

## CHARACTERIZATION OF ODOUR-ACTIVE VOLATILES AND SENSORY ANALYSES OF ROASTED OAK (*Quercus humboldtii* Bonpl.) ACORNS, A COFFEE SUBSTITUTE

CARACTERIZACIÓN DE LOS COMPUESTOS ACTIVOS OLFATIVAMENTE Y ANÁLISIS SENSORIAL DE LOS FRUTOS TOSTADOS DEL ROBLE (*Quercus humboldtii* Bonpl.), COMO UNA BEBIDA SUSTITUTA DE CAFÉ

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### ABSTRACT

**Background:** Oak acorns are roasted and grounded to produce a powder that is used to prepare a coffee substitute beverage. **Objective:** To identify the odour-active volatiles of the powder derived from roasted acorns of *Quercus humboldtii* and to perform a quantitative descriptive analysis (QDA) of its appearance, odour, and taste. **Methods:** The odour-active volatiles of roasted oak acorn powder were extracted by SDE and analyzed by GC/MS and olfactory analyses (GC/O). Sensorial analyses of this beverage were done by QDA (Quantitative Descriptive Analysis). **Results:** Nineteen compounds were identified, showing acid, buttery/caramel, smoke/roasted, and fruity odour notes, which are common to those of roasted coffee. Ketones, aldehydes, aliphatic acids, furanic alcohols and pyrazines were identified as relevant for this coffee substitute beverage. The major volatile compounds were furfural, 5-methyl furfural, furfuryl alcohol, and 3-hydroxy-2-butanone, some of them related to the sugar breakdown by heating. Odour attributes characterized by QDA, were in agreement with those detected by GC-O (Gas Chromatography coupled to Olfactometry), but some off-taste notes were detected. **Conclusions:** The results of molecular sensory approach confirmed that this product resembles coffee; however, QDA analyses showed the presence of undesirable taste. More studies are needed in order to improve the taste quality of this coffee substitute.

**Keywords:** SDE; QDA; odour-active volatiles; gas chromatography; food chemistry.

### RESUMEN

**Antecedentes:** los frutos del roble se tuestan y muelen para producir un polvo que es empleado en la preparación de una bebida sustituta del café. **Objetivos:** identificar los compuestos volátiles activos olfativamente presentes en el polvo proveniente de los frutos tostados de *Quercus humboldtii*; así como llevar a cabo un análisis cuantitativo descriptivo (QDA) de la apariencia, olor y sabor de este polvo. **Métodos:**

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los compuestos volátiles activos olfativamente del polvo de los frutos tostados del roble se extrajeron por DES y se analizaron por CG-EM y análisis olfativo (CG-O). La caracterización sensorial de esta bebida sustituta de café fue realizada mediante el empleo de QDA. **Resultados:** se identificaron diecinueve compuestos y se evidenció la presencia de notas de olor como manteca/caramelo, ahumado/tostado y frutal, similares a las encontradas en el café tostado. Los componentes mayoritarios identificados para este sustituto de café fueron cetonas, aldehídos, ácidos alifáticos, alcoholes furánicos y pirazinas. Los compuestos mayoritarios detectados fueron furfural, 5-metil furfural, alcohol furfúrico y 3-hidroxi-2-butanona, algunos de ellos relacionados con la descomposición térmica de azúcares. Los atributos de olor caracterizados por QDA fueron similares a los detectados por CG-O, aunque se detectaron algunas notas de sabor desagradable. **Conclusiones:** los resultados del *molecular sensory approach* confirmaron que este producto se parece al café; sin embargo el QDA mostró la presencia de un sabor desagradable. Estudios adicionales son necesarios para mejorar la calidad del sabor de este sustituto de café.

**Palabras clave:** DES; QDA, compuestos volátiles activos olfativamente; cromatografía de gases; química de alimentos.

## INTRODUCTION

The *Quercus* genus (Oaks), belongs to the family Fagaceae, and is worldwide renowned by its social and economic value, mostly in the wood processing industry (1, 2). Their species, which are approximately 400, mainly occurs in the northern hemisphere as big trees, shrubs or small trees, occupying different kinds of habitats (3). In addition to the use of the cortex in the timber industry, the fruits (acorns) from oak have been part of the human diet since ancient times, due to their great nutritional value. Acorns have been used as food resource or as an ingredient for the elaboration of food and beverages. Products from acorns, such as flour, bread, soup, oil, coffee substitute, among others, have been reported elsewhere (4-9). Despite its high nutritional value, a decline in consumption of food and beverage products based on acorns has been evident, but with the development of organic, functional and wild collected food, this condition is currently changing (4, 10-11).

