

Volatile compounds of ditax fruit (*Detarium senegalense* J.F. Gmel) from Senegal

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Volatile compounds of ditax fruit (*Detarium senegalense* J.F. Gmel) from Senegal.

Abstract – Introduction. *Detarium senegalense* J.F. Gmel is a forest tree found in Senegal whose fruits are locally called ditax in Wolof. It is eaten fresh but it is widely used as nectar, which is one of the most popular beverages in Senegal. However, the chemical characterization of ditax pulp remains incomplete. This paper describes the volatile compounds of ditax to assess its organoleptic qualities. **Materials and methods.** Free volatile compounds of fresh ditax pulp were isolated by solvent-assisted flavor evaporation and analysis by GC-MS. **Results and discussion.** Among the 53 compounds tentatively identified, 49 are reported for the first time in this fruit. In total, 17 aldehydes, 11 aliphatic alcohols, 1 terpene alcohol, 7 free fatty acids, 3 unsaturated hydrocarbons, 1 terpene hydrocarbon, 7 sesquiterpene hydrocarbons, 1 phenol, 2 ketones, 2 esters and 1 organic acid compound were tentatively identified in ditax fresh pulp. The main volatiles identified in fresh ditax pulp were *trans*, *cis*-2,6-nonadienal (2.47 mg·kg⁻¹), *cis*-2-heptenal (1.93 mg·kg⁻¹), *trans*- α -bergamotene (1.11 mg·kg⁻¹), bicyclo [2,2,0] hexane-1-carboxaldehyde (0.80 mg·kg⁻¹), butyl octadecanoate (0.55 mg·kg⁻¹) and *trans*-2-nonenal (0.47 mg·kg⁻¹ fresh pulp). **Conclusion.** Among the volatile compounds identified, aldehyde compounds were widely predominant. To assess the aromatic qualities of ditax pulp, the primary impact aromas should be determined by identifying the aroma-active compounds by GC-olfactometry.

Senegal / *Detarium senegalense* / fruits / flavor / volatile compounds / solvent extraction

Les composés volatils du fruit de ditax (*Detarium senegalense* J.F. Gmel) au Sénégal.

Résumé – Introduction. *Detarium senegalense* J.F. Gmel est un arbre forestier, trouvé au Sénégal, dont les fruits sont localement appelés ditax en Wolof. Le fruit est directement consommé mais il est largement utilisé en nectar, l'une des boissons les plus populaires au Sénégal. Cependant, la caractérisation chimique de la pulpe de ditax reste incomplète. Cette étude décrit les composés volatils de la pulpe de ditax afin d'évaluer sa qualité organoleptique. **Matériel et méthodes.** Les composés volatils libres de la pulpe de ditax fraîche ont été isolés par la technique d'évaporation assistée par solvant et analysés par GC-MS. **Résultats et discussion.** Parmi les 53 composés identifiés, 49 sont signalés pour la première fois dans ce fruit. Au total, 17 aldéhydes, 11 alcools aliphatiques, 1 alcool terpénique, 7 acides gras libres, 3 hydrocarbures insaturés, 1 hydrocarbure terpénique, 7 hydrocarbures sesquiterpéniques, 1 phénol, 2 cétones, 2 esters et 1 acide organique ont été identifiés dans la pulpe de ditax fraîche. Les principaux composés volatils identifiés dans la pulpe de ditax fraîche ont été le *trans*, *cis*-2,6-nonadiénel (2,47 mg·kg⁻¹), le *cis*-2-hepténel (1,93 mg·kg⁻¹), le *trans*- α -bergamotène (1,11 mg·kg⁻¹), le bicyclo [2,2,0] hexane-1-carboxaldéhyde (0,80 mg·kg⁻¹), l'octadecanoate de butyle (0,55 mg·kg⁻¹) et le *trans*-2-nonénel (0,47 mg·kg⁻¹ de matière fraîche). **Conclusion.** Parmi les composés volatils identifiés, les aldéhydes sont les composés majoritaires. Pour évaluer la qualité aromatique de pulpe de ditax, l'identification des composés d'impacts notamment par GC-olfactométrie serait une perspective intéressante.

