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Volatile and Flavonoid Composition of the Peel of *Citrus medica* L. var. *Corsican* Fruit for Quality Assessment of Its Liqueur

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Summary

The volatile and flavonoid compositions of the peel of *Citrus medica* L. var. *Corsican* fruits cultivated in Corsica were studied according to the maturity of the citron fruits measured using growing degree-days. Quantitative variation with the stage of development of the fruit was observed using gas chromatography, gas chromatography-mass spectrometry, and liquid chromatography-mass spectrometry/mass spectrometry. Thirty volatile compounds were identified in the peel essential oil. Limonene and γ-terpinene were the major compounds. The volatile compositions of commercial citron liqueurs were also characterized by high amounts of monoterpene hydrocarbons with the same two major components. The main flavonoid components of citron fruits and derived liqueurs were rutin and neohesperidin. This chemical characterization can be used for quality assessment of food products from *C. medica* var. *Corsican*.

Key words: Citrus medica L. var. *Corsican*, citrus peel, flavonoid compounds, essential oil, commercial citron liqueur

Introduction

Citrus fruits have an important role in the world economy, and in local regions of the Mediterranean area, such as Corsica, they are consumed either directly or processed. The citron (*Citrus medica* L.) belongs to the family Rutaceae, subfamily Aurantiaca, tribe Citrae (*1*). Systematic studies highlight the major role of citron in the phylogeny of several varieties of the *Citrus* genus as the pollinator parent (*2–6*). All taxonomists recognize the contribution of the citron to the development of several important cultivated genotypes. For instance, citron combined with sour orange (*C. aurantium*) and *C. micrantha* is phylogenetically the ancestor of lemon (*C. limon*) and lime (*C. aurantifolia*), respectively (*7,8*). Citron trees are small, 3 to 5 m high, with thorny branches and oval, elongated leathery leaves. Considered indigenous to northeastern India, Myanmar, and Yunnan province of China, the citron was the first *Citrus* species introduced to the Mediterranean basin.

During the 1890s, Corsica was the world's leading citron producer, exporting 1700 tonnes of fruit per year. The brined fruits were shipped mainly to northern Europe and used as candied peel in traditional Christmas cakes. In Corsica, only 5 or 6 ha of citron were still cultivated in 2008; the variety grown is C*orsican*. The flowers of this variety are white, while those of other citron varieties are usually purple or pink. The Corsican variety is the only one of the citron group that has acid-free pulp (juice vesicles) and sweet albedo. The fruit of the Corsican variety is generally light orange-yellow to cadmium yellow, large and elliptical in shape, 7–10 cm i.d., 8–14 cm in length, with a rind about 3–4 cm thick. The citron's highly scented peel is attributed to its external vesicles, which contain es-

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sential oils, rather than its pulp, which is seldom consumed. Its skin is thick, and its white inner tissue, called albedo, is consumed usually after being processed into jam or candied fruit. In Corsica, the main product from the peel is a local liqueur (called Cédratine in French or Aliméa in Corsican language). For the production of this alcoholic beverage, the harvest of citron fruits runs usually from September to November. A second set of fruits can be also collected from January to February.

Several chemical studies have reported the volatile composition of peel from *C. medica*. The peel essential oil composition from various citron varieties has also been investigated: *Etrog*, *Diamante*, *Rhobs el Arsa*, *Buddha's hand* and *Corsican* (*9–14*). These studies showed quantitative differences with regard to the major constituents: limonene, γ-terpinene, geranial and neral. Thus, several chemical compositions of the same variety have been reported according to their geographical origins. Venturini *et al.* (*15*) reported the chemical compositions of peel and leaf oils from 17 citron cultivars. According to the levels of seven components (limonene, β-pinene, γ-terpinene, neral, geranial, nerol and geraniol), the citron cultivars were classified in four main oil chemotypes. To our knowledge, there have been no studies on the polyphenolic composition of *C. medica* var. *Corsican*. However, some articles have dealt with flavonoid components in other varieties of *C. medica* (*16–18*). The flavonoids reported in the fruits are rutin and hesperidin derivatives (sakuranetin, 7-*O*-methyl-aromadendrin, dihydrokaempferide). High amounts of glycosylated flavones, flavanones, and dihydrochalcone were also identified; the major compounds were *C*- and/or *O*-glycosides of apigenin, phloretin, diosmetin and hesperetin.