In Colombia, there is only a single oak species, *Quercus humboldtii* Bonpl. This endemic species, is commonly known as “roble de tierra fría”, “roble”, “roble blanco”, “roble negro” and “encino” and is widely distributed in the Andean region with altitudes ranging between 1600 and 3500 meters above sea level (12-14). Traditionally, wood obtained from *Q. humboldtii* is used in craft making, wood for barrels, and firewood as well as in the furniture production and leather industry because its high content of tannins (12). However, rural communities from Santander and Cauca States in Colombia, dry the acorns of *Q. humboldtii* under

sunlight, then roast and ground to produce a powder (so called “café roble”), which is used to prepare a hot infusion beverage that is consumed as coffee substitute beverage (15). The use of roasted oak nuts in the elaboration of a coffee substitute is not a new practice and the utilization of *Quercus* acorns from different species has been reported (5). Local population refers such obtained *Q. humboldtii* product has similar organoleptic properties to those exhibited by roasted coffee (*Coffea arabica*) beans and exhibited a sweet chocolate-like aroma.

Based on this information, the aim of the present work was to characterize the sensory properties of a hot beverage obtained from roasted *Q. humboldtii* acorns that is consumed as a coffee substitute. Thus, the odour-active volatiles of the powder derived from roasted *Quercus humboldtii* acorns were identified by using the combination of GC-O (Gas Chromatography coupled to Olfactometry), and GC-MS (Gas Chromatography coupled to Mass Spectrometry); and appearance, odour, and taste of the hot beverage was evaluated by a QDA (Quantitative Descriptive Analysis). With these data the quality of this added-value product could be improved, to achieve a medium-term goal to commercialize it as functional food.

## MATERIALS AND METHODS

### Plant material

The study was carried out on a 500 g sample of roasted *Q. humboldtii* acorn powder acquired in the local markets of Suaita (Santander, Colombia). For that purpose, the *Q. humboldtii* acorns are sun

dried to extract the kernels, that are uniformed roasted at medium temperature (190–200°C) and then pulverized to get the powder (15), in an artisan process.

### Chemicals

Diethyl ether, *n*-pentane, and anhydrous sodium sulfate were acquired from Merck (Darmstadt, Germany). All solvents were freshly distilled prior to use. An *n*-Alkane mix (C<sub>8</sub>–C<sub>26</sub>) was purchased to Laboratory Dr. Ehrenstorfer GmbH, Augsburg, Germany.

### Sample preparation

A Likens–Nickerson type SDE (Simultaneous steam Distillation-solvent Extraction) apparatus (16) was used to extract volatile compounds of the sample. For the analysis, 200 g of the sample were dissolved in 500 mL of distilled-deionized water and loaded in a 2 L round bottom flask. The extraction was carried out with 320 mL of *n*-pentane-diethyl ether (1:1, *v/v*) during 2.5 h. The extract was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to 1.0 mL using a *Vigreux* column.

### Analyses of odour-active volatiles by GC-FID and GC-O

GC-O analyses were performed in a gas chromatograph HP 5890 Series II (Hewlett-Packard, USA) equipped with FID and operated in split mode (1:10, injected volume, 1 μL). The injection port was set at 230°C and helium was used as carrier gas at 1.0 mL/min. Two capillary columns, DB-FFAP and DB-5 (each 30 m × 0.32 mm i.d., 0.25 μm film thickness; J&W Scientific, Chromatographie-Handel Müller, Fridolfing, Germany, and Restek, USA, respectively) were used for the volatile analyses. The column oven was programmed at 40°C for 4 min, followed by an increasing until 180°C, at 6°C/min, then at 12°C/min until 230°C for the DB-FFAP and 300°C for the DB-5, and finally the columns were maintained for 10 min at the maximum temperatures. The end of the FFAP column was connected to a deactivated Y-shaped glass splitter (Chromatographie Handel Mueller, Fridolfing, Germany), which divides the effluent into two equal parts, one for FID (230°C) and the other for heated sniffing port (200°C) by using deactivated fused silica capillaries of the

same length (50 cm × 0.32 mm i.d.). Sniffing port consisted of a self-made elbow-shaped aluminum tube (80 × 5 mm i.d) where the trained panelists (3) located the odour-active zones of the SDE extracts by GC-O (17).

