FATTY ACIDS CONTENT OF KAHAI (CARYODENDRON ORINOCENSE KARST) SEEDS CULTIVATED IN AMAZONIAN OF ECUADOR

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INTRODUCTION

Caryodendron orinocense Karst, named kahai, inchi, mani de arbor, and nuez de barinas in Ecuador, Colombia, and Venezuela. This plant belongs to the Euphorbiaceae family; it is cultivated to obtain kernel oil. There are very few studies about the composition of fatty acids of kahai oil [1]. Kahai fruit grows from November to June in the Amazonian region in Ecuador [2]. In the Amazonian region, there are a high number of plants with high lipid contents in the mesocarp and kernel seeds that can represent a good alternative to economy to the indigenous communities. Many crops can be exploited to this purpose as sacha inchi (Plukenetia volubilis) and Ungurahui (Onecorpus bataua). Kahai seeds have a high content of lipids and proteins with approximately 29–50% of lipid content with a high content of polysaturated fatty acids, especially linoleic acid (omega 6) [1]. Radice et al., have reported a lipid content of 46.9% in kahai seeds out of which a value of 10.3% of palmitic acid (C16:0), 3.4% of stearic acid (C18:0), and a value of 85.59% of linoleic acid (C18:2) [3]. Alfaro et al., have reported a value of 30% of lipid content in kahai seeds out of which a value of 9.52% of palmitic acid (C16:0), 2.17% of stearic acid (C18:0), 11.80% of oleic acid (C18:1), 75.13% corresponding to linoleic acid (C18:2), and 0.92% of linolenic acid (C18:3) [4]. This oil type has a good acceptance of the cosmetic and pharmaceutical industry to elaborate different formulations [5]. The aim of this work was to identify the fatty acids composition present in kahai (C. orinocense Karst) kernel from Ecuador using the gas chromatography–mass spectrometer (GC–MS).

METHODS

Total lipid extraction
Kahai walnuts were obtained in the Amazonian of Ecuador. Kahai oil sample was obtained from kahai seeds using the cold pressing method. Oil was then stored at 4.0 ± 2°C. Oil extraction was conducted using a Soxhlet apparatus for approximately 5 h with hexane as solvent, with a solid-to-solvent ratio of 1/7 m/v. After the extraction process, the flask contents were filtered, and the liquid fraction containing the lipid extract and solvent was poured into a 250-mL flask of a rotary film evaporator to remove the solvent. The obtained oil was collected, evaporated under nitrogen, weighed, and stored in sealed amber glass vials at −20°C until analysis [6].

Methyl esters fatty acids (FAME)
Methyl esters (FAME) were prepared from 3 to 5 mg of total lipids using the two-step methylation method [1% NaOH3/MeOH] followed by 5% HCl/MeOH); both steps were performed at 60°C, 20 min [7]. FAME were extracted with 6 ml of hexane.

Analysis of FAME from kahai oil by GC–MS
The fatty acid composition of oil extracted from kahai walnut seeds was analyzed by injecting fatty acid methyl esters [8] into an Agilent Technologies 7890A system gas chromatography (Agilent, Santa Clara, CA) equipped with a mass selective detector 5977A GC/MSD, an auto-sampler 7693, column (60 m × 250 µm × 0.25 µm, DB-WAX Agilent Technologies, 7980A system gas chromatography (Agilent, Santa Clara, CA) equipped with a mass selective detector 5977A GC/MSD, an auto-sampler 7693, column (60 m × 250 µm × 0.25 µm, DB-WAX Agilent Technologies 122–7062). The oven temperature was programmed as follows: From 80°C; ramp 1: To 100°C at 20°C/min during 1 min; ramp 2: At 200°C at 25°C/min during 10 min; and ramp 3: At 250°C at 2°C/min. The injector and detector temperatures were set at 250°C. Helium was used as carrier gas at a linear flow velocity of 1.4 mL/min.