As part of our investigations on the chemical characterization of citron varieties (*15*) and alcoholic beverages from Corsica (*19*), the aim of this work is to provide a better knowledge of the chemical characteristics (volatile and polyphenolic compositions) of typical commercial liqueurs from *C. medica* var. *Corsican*. This characterization should lead to a better valorization of these products by standardization of their quality.

Materials and Methods

Sampling of plant material

One thousand and five hundred fruits of *Citrus medica* var. *Corsican* (bourgeoning stage) were selected from 100 trees grown under the same pedoclimatic conditions. All trees were grown on a plantation at Vescovato, Corsica, France (latitude 42° 29′ N, longitude 9° 30′ E, Mediterranean climate, soil derived from alluvial deposits and classified as fersiallitic). Citron fruits were collected each month from July to January. Phenotypic characteristics of fruits such as mass, length, width, and colour were measured each month. These fruit samples were also used for the preparation of peel essential oil and solvent extracts at each harvest.

Determination of fruit maturity

The degree of maturity of Corsican citron was evaluated using the method described by Stenzel *et al.* (*20*) and based on the calculation of growing degree-days (GDD). It should be noted that this procedure provides results independent of interannual variations of temperature, but dependent on the species or varieties and environmental factors. The flowers of *C. medica* var. *Corsican* were scored at the date of anthesis (flowers blooming on April 15, 2010). From this date until the final harvest of the fruit (January 4, 2011), the air temperature was measured daily by recorders placed on the canopy of three trees (facing east).

Isolation of essential oil

The peel of *C. medica* L. var. *Corsican* was water distilled (5 h) using a Clevenger-type apparatus (Midisciences, Fuveau, France) according to the method recommended in the European Pharmacopoeia and the essential oil yields were 0.40–0.72 %. Each distillation was performed in triplicate.

Preparation of solvent extracts

A mass of 100 grams of fresh peel was lyophilised and extracted at room temperature with ethanol (1 L). The solution was filtered on a Büchner funnel and extracted with ethyl acetate (3×100 mL). The solution was evaporated, recovered in methanol (50 mL) and filtered through a 0.45-μm membrane (Phenomenex, Le Pecq, France). Each polyphenol extraction was performed in triplicate.

Essential oil analysis

Gas chromatographic analysis was carried out using a Perkin-Elmer (Waltham, MA, USA) Autosystem XL apparatus equipped with dual flame ionization detection (FID) systems and two fused silica capillary columns (60 m×0.22 mm i.d.; film thickness 0.25 μm) coated with Rtx-1 (polydimethylsiloxane) and Rtx-Wax (polyethylene glycol). The oven temperature was programmed from 60 to 230 °C at 2 °C/min and then held isothermally at 230 °C for 35 min. The injector and detector temperatures were maintained at 280 °C. Samples were injected in the split mode (1/50) using helium as carrier gas (1 mL/min); injection volume of pure oil was 0.2 μL. For headspace sampling by solid-phase microextraction and gas chromatography (HS-SPME-GC) analysis, only the Rtx-1 column was used and volatile components were desorbed in a GC injector with an SPME Intel liner (0.75 mm i.d.; Supelco, Bellefonte, PA, USA). Samples were also analyzed with a Perkin-Elmer Turbo mass detector (quadrupole), coupled to a Perkin-Elmer Autosystem XL, equipped with fused silica capillary columns Rtx-1 and Rtx-Wax. Ion source temperature was 150 °C, energy ionization was 70 eV, electron impact mass spectra were acquired over the mass range of 35–350 Da (scan time: 1 s). Other GC conditions were the same as described above except for the use of a split ratio of 1/80. For HS-SPME-GC-mass spectrometry (MS) analysis, only the Rtx-1 column was used and volatile components were desorbed in a GC injector with an SPME Intel liner (0.75 mm i.d.; Supelco). The identification of individual volatiles was based on comparison of calculated retention indices (apolar and polar columns) and mass spectra with those of our own library of authentic compounds or literature data (I_l) (*21*). Retention indices (I_a and I_p, respectively) of the compounds were determined relative to the reten-

tion times of series of *n*-alkanes (C_5–C_{30}) with linear interpolation, using the Van den Dool and Kratz equation (*22*) and software from Perkin-Elmer. The relative concentrations of components were calculated based on the GC peak areas (apolar column Rtx-1) without using correction factors.

