvest and conservation conditions. Studies reported that *Hibiscus sabdariffa* are rich in protein, fat, carbohydrates, fibre, vitamin C, β -carotene, calcium and iron. Therefore *Hibiscus sabdariffa* is an important source of essential nutrients. The seeds are also potential source of nutrients essential oil, protein, carbohydrate, crude fibre, ash and minerals. The most important identified saturated fatty acids in the seed oil are palmitic and stearic acid, and the main unsaturated fatty acids are linoleic and oleic acid (Nzikou et al., 2011). It has been mentioned in the literature that *Hibiscus sabdariffa* extracts have bioactive properties that may play an important role in the prevention of chronic diseases such as hypertension, hepatic disease, cardiovascular disease, atherosclerosis and diabetes (Ali et al., 2005; Maganha et al., 2010; McKay et al., 2010; Prenesti et al., 2007; Chen et al., 2004 and Agoreyo et al., 2008). *Hibiscus sabdariffa* extracts are effective agents to diminish high cholesterol to vital diseases and they have anticancer, mutagenic and proliferative properties (Hainida et al., 2008). It has also been shown that extracts of *Hibiscus sabdariffa* have antimicrobial activities and can increase reproductive function (Amin et al., 2008; Maganha et al., 2010). Roselle is largely consumed in the world especially in tropical countries as processed food, flavoring agent, traditional hot or cold beverage as well as a natural coloring (Hopkins et al., 2013; Cisse et al., 2009b). It is also suggested as a hypotensive (Morton, 1987). Medicinal value of *Hibiscus sabdariffa* has been greatly studied and the results revealed that this plant has hepatoprotective, antioxidant, anti-inflammatory, antibacterial, cytotoxic properties. Furthermore, *Hibiscus sabdariffa* extract is used to stabilize body temperature, to treat drunkenness, sore throats coughs as well as genital troubles, kidney disease and bladder stones (Leung and Foster, 1996; Morton, 1987; Neuwinger, 2000; Maganha et al., 2010). The decoction from the seeds can relieve pain in urination, indigestion and to enhance or induce lactation in cases of poor milk production, poor letdown and maternal mortality, treat stomachic, liver and high pressure problems (Gaya et al., 2009; Morton, 1987).

The calyces are processed for several purposes. Indeed, Fresh or dried calyces of *Hibiscus sabdariffa* are used in the preparation of drinks (hot and cold beverages and fermented drinks), wine, jam, jellied confectionaries, ice cream, chocolates, flavouring agents, puddings, cakes and in dairy products. In Benin, the dried calyces are used to make juice called " jus de bissap" and syrup. The seeds are eaten roasted or ground in meals, used as a substitute for coffee and processed for oil, while the leaves and shoots are eaten raw or cooked simply or cooked with onions and groundnuts, as a sour-flavoured vegetable or condiment (Wilson and Menzel, 1964; Da-Costa-Rocha et al., 2014; Ismail et al., 2008; Wong et al., 2002).

Several studies have been consecrated to analysis of bioactive properties of roselle. Roselle dried calyces and decoction residues are known to be good source of polyphenols and anthocyanins with great antioxidant capacities (Christian and Jackson, 2009, Abdullakassim et al., 2007). It has been affirmed that *H. sabdariffa* is great source of antioxidant and can be an a suitable alternative to grape and biberries (Leyla et al., 2012). Delphinidin-3-sambubioside, cyanidin-3-sambubioside, cyanidin-3-*O*-rutinoside, chlorogenic acid, are important individual anthocyanins identified from *H. sabdariffa* (Hong and Wrolstad, 1990, Segura-Carretero et al., 2008, Sinela et al., 2009). In addition, roselle displayed a total phenolic compound higher than the one found in *Prunus armeniaca*, *Ficus carica* and *Curcuma longa* (Leyla et al., 2012).

The flavor, consist of aroma (odor) and taste, is one of the factors which determine the decision of consumer. The perception of flavor is strongly linked to a chemical phenomenon and the acceptability and desirability of a flavor is highly affected by social context, physical environment and ethnic diversity of the consumers (Shahidi and Ho, 1999; Eertmans et al., 2001). A survey showed that the consumers consider sensory perceptions of food more than other values which can influence their food choices (Furst et al. 1996). As foodstuff, roselle's flavor can be originated by enzymes, fermentation, lipid oxidation, Maillard reaction or processing operations and also reaction of amino acids and proteins with

carbohydrates. In the same way, its aroma is generated by numerous volatile compounds. However, the participation of each volatile compound of all aroma profile is different from each other. Aroma-active, also named potent odorant or key compound is the odorants which creates the olfactory perceptions that affect memory, attention and behavior (Tan, 1998). Even though aroma-active compounds are in very low concentration around ppm and ppb, they can be perceived by human nose. Since these compounds are in trace amounts in the food, a suitable extraction method has to be chosen. Furthermore, the solvent choice is a determinant step during aroma extraction. After aroma isolation, the isolates are analysed depending on the objective. Thus, if the objective is to evaluate the amount of an aroma compound(s) in a food, gas chromatography (GC) may be enough or if the target is odorous compounds in a food, then GC/Olfactometry will be used. If the aim consists of identification of the aroma compounds in a sample, this would require GC and mass spectrometry (or GC/Olfactometry/MS) (Reineccius, 2006).

Despite health benefits and commercial opportunities of roselle very few flavor studies have been done. This study has potential of bringing more informations on roselle's aroma compounds.

The aim of this study consists of the isolation and analysis of roselle (*Hibiscus sabdariffa* L.)'s teas aroma and aroma-active compounds obtained by three different infusion methods. The volatile compounds were extracted liquid-liquid extraction (LLE) technique and analyzed by GC-MS-Olfactometry. In addition, phenolic compounds of samples were analysed by Folin-ciocalteu reagent and antioxidant activities was determined by DPPH method.



2. LITERATURE OVERVIEW

2.1. Nutritional composition of *Hibiscus sabdariffa* and other properties

Many studies have been consecrated on compositional value of *Hibiscus sabdariffa*. It has been figured out that this plant is useful from the roots to the leaves, regarding its nutritional value physico-chemical properties and medicinal potential.

Samy (1980) extracted the oil from *Hibiscus sabdariffa* seeds and analyzed the phytochemical of the oil in comparison with crude seeds (unextracted). The chemical composition, including moisture, crude protein, ether extract, nitrogen-free, crude fiber, ash, calcium, phosphore and sulfur were on one hand, 7.58 (%), 23.95 (%), 22.34 (%), 23.81 (%), 15.30 (%), 7.02 (%), 0.31 (%), 0.60(%) and 0.35 (%) for unextracted seeds, respectively and on other hand 8.18 (%), 29.04 (%), 0.69 (%), 20.04 (%), 9.19 (%), 0.41 (%), 0.79 (%) and 0.37 (%) for the extracted seeds, respectively.

Duke (1983) studied nutritional value of some parts of *Hibiscus sabdariffa*. It has been revealed in the work that for 100 g, the fruit possesses 49 calories and 84.5% of water, and 1.9g protein, 0.1g fat, 12.3g carbohydrate, 2.3g fiber, 1.2g ash, 1.72mg calcium, 57mg phosphore, 2.9mg iron, 300 μ g β -carotene and 14mg ascorbic acid. Regarding 100g of the leaves, 43 calories, 85.6% water rate, 3.3mg protein, 0.3mg fat, 9.2mg carbohydrate, 1.6mg fiber, 1.6mg ash, 213mg calcium, 93mg phosphore, 4.8mg iron, 4135 μ g β -carotene, 0.17mg thiamine, 0.45mg riboflavin, 1.2mg niacin and 45 mg ascorbic acid.

Duke and Atchley (1984) have studied proximate value of *Hibiscus sabdariffa* flower and declared for 100g of flower to contain 44 calories, 86.2% H₂O, 1.6g protein, 0.1g fat, 11.1g total carbohydrate, 2.5g fiber, 1.0g ash, 160mg Ca, 60mg P, 3.0 mg Fe, 285mg β -carotene, 0.04mg thiamine, 0.6mg riboflavin, 0.5mg niacin, and 14 mg ascorbic acid.

Gabb (1997) has investigated nutritional importance of Sudan *Hibiscus sabdariffa* calyces and concluded that moisture value, protein, total hydrocarbon, fiber and ash were in proportion of 15%, 5%, 15%, 12% and 7%, respectively.

Rao (1996) searched about proximate composition of two Indian varieties of *Hibiscus sabdariffa* seeds. Protein, fat, fiber, phosphore, calcium, zinc, manganese, copper, magnesium, chromium, riboflavin, nicotinic acid have been ranged from 6.7-8.6 %, 18.8-22.3 %, 19.1-22.8 %, 5.4, 39.5-42.6 %, 576-672 (mg/100), 119-128 (mg/100), 4.0-4.3 (mg/100), 5.9-7.4 (mg/100), 2.8-3.1 (mg/100), 369-393 (mg/100), 0.08-0.18 (mg/100), 0.36-0.51 (mg/100), 0.9-1.0 (mg/100) for both varieties, respectively. It has been concluded that *Hibiscus sabdariffa* seeds are rich in protein, fatty acids (unsaturated), minerals and amino-acid (lysine and thryptophan).

Hainida et al. (2008) studying effet of non-fat *Hibiscus sabdariffa* seed powder on hypercholesterolemia, have been interested in analysis of nutritional composition of seed powder and deffated seed powder. Protein, lipid and total fiber of normal seed powder were 334.5 ± 0.45 g/kg, 221.3 ± 0.39 g/kg and 182.6 ± 0.3 g/kg, respectively, while 359.4 ± 0.86 g/kg, 54.0 ± 1.1 g/kg and 184.3 ± 0.14 g/kg were the values of the same elements for deffated seed powder. The study indicated that non-fat seed powder displayed a great hypocholesterolemia potential.

Liu et al. (2005) conducted a study in order to determine antibacterial effect of *Hibiscus sabdariffa*. The study focused on inhibition of *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acetobacter baumannii*. The results have shown that these bacteria stopped growing with the addition of roselle calyx extract. Moreover it was indicated that protocatechiuc acid had displayed more effect on the bacteria inhibition than simple *Hibiscus sabdariffa* calyces.

Ismail et al. (2008) investigated the qualities of roselle seeds. It has been resulted from the work that roselle seeds are not only rich in nutrients but they have also health properties. Roselle seeds were reported to contain protein, oil, dietary

fiber, lysine, phenylalanine, arginine, glutamic acid and antioxidant properties. Additionally, roselle seeds showed high anti-hypercholesterolemia effects.

Beltran-Debon et al. (2010) have used *Hibiscus sabdariffa* calyces as additional food or diet supplementation in purpose to evaluate the improvement of human health. They assessed that *Hibiscus sabdariffa* calyces have great antioxidant activity originated by anthocyanins and phenolic compounds like hydroxycitric acid and chlorogenic acid. The antioxidant potential of hibiscus extracts allowed the inhibition of mononuclear cells death caused by H₂O₂. Then, it has been concluded that *Hibiscus sabdariffa* calyces are very worthy for the treatment of chronic inflammatory illnesses.

Nwaiwu et al. (2012) have a research on antibacterial effect of seeds of *Hibiscus sabdariffa*, *Moringa oleifera* and *Hibiscus esculentus*. Salmonella, Shigla and Enterobacter species have used for the test. The result showed that the seeds of *Hibiscus sabdariffa*, *Moringa oleifera* and *Hibiscus esculentus* were reported to have significant antimicrobial effect on the tested organisms. It was suggested that the seeds of *Hibiscus sabdariffa* and *Hibiscus esculentus* could be used for the medicinal purpose.

Sirag et al. (2013) have undertaken the microbiological analysis to determine antibacterial effect of *Hibiscus sabdariffa*. The results showed that *Hibiscus sabdariffa* calyces have similar effect with cefuroxime, which is the drug capable of inhibiting *Klebsiella pneumoniae*. The *Hibiscus sabdariffa* calyces showed also remarkable antibacterial effect against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*.

Dhar et al. (2015) investigated on the chemical, phytochemical and medicinal properties of *Hibiscus sabdariffa* and *Hibiscus cannabinus* seeds. The seeds of these plants displayed high amounts of polyphenols and phenolic acids. Furthermore, nutritional and medicinal properties have been figured out from seeds of *Hibiscus sabdariffa* and *Hibiscus cannabinus*. The oil extracted from the seeds of these plants, contained diversified fatty acids. It has been suggested in

conclusion that *Hibiscus sabdariffa* and *Hibiscus cannabinus* could be used as a functional food and a natural pharmaceutical plant.

2.2. Aroma profile of *Hibiscus sabdariffa*

Jirovetz et al. (1992) have used GC-MS and GC-FTIR to evaluate and identify aroma compounds of roselle seed oil. More than 25 volatile compounds have been identified and ranged mainly as unsaturated hydrocarbons (with one or two double bonds), alcohols and aldehydes. They reported that *Hibiscus sabdariffa* seed oil contains several fatty acids including linolic acid (the most abundant), oleic acid, palmitic acid, sterculic acid, linoleic acid, myristic acid, palmitoleic acid, epoxyoleic acid, steric acid, malvalic acid, dihydrosterculic acid and eicosenoic acid.

Chen et al. (1998) using the Likens-Nickerson steam distillation procedure researched about volatiles in roselle tea. Roselles were prepared separately according to drying processes. Researchers were applied hot air drying at two different temperatures (50 and 75°C). After drying and freezing, roselles teas were prepared and investigated in terms of volatile composition. Up to 37 aroma compounds were characterized and classified into four groups such as fatty acid derivatives, sugar derivatives, phenolic derivatives, and terpenes. A huge concentration of the high aliphatic lipid derivatives (hexanol, (*E*)-2-hexenal, (*Z*)-3-hexenol and 2-hexanol) have been detected in the fresh roselle, while a small amount has been found dried and frozen teas. Authors indicated that these volatile compounds could be formed by lipoxygenase action and thermal decomposition. This work revealed that freezing and hot-air-drying seemed to inactivate the lipoxygenase activity. Furfural and 5-methyl-2-furfural were found in high amount in dried roselle tea however, these volatiles compounds showed low concentration in fresh roselle.

Pino et al. (2006) investigated on volatile compounds of roselle grown in Cuba. Likens-Nickerson steam distillation method was used to isolate aroma

compounds. A total of 81 aroma compounds have been quantified and identified from roselle tea. Among these, terpenoids such as linalool and α -terpineol were found to be the main odorants. Fatty acids, alcohols, furans, aldehydes, esters and volatile phenols have been detected in minor amount in roselle samples. They also mentioned that furfural and 5-methyl-2-furfural could be issued from degradation of ascorbic acid and sugars.

Gonzalez-Palomares et al. (2009) studied effect of spray drying temperature on the aroma compounds of *Hibiscus sabdariffa* powder. Roselle samples were spray dried at seven different temperatures. Aroma compounds were extracted with Solid Phase Micro Extraction (SPME) and analyzed with GC-MS. A total of twenty volatile compounds have been identified and listed in the aroma groups as: terpenoids, esters, aldehydes and hydrocarbons. The research displayed that during the passage from fresh fruit to powdered stage with application of spray drying, some compounds were lost while some of them were newly generated as a result of temperature rising.

Ramirez-Rodrigues et al. (2011) studied teas obtained from fresh and dried roselle's calyxes in terms of volatile aroma compounds by using Solid Phase Micro Extraction (SPME) method for extraction and GC-MS for the identification. Teas from roselle were prepared with hot (98 °C for 16 min) and cold (22 °C for 240 min) infusions. A total of 32 aroma compounds, classified as aldehydes, alcohols, terpenes, and acids, have been quantified and identified in all samples. Alcohols were found as qualitatively the most abundant volatile compounds in the infusions. Nonanal, decanal, octanal, and 1-octen-3-ol were the common major volatile compounds found in all beverages. Researchers also indicated that hot infusion process increased the quantity and intensity of aroma compounds as dried roselle tea with hot infusion had the highest amount of volatiles. The most intense aroma volatiles were 1-octen-3-one and nonanal with a group of 4 aldehydes and 3 ketones common to all samples.