Spectra were compared with the NIST 14L library and the fatty acids mass spectra archive [9]. All GC analyses of the fatty acids were carried out in triplicate, and the results were expressed as the mean ± standard deviation.

RESULTS AND DISCUSSION

Methyl esters fatty acids (FAME) were analyzed with the GC–MS. The GC chromatogram of methyl esters fatty acids from kahai oil present five majoritarian peaks that were separated with a column Agilent DB-WAX 122–7062. These peaks were identified with the help of a
The quantification of fatty acids was obtained using the peak area ratio. Fig. 1 shows five abundant peaks with a good separation and definition; these peaks were identified with retention times of 19.321 min, C18:0 with a retention time of 25.995 min, C18:1 with a retention time of 26.856 min, C18:2 with a retention time of 28.628 min, and finally C18:3 with a retention time of 31.092 min.

The concentration of methyl esters fatty acids (FAME) was determined using the peak area ratio. Table 1 summarizes C16:0 with 7.0%±0.90 of fatty acid total content, C18:0 with 3.47%±0.16 of fatty acid total content, C18:1 with 18.59%±0.44 of fatty acid total content, C18:2 with 68.04%±1.14 of fatty acid total content, and C18:3 with 2.90%±0.26 of fatty acid total content of kahai oil. The total lipid of kahai seeds was of 62.36% of lipid in fresh weight (Table 1). The mass spectrum of palmitic acid obtained from kahai oil is shown in Fig. 2. The identity and structures of these fatty acids were confirmed by the GC-MS method. Palmitic acid (C16:0) was identified using the mass spectrum with ions m/z between 55 and 270 m/z. In the previous range, the ions 74 and 87 m/z were the most abundant in the mass spectrum (Fig. 3).

Stearic acid (C18:0) was identified using the mass spectrum with ions m/z between 55 and 298 m/z. In the previous range, the ions 74 and 87 m/z were the most abundant in the mass spectrum (Fig. 3).

Oleic acid (C18:1) was identified using the mass spectrum with ions m/z between 55 and 296 m/z. In the previous range, the five ions with the highest abundance were the 55, 74, 83, 97, and 264 m/z (Fig. 4).

Linoleic acid (C18:2) was identified using the mass spectrum with ions m/z between 55 and 294 m/z. In the previous range, the three most present ions were the 55, 67, 81, and 95 m/z (Fig. 5).

When the kahai fatty acid composition is compared to some other common vegetable oils, it can be seen that olive oil has a high content of monounsaturated fatty acids; C18:1 named oleic acid with 77.6% of oleic acid. It can be seen that kahai oil from Ecuador has a good content of monounsaturated fatty acids with a content of 18.59% of oleic acid. Kahai oil has also a high content of polyunsaturated fatty acids with 68.04% of linoleic acid. Olive oil contains few omega 6 and omega 3 fatty acids with 9.0% and 1.0%, respectively, and macadamia oil contains 3.22% of omega 6 and 1.79% of omega 3 (Table 2). Kahai oil has a similar profile of fatty acids when it is compared to walnut Juglans regia with a high content of omega 6 and a good content of omega 9, both oils presenting a few content of omega 3 [10,11]. Walnut has been considered as a healthy food due to its capacity to reduce cardiovascular diseases risk. Food and Drug Administration

### Table 1: Total lipid and fatty acids composition of tocte oil sample from Ecuador by GC–MS analysis and their percentage

| Retention time | Peak area ratio % | Carbon number: Double bound | FAMEs name   |
|---------------|------------------|-----------------------------|--------------|
| 19.321 min    | 7.00             | C16:0                       | Palmitic acid|
| 25.995 min    | 3.47             | C18:0                       | Stearic acid |
| 26.856 min    | 18.59            | Δ9 C18:1                    | Oleic acid   |
| 28.628 min    | 68.04            | Δ9, 12 C18:2                | Linoleic acid|
| 31.092 min    | 2.90             | Δ9, 12, 15 C19:3            | Linolenic acid|
| Total lipid   | 62.36            |                             |              |