Analysis of solvent extract

Solvents and reagents used for sample preparation and chromatography were LC-MS grade acetonitrile (ACN) and ammonium acetate (NH_4OAc, LC-MS grade) obtained from Fisher Scientific (Illkirch, France). Deionized water was purified using a MilliQ water (Millipore, Bedford, MA, USA) purification system. Reference flavonoids (99 % purity determined by high-performance liquid chromatography (HPLC) were purchased from Extrasynthese (Geney, France). Solutions of reference compounds were prepared by dissolving the compounds in ACN at 1 mg/mL and then filtered through a 0.2-μm polytetrafluoroethylene (PTFE) filter. Direct-infusion MS analyses were performed with reference solutions at a concentration of 0.1 mg/mL in volume ratio of ACN/H_2O=5:5 and 0.1 % NH_4OH. Prior to LC-MS/MS analysis, the mixture was diluted in the initial mobile phase to obtain the desired concentrations.

The LC system consisted of a Flexar ultra-high-performance liquid chromatography (UHPLC; Perkin-Elmer) with two Flexar FX-10 UHPLC pumps, a Flexar solvent manager, a 275-Flexar autosampler, and a Flexar LC PE200 column oven. UHPLC analyses were performed on a 100 mm×2.1 mm i.d., 3 μm, LUNA 3U C18 column (Phenomenex) and the column temperature was set at 30 °C. A volume of 10 μL of sample was injected using an injection loop of 15 μL in full loop mode. The mobile phase consisted of MilliQ water (solvent A) and ACN (solvent B) with 0.1 % (by volume) NH_4AcO acetate buffer at a flow rate of 500 μL/min. The column was equilibrated in 90 % A and 10 % B for 5 min, and elution was carried out with the following linear gradient from 90 to 10 % A in 2 min, an increase from 10 % A to 100 % in 14 min, and 100 % A for 6 min.

Mass spectra were acquired using an AB Sciex (Toronto, Canada) 3200 QTRAP linear triple quadrupole fitted with an electrospray ionization (ESI) source operating in positive or negative mode. High purity nitrogen was used as both nebulizer and turbo gas. The ESI source was operated with following settings in positive mode: curtain gas (CUR) 25 psi, nebulizer gas (GS1) 31 psi, heater gas (GS2) 65 psi, ion spray voltage (IS) 5000 V and temperature 550 °C. The following settings were used in negative mode: CUR 25 psi, GS1 41 psi, GS2 65 psi, IS –4200 V and temperature 550 °C. For enhanced product ion (EPI) experiments, the MS parameters were set as follows in positive ion mode: declustering potential (DP) 50 V, entrance potential (EP) 10 V, collision energy (CE) –35 V, and collision energy spread (CES) ±15 V, and in negative mode: DP –80 V, EP –10 V, CE –35 V, and CES ±15 V. The software used for data acquisition and data analysis was Analyst v. 1.5.1 (AB Sciex). For each reference compound, a relevant transition of the pseudomolecular ion was selected and the mass parameters were optimized in direct infusion (flow rate: 10 μL/min) using the automated component optimization function of the Analyst software. Multiple EPI spectra were recorded in our MS spectral library for each compound. Data acquisition was performed in the multiple reaction monitoring (MRM) mode, followed by an EPI scan (MRM-EPI). EPI mass spectra were recorded in the range of m/z=50–1000 at 4000 Da/s. Compound identification was allowed by comparison of retention time, MRM transition and the EPI mass spectrum of reference compounds. An external standard method was used for quantification of flavonoid compounds. The quantification was performed using a calibration curve obtained by serial diluted reference solution (six levels of concentrations with three injections per level) of each compound in our chromatographic conditions.