Ramirez-Rodrigues et al. (2012) investigated the change in phytochemical and sensory attributes of roselle beverages treated with dense phase carbon dioxide (DPCD) and conventional thermal method. They reported that negligible changes were recorded during five weeks of storage in the samples treated with DPCD. In the study, isolation of aroma compounds were performed with SPME method and identification were carried out using GC-MS. A total of 13 compounds have been reported and the main aroma compounds in hibiscus tea constituted of alcohols and aldehydes. 1-Octanol, decanal, octanal, 1-hexanol and nonanal were the most abundant compounds in *Hibiscus sabdariffa* tea. It is also reported that DPCD process retained more volatiles in comparison with conventional thermal treatment. Tea samples treated with DPCD were observed that they are more durable in storage than thermally treated samples.

Camelo-Mendez et al. (2013) compared aroma and compounds of four varieties of *H. sabdariffa* (Rosa, Negra, Sudan and Blanca). SPME and GC-MS were used for isolation and identification of aroma compounds, respectively. Water and ethanol were used for extractions and ethanol was reported to exhibit more efficacies in both anthocyanin and volatile retaining. Rosa variety was reported to have the highest volatile quantity compared other cultivars. Geraniol was detected as the most abundant volatile compounds in all varieties. A total of nine aroma compounds including geraniol, menthol, benzaldehyde, linalool, gamma-undecalactone and ethyl methyl phenylglycidate have been detected in all samples.

Farag et al. (2015) used SPME coupled to GC-MS to extract and profile aroma compounds and primary metabolites of two *Hibiscus sabdariffa* cultivars (viz. Aswan and Sudan-1). Aswan cv. had higher volatiles with a number of 74 compounds compared to Sudan variety having 71 compounds. The authors detected that two varieties are very similar qualitatively with having a difference quantitatively. The main volatile classes were found as sugar and fatty acid derived compounds. There were also terpenoids (α -terpinene, β -phellandrene and (Z)-geranylacetone), phenylpropanoids and aromatic derivatives (E)- cinnamaldehyde,

p-cresol and eugenol); and amino acids derived volatiles. Generally, furfural, aldehydes (cinnamaldehyde), acids (acetic acid) and the short chain alcohol (1-octen-3-ol) appeared as the important compounds accounting for the differences among the 2 cultivars. The primary metabolites of both cultivars were mono- and disaccharides, in addition to fatty, amino- and organic-acids.

Juhari et al. (2015) investigated on the effect of sample preparation's methods on the aroma profiles of dried *H. sabdariffa* using Dynamic Headspace Sampling for the isolation and GC-MS for the identification. A total of 125 aroma compounds have been revealed and classified as terpenes, aldehydes, esters, ketones, alcohols and furans, acids, sulphurs, lactones and others. Within these, terpenes and aldehydes were reported to show the highest amounts. The greatest amount of aroma compounds was discovered in roselle treated in water bath (2 h/40°C).

Tahir et al. (2017) searched about aroma compounds of red and white *Hibiscus sabdariffa* using Solid Phase Micro Extraction (SPME) and GC-MS to isolate, quantify and identify them. They reported (19) volatile compounds from white and red *Hibiscus sabdariffa* extract classified as aldehydes, alcohols, ketones, esters and volatile phenols. Furfural, hexanal, ethyl acetate, 2-methylpropyl acetate, 3-methyl butanol acetate, 1-hexanol, phenylethyl alcohol, 2-ethyl and 1-nonanol, 6-methyl-5-hepten-2-one, 1-(2-methyl-1-cyclopenten-1-yl) ethanone, 2,4-di-tert-butyl phenol were found as the main contributors of roselle aroma profile.

Aroma-active compounds are one or many aroma compounds that can be responsible for the odor of foods. Several researches have been consecrated to determine these compounds. Gas Chromatography-Olfactometry (GC-O) is the system used for the implementation of aroma-active analysis. GC-O includes Gas Chromatography (GC) and human nose which is used as a detector in order to define the quality and intensity of the sensation. Gas Chromatography-Olfactometry has been suggested for the first time by Fuller et al. (1964). The

system they had proposed was difficult to be reproduced due to the malaise caused by sniffing hot dry effluent gases and sensitivity of volatile compounds. Latter, Dravnieks and O'Donnell (1971) had fixed partially the problem by incorporating humidified air to GC-O system.

For each single aroma-active compound from GC, human nose is capable to detect time of odor activity, quality and intensity of odor of each compound. Since the first time GC-O had been experimented, numerous techniques have been extended to gather and provide GC-O data and to evaluate the sensory contribution of single potent aroma-active compounds, which can be categorized in the following three categories: Dilution analysis, detection frequency method and time-intensity method.

Dilution analysis is the most used technique for the aroma-active analysis. This technique consists of the determination of the relative power, regarding to the presence of the odor of aroma compounds in an extract (Saskia and Ruth, 2001). Two research groups have investigated in the development of this technique. The first group developed Charm analysis (Acree et al., 1984) and the second group established Aroma Extract Dilution Analysis (AEDA) (Grosch et al., 1987). During Charm analysis, series of dilutions of aromatic extract are randomly sniffed and graphed according to FD (dilution factors) to retention time or indice, while during AEDA, the concentrated extract is diluted step by step and, each diluted extract is injected and sniffed until the odor is not perceived and the flavor dilution value. AEDA is the most preferred technique, easy to use and the dilutions' results reflect the final result, however this technique takes a lot of times.

Detection frequency method had been suggested by Linssen et al. (1993). However, the method has been developed latter by Pollien et al. (1997). In this method, GC-Olfactometric analysis is executed simultaneously by many panelists (up to 12) on the same sample. The panelist reports the intensity and the duration of the aroma-active compounds and the compounds which appear regularly have

relatively high importance. This method is simple and reproducible but does not reflect the accurate intensities of the compounds.

Time-intensity also named Osme has been investigated and developed by McDaniel et al. (1992). The technique requires the assistance of computer tools, trained participants to sniff and report intensities and duration of the odor of aroma-active compounds coming from GC device. Four participants are generally involved to perform GC-Olfactometric analysis with this technique. Although the results are valuable, accuracy of the representativity is still problematic due to a few numbers of the panelists. In the existant literature, only few studies have been conducted using Osme technique because parameters of time and intensity have been mastered. Further studies are needed in order to improve this olfactometric technique.

Ramirez-Rodrigues et al. (2011) have used Osme to performed olfactometric analysis of roselle. In this study, total of 22 aroma-active compounds have been revealed. Among these, 1-octen-3-one (mushroom) and nonanal (green) were the abundant aroma-active compounds found in the fresh and dried roselle.

In our work, olfactometric analysis was performed using GC-O with application Aroma Extract Dilution Analysis (AEDA). During AEDA, successive dilutions up to 1:1024 were made to determine the flavor dilution factors (FD factor) of each aroma-active compound.

2.3. Phytochemical properties of *Hibiscus sabdariffa*

The phenolic compounds are natural antioxidants which help to prevent or treat cancer, cardiovascular, inflammatory and neurodegenerative diseases. They are also used as additives in agro-business, pharmaceutical and cosmetical industries. Dried roselle calyces and decoction residues are known as good source of polyphenols with antioxidant capacity.

Christian and Jackson (2009) investigated the total phenolic composition of three varieties of *Hibiscus sabdariffa* using Folin-Ciocalteu reagent. The obtained

total phenolic amounts were ranged between 5 and 24 mg GAE/g and the highest value has been discovered in TRED and ERED species while the lowest amount has been found in the white variety. The authors suggested the valorization of *Hibiscus sabdariffa* because this plant displayed high value of phytonutrients.

Leyva et al. (2012) have used Folin-Ciocalteu colorimetric method to determine the total phenolic compounds of roselle samples dried on a fixed bed, the values were reported to range from 435.03-637.65 mg GAE/100 g residue. This study revealed that total phenolic compounds increase with decrease of both temperature and air velocity. Furthermore roselle displayed a total phenolic compound higher than the one found in *Prunus armenidca*, *Ficus carica* and *Curcuma longa longa* samples. In the work, the analysis of the antioxidant activity has been performed with ABTS assay. The antioxidant activity *Hibiscus sabdariffa* calyces have been ranged from 181.13 to 288.44 $\mu\text{mol TE/g}$ of dried extract. It has been concluded that with such antioxidant potential, *Hibiscus sabdariffa* can be an alternative to grape and bilberries.

Salazar-González et al. (2012) also used Folin-Ciocalteu to investigate total phenolic compounds in roselle with the use of two different solvents and assessed that the phenolic content of *Hibiscus sabdariffa* is ranged from 1,067 (in ethanol) to 2,649 (in ethanol:water 70:30%) mg of gallic acid/100 g of dried calyces. In this work, water extract displayed a total phenol compound less than the ethanol extract of roselle. They concluded then that the total phenolic compound can also be affected by the extraction method and the utilized solvent. The authors were also interested in the antioxidant activity of *Hibiscus sabdariffa* and found out that the antioxidant capacity of *Hibiscus sabdariffa* is ranged between 3.11 (in ethanol) to 8. mmoles of Trolox/100 g of calyces.

Jung et al. (2013) determined the total phenol compounds in dried roselles by Folin-Ciocalteu reagent. At 760 nm, the total phenol compounds in roselle were found as 44.18 and 48.42 mg gallic acid/ 1000g in water extract and ethanol extract respectively. Similar with the previous study, ethanol displayed higher efficiency in

obtaining phenolics of dried roselle samples as it yielded higher amount of total phenolics. The authors also investigated the antioxidant and antibacterial properties of samples and determined that dried roselles possess high antioxidant activity and can be used as a source of natural antioxidants. They also detected that, roselle extracts showed an inhibition on *Bacillus subtilis* and *Staphylococcus aureus* in addition to *E. coli* with a slightly less effect.

Leyva et al. (2013) investigated on polyphenolic compounds of lixiviated *Hibiscus sabdariffa* samples using Folin-Ciocalteu. The data collected from analysis showed that phenolic compounds dried roselle is ranged from 430.48 to 716.64 mg GAE/ 100 g residue d.b. They also investigate the usage of lixiviated roselles in the production of yoghurt and indicated that the usage of roselles in dairy products can enhance the bioactive properties of beverages.

Lukmanto et al., (2013) used supercritical CO₂, one of sophisticated analysis tools to evaluate polyphenolic compounds of *Hibiscus sabdariffa* and they found out the value of phenolic content is 8.63 mg GAE/g dried roselle. They also assessed that the amount of extracted phenolic compounds rose with an increase in pressure and temperature. The solubility data of phenolic compounds in supercritical CO₂ were correlated using density-based models.

Sindi et al. (2014) have used Folin-Ciocalteu to determine polyphenols in *Hibiscus sabdariffa*. The results revealed that the water extracts with and without formic acid, provided the greatest content of total polyphenols (21.67 and 17.94 mg/g gallic acid equivalent, respectively) and the extract with ethyl acetate and hexane with and without formic acid, presented the lowest polyphenolic content. . In addition, they extracted *Hibiscus sabdariffa* with solvents such as water, methanol, ethyl acetate and hexane in the presence or absence of formic acid. Then they extracted using FRAP and acidified and non-acidified aqueous extracts. The obtained values are 38.18 and 39.14mg/g Trolox equivalent, respectively whereas acidified and non-acidified hexane and ethyl acetate extracts made at boiling point had the lowest values (0.03 and 0.15 mg/g Trolox equivalent, respectively). It has

been concluded that the water extract (10min / 100 °C) with or without formic acid presented the greatest antioxidant activities.

Bergmeier et al. (2014) use Folin-Ciocalteu to quantify the total phenolic compounds *Hibiscus sabdariffa*. The total phenolic contents were 460.48 and 5012.54 mg/g gallic acid equivalent in ethanol and acetone, respectively. It has been showed in this work that experiment affect the amount of the total phenolic compounds and antioxidant. It has been also assessed that *Hibiscus sabdariffa* could be an important source of total phenolic compounds and therefore antioxidant.

Borras-Linares et al. (2015) investigated in the phenolic compounds, flavonoids, anthocyanins and antioxidant activity of *Hibiscus sabdariffa* using HPLC-DAD-ESI-TOF-MS. The value of phenolic compounds found in different varieties of roselle, was ranged from 2400 to 10000 mg gallic acid equivalent (GAE)/100 g dry calyx (dc). They concluded that the total phenolic compound of *Hibiscus sabdariffa* varied among varieties. In addition the authors reported that the anthocyanidin contents of *Hibiscus sabdariffa* extracts varied from 0 to 4408 mg/100g dc with different ratios of delphinidin: cyaniding. Furthermore, it has been affirmed that the amounts of anthocyanins vary significantly between extraction procedures of *Hibiscus sabdariffa*. Furthermore, antioxidant activities of *Hibiscus sabdariffa* have seen to vary from 27.4 to 112 mol equivalent Trolox/g dc. The authors indicated that there is a high difference between antioxidant activities of *Hibiscus sabdariffa* varieties. Hence, *Hibiscus sabdariffa* cultivars can influence antioxidant activities of *Hibiscus sabdariffa*.

Mercado-Mercado et al. (2015) showed that decoction residues from calyces released high quantities of polyphenol content (296.03 mg GAE/g), whereas complete calyces had higher polyphenol content associated with soluble dietary fiber (117.31 mg GAE/g db). Released polyphenol being higher than that obtained to convectional aqueous-organic extraction. Gallic (46.95%), syringic (42.93%) and caffeic (10.12%) acids were found in polyphenol released from

complete calyces. However, caffeic (75.28%), gallic (14.09%), and chlorogenic (10.62%) acids were identified in decoction residues. polyphenol bioaccessibility in decoction residues and complete calyces was 71.72 and 26.68%, respectively. Released Polyphenol from decoction residues are bioaccessible in the gastrointestinal tract.

Formagio et al. (2015) have utilized Folin-Ciocalteu to investigate the total phenolic compound of *Hibiscus sabdariffa*. They assessed that the greatest amounts of phenolic were found in *Hibiscus sabdariffa* leaves and calyces with 389.98 and 474.09 mg g⁻¹, respectively.

Arenas et al. (2016) have utilized Folin-Cocalteu to determine total polyphenol of *Hibiscus sabdariffa*. The results showed that the total polyphenolic content in leached roselle calyxes was 913 mg GAE per 100 g db and from 876 mg GAE per 100 g residue db and 1013 mg GAE per 100 g residue db after drying. Additionally, it has been reported that the initial content of monomeric anthocyanin before drying procedure was 227 mg delphinidin-3-sambubioside per 100g db and the values obtained during drying, were ranged from 212.9 mg to 280.4 mg delphinidin-3-sambubioside. The authors concluded that the amounts of anthocyanins rose during the drying process.

Camelo-Méndez et al. (2016) used chromatographic method to separate up to 24 phenolic compounds of *Hibiscus sabdariffa*. Among these, there were two hibiscus acids, six phenolic acids, six flavanols, eight flavonols in Rosa, negra, Sudan and Blanca varieties. Two anthocyanins have been found in Rosa, negra and Sudan, however no anthocyanin has been found in Blanca variety. It has also concluded that Sudan cultivar presented the biggest amount of anthocyanin followed Negra and Rosa cultivars.