Sénégal / *Detarium senegalense* / fruits / flaveur / composé volatil / extraction par solvant

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1. Introduction

The *Detarium senegalense* tree from the Ceasalpiniaceae family, an arboreal fruit species, is still found wild in Senegal (West Africa), in the Sine-Saloum islands and in Casamance [1]. Reaching (15 to 40) m, it is also found in the wet dense forest borders, the coastal and septentrional regions, and the Sudan-Guinean zone. The *Detarium senegalense* fruit, locally called ditax in Wolof, is one of the most important forest fruit-bearing species with great economic importance. The fruit (*figure 1*) appears in the form of a drupe with a diameter from (3 to 8) cm and a mean weight of (49.2 ± 11.2) g. The epicarp (13% of the fruit weight) is dark green and hard in unripe fruits, and light green, brown and brittle in ripe fruits. The pulp (34% of the fruit weight) is green and entangled in a high-fiber network firmly inserted on the stone (53% of the fruit weight), which contains one single dark brown seed [1–4]. The pulp is very rich in ascorbic acid [from (1.2 to 2.2) $\text{g}\cdot 100\text{ g}^{-1}$ of fresh weight according to geographical area]; sucrose (18–20% dry weight) is the main component of the total sugars (23% dry weight) and pheophytin a ($128\text{ mg}\cdot\text{kg}^{-1}$ fresh weight) is the major pigment of ditax pulp [5, 6]. The fruit is eaten fresh but it is widely used as nectar (fruit pulp with water and sugar), which is one of the most popular beverages in Senegal. However, the chemical characterization of ditax pulp remains incomplete. To the best of our knowledge, only Haddad makes reference to the aroma volatiles of ditax [3]. Therefore, our paper describes for the first time the volatile compounds of ditax to complete the chemical characterization data of this forest fruit and to assess its organoleptic qualities.

2. Materials and methods

2.1. Fruits

Fresh ripe fruits were bought at the market in September 2012, and after one week of storage at $-18\text{ }^{\circ}\text{C}$, pulps were extracted just before analysis.

2.2. Solvent-assisted flavor evaporation (SAFE) of ditax pulp volatiles

The solvent-assisted flavor evaporation (SAFE) technique was applied for careful extraction of the ditax pulp volatiles. Volatiles, preliminarily extracted in a solvent, were evaporated at low temperature in high vacuum conditions (10^{-3} mbar) and condensed with a liquid nitrogen trap.

Forty g of fresh ditax pulp and 50 μL of internal standard solution (20 $\mu\text{L}\cdot 20\text{ mL}^{-1}$ H_2O) of nonan-4-ol were combined with 40 mL of distilled water and 80 mL of dichloromethane, and stirred for 30 min in an iced water bath under a nitrogen atmosphere. The supernatant was collected and the residue was reextracted with 80 mL of dichloromethane and 40 mL of distilled water for 30 min again to achieve complete transfer of the volatile compound from the pulp. Organic phases were combined, dried over anhydrous sodium sulfate and distilled at $30\text{ }^{\circ}\text{C}$. After complete distillation, the system was left for 1 h under vacuum. The distillate was then concentrated in a water heating bath at $40\text{ }^{\circ}\text{C}$ using a Kuderna-Danish apparatus. Preliminary extractions were realized using a mixture of pentane-diethyl ether (50/50).

2.3. GC-MS analysis of safe extract

A tandem gas chromatograph 6890 / MSD 5973 / Gerstel Multipurpose Sample MPS-2 (Agilent Technologies, Palo Alto, USA) was used for volatile compound analysis. Injection (1 μL) was carried out in split 1/10 mode at $250\text{ }^{\circ}\text{C}$ using a DB-WAX polar column (J&W, $30\text{ m} \times 0.25\text{ mm} \times 0.25\text{ }\mu\text{m}$) and a DB-1ms nonpolar column (J&W, $0.25\text{ mm} \times 30\text{ m} \times 0.25\text{ }\mu\text{m}$) (Agilent Technologies, Palo Alto, USA). Helium was used as the carrier gas at $1\text{ mL}\cdot\text{min}^{-1}$. Elution was realized with the following temperature program: $3\text{ }^{\circ}\text{C}$ per min from $40\text{ }^{\circ}\text{C}$ to $170\text{ }^{\circ}\text{C}$, then $10\text{ }^{\circ}\text{C}$ per min up to $240\text{ }^{\circ}\text{C}$ and held for 10 min. Mass spectra were recorded in EI+ mode at 70 eV within (15 to 300) Da. The analyzer and source temperatures were $150\text{ }^{\circ}\text{C}$ and $250\text{ }^{\circ}\text{C}$, respectively.