Identification of flavonoid components

The optimized MS/MS parameters (declustering potential, entrance potential, collision cell entrance potential, collission energy, collision cell, exit potential) and MRM transitions for each flavonoid component of *C. medica* var. *Corsican* extract were described in Table 1. According to the data previously reported in literature (*23–26*), the fragmentation mechanism of molecular ions to fragment ions used for MRM transitions was indicated in Table 1.

Analysis of commercial liqueur

Commercial citron liqueur prepared with *C. medica* var. *Corsican* peel (fruits harvested in November 2010) was provided by the Mavela distillery (Aleria, Corsica, France). These commercial liqueurs (10 mL of sample in a 20-mL vial) were subjected directly to HS-SPME. The SPME device (Supelco) coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (fibre 2 cm, 30 μm thickness) was used for the extraction of volatile compounds from the liqueur. The extraction required the optimization of HS-SPME parameters (temperature and extraction times), based on the sum of total peak areas. The equilibrium time was 120 min. The temperature and time of extraction were selected after three different experiments at 20, 50 and 70 °C, respectively, for each different extraction time (15, 30, 60 and 120 min). The maximum sum of total peak areas was obtained at 20 °C for 30 min for liqueurs. After sampling, the SPME fibre was inserted into the GC and GC-MS injection ports for desorption of volatile components (5 min at 250 °C), both using the splitless injection mode. Before sampling, each fibre was reconditioned for 5 min in the GC injection port at 260 °C. HS-SPME and subsequent analyses were performed in triplicate. Volatile and flavonoid analyses of commercial citron liqueurs were performed using the same experimental parameters as those described for essential oil and solvent extractions, respectively.

Results and Discussion

Fruit maturity according to growing degree-days

Table 2 shows the number of days per month for which the formulae A, B and C described by Stenzel *et al.* (*20*) were applied, the GDD for each month (April to De-

Table 1. MS/MS detection parameters of 13 flavonoid components of *Citrus medica* var. *Corsican*

Elution order	Compound	Type	t / min	Transition/(m/z)*		Optimized MS parameters				
				Q1 mass / Da	Q3 mass / Da	DP / V	EP / V	CEP / V	CE / V	CXP / V
1	Flavanomarein	monoglycosylated flavanone	6.7	449.1	287.1	–70	–10	–28	–50	–3
2	Hyperoside	monoglycosylated flavonol	8.1	463.0	300.1	–105	–10	–18	–28	–4
3	Narirutin	diglycosylated flavanone	8.5	579.1	270.4	–80	–8.5	–32	–30	–4
4	Diosmin	diglycosylated flavone	7.5	607.1	299.1	–80	–10	–34	–35	–3
5	Neohesperidin	diglycosylated flavanone	7.5	609.1	301.1	–80	–10	–34	–35	–3
6	Rutin	diglycosylated flavonol	7.6	609.1	300.1	–80	–10	–34	–35	–3
7	Retusin	polymethoxylated flavonol	15.5	357.1	299.3	–45	–9	–24	–20	–10
8	Robinetin trimethylether	polymethoxylated flavonol	7.7	343.1	313.1	–70	–10	–24	–40	–3
9	Luteolin	flavone	9.9	287.0	153.1	20	10	17	35	6
10	Sinensetin	polymethoxylated flavonone	9.7	373.1	343.3	71	4.5	30	27	6
11	Casticin	polymethoxylated flavonol	10.1	375.1	359.1	50	10	20	35	3
12	Nobiletin	polymethoxylated flavone	13.4	403.1	373.2	56	4.5	16	27	6
13	Tangeretin	polymethoxylated flavone	14.3	373.1	343.3	81	8.5	28	25	14

DP=declustering potential, EP=entrance potential, CEP=collision cell entrance potential, CE=collission energy, CXP=collision cell exit potential