Owoade et al. (2016) in purpose to identify phenolic compounds in *Hibiscus sabdariffa* used Folin-Ciocalteu method and concluded that the total amount of phenolic compounds was 97.9 mg/g. In this work, chromatografic analysis has been used to identify five individual phenolic compounds including

ferulic acid, chlorogenic acid, naringenin, rutin and quercetin which are currently used for pharmaceutical purpose.

Zhen et al. (2016) using Folin-Ciocalteu to determine phenolic compounds of roselle. The value of the total phenolic compounds found in this study was ranged from 18.98 to 29.9 mg GAE/g. Ten individual polyphenolic compounds including neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, quercetin, kaempferol and their glycosides together with 5-(hydroxymethyl) furfural were identified by analyzing HPLC-MS data.

Ochao-Velasco et al. (2017) have used Folin-Ciocalteu to analyze the total phenolic compounds of *Hibiscus sabdariffa*. The extract has been obtained using 50:50 (v/v) mixture of ethanol: water solution. The results showed that values of the total phenolic of encapsulated powders and concentrate-gum were 3.43 and 15.27, respectively. It has been concluded from this work that *Hibiscus sabdariffa* powders could be used not only as additives in reason of its flavoring and colour properties but also as source of antioxidant.

Tahir et al. (2017) searched about the total phenolic compounds four varieties *Hibiscus sabdariffa* using Folin-Ciocalteu reagent. All the varieties have been ground prior the analysis. The total phenolic compounds were ranged from 79.84 to 148.17 mg GAE/100g. The lowest amount has been discovered in white variety.

The current interest in natural antioxidants from plant sources has become overwhelming, particularly in bioactive antioxidants such as polyphenols and flavonoids (Sindi et., 2014). Numerous studies both in vitro and in vivo have revealed that extracts of *Hibiscus sabdariffa* have a potent antioxidant effect. The antioxidant activity of the extract is due to its strong scavenging effect on reactive oxygen and free radicals (Farombi & Fakoya, 2005; Mohd-Esa et al., 2010; Olalye & Rocha, 2007; Sayago-Ayerdi et al., 2007; Tseng et al., 1997), inhibition of xanthine oxidase activity, protective action against tert-butyl hydroperoxide (t-BHP)-induced oxidative damage (Tseng et al., 1997), protection of cell from

damage by lipid peroxidation (Farombi & Fakoya, 2005; Olalye & Rocha, 2007), inhibition in Cu²⁺-mediated oxidation of LDL and the formation of thiobarbituric acid reactive substances (TBARs) (Hirunpanich et al., 2005; Ochani & D'Mello, 2009; Olalye & Rocha, 2007), inhibition of the formation of malondialdehyde content (100-300 mg/kg) (Farombi & Fakoya, 2005; Usoh et al., 2005), reduction of glutathione depletion, increase of the liver and decrease blood activity of superoxide dismutase and catalase (Usoh et al., 2005) while in the liver it increased superoxide dismutase, catalase and glutathione and decreased malondialdehyde (Mossalam et al., 2011). The effects were observed for both water and ethanolic extracts from flowers of *Hibiscus sabdariffa* L., as well as from the seeds or leaves (Mohd-Esa et al., 2010). A wide range of extraction conditions have been used in roselles to evaluate the antioxidant activity as seen in Table 2.1.

Table 2.1. Examples of different extraction conditions used in different *Hibiscus sabdariffa* (1g) samples (Sindi et al., 2014).

Solvent	Solvent volume (ml)	Time (min)	Temperature °C	Study
Water	50	60	25	Wong et al., 2006
Water	100	5	100	Oboh and Rocha, 2008
Water	100	600	25	Cisse et al., 2009
Acidified methanol	50	180	64	Farombi and Fakoya, 2005
%80	1000	120	25	Mohd-Esa et al., 2010
%70 Ethanol	10	2	Microwave 150 W	Amin et al., 2008

Tsai et al. (2002) used three methods such as ferric reducing ability of plasma, oxygen radical absorbance capacity and total antioxidant status to evaluate the antioxidant capacity of *Hibiscus sabdariffa*. The result showed that antioxidant increase with increase of the extraction time and the masse of the calyces.

Abdullakasim et al. (2007) researched antioxidant capacity of twelve selected Thai health beverages including roselle using DPPH and photochemiluminescence methods. The antioxidant activity of roselle drink was 59.4 $\mu\text{mol TE}/100\text{ ml}$ and 68.9 $\mu\text{mol AAE}/100\text{ ml}$ in both equivalents to Trolox and ascorbic acid, respectively. The greatest antioxidant activity of roselle drink has been found with photochemiluminiscence in both water and lipid soluble substances (341.2 to 7 59.4 $\mu\text{mol TE}/100\text{ ml}$ and 671.2 $\mu\text{mol AAE}/100\text{ ml}$).

Sáyago-Ayerdi et al. (2007) measured using ABTS roselle flower beverage's antioxidant and reported that its antioxidant capacity is 335 $\mu\text{mol Trolox equivalents}/100\text{ mL}$. This research group concluded that *Hibiscus sabdariffa* could be a great source of polyphenols and consuming this plant could be very benefict of the health

Mohd-Esa et al. (2010) have used β -carotene and DPPH assays to analyze the antioxidant activity of different parts of *Hibiscus sabdariffa*. The results showed that the antioxidant activity of roselle's calyce in water and methanol was 54.1 and 61.5 % respectively and *Hibiscus sabdariffa* seed extracts the most antioxidant activity and radical - scavenging activity. They concluded that *Hibiscus sabdariffa* seeds showed the greatest lipid oxydation activity.

Akim et al. (2011) evaluated the antioxidant activity of *Hibiscus sabdariffa* juice during three periods: one week, one month and 1 year. The found EC50 values of one week, one month and one year were 3.733, 3.717 and 3.383 respectively. These values were compared to 0.217 % for positive control. They concluded that the variation in antioxidant activity between different storage periods of *Hibiscus sabdariffa* juice was not significant but all samples exhibited increasing activity with increasing concentrations.

In the extant literature, it has been demonstrated that *Hibiscus sabdariffa* overflowed great amounts of bioactive compounds including anthocyanins. Indeed, chemically, anthocyanins are glycosides of polyhydroxy and polymethoxy derivatives of flavylum salts. Besides their vibrant colors, anthocyanins also have bioactive properties which can be explained the health benefits these phytochemicals provide. Some of the researches found in the existent literature and conclusions are listed bellow:

Hong and Wrolstad (1990) studied individual anthocyanins of *Hibiscus sabdariffa*. It has been resulted from this work that delphinidin-sambubioside, cyanidin-3-sambubioside, delphinidin-3- glucoside and cyanidin-3-glucoside were the four identified anthocyanins in *Hibiscus sabdariffa*. In addition the authors assessed that delphinidin-sambubioside and cyanidin-3-sambubioside were the most abundant while delphinidin-3- glucoside and cyanidin-3-glucoside were found in few quantities (less than 7 %).

Segura-Carretero et al. (2008) investigated the selective extraction, separation and identification of roselle by using solid phase extraction-capillary electrophoresis-mass spectrophometry. The results showed that dephinidin-3-sambubioside and cyanidin-3-sambubioside are the most important anthocyanin components found in *Hibiscus sabdariffa* extracts. Furthermore, the authors discovered some minor anthocyanin components including cyanidin-3-O-rutinoside, delphinidin-3-O-glucoside and cyanidin-3, 5-diglucoside and chlorogenic acid.

Sinela et al. (2009) investigated degradation rates of the anthocyanins of roselle extracts during the storage with metal, oxygen concentration and polyphenolic fraction. The findings indicated that the concentration of oxygen did not significantly influence the degradation of the main anthocyanins of roselle. Degradation rate of delphinidin 3-O-sambubioside increased 6-fold when *Hibiscus sabdariffa* extracts are mixed with iron from 1 to 13 mg/kg but decreased when the mixture *Hibiscus sabdariffa* extracts is done with chlorogenic and gallic acids. The

authors added that degradation rate of cyanidin 3-O-sambubioside was not affected by polyphenols but increased by 3-fold with increasing iron concentration with a concomitant yield decrease of scission product, protocatechuic acid. Moreover, two pathways of degradation of anthocyanins were identified. The first degradation pathway of anthocyanins in *Hibiscus sabdariffa* extracts is a major metal-catalyzed oxidation followed by condensation and the second pathway of degradation of anthocyanins in *Hibiscus sabdariffa* extracts is a minor scission which represents about 10% of degraded anthocyanins.

Grajeda-Iglesias et al. (2016) use lipophilization and MS characterization methods to isolate and to characterize anthocyanins from *Hibiscus sabdariffa* flowers. It resulted from this work that *Hibiscus sabdariffa* calyces are an important source of natural pigments. It has also showed that the anthocyanins of *Hibiscus sabdariffa* are significantly hydrosoluble and unstable bioactive compounds. Basing on the mass spectra of the individual anthocyanins found in *Hibiscus sabdariffa* calyces, delphinidin-3-o-(2"-xylosyl) glucoside (delphinidin-3-o-sambubioside, Dp-samb) and cyanidin-3-o-(2"-xylosyl)- glucoside (cyanidin-3-o-sambubioside, Cy-samb), with [M+] at m/z 597 were identified. The authors concluded that delphinidin-3-o-sambubioside corresponded to the most abundant compound (46% of total area).

Guzmán-Figueroa et al. (2016) investigated the anthocyanins of *Hibiscus sabdariffa* and synthesis of pyranoanthocyanins. It has been demonstrated that delphinidin-3-sambubioside and cyanidin-3-sambubioside can be mixed to produce pigments like pyranoanthocyanins. The authors concluded that pyranoanthocyanins are more stable than anthocyanins and also they show more colours properties.

3. MATERIAL AND METHODS

3.1. Material

3.1.1. Raw material

Hibiscus sabdariffa cultivated in Benin Republic was investigated in this study. Roselles were harvested in 2016 from University of Agriculture of Ketou's experimental field. After harvesting, they were decorticated and sun dried for 6-8 days (24-32 °C).



Figure 3. 1. Dried *Hibiscus sabdariffa* samples

3.1.2. Sample preparation

Roselle tea samples were prepared by three different infusion procedures: Two methods traditional of Benin (TI₁ and TI₂) and one other method used by Ramirez-Rodrigues et al. (2011) (IR). The sample preparation parameters are mentioned below in detail:

- ü 50g of roselle's calyces were sampled and mixed with 1L of distilled and boiled at 100 °C for 40 minutes. After cooling, the infusion was filtered and the obtained tea (coded as TI₁) was transferred to a flask.
- ü 50g of roselle calyces were soaked in 1L of distilled water for 24 hours at ambient temperature. The infusion was filtered and the obtained tea (coded as TI₂) was transferred to a flask.
- ü 1g of roselle's calyces was mixed with 40ml of distilled water in a ratio and boiled at 98 °C for 16 min (Ramirez-Rodrigues et al., 2011). After cooling, the infusion was filtered and the filtered tea (coded as IR) was transferred to a flask.



Figure 3. 2. *Hibiscus sabdariffa* infusions

3.2. Methods

3.2.1. Physico-chemical properties of *Hibiscus sabdariffa* infusions analysis

Colour measurement of *Hibiscus sabdariffa* infusions were performed by registering the values of L*, a* and b* (Uckun and Selli, 2017). Hunter Lab Scan

(Hunter Associates Laboratory, Inc., Reston, VA, USA) apparatus was used to measure L^* , a^* , b^* values. L^* value refers to brightness (white darkness or lightness); $+a^*$ indicates the value of red; $-a^*$ is the value of green while $+b^*$ value means of the yellow and $-b^*$ is the value of blue. The pH of samples was directly measured with a glass electrode pH meter (ORION 3-Star, USA). Total soluble solids were measured with a handheld refractometer (Westover,USA).

3.2.2. Determination of total phenolic content

The total phenolic content of *Hibiscus sabdariffa* tea was determined using the method Mohd-Esa et al (2010) used with some modification. Briefly, 1 ml of each sample extract was mixed with 60 ml of pure water, 5 ml of Folin-Ciocalteu solution and 15 ml of sodium bicarbonate solution, and allowed to stand at ambient temperature for 2 hours. The absorbance was read in a UV-Visible spectrophotometer at 765 nm. The phenolic content was expressed as gallic acid equivalents (GAE) in milligram per kg of dry *Hibiscus sabdariffa*.

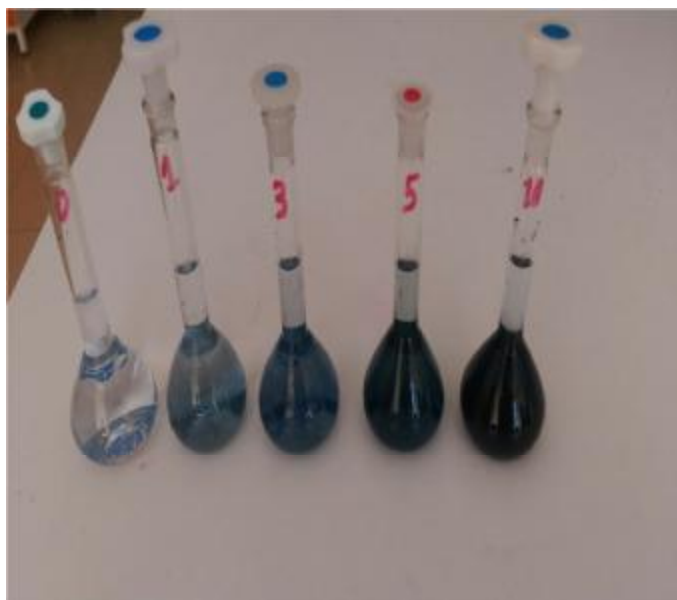


Figure 3.3. Preparation of standard phenolic solutions to obtain calibration curve

3.2.3. Analysis of antioxidant activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay was used to evaluate roselle infusion's antioxidant activity. The electron donation ability of the samples was determined by whitening of the purple-colored solution of DPPH radical by the method of Kelebek et al. (2013). Three different dilutions of each extract were processed in ethanol/ water (v/v). An aliquot of 0.1 mL of diluted tea was appended to 3.9 mL of DPPH solution in methanol (6×10^{-5} M). The ensemble was mixed vigorously. The absorbance was read with a UV- visible spectrophotometer at 515 nm.



Figure 3.4. Preparation of standard DPPH solutions to obtain calibration curve

3.2.4. Aroma analysis of *Hibiscus sabdariffa* teas

3.2.4.1. Liquid liquid extraction (LLE)

LLE is a separating technology that is based on distribution of one or more components between two immiscible or almost immiscible liquids. One of the liquids can be water and the other an organic solvent or the both phases can be organic or organic mixtures. LLE has often been a favored choice of the process

engineer for the development of separation processes (Lo, 1996). The volatile compounds of roselles tea samples were isolated with the use of dichloromethane which showed its efficiency as solvent for volatile compounds isolation from fruits and plants (Kelebek & Selli, 2011).



Figure 3.5. Extraction of aroma compounds

The extraction method was derived from existent procedures (Aubert et al., 2005; Amanpour et al., 2015). For the extraction, 200 ml of roselle's tea (sample), 160 ml of dichloromethane and 5 μ L of 2-octanol as internal standard were mixed in a 500 ml flask. Under nitrogen, the assembly was stirred at 4°C for 40 min and then centrifuged at 4°C at 4500 rpm for 15 min. The solvent phase was separated from the soluble phase. The pooled aromatic extract was dehydrated with

anhydrous sodium sulfate and reduced to 0,5 ml in a concentrator. The volume of extract was reduced again to 200 μ L under a gentle stream of purified nitrogen. The concentrated extract was injected to the GC-MS for aroma compounds determination. Three repetitions of liquid liquid extraction were performed.