2.4. Identification of volatile compounds

Data were analyzed with the MSD Chem revD1 acquisition and data retreatment software. Peak identification was done by comparing mass spectra with those of the NIST 2008 (National Institute of Standard Technology) database [7]. Injection of n-alkane series C₈-C₂₀ (Sigma-Aldrich) was used for retention index (RI) calculations and their comparison with those found in the literature, and on the Flavornet¹ and Pherobase² websites.

2.5. Quantification of volatile compounds

Semi-quantitative determinations were carried out by adding nonan-4-ol (Sigma Aldrich) as an internal standard at a concentration of 20 µL:20 mL⁻¹ H₂O. The ratio of the response factor was considered to be equal to 1, thus allowing semi-quantification in µg·kg⁻¹ fresh pulp using the equation:

$$m_i (\mu\text{g}\cdot\text{kg}^{-1} \text{ fresh pulp}) = 1000 \times \frac{A_i m_{IS}}{A_{IS} m_p}$$

with A_i = peak area of volatile compound i ; A_{IS} = peak area of nonan-4-ol; m_{IS} = quantity of nonan-4-ol; m_p = pulp quantity.

3. Results and discussion

The aroma compounds were separated on two columns of different polarity (DB-WAX and DB-1ms) (table D). Quantification on both the DB-WAX and DB-1ms columns, (11 769 and 10 200) µg·kg⁻¹, were quite similar. This slight difference could be

¹ Acree T., Arn H., Flavornet and human odor space, Gas chromatography-olfactometry (GCO) of natural products, Datu Inc. 2004, www.flavornet.org.

² El-Sayed A.M., The Pherobase : Database of Pheromones and Semiochemicals, www.pherobase.com, 2003–2012, The Pherobase.



Figure 1. Fruit of *Detarium senegalense* J.F. Gmel: whole fruit, husked fruit (green pulp), fruit after pulp extraction (stone with fibrous network).

explained by a longer storage in the freezer (17 d at -18 °C) before analysis on the DB-1ms. Among the 53 compounds tentatively identified, forty-nine are reported for the first time in this fruit. In total, seventeen aldehydes, eleven aliphatic alcohols, one terpene alcohol, seven free fatty acids, three unsaturated hydrocarbons, one terpene hydrocarbon, seven sesquiterpene hydrocarbons, one phenol, two ketones, two esters and one organic acid compound were identified or tentatively identified in ditax fresh pulp (table D). Aldehydes known to impart “green” and “grassy” notes were qualitatively and quantitatively the most important class of compounds in ditax fresh pulp, with a total concentration of 6 704 µg·kg⁻¹ of fresh pulp (57% of the total volatile fraction). Among them, *trans*, *cis*-2,6-nonadienal was the main volatile, representing 21% of the total volatile fraction. Then followed *cis*-2-heptenal (16.4% of the total volatile fraction), bicyclo [2,2,0] hexane-1-carboxaldehyde (6.8%), *trans*-2-nonenal (4%) and *trans*-2-hexenal (3.8%).

Table 1. Volatiles compounds identified or tentatively (*) in fresh ditax pulp after solvent-assisted flavour evaporation (SAFE) extraction and analysis on polar and non polar column.