*MRM=Q1 [molecular ions] and Q3 [fragment ions]
Flavanomarein: Q1 $[M–H]^-$ / Q3 $[Yo]^- = [M–C_6H_{11}O_5]^-$
Hyperoside: Q1 $[M–H]^-$ / Q3 $[Yo–H]^- = [M–C_6H_{12}O_5]^-$
Narirutin: Q1 $[M–H]^-$ / Q3 $[Yo–H]^- = [M–C_{12}H_{22}O_9]^-$
Diosmin: Q1 $[M–H]–$ / Q3 $[Yo]^- = [M–C_{12}H_{21}O_9]^-$
Neohesperidin: Q1 $[M–H]–$ / Q3 $[Yo]^- = [M–C_{12}H_{21}O_9]^-$
Rutin: Q1 $[M–H]–$ / Q3 $[Yo–H]^- = [M–C_{12}H_{22}O_9]^-$
Retusin: Q1 $[M–H]^-$ / Q3 $[M–H–2CH_3–CO]^-$
Robinetin trimethylether: Q1 $[M–H]^-$ / Q3 $[M–H–2CH_3]^-$
Luteolin: Q1 $[M+H]^+$ / Q3 $[^{1,3}A]^+ = [M+H–C_8H_6O_2]^+$
Sinensetin: Q1 $[M+H]^+$ / Q3 $[M+H–2CH_3]^+$
Casticin: Q1 $[M+H]^+$ / Q3 $[M–CH_3]^+$
Nobiletin: Q1 $[M+H]^+$ / Q3 $[M+H–2CH_3]^+$
Tangeretin: Q1 $[M+H]^+$ / Q3 $[M+H–2CH_3]^+$

cember), and the total GDD from the date of anthesis (April 15) to the harvest (July to January). The monthly GDD increased between April (68.2) and August (434.1), then decreased in the following months to reach 31.6 in December. The total GDD until the harvest date increased from June (705.0) until September (1927.7) and then took an asymptotic value (2105.5–2204.9) in the following months (October–December). Fruit maturity was obtained at a value of total GDD in the order of 2000. Stenzel *et al.* (*20*) showed that orange (*C. aurantium*) from Brazil requires 2500 to 3600 of total GDD to reach full maturity. This level of GDD does not seem to be necessary to obtain the maturity of the Corsican citron variety. This could be explained by the difference of climatic factors between Corsica (Mediterranean area) and Brazil (tropical area) or by the difference of metabolism between species (*20*).

The morphological characteristics (mass, length, and width) were measured at each harvest (Table 2), and they increased from July to October, after which they remained stable from October to January. Immature fruits had a dark green colour (July–August), which changed to green–yellow (September–November) and to yellow (November––December) of mature fruits. After maturity, citron fruits take a yellow–orange colour. Corsican citron fruit is traditionally collected in November. The mature fruit (November) has a mass of 1100–1300 g, length of 12–13 cm, and width of 12.5–13.5 cm.

Analysis of volatile components

The essential oil composition of the peel of *C. medica* var. *Corsican* according to the development stage of fruit was studied monthly from July to January (Table 3). Thirty compounds were identified in the peel essential oil (97.6–99.5 % of the total composition). Twelve monoterpene hydrocarbons, twelve oxygenated monoterpenes, four sesquiterpene hydrocarbons, and two linear oxygenated components were reported. Essential oil was dominated by monoterpene hydrocarbons (66.8–82.5 %). Limonene was the major compound (54.2–60.6 %) followed by

Table 2. Growing degree-days (GDD) and phenotypic characteristics of *C. medica* var. *Corsican* according to fruit development

	April	May	June	July	August	September	October	November	December	January
Formula A*	–	15	25	17	27	24	14	–	–	–
Formula B*	15	14	–	–	–	–	17	30	31	–
Formula C*	–	2	5	14	4	6	–	–	–	–
Monthly GDD	68.2	300.1	336.7	444.9	434.1	343.7	177.8	67.8	31.6	–
Total GDD	–	–	**705.0**	**1149.9**	**1584.0**	**1927.7**	**2105.5**	**2173.3**	**2204.9**	–
Mass/g	–	–	–	119.1±33.5	349.2±62.7	821.4±201.1	1218.6±187.1	1122.6±272.2	1203.0±146.3	1289.1±281.7
Lenght/cm	–	–	–	6.9±0.8	9.3±1.0	12.0±1.8	12.4±1.5	13.0±1.0	12.4±1.3	12.2±1.1
Width/cm	–	–	–	6.0±0.6	9.1±0.7	11.7±1.4	13.5±1.1	12.5±1.3	13.3±0.5	13.3±1.2
Colour	–	–	–	dark green	dark green	green-yellow	green-yellow	pale yellow	yellow	yellow-orange