### Gas chromatography-mass spectrometry analyses

GC-MS (EIMS) analyses of aroma active compounds were carried out on a GC Agilent 7890B gas chromatograph coupled to a mass spectrometry 5977A (Agilent Technologies Inc. Wilmington, DE, USA). MS data were recorded between 40–350 u, with an electron energy of 70eV and processed by Mass Hunter software. Chromatographic conditions were the same that those above-mentioned for GC-FID analyses for FFAP column. Data acquisition was carried out with Qualitative Analysis Software (database NIST/EPA/NIH Mass Spectral Library 2014 (2.2)).

### Identification of odour-active volatiles

Linear retention indexes (LRI) of the odour-active compounds were calculated by using a mixture of normal paraffin (C<sub>8</sub>–C<sub>26</sub>) as external references (18). The identification of volatile compounds was completed by comparison of their retention indexes (in the two columns), mass spectra, and odour notes, with those reported in databases (19), and the above-mentioned NIST database.

### Sensory analyses: Quantitative Descriptive Analysis

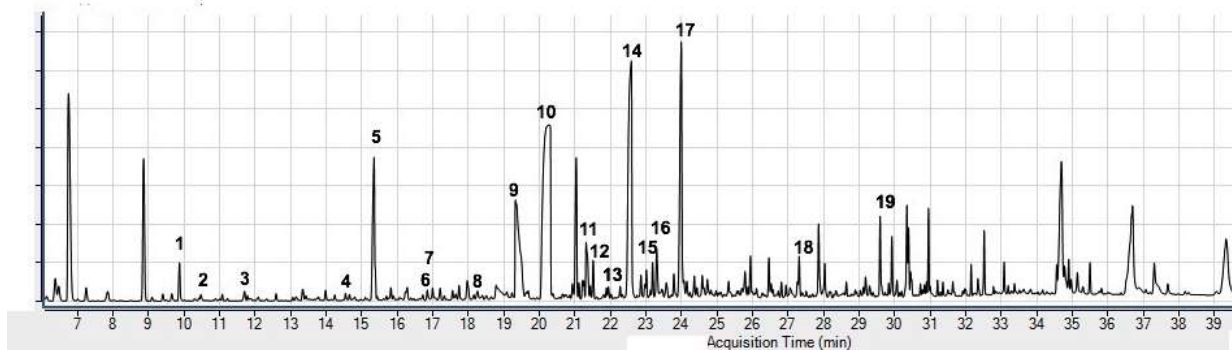
The appearance, aroma, and taste profile of coffee substitute was evaluated by the sensory panel from the staff of the “Laboratorio de Análisis Sensorial de Alimentos, Universidad de Antioquia (Medellín, Colombia)”. It was composed of 10 trained judges (men and women with ages ranging from 25 to 60), who were trained in several sessions prior to analysis, in which the sensory relevant attributes of coffee powder were defined and evaluated (20). Roasted *Q. humboldtii* acorn powder (5.5 g) was dissolved in 100 mL of water at 90°C and then homogenized. The panelists evaluated the sample at 58°C, ranking the attributes for appearance (3), odour (16), and taste (14), from ten (10) to zero (0) according to the intensity of each attribute.

## RESULTS

### Odour-active volatiles

The volatile extract obtained by SDE from powder of roasted oak acorns was analysed by GC-FID and GC-O to detect the odour-active volatiles, which were the only identified compounds, according to the well-known molecular sensory approach (17). Thus, nineteen odour-active volatile compounds were identified in the roasted oak acorn beverage (Table 1, Figure 1). Different odour notes were detected, all of them related to the usual notes of coffee (21): acid (acetic, and propanoic acids), buttery/caramel (2,3-pentanedione,

hexanal, 3-hydroxy-2-butanone, nonanal, furfural, 2,3-dimethyl-2-cyclopenten-1-one, 5-methyl furfural, butanoic acid, isomaltol, 1-furfuryl pyrrole, and 2-acetyl-1H-pyrrole), smoke/roasted (furfuryl methyl ether, dimethyl pyrazine, 2-ethyl pyrazine, and furfuryl alcohol), and fruity (3-penten-2-one, and benzaldehyde) odour notes. Among them, 2,3-pentanedione, hexanal, dimethyl pyrazine, 2-ethyl pyrazine, acetic acid, furfural, 5-methyl furfural, and butanoic acid have been reported as odorant compounds of roasted coffee (21, 22). Furfural, 5-methyl furfural, and furfuryl alcohol were predominant in the SDE extract of roasted oak acorns (Figure 1).