GC-MS: Gas chromatography–mass spectrometer

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Fig. 1: Analysis by gas chromatography of methyl esters fatty acid (FAME) from kahai oil

Fig. 2: Mass spectrum of palmitic acid from kahai oil

Fig. 3: Mass spectrum of stearic acid from kahai oil

Fig. 4: Mass spectrum of oleic acid from kahai oil
Table 2: Fatty acid composition (%) of 10 vegetable oils

| References | Vegetal oil | C16:0 | C16:1 | C18:0 | C18:1 | C18:2 | C18:3 |
|------------|-------------|-------|-------|-------|-------|-------|-------|
| [18,19]    | Olive oil   | 13.8  | 1.4   | 2.8   | 71.6  | 9.00  | 1.0   |
| [18]       | Sunflower oil | 5.2   | 0.1   | 3.7   | 33.7  | 56.5  | 0.0   |
| [18]       | Palm oil    | 44.8  | 0.0   | 4.6   | 38.9  | 9.5   | 0.4   |
| [18]       | Soybean oil | 10.1  | 0.0   | 4.3   | 22.3  | 5.37  | 8.1   |
| [19]       | Corn oil    | 11.6  | 0.0   | 2.5   | 38.7  | 44.7  | 1.4   |
| [19]       | Sacha inchi oil | 3.98 | 0.0  | 3.12  | 8.58  | 34.98 | 47.04 |
| [20]       | Sambo oil   | 9.33  | 0.0   | 6.84  | 41.36 | 33.98 | 0.0   |
| [21]       | Macadamia oil | 9.11 | 12.48 | 3.93  | 63.36 | 31.22 | 1.69  |
| [22]       | Walnut Juglans regia | 7.90 | ND   | 2.30  | 23.10 | 66.54 | ND    |
|            | Kahai oil   | 7.0   | 0.0   | 3.47  | 18.59 | 68.04 | 2.90  |

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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Fig. 5: Mass spectrum of linoleic acid from kahai oil

of US in the year 2004 have accepted the claim about the consume of walnuts to reduce and prevent cardiovascular diseases. It is also known that walnut inclusion in diets brings benefits in the human health for their good lipid composition [12]. Around 1 billion people are affected by hypertension disease and 7 million annual deaths are attributed to this disease. The type 2 diabetes mellitus is a disease rapidly increasing in the world and is directly implicated in the risk of cardiovascular diseases. Fortunately, hypertension and diabetes prevention trials in high-risk groups have shown that weight loss among obese individuals, physical activity, adoption of a diet rich in fresh fruit, vegetables, potassium, and a reduced sodium content may reduce the incidence of hypertension. Nuts have long been part of human diet since pre-agricultural times, supply food rich in a number of nutrients, and phytochemicals components that may reduce risks of cardiovascular disease (diabetes and hypertension) [13,14]. Nuts have saturated fatty acids and high in monounsaturated and polyunsaturated fats. Omega 9: Oleic acid, omega 6: Linoleic acid, and omega 3: Linolenic acid. Nuts are also a good source of vegetable protein, fiber, phytosteryl, polyphenols, vitamins, and minerals [15]. These bioactive compounds can serve as antioxidants, are anti-inflammatory, and may improve insulin resistance, and thus nuts may lower diabetes and hypertension risk. Nuts protein can be a source of bioactive peptides that can be free after human digestion. When people include nuts in their diet, they can reduce and prevent cardiovascular diseases [16,17].

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AUTHOR CONTRIBUTIONS

Greffs J, Vinueza D, Carpio C, Morales D and Carrillo W conceived and designed the experiments. Silva M and Alvarez M performed the gas chromatography analyses. Carrillo W wrote the paper.
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