*according to Stenzel *et al.* (*21*)

γ-terpinene (6.7–15.2 %). The oxygenated monoterpenes nerol, neral, geraniol and geranial were present in relatively high concentrations (2.9–8.2, 2.1–6.6, 1.8–5.9, and 2.1–5.7 %, respectively). These results are in accordance with those of Lota *et al.* (*13*) and Venturini *et al.* (*15*). Indeed, these authors have reported a chemotype limonene/γ-terpinene of the Corsican citron variety. This combination had previously been observed in various *Citrus* species including limes (*C. latifolia* and *C. aurantifolia*), tangerine (*C. reticulata* × *C. sinensis*), mandarin (*C. reticulata Blanco*) and lemon (*C. limon*) (*27–29*).

During fruit development, the yield and the chromatographic profile of essential oil exhibited significant differences between immature fruits (July) and later harvested fruits (August to January). Fruit samples from July had an oil yield of 0.4 %, while the other samples exhibited yields near 0.7 %. The peel essential oil of *C. medica* var. *Corsican* showed a relatively stable chemical composition (Table 3) from August to January with a chromatographic profile dominated by limonene (57.8–54.6 %) and γ-terpinene (11.5–15.2 %). The content of the major component, limonene, can be considered stable (54.2 % in July, 54.6 % in January). A significant difference between immature and mature fruits was only observed in the content of γ-terpinene. Relative percentages of γ-terpinene increased between July and the following months, whereas the content of nerol, neral, geraniol and geranial decreased in the same period. However, the decrease of nerol (5.9 % in July to 4.3 % in November) and neral (from 5.7 % in July to 4.7 % in January) is not related to fruit development (Table 3).

The HS-SPME volatile fraction of the commercial liqueur had seventeen components. All these compounds were also reported in Corsican citron essential oil. From a quantitative point of view, the volatile composition of citron peel oil and commercial liqueur showed several similarities, except for the essential oil from immature fruits (July). For instance, the major components were always limonene (60.5 %) and γ-terpinene (21.8 %). However, it was not possible to establish direct correlation between the chemical composition of the peel essential oil and that of the volatile fraction of liqueur obtained by HS-SPME. The two extraction processes (hydrodistillation *vs*. headspace) and HS-SPME parameters (particularly the temperature) could be responsible for the difference in the contents of oxygenated compounds.

Analysis of flavonoid compounds

As far as we know, the flavonoid composition of *C. medica* var. *Corsican* species has not been reported previously. Thirteen compounds were identified including three glycosylated flavanones, two glycosylated flavonols, one glycosylated flavone, one aglycone flavone, three polymethoxylated flavonols and three polymethoxylated flavones (Table 4). The main chemical classes determined on dry mass basis were the glycosylated flavonols (10.0–14.3 mg/g) followed by glycosylated flavanones (1.1–10.2 mg/g) and flavones (0.5–9.9 mg/g).

The main components were identified as rutin, diosmin, and neohesperidin. Mass fractions of flavonoids changed differently with fruit maturity. The rutin mass fraction was stable from July to January between 8.0 and 11.2 mg/g. The mass fraction of diosmin decreased gradually from July to January (9.9 to 0.5 mg/g, respectively). Conversely, the mass fraction of neohesperidin increased during fruit development from 0 (July) to 8.7 mg/g (January). The total concentration of flavonoids decreased from July to September (total GDD <2000) changing from 26.5 to 13.8 mg/g and then increased in the following months (October–January, total GDD >2000), reaching values between 18.6 and 25.3 mg/g. From this result, it can be seen that the content of polyphenolic compounds varied during fruit development. Secondary metabolites may play a role in plant protection against photooxidative stress, in mediating thermotolerance and in direct defense against microbes and insects (*30–32*).