**Figure 1.** Total ion chromatogram of SDE extract of volatile from roasted oak acorns using FFAP column. The numbers correspond to the compounds in Table 1.

**Table 1.** Odour-active volatile compounds detected in the SDE extract from coffee substitute obtained from roasted *Q. humboldtii* acorn powder.

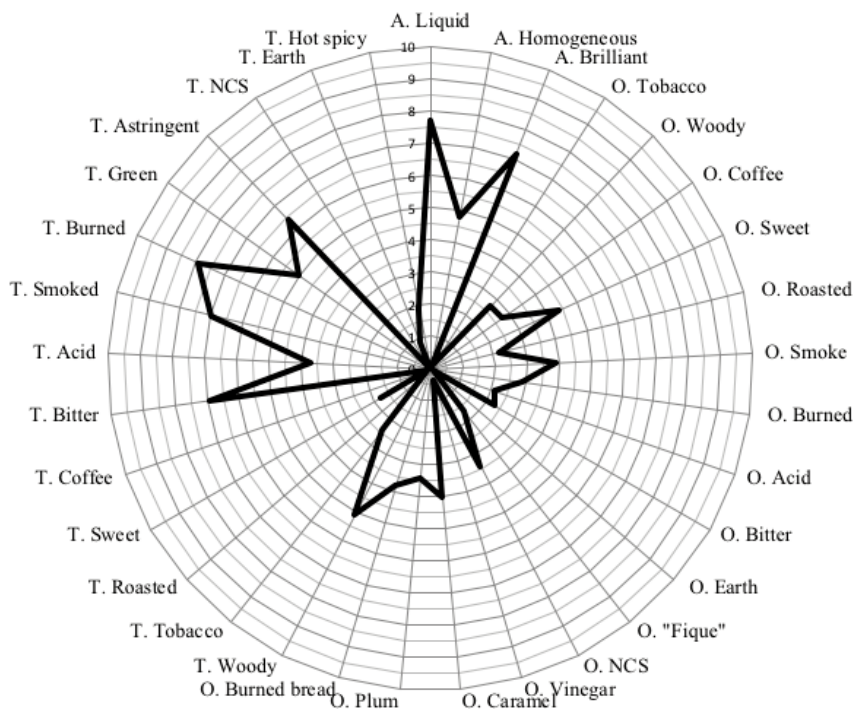
No.	Compound	Odour quality	RI		Amount* (Area percentage $\pm$ SD)
			FFAP column	DB-5 column	
1	2,3-Pentanedione	Sweet, caramel	1084	-	1.43 $\pm$ 0.12
2	Hexanal	Green, fatty	1104	-	0.27 $\pm$ 0.00
3	3-Penten-2-one	Fruity, metallic	1117	-	0.43 $\pm$ 0.02
4	Furfuryl methyl ether	Coffee	1268	820	0.31 $\pm$ 0.03
5	3-Hydroxy-2-butanone	Fatty	1300	711	7.41 $\pm$ 0.02
6	Dimethyl pyrazine	Roasted	1321	-	0.60 $\pm$ 0.07
7	2-Ethyl pyrazine	Coffee, roasted	1350	906	0.47 $\pm$ 0.05
8	Nonanal	Fatty, chocolate	1408	-	0.48 $\pm$ 0.04
9	Acetic acid	Vinegar	1428	-	11.50 $\pm$ 0.16
10	Furfural	Coffee, sweet	1498	-	30.81 $\pm$ 1.15
11	Propanoic acid	Rancid, green	1521	-	2.95 $\pm$ 0.04
12	Benzaldehyde	Fatty, fruity	1546	-	1.26 $\pm$ 0.07
13	2,3-Dimethyl-2-cyclopenten-1-one	Floral, sweet	1596	-	0.39 $\pm$ 0.03
14	5-Methyl furfural	Roasted, caramel	1611	978	20.19 $\pm$ 0.35
15	Butanoic acid	Rancid, papaya, fruity	1614	811	1.10 $\pm$ 0.06
16	Isomaltol	Caramel, sweet	1654	-	2.17 $\pm$ 0.07
17	Furfuryl alcohol	Roasted, burned	1665	851	13.50 $\pm$ 0.39
18	1-Furfuryl pyrrole	Roasted, sweet	1869	-	1.93 $\pm$ 0.17
19	2-Acetyl-1H-pyrrole	Roasted, nutty, sweet	2020	1060	2.80 $\pm$ 0.16

-: Not detected; RI: Retention Index; \*: average of odour-active volatiles ( $n = 2$ ).