Compounds	Retention indice polar ^a		Retention indice non polar ^b		Concentration ($\mu\text{g}\cdot\text{kg}^{-1}$ of fresh pulp)	Odor
	Experienced	Literature ^c	Experienced	Literature ^c		
Aliphatic alcohols						
1-Butanol	1144	1145	–	658	12.34 ± 0.66	Medicine, fruit ¹ [8]
1-Penten-3-ol	1155	1181	–	669	77.87 ± 2.76	–
1-Pentanol	1242	1255	814	730	52.60 ± 3.75	Balsamic ¹
Cis-2-penten-1-ol	nd	1313	814	740	112.12	–
Cyclopentanol	1314	1323	–	774	159.99 ± 4.43	–
Cis-3-hexen-1-ol	1384	1401	859	844	67.50 ± 5.05	Leaf, fatty grass [9]
1-Hexanol	1353	1360	870	858	332.18 ± 10.98	Fruit [9]; resin, flower, green ¹
Trans-2-hexen-1-ol	1410	1400	867	870	49.14 ± 0.34	Fruit, leaf [9]; green [8]
1-Heptanol	1469	1467	950	945	67.11 ± 0.37	Chemical, green ¹
6-Hepten-1-ol	1530	–	948	950	35.82 ± 0.16	–
Cis-6-nonen-1-ol	1756	1714	1151	1171	73.64 ± 0.60	–
Terpenic Alcohols						
1,8-Cineole	1194	1214	1009	1015	31.85 ± 1.27	Mint, sweet ¹
Aldehydes						
Pentanal	937	935	–	674	105.79 ± 6.94	Almond, malt, pungent ¹
Hexanal	1096	1093	824	773	199.46 ± 9.40	Green [8]; grass, tallow, fat ¹ ,
Trans-2-pentenal	1126	1117	804	724	18.08 ± 0.49	Strawberry, fruit, tomato ¹
Cis-3-hexenal	1137	1132	823	801	12.13 ± 0.14	Leaf, green ¹
Heptanal	1172	1174	887	885	72.97 ± 3.06	Fat, citrus, rancid ¹
Cis-2-hexenal	1186	1187	847	815	Traces	–
Trans-2-hexenal	1201	1220	851	826	452.18 ± 19.58	Green [8]
Cis-4-heptenal	1220	1230	878	885	20.31 ± 0.79	Biscuit, cream ¹
Cis-2-heptenal	1310	1319	931	927	1929.56 ± 21.96	Green ¹
Bicyclo [2.2.0] hexane-1-carboxaldehyde*	1372	–	916	–	803.85 ± 1.68	–
Nonanal	1387	1385	1078	1079	29.98 ± 0.11	Fatty, citrus, green ¹
Trans-2-octenal	1425	1442	1024	1031	16.00 ± 0.84	–
Cis, cis-3,6-nonadienal	nd	1444	1065	1083	34.17	–
Cis-6-nonenal	1449	1469	nd	1112	27.34 ± 4.58	–
Trans, cis-2,4-heptadienal	1463	1480	nd	921	37.60 ± 1.08	–
Trans-2-nonenal	1545	1527	1132	1130	470.97 ± 1.96	Cucumber, fatty, green ¹
Trans, cis-2,6-nonadienal	1602	1605	1125	1137	2474.08 ± 71.11	Cucumber, waxy, green ¹

Table I.
continued.