Figure 3.6. Separation of aromatic extract of *Hibiscus sabdariffa*

3.2.4.2. GC-MS-O conditions

The GC system consisted of an Agilent 6890 chromatograph fitted with a flame ionization detector (FID) (Wilmington, DE), an Agilent 5973N-mass selective detector (MSD), and a Gerstel ODP-2 (Linthicum, MD) sniffing port using deactivated capillary column (30 m - 0.25 mm) heated at 240 ° C and supplied with humidified air. This system led us to obtain an FID signal for the quantification, an MS signal for the identification at the same time, and the odour

characteristics of each compound detected by the sniffing port. GC effluent was divided 1:1:1 between the FID, MSD, and olfactometer via a Dean's switch. Volatile compounds were isolated on a DB-Wax (30 m × 0.25 mm, 0.5 μm thickness; J&W Scientific, Folsom, CA) column. 3 uL of extract was squirted each time in pulsed splitless (40 psi; 0.5 min) mode. The injector and FID were fixed at 270 and 280 °C, respectively. The flow rate of carrier gas (helium) was 1.5 mL min⁻¹. The oven temperature was first raised from 50 to 200 °C at an incidence of 5 °C/min and then to 260 °C at 8 °C/min and finally kept at 260 °C for 5 min. The mass detector was run in scan mode, with electronic impact ionization energy of 70 eV. The temperatures of GC-MS interface and ionization were kept at 250 °C and 180 °C, respectively. Identification and quantification were carried out in full scan mode scanning a mass range of m/z 30-300 at 2.0 scan/s. The volatile compounds were identified by confronting their mass spectra with those in Wiley 9 and NIST 11 mass spectral data libraries and an in-house library made from previous laboratory works. Some of the identifications were confirmed by the injection of chemical standards into the GC - MS system. Retention indices of the compounds were calculated by using the retention data of a linear alkane series. The concentrations of volatiles compounds were computed by GC-FID according to the internal standard (40 μg of 4-nonanol) (Amanpour et al., 2015).



Figure 3.7. Gas Chromatography-Mass Spectrometry-Olfactometry

3.2.4.3. Aroma extract dilution analysis (AEDA)

GC-MS-Olfactometry analysis was achieved on a precise (Agilent-USA) device. Three experienced sniffers took part to analyze the original aroma by GC-MS-O. For AEDA, the concentrated aromatic extract (200 μ L) of roselle was diluted step by step using dichloromethane as diluent to get dilutions of 1:1, 1:2, 1:4, 1:8, 1:16, and up to 1:4096 of the initial extracts (Amanpour et al., 2015; Fickert and Schieberle, 1998; Schieberle and Grosch, 1987). Sniffing of obtained dilutions was pursued until the odor of GC-MS-O effluent could not be perceived by sniffer. Each odor was then attributed a flavor dilution factor (FD factor) which represents the last dilution in which the odor was still perceptible. To avoid nose drying during the sniffing, a special device was used to damp olfactometry port and this increases panelist sensitivity. Each sniffing was done by trained panelist for 20 minutes. Before the GC-MS-Olfactometry analysis, roselle's standard aroma compounds were taught to the panelists. After identification of the peaks, aroma

compounds concentration was calculated with internal standard method (Selli et al, 2008).

3.2.4.4. Calculation of aroma compounds quantities

After identification of aroma compounds, their concentration was calculated by internal standard method like described bellow:

$$C_i = (A_i / A_{st}) \times RF \times C_{st} HF$$

C_i: Concentration of the compound

A_i: Peak area of the compound

A_{st}: Peak area of internal standard

C_{st}: Concentration of internal standard (40 µg)

RF: Response factor

HF: Calculation factor (factor of conversion of 1 kg of sample)

3.2.5. Sensory Analysis

In sensory evaluations; taste, aroma and overall likeability of samples were investigated according to nine different criteria. Evaluations were made by putting 15 ml of roselle tea sample at 24 ± 2 °C into transparent glasses which were coded with three different numbers (Zhu et al., 2017). The analysis of tea samples was carried out by a group of 10 previously trained panelists (between 25 and 47 years old). Each panelist was asked to mark the intensity of perceived attribute on a 100 mm scale on the profile sheet by smelling and tasting. The profile sheet used by the panelists is shown in Figure 3.8. The results of the evaluations were displayed in both table and spider plot.

Ad Soyadı:	DUYUSAL ANALİZ FORMU	Tarih:
Aroma	_____→	
Meyvemsi Koku	_____→	
Tatlı/Karamelize koku	_____→	
Bitki (Herbal) kokusu	_____→	
Tat	_____→	
Asitlik	_____→	
Bırcılık	_____→	
Renk	_____→	
Genel Beğenililik	_____→	

Figure 3.8. Profile sheet used for sensory analysis

3.2.6. Representativeness of aroma extract

In this research, the aroma of *Hibiscus sabdariffa* infusions and obtained the aromatic extracts were characterised using the representative test. Sensory evaluations were performed at Department of Food Engineering of the Faculty of Agriculture, Cukurova University. Sensory analysis of *Hibiscus sadariffa* infusions and their aromatic extracts was achieved by a panel composed of 7 people.

The representative test has been successfully performed in orange juice (Selli and Kelebek, 2011), cherry tomato extracts (Selli et al., 2014) and saffron extracts (Amanpour et al., 2015). Dichloromethane was used to extract the aroma compounds of *Hibiscus sadariffa* infusions. 10 ml of *Hibiscus sadariffa* infusions was put in a 25 ml brown coded flask and presented to panelist as reference. The aromatic extracts obtained from different infusions were absorbed onto special cardboard (SARL H.Granger-Veyron, France) and then left 1 minute allowing the solvent to evaporate. Later, these cardboards were placed into two different 25 ml brown bottles and presented to the panelists. *Hibiscus sadariffa* infusions and their

respective aromatic extracts were then compared by panelists. As result, reliability of the solvent was tested simultaneously for aromatic extracts. Similarity test and aroma intensity test were used to determine representative test values.

3.2.6.1. Similarity test

Panelists were asked to determine the closeness between the odours of the *Hibiscus sadariffa* infusions and aromatic extracts. For this purpose, a scale of 100 mm was used (Figure 3.4) (Van Ruth et al., 1995).

3.2.6.2. Aroma intensity test

In this test, the panelists were asked to compare the intensities of the *Hibiscus sadariffa* infusions and their respective aromatic extracts. For this purpose, a scale of 100 mm in was used (Figure 3.5) (Van Ruth et al., 1995).



Figure 3.9. The scale used in the similarity test

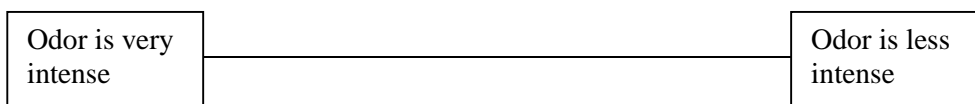


Figure 3.10. The scale used in the aroma intensity test

3.2.7. Statistical Analysis

The statistical analysis of the data obtained from the analysis of *Hibiscus sabdariffa* teas were performed by variance analysis and Duncan multiple comparison test using SPSS 16 software package (Özdamar, 1999).



4. RESULTS AND DISCUSSION

4.1. Physico-chemical properties of *Hibiscus sabdariffa* infusions

Roselle tea samples were investigated in terms of total soluble solids (TSS), pH and colour parameters. pH values were found similarly in all three samples. Previous studies reported that pH values of aqueous roselle extracts may vary between 2,2 and 2,6 (Bechoff et al., 2014; Cisse et al., 2009; Ramírez-Rodrigues et al., 2011; Ramirez et al., 2010). Roselle teas obtained from different infusions had slightly higher pH values changing between 2.79 and 2.95 (Table 4.1). This may be caused from cultivar, post-harvest operations, and calyx extraction conditions differences. Regarding TSS, highest value (3.4 °Bx) was recorded in TI₁ sample which prepared with hot infusion. Alobo et al. (2009) reported that aqueous hibiscus extracts had a total soluble solid value of 2 °Bx. The difference in TSS values may be caused from both applied heat during infusion and total time of contact with water as these two parameters may increase the soluble solid amounts. Colour properties of *Hibiscus sabdariffa* infusions are one of determinant factors which can influence consumer decision. L* values found in infusions TI₁, TI₂ and IR were 1.13, 1.41 and 5.65, respectively. The value of a* was higher in IR (30.6) sample that TI₁ (7.41) and TI₂ (9.22). In present work, infusion IR displayed the highest of b* values (9.58) as compared to infusions TI₁ (2.00) and TI₂ (2.28). It is resulted from the colour analysis that L*, a* and b* values are higher in IR infusion, while lower in TI₁ and TI₂ infusions (p<0, 05).

Table 4.1. Physico-chemical properties of *Hibiscus sabdariffa* infusions

Parameters	TI ₁	IR	TI ₂
Total soluble solids (°Brix)	3,4±0,25 ^c	1,7±0,2 ^a	2,7±0,3 ^b
pH	2,79±1,3 ^a	2,95±1,5 ^a	2,88±0,95 ^a
L*	1,13±0,7 ^a	5,65±1,3 ^c	1,42±1,4 ^b
a*	7,41±1,0 ^a	30,6±1,0 ^c	9,22±0,8 ^b
b*	2,0±0,2 ^a	9,58±1,2 ^b	2,28±0,1 ^a

The results are indicated as mean values of evaluations.

a, b, c: Same letters mean no significant differences between samples.

Examining these values, it can be assumed that the colours of the infusions (TI₁, TI₂ and IR) are a mixture of dark, red and yellow attributes. Having higher value as L* parameter, IR sample can be commented as brighter than other ones. The found colour values in our work are lower (and darker) as compared to the colour values revealed by Ramirez-Rodrigues et al. (2011) in cold and hot *Hibiscus sabdariffa* infusions. Liang et al. (2005) investigated the quality of three Chinese teas (green, black and oolong teas) based on the colour analysis. The authors reported very low L* and a* values (negative values) in all teas whereas higher b* values in teas (ranged from 22.64 to 65.05).

4.2. Total phenolic content of tea samples

The Total phenolic compounds of roselle tea were analyzed using Folin-Ciocalteu reagent. The amounts of total phenolic compounds obtained from Beninese roselle teas were found as 2135, 1556 and 2201 mg gallic acid equivalent (GAE)/kg in TI₁ (Traditional Infusion obtained at 100 °C/40 minutes), TI₂ (Traditional Infusion obtained at ambient temperature/24 hours) and IR (Infusion obtained by heating *Hibiscus sabdariffa* calyces at 98 °C/16 minutes) respectively as seen in Table 4.2. The highest value of the total phenolic compounds was in IR

extract, followed by TI₁ and TI₂ extracts. There were significant differences ($p < 0.05$) in phenolic compounds of the infusions (TI₁, TI₂ and IR).

The values of the total phenolic compounds detected in the infusions (TI₁, TI₂ and IR) were higher than the one found by Tahir et al. (2017) in four species of Egyptian roselle teas which was ranged from 79.84 to 148.17 mg GAE/100g. Ochoa-Velasco et al. (2017) reported 3.43 mg GAE/100g which is less than the value found in the present work. However, it is found that teas obtained from Beninese roselles, with some exceptions, displayed slightly lower total phenolic amounts than roselles harvested from other countries. In the work conducted by Arenas et al. (2016), the analysis showed 913 mg GAE/100 g, 876 mg GAE/100g and 1013 mg GAE/100g in washed calyces, residue and dried calyces of Mexican roselles, respectively. Mercado-Mercado et al. (2015) found that total phenolic compounds ranged between 117.31 to 296.03 mg GAE/g in calyces residue and complete calyces. Furthermore, Zhen et al. (2016) and Borrás-Linarez et al. (2015) reported that they determined total phenolic amounts between 18.98-29.9 mg GAE/g in dried leaves of roselles and 2, 4-10 g GAE/100g in Mexican roselle calyces.

The total phenolic content of the infusions obtained by heating process (TI₁ and IR) presented higher values than the infusion obtained by soaking roselle's calyces in water at ambient temperature for 24 hours (TI₂). IR and TI₁ infusions were prepared at the water boiling temperature (98-100 °C). The heating process may induce the release of the phenolic compounds. Prenesti et al. (2007) and Ramirez-Rodriguez et al. (2011) assessed that the more the extraction temperature increases more the yield of the total phenolic compounds has been increases. This has been confirmed by the work of Sindi et al. (2014) in which the highest amount of the total phenolic compounds has been observed in the roselle extracts obtained with water at boiling temperature. Mercado-Mercado et al. (2015) found also a high value of the total phenolic compounds from the extract obtained 85 °C. However, a slight decrease of the total phenolic compounds in TI₁ extract was

noted when it is compared to IR. This may be explained with the long time (40 minutes) of hot infusion applied during the preparation of teas. Thus, the time is a determinant factor to optimize the extraction of total phenolic compounds. This has been confirmed by the study of Sindi et al. (2014) which suggested 10 minutes as the optimal extraction time.

In addition, the variations observed through different studies on total phenolics of *Hibiscus sabdariffa* were somehow linked either to the extraction temperature, time and solvent used for the extraction or the analysis assay and raw material origin (Sindi et al. 2014; Prenesti et al. 2007; Ramirez-Rodrigues et al. 2011). Arenas et al. (2016) revealed that total phenolic compounds of *Hibiscus sabdariffa* could also be influenced by drying process depending on air velocity, air temperature and relation between air and temperature.

Table 4.2. Phenolic compounds and antioxidant activities of *H. sabdariffa* teas

Sample preparation methods	Total Phenolic Content (mg/kg gallic acid equivalent)	Antioxidant Activities (mM/kg of trolox equivalent)
TI ₁	2135±9 ^b	714±34 ^b
TI ₂	1556±5 ^a	358±20 ^a
IR	2201±32 ^c	761±34 ^b

4.3. Antioxidant activity of tea samples

The antioxidant activities of the samples were determined with the application of DPPH assay. The results were found as 714, 358 and 761 mM Trolox equivalents (TE)/kg of db, in TI₁, TI₂ and IR infusions respectively (Table 4.1.). Statistical analysis showed significant differences ($p < 0.05$) between TI₂ and other infusions (TI₁ and IR), however no difference was found between IR and TI₁. These values were in accordance with the results of some other studies found in

literature. Borrás-Linares et al. (2015) reported an antioxidant activity value between 27.4 and 112 mol Trolox equivalents (TE)/g db from calyces of 25 varieties *Hibiscus sabdariffa*. Ochoa-Velasco et al. (2017) reported 9.34 mol Trolox equivalents/100g in *Hibiscus sabdariffa* calyces, which was less than the found values in TI₁, TI₂ and IR infusions. In the work conducted by Leyva et al. (2012), the antioxidant activities of calyces upon different drying conditions, displayed a result ranged from 88.44 to 181.13 µmol Trolox equivalent/g db. Furthermore, Salazar-Gonzalez et al. (2012) investigated the antioxidant activity of dried *Hibiscus sabdariffa* calyces and the results have been ranged from 3.11 to 8.0 mmole Trolox/100 g calyces. Moreover, Abdullakasim et al. (2007) reported that they determined the antioxidant activity of *Hibiscus sabdariffa* drink as 59.4 µmol Trolox equivalents/100 ml.

The variations of antioxidant activities observed in *Hibiscus sabdariffa* calyces, extract or leaves in both present and existent works, could be explained by factors such as extraction process (Borrás-Linares et al., 2015; Sindi et al., 2014), inconsistencies of the methodology (Wang et al., 2014), varieties of the genotype (Borrás-Linares et al., 2015), growth conditions such as temperature and soil moisture (Ottai et al., 2004) and infusion preparation conditions.