## Sensory analyses

The results of QDA are shown in Figure 2. The evaluated attributes were the characteristic of roasted coffee bean powder. The most significant odour notes were: sweet, smoke, NCS (non-centrifugal cane sugar, so called “panela”), caramel,

and burned bread, in agreement with the odour notes perceived by GC-O from volatile extract obtained by SDE. As part of QDA, many taste notes were evaluated; thus, bitter, smoke, burned, and astringent taste notes were ranked with the highest scores.



**Figure 2.** Sensory evaluation (QDA) results of infusion prepared with roasted oak acorn powder.

## DISCUSSION

SDE technique, that includes the heating of the sample, was chosen to obtain the volatile extract from roasted oak acorn powder because this coffee substitute beverage is consumed after the oak powder is mixed with hot water for a certain time, in a similar way that common coffee is prepared. Among nineteen odour active volatiles, furfural, 5-methyl furfural, and furfuryl alcohol were the major constituents. These volatile compounds are usually produced by sugar thermal breakdown, which is likely to happen during the acorn roasting. It is known that roasting is a process that favors the development of Maillard reaction, lipid thermal oxidation, Strecker degradation, or caramelisation, according to the composition of original material (23). Additionally, the influence of furfuryl compounds in the flavor of oak-aged wines has been studied and is influenced by the wood's origin

of barrels (24), however furfuryl alcohol does not have any significance in the aroma of wines, in contrast with the results here obtained for the coffee substitute beverage.

It is important to point out, that the other volatile compounds present in the SDE volatile extract (Figure 1) from roasted oak acorn were not reported in this study because they do not have any influence on the aroma. But their mass spectra showed the presence of many fatty acids, some volatile phenolic compounds, such as, guaiacol and phenol, and aromatics such as, 1, 2, 4-trimethoxy benzene. The phenolic compounds and fatty acids from acorns of other *Quercus* species (*Q. ilex*, *Q. rotundifolia*, and *Q. suber*) have been identified (25), showing the predominance of oleic, palmitic, and linoleic acids. Some phenolic compounds were also characterized, mainly gallic acid derivatives, suggesting that they are responsible for the antioxidant activity of oak acorns (11).

The results of sensory analyses are interesting because 2,5-dimethyl pyrazine, furfural, propanoic acid, and furfuryl alcohol (2-furanmethanol) were also identified as odour-active volatiles in non-centrifugal cane sugar (NCS) beverage (26). Despite odour notes resemble those of coffee, the trained panel concluded that the taste of this coffee substitute is not well equilibrated, because the presence of off-flavors, such as, astringency and burning taste notes. The next step is to characterize phenolic compounds that could be responsible for these undesirable taste notes in order to equilibrate and improve its flavor. Rakić et al, (11) found that roasted oak acorns from *Quercus robur* contain 14.93% of polyphenols and 14.06% of proteins, among other constituents. In that work, the content of biofunctional compounds showed that processing of oak acorns improves their functional properties (antioxidant activity). Despite other food product develop from *Quercus acorns* have been studied before (4-9), it is important to point out that this is the first study of aroma volatile compounds of this coffee substitute beverage.

## CONCLUSIONS

A coffee substitute beverage from roasted oak acorns was sensory evaluated. The results of molecular sensory approach focused on odour notes, confirmed that this product resembles coffee; however, QDA analyses performed by a trained panel showed the presence of undesirable taste. Despite this fact, this coffee substitute is widely consumed in some regions of Colombia. This is the first approach to evaluate sensory properties of this promising added-value product obtained from oak, with potential to be commercialized as functional food. This coffee substitute exhibits coffee-like sensory properties without having caffeine; however, more sensory studies as well as, the characterization of non-volatile compounds related to taste notes (phenolics and alkaloids) are need in order to improve the taste quality of this coffee substitute.

## CONFLICTS OF INTEREST

The authors declare that there is no any conflict of interest.

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## AUTHOR'S CONTRIBUTIONS

LDR, NGB and DPF performed experimental work. ELL and JCML contributed with conception and design of the study. JCML and CO performed the experimental designed, supervised the work at lab, interpreted the data and wrote the manuscript. All the authors have read the final version of the manuscript and approved it.

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