Compounds	Retention indice polar ^a		Retention indice nonpolar ^b		Concentration ($\mu\text{g}\cdot\text{kg}^{-1}$ of fresh pulp)	Odor
	Experienced	Literature ^c	Experienced	Literature ^c		
Free fatty acids						
Hexanoic acid	1879	1872	1004	890	273.24 \pm 0.96	Fat, sweat ¹
Heptanoic acid	1985	1990	1125	1064	82.96 \pm 4.24	Rancid ¹
<i>Trans</i> -2-hexanoic acid	1996	1962	nd	-	20.02 \pm 1.20	-
Nonanoic acid	2183	2202	1280	1258	26.73 \pm 4.10	Rancid ¹
Dodecanoic acid*	2613	2517	1501	1556	33.17 \pm 0.03	Metal ¹
Hexadecanoic acid	2936	2940	1955	1950	202.39 \pm 13.24	-
Octadecanoic acid	3184	3181	2093	2124	200.59 \pm 41.76	-
Ketones						
1-Penten-3-one	1063	1034	nd	673	87.76 \pm 2.99	Fish, pungent ¹
3-Hydroxy-2-butanone	1266	1272	nd	678	81.84 \pm 5.97	-
Esters						
Butyl hexadecanoate*	2573	-	2110	1977	367.87 \pm 3.11	-
Butyl octadecanoate*	2733	-	2257	2374	552.07 \pm 6.92	-
Unsaturated hydrocarbons						
Toluene	1071	1070	nd	756	39.54 \pm 1.62	Paint ¹
Ethylbenzene	1121	1124	864	846	5.63 \pm 0.03	-
Styrene	1234	1261	882	876	154.47 \pm 9.88	Balsamic ¹
Terpene hydrocarbons						
Limonene	1182	1178	1012	1022	5.5 \pm 1	Citrus, mint ¹
Sesquiterpene hydrocarbons						
α -Copaene					11.21	Woody, spicy ¹
<i>Cis</i> - α -bergamotene	1580	1580	1404	1414	114.07 \pm 3.06	-
α -Santalene	1581	1574	1409	1419	96.30 \pm 6.15	-
<i>Trans</i> - α -bergamotene	1602	1779	1425	1431	1112 \pm 6.17	Woody, tea, warm ^{1,2}
<i>Trans</i> - β -farnesene*	1692	1674	1441	1448	98.83 \pm 7.75	Woody, citrus, sweet ¹
β -Bisabolene*	1750	1726	1465	1513	35.02 \pm 4.31	Balsamic ¹
β -Cadinene*	1777	1982	1497	1440	60.41 \pm 0.94	-
Phenol						
Phenol	2026	1479	958	961	43.52 \pm 1.51	Phenol ¹
Acid						
Acetic acid	1450	1452	nd			Acid ¹

^a linear retention index on DB-wax.

^b linear retention index on DB-1ms.

^c linear retention index from Flavornet¹, Pherobase² and NIST 2008 [7].

trace: < 5 $\mu\text{g}\cdot\text{kg}^{-1}$ fresh pulp.

nd: Retention indice (RI) experimented < 800 or not detected.

Haddad reported that 2,6-nonadienal (42.3%), *trans*-2-heptanal (13.5%), *cis*-6-nonenal (7.5%), *trans*-2-nonenal (7.4%), caryophyllene oxide (2.5%), humulene oxide (1.8%) and tetradecane (0.7%) were the main volatiles of essential oil extract of ditax pulp obtained by hydrodistillation [3]. The following different flavors confirmed our perception; *trans*, *cis*-2,6-nonadienal is cucumber, violet, green and waxy; *trans*-2-nonenal is fatty, penetrating and waxy; melon or green melon and citrus are characteristic of *cis*-6-nonenal and green and watermelon-like of *cis*, *cis*-3,6-nonadienal [10–15]. Hexanal and *trans*-2-hexenal odor is green [8]. It has been widely reported that C₉ odorant compounds were the result of lipoxygenase degradation of linoleic and linolenic acid, which occurs rapidly after tissue disruption [16, 17].

Sesquiterpene hydrocarbons represent the second class of compounds (12.9% of total volatile compounds), with *trans*- α -bergamotene being the major component, with 73% of total sesquiterpene hydrocarbons and 9.4% of the total volatile fraction.

Aliphatic alcohols were the third class of compounds, with 8.8% of the total volatile fraction for a total amount of 1 040 $\mu\text{g}\cdot\text{kg}^{-1}$ of fresh pulp. Among them, 1-hexanol (floral, green notes) was the main volatile (2.8% of the total volatile fraction) followed by cyclopentanol (1.4%). However, other alcohols such as 1-butanol, *cis*-3-hexen-1-ol and *trans*-2-hexen-1-ol have also been identified in ditax pulp. They have already been reported in many other fruits such as apricot [18–21], copoazù [22] and acerola [23].

1,8-Cineole (mint, sweet flavor) was the only terpene alcohol identified in ditax pulp (0.3% of the total volatile fraction). 1,8-Cineole has also been identified in cantaloupe [24]. Alcohols and aldehydes of six carbon atoms are responsible for the herbaceous odor of several fruits and vegetables [20, 25].