In the present study, the highest level of antioxidant activity was found in IR extract followed by TI₁ and TI₂ infusion extracts. TI₁ and RI which were obtained by heating process showed the highest levels of antioxidant activity. High temperature and the length of applied time during infusion preparation may induce the release of compounds having antioxidant activity present in *Hibiscus sabdariffa* calyces. This is supported by Sun et al. (2017), who investigated the phytochemical compounds of wolfberry infusion. In that study, Sun et al. (2017) revealed that the highest amount of antioxidant activity has been found in wolfberry tea at 100°C/150 minutes. Hence, they assumed that the higher the infusion temperature and the length of time are, the more release of the compounds responsible of antioxidant activity is observed. Similarly, Balci and Ozdemir (2016) mentioned

that the antioxidant capacity of Turkish green teas increased as infusion temperature and time were augmented. Furthermore, in the study conducted by Kelebek (2016), the analysis of antioxidant activity of Turkish black teas prepared with different temperature and time combinations, showed more important antioxidant activity in the high temperature and time. Many other work found in the existent literature reported the increase in infusion temperature and time associated with the increment of antioxidant capacity (Sharpe et al., 2016; Hajiaghaalipour et al., 2016). However, the infusion preparation conditions have to be carefully monitored because an excessive high temperature and time can be harmful to the compounds responsible of antioxidant activity. It has been confirmed by Leyva et al. (2014) who affirmed that when the sample is heated for an unnecessary long time, the compounds responsible of antioxidant capacity can be negatively affected.

TI₂ infusion presented the lowest value antioxidant of *Hibiscus sabdariffa*. This result may be explained by the fact the sample was not heated. It has been said that when the heating is done in the better conditions of time and temperature, the yield of polyphenols can increase with no significant losses of antioxidant compounds (Prencesti et al., 2007; Ramirez-Rodrigues et al. 2011). In addition, Damiani et al. (2014) suggested roselle tea obtained at room temperature for two hours as an alternative to the generally consumed hot teas.

4.4. Aroma profile of *Hibiscus sabdariffa* teas

The dainty and delicate aroma of *H. sabdariffa* was reported to generally derive from fatty acids and sugars. Although there are few studies about roselle aroma, it was determined that mostly furans, alcohols, aldehydes, and ketones compounds (Chen et al., 1998; Pino et al., 2006). The effect of different infusion procedures on volatile composition of roselle teas were investigated in present study by GC-MS with the aid of liquid liquid extraction (LLE).

Table 4.3. Overall aroma groups of three different *Hibiscus sabdariffa* infusions and their concentrations

Group	TI ₁	IR	TI ₂
Furans	110560,1	72121,7	148415,4
Alcohols	8800	5432,1	11731
Acids	1549,5	2408	3104,4
Aldehydes	822	868	2252
Ketones	1863,7	1958,2	2365,1
Volatile phenols	4862	1960	2656
Lactones	2010	2010	2490
Pyranone	2261	838	1278
Pyrrole	1032	878	611
Ester	936	865	1496
Terpene	135	228	146

Concentration: Means of three repetitions as $\mu\text{g}/\text{kg}$

The aromatic extract obtained from LLE was evaluated with representative tests. As a result of the representative tests, it was determined that the extracts showed a similarity of 60.71% with original sample and the odor intensity was found as 87%. Hence, the method and dichloromethane solvent were found reliable.

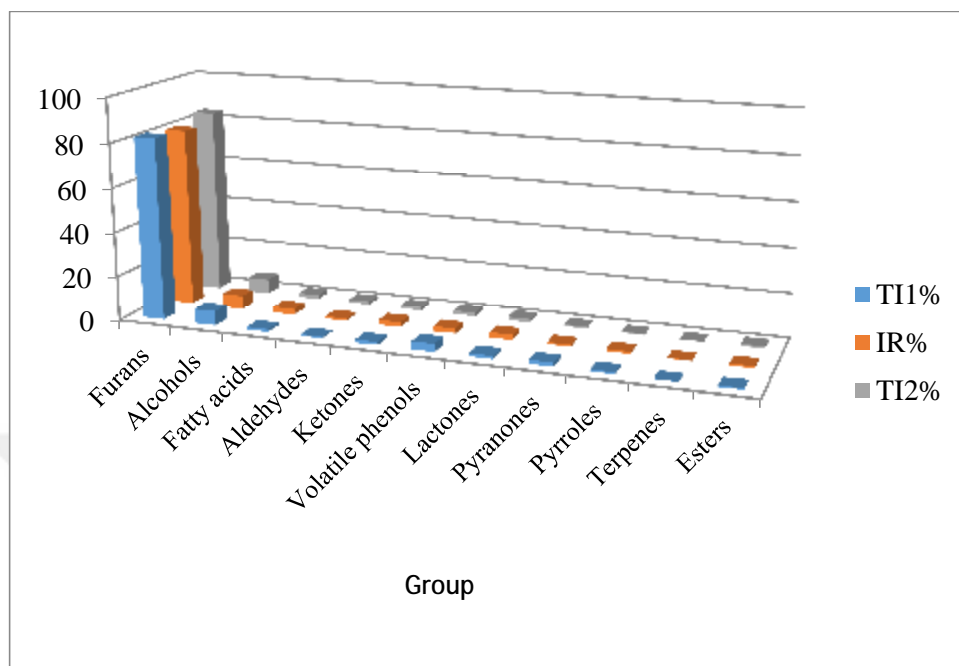


Figure 4.1. Overall aroma groups of three different *Hibiscus sabdariffa* infusions

4.4.1. Aroma profile of *Hibiscus sabdariffa* tea extracts obtained with different infusions

The composition and amounts of volatiles determined in Beninese roselle tea samples obtained by different infusions (TI₁, TI₂ and IR) were displayed in Table 4.3. A total of 39 compounds were identified in the samples mainly including the groups of furans, alcohols, acids, ketones, volatile phenols, lactones, and etc. The volatile composition of each sample showed similarity in between and the effects of infusion techniques were clearly observed on the amounts of compounds.

Table 4.4. Aroma compounds of roselle teas from three different infusions

No	LRI	Compounds	TI ₁	IR	TI ₂	Identification
Furans						
1	1238	2-Pentyl furan	74.1±8 ^b	56.67±3 ^a	49.4±1 ^a	LRI, MS, Std
2	1469	Furfural	50822±1396 ^a	45802±1089 ^a	110168±9970 ^b	LRI, MS, Std
3	1536	2- Acetylfuran	342±33 ^a	331±28 ^a	649±40 ^b	LRI, MS, Std
4	1575	5-Methyl-2-furfural	1649±4 ^a	1692±93 ^a	3767±169 ^b	LRI, MS, Std
5	1608	2-Acetyl-5-methylfuran	102±6 ^a	104±10 ^a	106±10 ^a	LRI, MS, Std
6	1761	2(5H)-Furanone	448±27 ^b	322±18 ^a	545±9 ^c	LRI, MS, Std
7	1879	Furfural acetone	246±6 ^b	162±1 ^a	139±12 ^a	LRI, MS, Tent
8	2395	5-Hydroxymethylfurfural (HMF)	56877±2802 ^c	23652±2352 ^b	32992±939 ^a	LRI, MS, Std
Total			110560,1	72121,7	148415,4	
Alcohols						
9	1026	2-Methyl-3-buten-2-ol	926±76 ^a	872±29 ^a	1278±139 ^b	LRI, MS, Std
10	1170	3-Penten-2-ol	6926±244 ^b	3416±325 ^a	8474±867 ^b	LRI, MS, Std
11	1187	2-(2-Butoxyethoxy) ethanol	162±7 ^a	322±9 ^a	508±50 ^b	LRI, MS, Tent
12	1200	3-Hexen-1-ol	170±11 ^a	201±6 ^a	277±28 ^b	LRI, MS, Std
13	1230	2-Phenoxy ethanol	165±7 ^a	181±16 ^{ab}	208±14 ^b	LRI, MS, Std
14	1661	2-Furan methanol (Furfuryl alcohol)	100±3 ^a	56,1±1 ^a	273±24 ^b	LRI, MS, Std
15	1867	Phenylethyl alcohol	118±2 ^a	173±8 ^b	192±16 ^b	LRI, MS, Std
16	1877	Benzyl alcohol	233±7 ^a	211±23 ^a	121±12 ^a	LRI, MS, Std
17	1949	Isophytol	Nd	Nd	400±18	LRI, MS, Tent
Total			8800	5432,1	11731	
Acids						
18	1446	Acetic acid	757±11 ^a	1416±61 ^b	2036±145 ^c	LRI, MS, Std
19	1667	2-Methyl butanoic acid	219±7 ^b	144±2 ^{ab}	120±1 ^a	LRI, MS, Std
20	1838	Hexanoic acid	157±3 ^c	106±1 ^b	65,4±5 ^a	LRI, MS, Std
21	2060	Octanoic acid	92,5±3 ^a	402±35 ^b	425±13 ^b	LRI, MS, Std
22	2158	Nonanoic acid	324±19 ^a	340±20 ^a	458±44 ^b	LRI, MS, Std
Total			1549,5	2408	3104,4	
Aldehydes						
23	1078	Hexanal	174±15 ^a	219±10 ^b	297±6 ^c	LRI, MS, Std
24	1396	Nonanal	385±20 ^a	396±40 ^a	645±10 ^b	LRI, MS, Std
25	1508	Benzaldehyde	263±1 ^a	253±8 ^a	1310±125 ^b	LRI, MS, Std
Total			822	868	2252	
Ketones						

Table 4.4. Continued

26	1006	3-Methyl-3-buten-2-one	1219±12 ^a	1435±129 ^a	1887±169 ^b	LRI, MS, Std
27	1243	Methylacetoin (2-butanone,3-hydroxy-3-methyl)	80,1±5 ^a	88.3±3 ^{ab}	99.2±7 ^b	LRI, MS, Std
28	1277	3-Hydroxy-2-butanone (acetoin)	107±10 ^b	74,0±1 ^a	61.0±6 ^a	LRI, MS, Std
29	1297	2-Octanone	38.6±4 ^a	32,9±1 ^a	38,9±3 ^a	LRI, MS, Tent
30	1321	1-Hydroxy-2-propanone (Acetol)	419±3 ^b	328±33 ^a	279±18 ^a	LRI, MS, Std
Total			1863,7	1958,2	2365,1	
Volatiles phenols						
31	2162	Eugenol	944±1 ^a	887±49 ^a	1190±90 ^b	LRI, MS, Std
32	2201	4-Vinylguaiacol	3202±285	487±52	1006±57	LRI, MS, Std
33	2379	4-Vinylphenol	716±5 ^c	586±7 ^b	460±22 ^a	LRI, MS, Std
Total			4862	1960	2656	
Lactones						
34	1628	γ -Butyrolactone	648±19 ^a	676±69 ^a	1046±107 ^b	LRI, MS, Std
35	2028	Pantolactone	1362±54 ^a	1394±122 ^a	1444±32 ^a	LRI, MS, Tent
Total			2010	2070	2490	
Pyranone						
36	1954	Maltol	2261±158 ^c	838±67 ^a	1278±108 ^b	LRI, MS, Std
Total			2261	838	1278	
Pyrrole						
37	2013	1H-pyrrole-2-carboxaldehyde (2-formylpyrrole)	1032±12 ^c	878±16 ^b	611±25 ^a	LRI, MS, Tent
Total			1032	878	611	
Terpene						
38	1206	<i>DL</i> -Limonene	135±5 ^a	228±15 ^b	146±6 ^a	LRI, MS, Std
Total			135	228	146	
Ester						
39	2025	Methyl-2-furoate	936±32 ^a	865±67 ^a	1496±99 ^b	LRI, MS, Tent
Total			936	865	1496	
General Total			134831.3	89627	176544.5	

LRI linear retention index calculated on DB-WAX capillary column.

a, b, c: Same letters mean no significant differences between samples.

Concentration: Means of three repetitions as $\mu\text{g}/\text{kg}$; *nd*: not detected.

Identification: Methods of identification; *LRI* linear retention index; *MS*: Identified by MS; *tent* tentatively identified by MS, *Std*: Identified by chemical standard.

Furans: Furans were determined as the major volatile group in all samples quantitatively as it can be seen in Table 4.2. The total concentration of furans was found as 110560,1 $\mu\text{g}/\text{kg}$ in TI_1 sample; 148415,4 $\mu\text{g}/\text{kg}$ in TI_2 sample and lastly 72121,7 $\mu\text{g}/\text{kg}$ in IR sample. Furfural, 5-hydroxymethylfurfural (HMF), 5-methyl-2-furfural, 2-acetylfuran, 2-pentyl furan, 2-acetyl-5-methylfuran, 2(5H)-furanone, furfural acetone were identified in the group of furans in all three samples as showed in Figure 4.2.

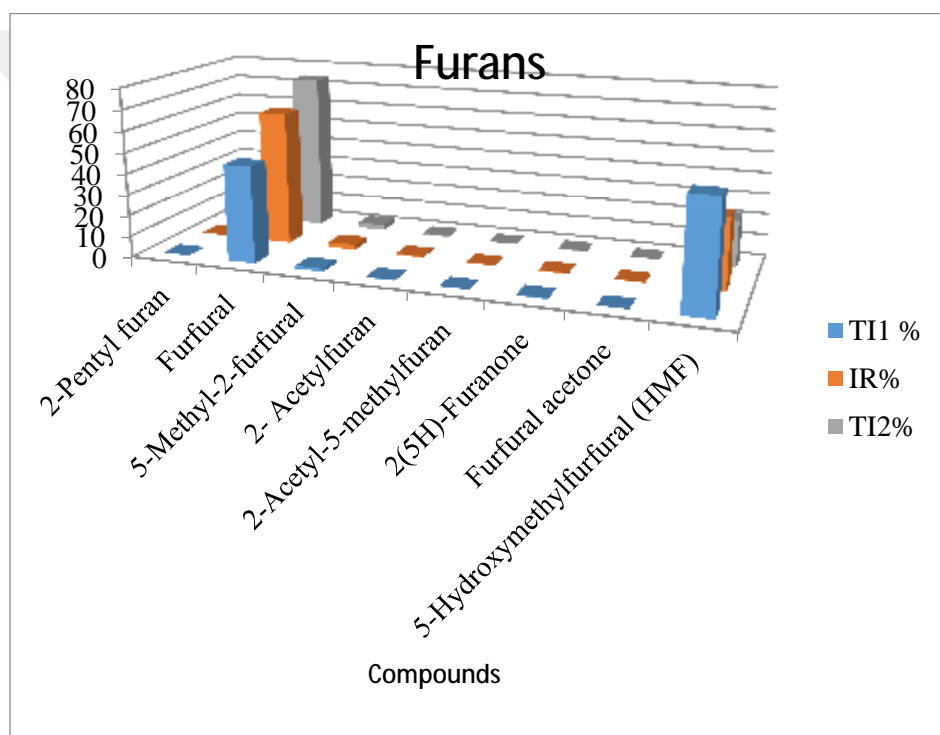


Figure 4.2. Furans compounds in three different *Hibiscus sadariffa* infusions
Furans compounds in three different *Hibiscus sadariffa* infusions

The development of furans are reported to generally occur during the drying period of *Hibiscus sabdariffa* through Maillard reaction (sugar-amino acid destruction) and their production may be sped up by reduction of moisture content to the water activity of 0.3-0.7 (Eichner and Karel, 1972; Ramirez-Rodrigues et al.,

2011). Furfural and 5-hydroxymethylfurfural (HMF) showed the highest concentration among furans in all three samples. Furfural was the main volatile of both TI₂ and IR samples while HMF had the abundance in TI₁ sample. Both furfural and 5-hydroxymethylfurfural are sugar derived compounds and they are reported to be formed during the drying process of roselle samples before infusion (Chen et al., 1988). It is also indicated in the literature that the decrease in moisture content of roselle samples may lead to an increase in the concentrations of these compounds (Eichner and Karel, 1972). Similarly, Chen et al. (1988) detected an increase in amounts of furfural and HMF after applying a drying process at 50 and 75°C. In addition, furfural was reported to be the most abundant volatile in roselle teas prepared from *H. sabdariffa* grown in Egypt and Sudan as it had about 32 and 39% of total peak areas in the chromatogram respectively (Farag et al., 2015). In another study conducted by Ramirez-Rodrigues et al. (2011), volatile compositions of roselle teas obtained from fresh and dried roselles were investigated and authors have detected furfural only in dried hibiscus teas. In the study, furans like furfural are indicated to be formed as a result of Maillard (amino-acid sugar) degradations and drying process of roselles were stated to be the reason for these compounds to occur that supports the hypothesis. In our samples, tea obtained from the traditional Beninese infusion at ambient temperature for 24 hour (TI₂) displayed the highest amount of furfural (110168 µg/kg) following by teas with other traditional (TI₁) and IR infusions (50822 and 45802 µg/kg respectively). Generally, it is expected that sample processed with higher temperature to comprise higher levels of furan compounds like furfural as their formation is related to Maillard degradation, yet these compounds are known to be highly polar and hydrophilic as they can be highly extracted by polar water (Kawata et al., 2001; Ramirez-Rodrigues et al., 2011). This information supports our results that as the infusion time increased, the obtained concentrations of compounds like furfural, 5-methyl-2-furfural, 2-acetylfuran have a statistically significant ($p < 0.05$) increase correlatively (Table 4.3). In addition, when the other samples are investigated, it can be clearly seen

that the infusion at higher temperature with longer time (i.e. TI₁: 100°C/40 min) had higher values of furfural in comparison with slightly lower temperature with less time (i.e. IR: 98°C/16 min). As the temperature rise supports the sugar-amino acid degradation, this result is like expected. Likewise, in a study investigating the aroma profile of Mexican roselle teas obtained by hot and cold water infusion, it is reported that furfural concentration increased with the increasing temperature of infusion (Ramirez-Rodrigues et al., 2011).

5-Methyl-2-furfural is reported to be an important contributor in overall aroma of *H. sabdariffa* (Chen et al, 1998; Pino et al., 2006; Ramirez-Rodrigues et al., 2011). This compound is also derived from sugar degradation similar to furfural and it was extracted in higher amounts with longer time of infusions. 5-Methyl-2-furfural concentrations were determined as 3767 µg/kg, 1692 µg/kg and 1649 µg/kg in TI₂, IR and TI₁ samples, respectively. Unlike this compound, an increase was observed in 2-pentylfuran's concentration with higher temperature infusions. 2-Pentylfuran is reported to be formed via thermal degradation of hydroperoxides or cyclic peroxides (Min et al., 1989; Belitz et al., 1986; Kroh, 1994). Thus, this information supports the rise in amount of this compound with increasing temperature.

Alcohols: Alcohols were the most abundant group qualitatively in *Hibiscus sabdariffa* teas. They represented approximately 6% of total aroma compounds found in the extracts obtained via different infusions. In another study performed on the hibiscus samples, the percentage of alcohols among total aroma profile was reported to be found in the same range (8.6%) (Pino et al., 2006).

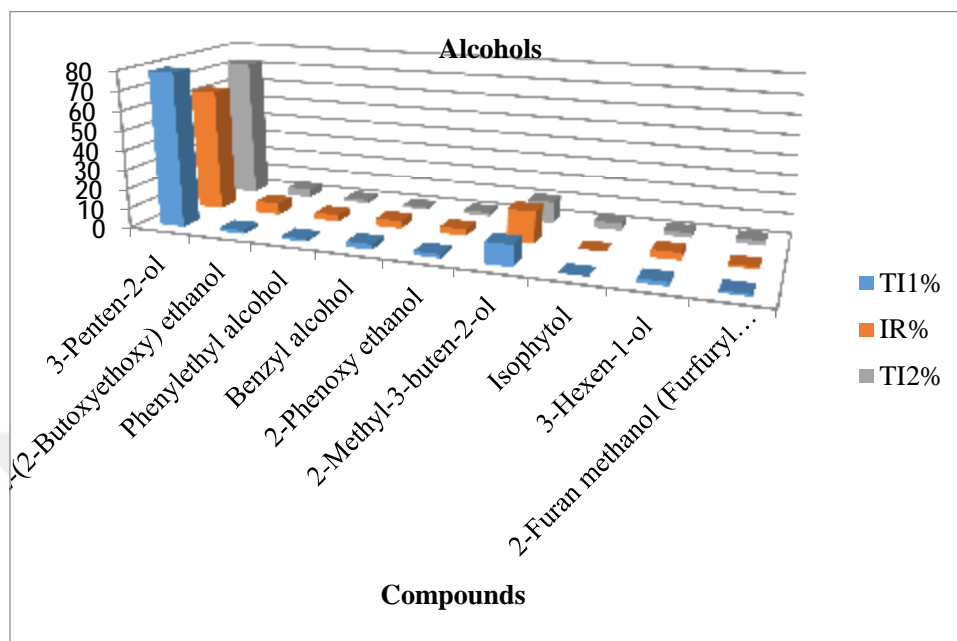


Figure 4.3. Alcohol compounds in three different *Hibiscus sadariffa* infusions

Alcohols are reported to be resulted from lipoxygenase action on unsaturated fatty acids and particularly when cell structure is disrupted in the presence of oxygen (Olias et al., 1993; Luning et al., 1995; Schlotzhauer et al., 1996). 3-Penten-2-ol and 2-methyl-3-buten-2-ol were determined as the major alcohols quantitatively identified in roselle teas and both of these compounds were detected for the first time (Figure 4.3). 3-Penten-2-ol is reported to be found in many other samples like banana, papaya, orange juice and olive oil (Selli and Kelebek, 2011; Selli et al., 2012; Kesen et al., 2013; Kelebek et al., 2015). This compound had the abundance in the group of alcohols of roselle teas with concentrations of 8474, 6926 and 3416 $\mu\text{g}/\text{kg}$ in TI_2 , TI_1 and IR samples respectively. Among the identified alcohols in our study, only the phenylethyl alcohol and isophytol have been previously identified in the extant literature. Phenylethyl alcohol exists in many foodstuffs such as defatted sesame, honey, kiwi puree, caju and others (Moreira et al., 2002; Laohakunjit et al., 2017; Uckun and

Selli, 2017). Isophytol has reported to be found in essential oils of *H. sabdariffa* (Inipki et al., 2014). This compound was found only in traditional cold infusion of roselle tea hence, it can be assumed that the temperature rise caused the loss of isophytol as it was not found in hot infusions. 3-penten-2-ol, 2-(2-butoxyethoxy) ethanol, benzyl alcohol and 2-phenoxy ethanol were found for the first time in *Hibiscus sabdariffa*. It is thought that the benzyl alcohol could be originated via glycosides under enzymatic action (Gunata et al., 1985; Guo et al., 1994; Guo et al., 1995). Benzyl alcohol is found in wide variety of natural samples like propolis, basil, thyme, mushroom, figs and etc. (Gibernau et al., 1997; Lee et al., 2005; Bankova et al., 2014; Pei et al., 2016). In hibiscus tea samples, an increase in the amount of this compounds was detected with risen temperature, yet it was determined that the increase is not statistically significant.

Acids: A total of five acids were determined with the total concentrations of 3104, 2048 and 1549,5 $\mu\text{g}/\text{kg}$ in TI_2 , IR and TI_1 samples, respectively. It has been observed that the amount of acids decreased with the increase in applied brewing temperature. Acetic, octanoic, nonanoic, hexanoic and 2-methyl butanoic acids were found in all three samples in the lead of acetic acid. In literature, acetic acid, 3-hexanoic acid and benzoic acid are reported to be found in *Hibiscus sabdariffa* calyces (Farak et al., 2015; Ramirez-Rodrigues et al., 2011; Chen et al., 1998; Ramirez-Rodrigues et al., 2012). Octanoic acid has been detected in both calyces and seeds oil while nonanoic acid has been reported only in the seeds oil (Jirovetz et al., 1992; Farag et al., 2015). Acetic acid was found in abundance in all three roselle tea samples.

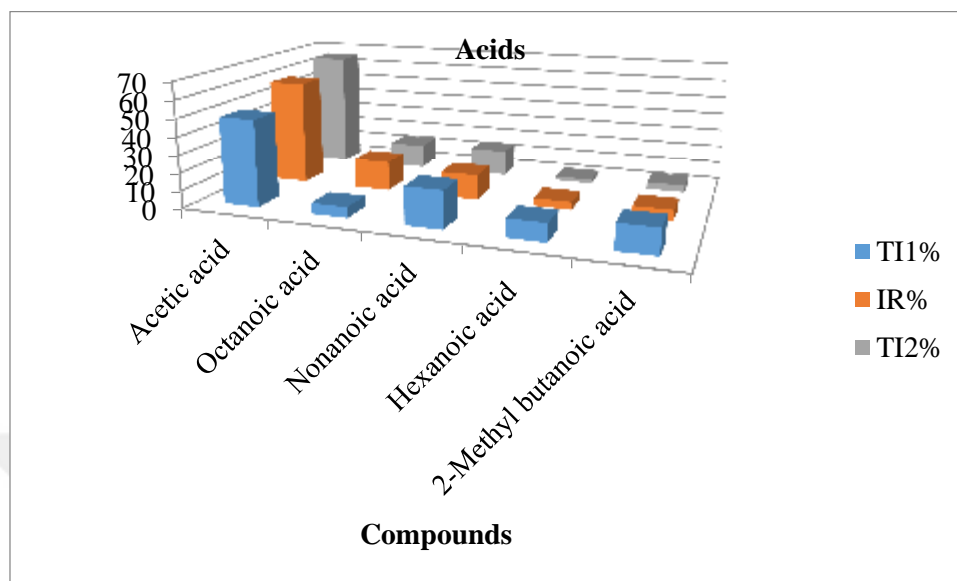


Figure 4.4. Fatty acids identified in *Hibiscus sabdariffa* infusions

Aldehydes: Nonanal, hexanal and benzaldehyde were identified in *Hibiscus sabdariffa* tea samples. Aldehydes are reported to be formed from the degradation of fatty acids and Stecker degradation of amino acids having a significant effect on the flavor of many different foods (coffee, wine, roasted cocoa, roasted peanuts and tea, etc.) (Hognadottir and Rouseff, 2003; van Boekel, 2006). In our study, aldehydes represent a small proportion of the detected volatile compounds, with concentrations of 2252, 868, 822 $\mu\text{g}/\text{kg}$ in TI_2 , IR and TI_1 samples respectively. The highest amount of aldehydes are found TI_2 sample, which has been obtained at the ambient temperature for 24 h. Our results are partially supported by the study of Shen et al. (2017), in which the amount and intensity of nonanal decrease when infusion temperature increased during *Camellia sinensis* tea preparation. In contrast, Ramirez-Rodrigues et al. (2011) assessed that aldehydes were in high amount in hot extracts of *Hibiscus sabdariffa*. Also it is stated in the same study that aldehydes are hydrophilic compounds and hence as

the extraction time increases with polar water, it is possible to obtain higher amounts of these compounds.

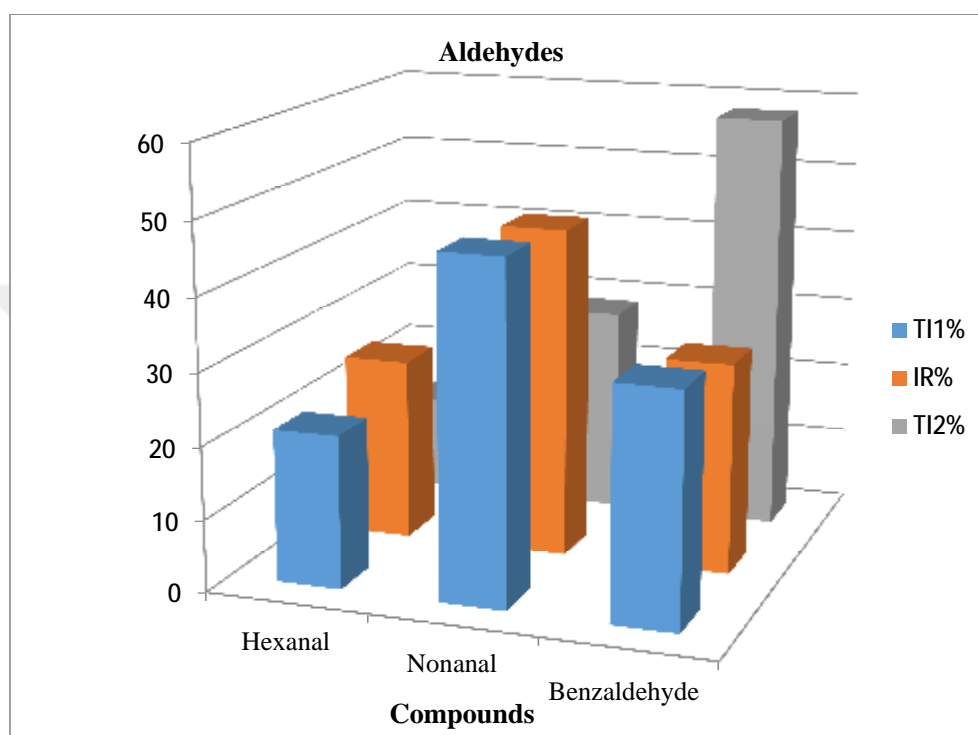


Figure 4.5. Aldehydes identified in *Hibiscus sadariffa* infusions

Benzaldehyde was found with the highest concentration (1310 $\mu\text{g}/\text{kg}$) in TI_2 infusion at ambient temperature while the other samples were rich in nonanal. Benzaldehyde has reported to result from oxidation of alcohols (Huang et al., 2013) and also enzymatic hydrolyzation of prunasin (Guo et al., 1998). This compound was also reported to be found in Mexican, Egyptian, Cuban and Sudanese fresh plant, teas and essential oils of *H. sadariffa* in addition to our Beninese samples (Pino et al., 2006; Gonzalez-Palomares et al., 2009; Farag et al., 2015; Tahir et al., 2017). According to our results, the infusion duration had a greater effect in obtaining benzaldehyde than temperature rise as the sample obtained with brewing

for 24 hour at room temperature had the highest amount. In the extant literature, nonanal, hexanal and benzaldehyde have been also identified in black and green teas (Qin et al., 2013). Hexanal has been found to be most abundant aldehyde in black tea (Qin et al., 2013) while nonanal displayed an abundance in oolong (Wang and Ruan, 2009), tartary buckwheat (Qin et al., 2011) and longjing tea infusions (Zhu et al., 2015).

Ketones: A total of five ketones, including 3-methyl-3-buten-2-one, methylacetoin (3-hydroxy-3-methyl-2-butanone), acetoin (3-hydroxy-2-butanone), 2-octanone and 1-hydroxy-2-propanone (acetol). These compounds were identified for the first time in *Hibiscus sabdariffa* teas. These ketones are widespread, as they have been found in several other products, such as brewed coffee (Akiyama et al., 2008), mango (Bonneau et al., 2016) and honeys (Uckun and Selli, 2017). Ketones have mentioned to be generated by thermal oxidation unsaturated fatty acids (Josephson and Lindsay, 1986), degradation of amino acids (Chung and Cadwallader, 1994), microbial damage (Pan and Kou, 1994) and maillard reaction (Chung et al., 2002).