Ester compounds were represented by butyl hexadecanoate (368 $\mu\text{g}\cdot\text{kg}^{-1}$) and butyl octadecanoate (552 $\mu\text{g}\cdot\text{kg}^{-1}$); these two compounds were tentatively identified because the experimental RIs (retention

indices) are different from those from the literature. These two compounds are esters of fatty acid and represent 7.8% of the total volatile fraction of ditax fresh pulp.

Free fatty acids represent 7% (839 $\mu\text{g}\cdot\text{kg}^{-1}$) of the total volatile fraction, where hexanoic acid (273 $\mu\text{g}\cdot\text{kg}^{-1}$), n-hexadecanoic acid (202 $\mu\text{g}\cdot\text{kg}^{-1}$) and octadecanoic acid (201 $\mu\text{g}\cdot\text{kg}^{-1}$) were the main compounds.

Acetic acid represents 2.45% of the total volatile fraction.

Other chemical compounds such as unsaturated hydrocarbons (1.7% of the total volatile fraction) with styrene as the main compound; ketones (1.45% of the total volatile fraction) and phenol (0.36% of the total volatile fraction) were also identified in ditax pulp.

4. Conclusion

The major volatiles present in ditax pulp could explain why ditax is very popular, especially for its strong cucumber-like aroma with gentle fruity, woody, herbal and green notes. Among the volatile compounds identified, aldehyde compounds were widely predominant. Five volatiles were responsible for over 38% of the total volatile fraction: *trans*, *cis*-2,6-nonadienal, (21%), *cis*-2-heptenal (16.4%), *trans*- α -bergamotene (9.4%) and bicyclo [2,2,0] hexane-1-carboxaldehyde (6.8%). To assess the aromatic qualities of ditax pulp, the primary impact aromas should be determined by identifying the aroma-active compounds by GC-olfactometry.

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Los compuestos volátiles del fruto de ditax (*Detarium senegalense* J.F. Gmel) en Senegal.

Resumen – Introducción. *Detarium senegalense* J.F. Gmel es una especie forestal que se encuentra en Senegal, cuyos frutos son denominados localmente ditax en Wólof. El fruto se consume directamente, pero también se emplea ampliamente en forma de néctar, una de las bebidas más populares de Senegal. Sin embargo la caracterización química de la pulpa de ditax permanece incompleta. El presente trabajo describe los compuestos volátiles de la pulpa de ditax con el fin de evaluar su calidad organoléptica. **Material y métodos.** Los compuestos volátiles libres de la pulpa de ditax fresca se aislaron mediante la técnica de evaporación asistida con solvente y se analizaron mediante GC-MS. **Resultados y discusión.** Entre los 53 compuestos identificados, se señalaron 49 por primera vez en este fruto. En total se identificaron 17 aldehídos, 11 alcoholes alifáticos, 1 alcohol terpénico, 7 ácidos grasos libres, 3 hidrocarburos sesquiterpénicos, 1 fenol, 2 cetonas, 2 éster y 1 ácido orgánico en la pulpa fresca de ditax. Los principales volátiles identificados en la pulpa de ditax fresca fueron el *trans*, *cis*-2,6-nonadienal ($2.47 \text{ mg}\cdot\text{kg}^{-1}$), el *cis*-2-heptenal ($1.93 \text{ mg}\cdot\text{kg}^{-1}$), el *trans*- α -bergamoteno ($1.11 \text{ mg}\cdot\text{kg}^{-1}$), el biciclo [2,2,0] hexano-1-carboxaldehído ($0.80 \text{ mg}\cdot\text{kg}^{-1}$), el octadecanoato de butilo ($0.55 \text{ mg}\cdot\text{kg}^{-1}$) y el *trans*-2-nonenal ($0.47 \text{ mg}\cdot\text{kg}^{-1}$ de materia fresca). **Conclusión.** Entre los compuestos volátiles identificados, los aldehídos son los compuestos mayoritarios. Para evaluar la calidad aromática de pulpa de ditax, una perspectiva interesante sería la identificación de los compuestos de impactos, sobre todo por GC-olfactometría.

Senegal / *Detarium senegalense* / frutas / sabor / compuesto volátil / extracción por disolventes