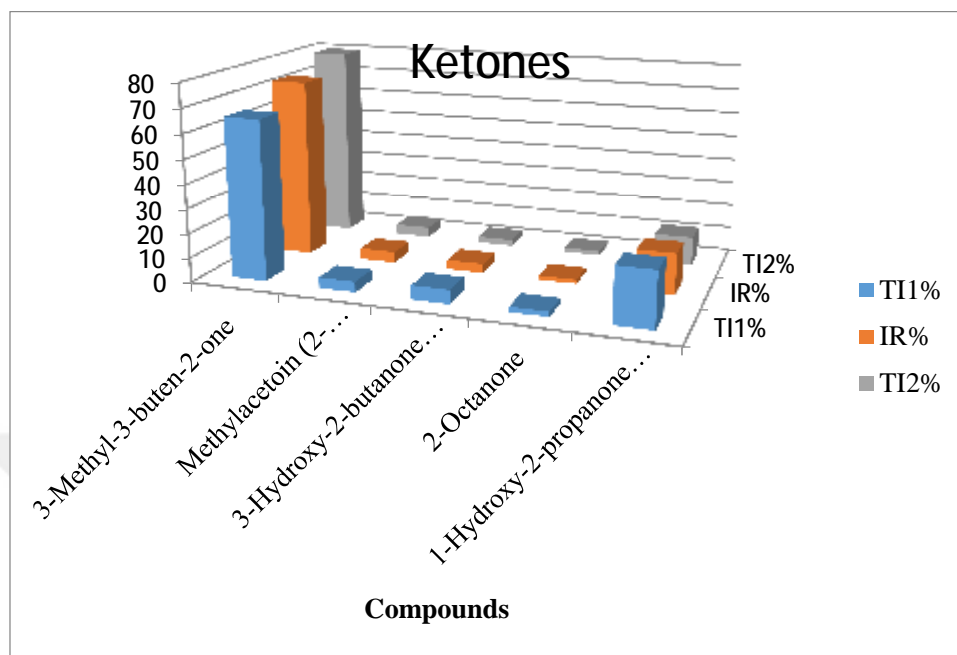


Figure 4.6. Ketones identified in *Hibiscus sadariffa* infusions

Ketones play an important role in overall aroma profile of teas (Alasalvar et al., 2012), probably due to their low threshold values (Josephson and Lindsay, 1986). The total concentrations of ketones were 1863.7, 1958.2 and 2365.1 in TI₁, IR and TI₂, respectively. Basing on these values, it can be affirmed that the infusion temperature may induce the loss of volatile ketones. 3-Methyl-3-buten-2-one was found in higher amounts in all three samples. This compound is detected in foods like kiwi fruit, pine mushroom and is reported to be one of the aroma-active compounds of lychee fruit (Jordan et al., 2002; Cho et al., 2006; Feng et al., 2017). It has been found that 3-hydroxy-2-butanone (acetoin) imparts the aroma profile of Turkish black teas (Alasalvar et al., 2012). Acetoin and 1-hydroxy-2-propanone have mentioned to be aroma-active compounds of Chinese congou black tea (Xiao et al., 2017). Another paper released acetoin to be one of important aroma compounds of green tea (Ochiai et al., 2015).

Volatile phenols: The existent papers revealed very few amount of volatile phenols in roselle (Tahir et al., 2017; Chen et al., 1998; Gonzalez-Palomares et al., 2009; Ramirez-Rodrigues et al., 2011). Nonetheless, an important concentration (ranging from 1960 to 4862 $\mu\text{g}/\text{kg}$) of volatile phenols was discovered in the present study. Similarly, phenolic compounds such as eugenol, 4-vinyguaiacol, phenol have seen to be augmented during infusion preparation of Rooibos (*Aspalathus linearis*) teas (Kawakami et al., 1993). Volatile phenols are well-known to be generated during the biosynthesis of lignin (Peterson and Reineccius, 2002). Among the volatile phenols, eugenol has revealed to be the most abundant compound in TI₂ sample obtained at room temperature. This is in accordance with the previous works, of which eugenol has detected to contribute greatly to aroma profile of *Hibiscus sabdariffa* (Ramirez-Rodrigues et al., 2011; Juhari et al., 2015). In addition, in the extant literature, eugenol has been mentioned in many teas, including *Aspalathus linearis* (Kawakami et al., 1993), green (Kumazawa and Masuda, 1999; Shen et al., 2017) and jasmine (Chen et al., 2017) teas. Another important volatile phenol, present in *Hibiscus sabdariffa* infusions (TI₁, TI₂ and IR samples) is 4-vinyguaiacol. The highest concentration of this compound is found TI₁ sample. This may be explained by the fact that higher temperature may promote the release of 4-vinyguaiacol during the infusion preparation, as previously stated by Kawakami et al. (1993). 4-Vinyguaiacol was found to be a contributor to overall aroma of the green and hard iced teas (Kumazawa and Masuda, 1999; He et al., 2018).

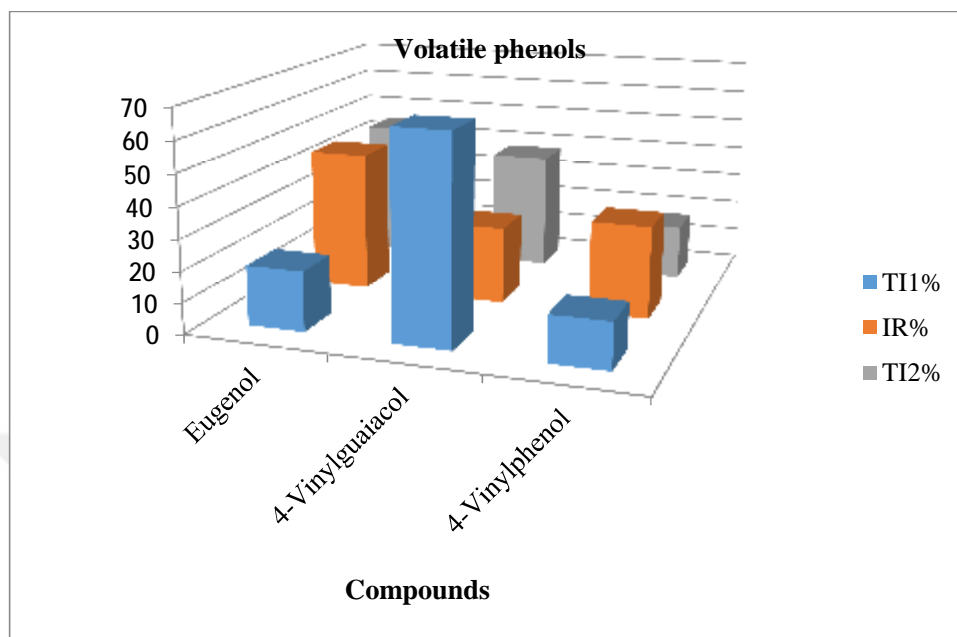


Figure 4.7. Volatile phenols identified in *Hibiscus sadariffa* infusions

Lactones: Pantolactone and γ -butyrolactone are the identified lactones in TI₁, TI₂ and IR samples. Pantolactone has been previously reported in *Hibiscus sabdariffa* as one of the fatty acid derived compounds (Farak et al., 2015), however γ -butyrolactone is identified for the first time. Lactones could be produced when normal metabolism of fatty acid is occurring (Peterson and Reineccius, 2002). In present work, pantolactone displayed a high concentration in *Hibiscus sabdariffa* infusions. This aroma compound has recognized to contribute to overall odor of aged oolong tea (Chen et al., 2013). It has been observed in the present study that the concentration of γ -butyrolactone decreased considerably during the heating. γ -Butyrolactone has been detected in green tea (Ochiai et al., 2015) and has mentioned to be one of the key aroma compounds of congou black tea (Xiao et al., 2017). These two lactones (pantolactone and γ -butyrolactone) have been also revealed in wine (Selli et al., 2006; Rocha et al., 2005; Ruiz et al., 2010; Ruiz and Zea, 2014).

Pyranone: Pyranones are one of the oxygen-containing heterocyclic aroma compounds and well-known to be caramelized and Maillard flavor contributor (Schieberle and Hofmann, 2002). Maltol (3-hydroxy-2-methyl-4H-pyran-4-one) is the only pyranone discovered in *Hibiscus sabdariffa* infusions. This aroma compound has been revealed as a sugar derived volatile in *Hibiscus sabdariffa* samples cultivated in both Egypt and Sudan (Frag et al., 2015). The odor of this compound is defined as caramel, sweet, fruity, hazelnut or burnt odor (Rios et al., 2007; Miyazoto et al., 2013). It was detected that the concentration of maltol was significantly higher in TI₁ sample. The high temperature (100 °C) and length of the time (40 minutes) may induce the release of maltol. This is supported by the work of Tressl et al. (1986), who revealed that maltol is issued from maltose under Maillard reaction when the sample is warmed up. In addition, Silwar and Lullmann (1993) found that the amount of maltol increased with increasing of roasting temperature and duration in coffee processing. These data are in accordance with our study. Maltol has been found in wide variety of teas, including tartary buckwheat tea (Qin et al., 2011; Peng et al., 2015) and green tea (Kumazawa and Masuda, 1999; Ochiai et al., 2015).

Pyrrrole: Pyrroles are nitrogen-containing heterocyclic compounds. Pyrroles and particularly 1H-pyrrole-2-carboxaldehyde (2-formylpyrrole) has been widely reported in teas. The generation of pyrroles is assumed to be derived from Strecker degradation of theanine and amino acid during infusion preparation (Sheibani et al., 2016). 1H-pyrrole-2-carboxaldehyde (2-formylpyrrole) is the only pyrrole identified in all infusions. The amount of this compound increased significantly with increment of temperature and duration of brewing. Similar results have been found by Yu et al. (1999), of which it has been revealed that pyrroles contribute in abundance to aroma profile of hot teas. In addition, Qiu et al. (2017) assumed that an increase of pyrrole scents has been observed in the long baked teas. In the extant data, 1H-pyrrole-2-carboxaldehyde has been revealed in oolong (Kuo et al., 2011; Chen et al., 2013) and green (Ochiai et al., 2015) teas.

Moreover, this compound was also mentioned in other foodstuffs, among them are Turkish and French coffees (Amanpour and Selli, 2016; Lopez-Galilea et al., 2006) and sesame seed oil (Shimoda et al., 1996).

Ester: Methyl-2-furoate is the only identified ester in *Hibiscus sabdariffa* infusions (TI₁, TI₂ and IR samples). Esters are known to be resulted from enzyme-catalyzed oxidation of unsaturated fatty acids (Buttery, 1981) and from decarboxylation and deamination of amino acids (Peterson, 2002). Methyl-2-furoate has been previously indicated in *Hibiscus sabdariffa* teas (Pino et al., 2006). Furthermore, methyl-2-furoate has been revealed in oolong infusion (Scheibani et al., 2016) and in many other food products, including honeys (Uckun and Selli, 2017), *Schisandra chinensis* Baillon fruit (Lee et al., 2011), *Rosa canina* L. (Ozyurt et al., 2016) fruit. It is worth noting that methyl-2-furoate displayed less amount in hot infusions (TI₁ and IR samples) whereas a high concentration was observed in non-heated infusion (TI₂).

Terpenes: Generally terpenes have been largely reported in *Hibiscus sabdariffa*; however there is a huge variation in regard to the type of terpene compounds. According to Gonzalez-Palomares et al. (2009), this might be due to *Hibiscus sabdariffa* variety and isolation method used. A small amount of terpenes resulted from the samples of the present work which includes only *DL*-limonene as a monoterpene. In general, this compound was frequently found in other hibiscus varieties like Cuban, Egyptian and Mexican roselles (Pino et al., 2006; Gonzalez-Palomares et al., 2009; Farag et al., 2015). Chen et al. (1998) found that the

terpenes are lost in dried roselles although it was found in fresh ones and they concluded that the drying process probably lead the loss of these compounds which may explain the low amount of terpenes in Beninese roselle teas.

4.5. Aroma-active compounds of *Hibiscus sabdariffa* tea samples

The aroma-active compounds of *Hibiscus sabdariffa* were identified using GC-Olfactometry technique. In order to identify the key odorants of the samples, flavor dilution factors (FD) were determined via a precise Aroma Extract Dilution Analysis (AEDA). This technique was also used in determining aroma-active compounds of wide variety of samples including green and black tea powders; coffee powders and brews; black and summer truffles; plants, fruits and fermented products (Guth and Grosch, 1993; Grosch, 1994; Semmelroch and Grosch, 1995; Cullere et al., 2010).

Table 4. 5. Aroma-active compounds of *Hibiscus sabdariffa*

No	LRI	Aroma compounds	Odor description	FD Factor		
				TI1	IR	TI2
1	1006	3-Methyl-3-buten-2-one	Fruity	128	128	128
2	1026	2-Methyl-3-buten-2-ol	Sweet, Fruity	16	16	32
3	1078	Hexanal	Green, Herbal	32	64	64
4	1147	Unknown	Sweet,Fresh	64	32	32
5	1170	3-Penten-2-ol	Oily, Herbal	256	128	256
6	1206	<i>DL</i> -Limonene	Citussy, Lemon	32	64	32
7	1243	Methyl acetoin	Fatty	16	16	32
8	1321	1-Hydroxy-2-propanone (Acetol)	Caramel	128	64	64
9	1340	Unknown	Green, Fatty	32	64	64
10	1396	Nonanal	Herbal, Grassy	64	64	128
11	1446	Acetic acid	Vinegary	16	64	512
12	1469	Furfural	Caramel, Bready	2048	2048	4096
13	1508	Benzaldehyde	Almond	16	16	128
14	1542	Unknown	Oily, Herbal	ND	ND	64
15	1575	5-Methyl-2- furfural	Caramel	256	256	1024
16	1628	γ -Butyrolactone	Caramel, Creamy	32	32	64
17	1667	2-Methyl butanoic acid	Cheesy, Acidic, buttery	64	32	32
18	1772	Unknown	Herbal	ND	ND	64
19	1877	Benzyl alcohol	Floral	8	8	4
20	2013	1H-Pyrrole-2- carboxaldehyde (2- formylpyrrole)	Tea, Herbal	64	32	32
21	2028	Pantolactone	Burnt, spicy	64	64	64
22	2025	Methyl-2-furoate	Caramel	64	64	128
23		Unknown	Floral	16	8	ND
24	2162	Eugenol	Spicy	64	64	128

The compounds obtained from olfactometric analysis, their given odors and dilution factors in the Table 4.4. The application of Aroma Extract Dilution Analysis (AEDA) on the TI₁ extract provided 22 aroma-active compounds, including 3 furans, 3 alcohols, 3 ketones, 2 fatty acids, 3 aldehydes, 2 lactones, 1 terpene, 1 volatile phenol, 1 pyrrole and 3 unknown compounds. 23 aroma-active compounds were revealed from TI₂ extract, ranged as furans (3), alcohols (3), ketones (3), fatty acids (2), aldehydes (3), lactones (2), terpene (1), volatile phenols (1), pyrrole (1) and 4 unknown compounds. 22 aroma-active were identified from IR extract, including 3 furans, 3 alcohols, 3 ketones, 2 fatty acids, 3 aldehydes, 2 lactones, 1 terpene, 1 volatile phenol, 1 pyrrole and 3 unknown compounds. The extracts TI₁, TI₂ and IR have 22 aroma-active compounds in common, however the flavor dilution factor (FD) of these compounds varied from an extract to another.

A total of two aroma-active furans compounds, including furfural (caramel, bready) and 5-methyl-2- furfural (caramel) were found in all infusions obtained from *Hibiscus sabdariffa* calyces. These furans have been produced during drying of *Hibiscus sabdariffa* (Ramirez-Rodrigues et al., 2011). Aroma-active compounds furfural and 5-methyl-2- furfural provide pleasant odors (caramel, bready) to *Hibiscus sabdariffa* infusions. Among all detected aroma-active compounds infusions TI₁, TI₂ and IR, furfural showed the highest FD factor, 2048, 4096 and 2048 in all TI₁, TI₂ and IR infusions, respectively. Furfural generated sweet and bready odors in the infusions; however, its intensity was stronger in TI₂. It has been mentioned in the existent literature that furfural could be one of the key aroma compounds in *Hibiscus sabdariffa* tea, providing sweet and baked bread odors (Ramirez-Rodrigues et al., 2011). Furfural was also reported to be the most important aroma-active compound in Tanyang congou black teas with an FD factor of 1024 (Xiao et al., 2017). The next most important aroma-active furan with regarding furan with regarding activity was 5-methyl furfural. The FD factors of this aroma-active compound were 256, 1024 and 256 in TI₁, TI₂ and IR *Hibiscus sabdariffa* extracts, respectively. The highest flavor dilution factors of the aroma-

active furans were found in TI₂ extract and, TI₁ and IR displayed less flavor dilution factors. The heating may decrease the intensities of furans in the infusions. Furfural and 5-methyl-2-furfural have been previously revealed in chinese longjing green tea as an important aroma-active compounds (Cheng et al., 2008).

A total of three aroma-active alcohols, including 2-methyl-3-buten-2-ol (sweet, fruity), 3-penten-2-ol (oily, herbal) and benzyl alcohol (herbal) were identified in *Hibiscus sabdariffa* infusions. It has been reported in the existent data that alcohols could be formed from unsaturated fatty acids under lipoxygenase action (Schlotzhauer et al., 1996, Luning et al., 1995). Among aroma-active alcohols, 3-penten-2-ol displayed the highest flavor dilution factor (FD= 256) in all infusions. This aroma-active compound was detected for the first time in *Hibiscus sabdariffa*. It has been reported that 3-penten-2-ol was one of the key odorants of kiwi and guava purees (Jordan et al., 2002; Jordan et al., 2003). Benzyl alcohol has been reported in Chinese different congou teas, providing strong sweet and flower notes (Xiao et al., 2017)

Acetic acid (vinegary) and 2-methyl butanoic acid (cheesy, acidic, and buttery) were aroma-active volatile acids detected in *Hibiscus sabdariffa* infusions. Acetic acid was the strongest aroma-active acid in all samples. Its highest flavor dilution factor has been found in TI₂ extract (FD=512), however the lowest was found TI₁ extract (FD=16). The temperature of the infusion preparation may have influence on this volatile acid. It has been mentioned in the existent literature that acetic acid and 2-methyl butanoic acid are two of the key aroma contributors of longjing green tea (Cheng et al., 2008). Additionally, acetic acid has been found to be key compound of black velvet tamarind spices (Lasekan and See, 2015), rice husk (Pino, 2014) and strawberry vinegars (Ubeda et al., 2016), providing sweet, vinegar and pungent notes, respectively.

Three aroma-active aldehydes including hexanal (green, herbal), nonanal (herbal, fatty) and benzaldehyde (almond) were found in *Hibiscus sabdariffa* extracts. Nonanal has been revealed as odor-active compound in cold and hot

infusions of Mexican dried and fresh *Hibiscus sabdariffa*, providing green and fruity notes (Ramirez-Rodrigues et al., 2011). The highest flavor dilution factors were found in TI₂ extract with 64, 128 and 128 for hexanal, nonanal and benzaldehyde, respectively. It worth noting that aroma-active aldehydes determined in the present work showed stronger intensity in the infusion without heating process (TI₂). It has been mentioned that nonanal provided green, floral and citrus notes to longjing green and chrysanthemum flowers teas (Cheng et al., 2008; Luo et al., 2017) while hexanal contributed to the green and leaf notes in Chinese congou black teas (Xiao et al., 2017).

A total of three ketones such as 3-methyl-3-buten-2-one (fruity), methyl acetoin (fatty) and 1-hydroxy-2-propanone (caramel) were aroma-active ketones identified in *Hibiscus sabdariffa* infusions. The most powerful aroma-active ketones were 3-methyl-3-buten-2-one which is responsible for the fruity odor and 1-hydroxy-2-propanone provided a sweet odor. FD factors of 3-methyl-3-buten-2-one were found as 128, whereas FD factors of 1-hydroxy-2-propanone were 128, 64 and 64, respectively. Aroma-active 1-hydroxy-2-propanone was more powerful in TI₁ while of 3-methyl-3-buten-2-one showed the same intensity in all the infusions. Xiao et al. (2017) have detected 1-hydroxy-2-propanone in four different Chinese congou black teas as one of the aroma-active compounds even though it has been in low intensity (FD=4-64).

Among identified volatile phenols, eugenol (spicy) was only aroma-active compound determined. This compound was detected in TI₁, TI₂ and IR with different intensities. The highest flavor dilution factor was found in TI₂ (FD=128), whereas the other infusions had the same factor (FD= 64). In the earlier study, eugenol has been reported as a key odorant in hot tea of dried *Hibiscus sabdariffa* (Ramires-Rodrigues et al., 2011). Eugenol has been widely reported as key odorant in numerous food products, as it has been notified to provide spicy note to Chinese syrah wine (Zhao et al., 2017), honeybush tea (Theron et al., 2014), aged red wine (Fernández de Simón, 2014). Furthermore, this compound has been detected as

aroma-active compound in rice husk and two strawberry vinegars, providing woody, sweet and clove notes (Pino, 2014; Ubeda et al., 2016).

Regarding the aroma-active lactones, γ -butyrolactone (caramel, creamy) and pantolactone (burnt, spicy) were detected in all *Hibiscus sabdariffa* infusions. The flavor dilution factor of pantolactone was FD=64 in all the infusions whereas the flavor dilution factor of γ -butyrolactone FD=32, FD=64 and FD= 32 for TI₁, TI₂ and IR, respectively. These compounds have been revealed in wine as key odorants (Selli et al., 2006; Rocha et al., 2005; Ruiz et al., 2010; Ruiz and Zea, 2014). It has been revealed that γ -butyrolactone contributed to the caramel and fruity odors of Chinese longjing and congou black tea infusions (Cheng et al., 2008; Xiao et al., 2017).

DL-limonene (citrusy, lemon) contributed slightly to aroma profile *Hibiscus sabdariffa* infusions (TI₁, TI₂ and IR). The flavor dilution factors of this aroma-active compound were FD=32 for TI₁ and TI₂, and FD=64 for IR. *DL*-limonene has been found in the chrysanthemum flower teas with more intensity (Luo et al., 2017). In the extant literature, *DL*-limonene and other isomers of limonene have been mostly mentioned to be to aroma-active compounds of many foodstuffs. Thus, *D*-limonene (citrusy, flowery), *R*-(+)-limonene (citrus) and limonene (fruity) have been indicated as key odorants in German rainbow trout (Mahmoud and Buettner, 2017), four varieties of mandarins (Xiao et al., 2017) and three different black velvet tamarind species (Lasekan and See, 2015), respectively.

1H-Pyrrole-2-carboxaldehyde (2-formylpyrrole) was the only pyrrole compound identified as aroma-active compound from the infusion TI₁, TI₂ and IR extracts. This compound was more powerful in the heated extract (TI₁). It has been revealed in the existent data that 1H-Pyrrole-2-carboxaldehyde contributes to the overall aroma profile of many food products including, oolong (Kuo et al., 2011; Chen et al., 2013), green (Ochiai et al., 2015) teas, Turkish and French coffees (Amanpour and Selli, 2016; Lopez-Galilea et al., 2006) and sesame seed oil (Shimoda et al., 1996). In addition, Qiu et al. (2016) affirmed that pyrroles could

be responsible of overbaking odor of black tea and this odor could be enhanced during the roasting.

Methyl-2-furoate (caramel) is aroma-active ester found in all infusions. This compound had the intensity in TI₁, TI₂ and IR extracts with 64, 512 and 64 FD factors, respectively. Methyl-2-furoate is found to be stronger in the infusion obtained at ambient temperature (TI₂ sample) while its intensities were lower in the hot infusions (TI₁ and IR samples). This may probably due to hydrophilic capacities of this compound that it extracted more efficiently with longer contact with water. Previously, methyl-2-furoate has been reported to contribute to overall aroma profile of red wine aged with different wood barrels (Fernández de Simón et al., 2014), rice husk (Pino, 2014) and *Ribes nigrum* L. juice (Varming et al., 2004). In addition, methyl-2-furoate has been revealed to be one of key aroma compounds of three different black velvet tamarind spices (Lasekan and See, 2015).

4.6. Sensory Analysis of Roselle Teas

The sensory profile of roselle teas was investigated with descriptive profile analysis by 10 trained panelists. The results of each perceived attribute was displayed in Table 4.5 and a spider plot (Figure 4.7) for a better visualization. The panelists were asked to evaluate the aroma, taste, and general acceptability of tea samples on a 100 mm scale.

According to results of sensory analysis regarding tea samples, in terms of taste, aroma (including fruity, sweet/caramel, herbal odors) and general acceptability, teas obtained at infusion for 24 hour at room temperature (TI₂) had the highest values following by TI₁ and IR samples respectively. Infusion prepared with boiling roselles for 40 minutes showed highest values in color and astringency. In comparison, the lowest values were recorded in IR sample which prepared at 98 °C with 16 minutes of infusion. Some of the attributes (e.g aroma, color, astringency, acidity) were also investigated in other hibiscus products (Bechoff et al., 2014; Monteiro et al., 2017), yet this is the first study in hibiscus

infusions, hence the comparison with other studies is infeasible. The results of sensory evaluation of these three infusions displayed that the sample obtained without heat application has more likeability than the heated ones and the statistical analysis displayed significant differences between samples ($p < 0,05$). In accordance with the suggestion of Damiani et al. (2014), roselle teas obtained at room temperature may become an alternative to the generally consumed hot teas.

Table 4. 6. Sensory evaluation of roselle tea samples

Sensory Attributes	TI ₁	IR	TI ₂
Aroma	5,92 ^b	5,25 ^a	6,69 ^c
Fruity odor	5,49 ^b	3,93 ^a	6,51 ^c
Sweet/Caramel odor	5,51 ^b	3 ^a	6,89 ^c
Herbal odor	4,69 ^a	4,31 ^a	5,21 ^b
Taste	5,48 ^b	4,9 ^a	6,18 ^c
Acidity	6,64 ^b	5,77 ^a	7,27 ^c
Astringency	7,25 ^c	3,83 ^a	6,82 ^b
Colour	8,4 ^c	6,43 ^a	7,42 ^b
General acceptability	5,47 ^b	4,52 ^a	7,49 ^c

*The results are indicated as mean values of evaluations.

* *a, b, c*: Same letters mean no significant differences between samples.

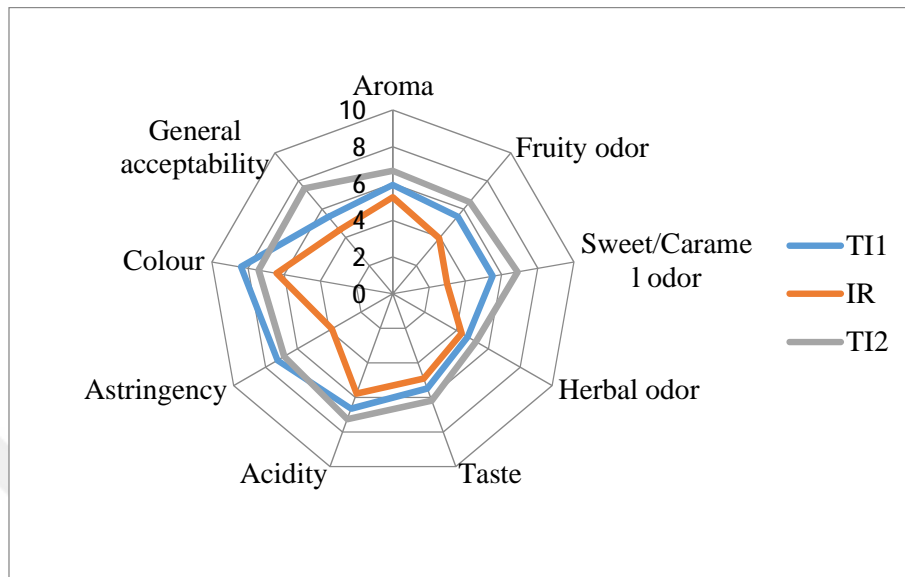


Figure 4. 8. Spider plot for three roselle tea

5. CONCLUSION

This study reports the total phenolic compounds, antioxidant activity, aroma and aroma-active compounds of *Hibiscus sabdariffa* tea obtained by two traditional infusion preparation methods (TI₁ and TI₂) of Benin and an alternative brewing method used previously by Ramirez-Rodriguez et al. (2011) (IR).

The results obtained from this work can be summarized in the following way:

- Total soluble solids of tea samples were found to be in the range of 1,7-3,4 °Bx, and there were no significant changes in pH values. In terms of colour properties, IR sample was determined to be brighter than the other infusions and all three samples had a colour combined with dark red and yellow attributes.
- The results showed that infusion preparation methods may influence the total phenolic compounds and antioxidant activities of *Hibiscus sabdariffa*. IR and TI₁ recovered the highest values of total phenolic compounds and antioxidant activity than TI₂ sample. This result can be assumed to be caused by the heat treatment applied on the samples which may induce the release of phenolic compounds. These obtained results are in accordance with the literature.
- A total of 39 aroma compounds are identified from *Hibiscus sabdariffa* infusions including furans, alcohols, acids, aldehydes, ketones, lactones, volatile phenols, etc. Furans were determined as the most dominant group in the samples and furfural exhibited the highest concentrations in all infusions.
- The amount of furans, alcohols, acids, aldehydes, ketones, lactones and esters raised with increase of infusion duration, while the amount of volatile phenols, pyranone and pyrrole have risen in the samples obtained at high temperature for longer time. Most of these compounds were

reported to be hydrophilic compounds as this information explains the increase in amounts with longer contact with water.

- Ü Olfactometric analysis revealed a total of 24 aroma-active compounds in *Hibiscus sabdariffa* infusions. Furfural was the strongest aroma compound in all *Hibiscus sabdariffa* extracts (FD=2048-4096), providing caramel-bready odors. This compound is thought to be produced during drying process of fresh calyces as it is formed generally as the result of Maillard reactions.
- Ü Compounds such as 5-methyl furfural (caramel), acetic acid (vinegary), 3-penten-2-ol (oily, herbal, sweet), nonanal (herbal, fatty), benzaldehyde (almond), eugenol (spicy) and 3-methyl-3-buten-2-one (fruity) showed relatively high FD factors. However, the FD factors of these aroma-active compounds were relatively higher in TI₂ probably due to the length of infusion time.
- Ü Sensory evaluation revealed that TI₂ is more preferred and displays significantly higher intensities of aroma, fruity, sweet and caramel attributes than TI₁ and IR. This sample was also had the highest acceptability from the panelists.
- Ü It has been resulted from the present study that all experimented infusion methods displayed good total phenolic compounds, antioxidant activity, aroma and aroma-active compounds and sensory properties. However, the infusion obtained at ambient temperature (TI₂) has revealed to be the best in respect with aroma and aroma-active compounds and sensory properties while the infusions obtained by heating method (TI₁ and IR) have revealed to be the best in total phenolic compounds and antioxidant activity probably because of the heat treatment. To sum up, hibiscus teas obtained without heat application appealed the most to the panelist and thus this sample preparation method can be used as an alternative to generally consumed hot teas.

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