All polyphenolic compounds detected in the peel extract were also reported in the commercial liqueur. The major flavonoid compounds of this product were rutin (16.8 mg/g) and neohesperidin (4.5 mg/g). Rutin and hesperidin were also reported as major compounds of solvent extract from *C. medica* var. *Etrog* (*18*). To the best of our knowledge, *C. medica* is the only *Citrus* species containing these as main components. Two main types of glycosylated flavanones, neohesperidosides and rutinosides, had previously been reported in the peel compositions of other *Citrus* species (*33*). These two forms were identified in *C. medica* extract with neohesperidin and rutin as major components. However, the flavonoid composition of Corsican citron is quite different from that of other *Citrus* species. Bergamot, grapefruit, mandarin, lemon, and orange

Table 3. Volatile composition of the essential oil and commercial liqueur from *C. medica* var. *Corsican* peel

Elution order	Compound	I_l	I_a	I_p	Y(essential oil)/%							φ(component in liqueur)*
					0.40	0.62	0.70	0.67	0.65	0.72	0.69	
					φ(component in essential oil)/%							%
					July	August	September	October	November	December	January	
1	α-Thujene	932	922	1023	0.1±0.1	0.2±0.1	0.3±0.0	0.3±0.0	0.3±0.1	0.4±0.1	0.4±0.1	1.2±0.3
2	α-Pinene	936	931	1022	0.4±0.2	0.7±0.1	0.9±0.1	1.0±0.1	0.9±0.4	1.1±0.3	1.0±0.3	3.6±0.5
3	6-Methylhept-5-en-2-one	963	963	1337	0.1±0.0	0.1±0.0	0.1±0.0	tr	tr	tr	0.2±0.1	–
4	Sabinene	964	969	1111	0.1±0.1	0.1±0.1	0.2±0.0	0.2±0.0	0.2±0.1	0.2±0.1	0.2±0.1	0.3± 0.2
5	β-Pinene	978	970	1110	0.4±0.2	0.7±0.1	0.8±0.1	0.9±0.0	0.9±0.3	1.0±0.2	1.0±0.2	2.8±0.3
6	Myrcene	987	979	1159	1.1±0.3	1.4±0.2	1.6±0.1	1.6±0.1	1.4±0.3	1.5±0.2	1.3±0.1	2.4±0.2
7	α-Terpinene	1013	1008	1178	0.1±0.1	0.2±0.0	0.3±0.0	0.3±0.0	0.3±0.1	0.2±0.1	0.3±0.1	–
8	*p*-Cymene	1015	1010	1169	2.4±1.1	1.4±0.7	0.5±0.1	0.7±0.4	0.6±0.3	2.0±0.6	1.7±0.5	1.3±0.2
9	Limonene	1025	1020	1199	54.2±4.0	57.8±1.7	60.3±1.0	60.6±1.2	56.1±1.9	57.9±3.0	54.6±1.1	60.5±1.3
10	(*Z*)-β-Ocimene	1029	1026	1230	0.2±0.1	0.3±0.1	0.2±0.0	0.2±0.0	0.1±0.1	0.2±0.1	0.2±0.0	–
11	(*E*)-β-Ocimene	1041	1043	1251	0.7±0.1	1.2±0.2	1.2±0.1	1.6±0.2	1.4±0.3	1.8±0.3	2.0±0.2	3.0±0.4
12	γ-Terpinene	1051	1047	1243	6.7±2.6	11.5±0.2	12.5±0.4	14.4±0.8	14.2±2.4	13.6±1.7	15.2±1.4	21.8±1.0
13	Terpinolene	1082	1078	1280	0.4±0.1	0.6±0.1	0.6±0.0	0.6±0.1	0.7±0.1	0.6±0.1	0.7±0.1	1.0±0.3
14	Nonanal	1084	1083	1388	0.1±0.1	tr	tr	tr	tr	tr	tr	–
15	Linalool	1086	1085	1544	0.6±0.1	0.5±0.1	0.4±0.1	0.3±0.1	0.5±0.1	0.4±0.2	0.5±0.2	–
16	Citronellal	1129	1132	1479	0.1±0.1	0.1±0.0	0.2±0.1	0.1±0.0	0.1±0.1	0.2±0.1	0.5±0.1	–
17	Isoneral	1140	1142	1556	0.5±0.1	0.3±0.1	0.4±0.1	0.2±0.0	0.3±0.2	0.1±0.0	0.1±0.1	–
18	Isogeranial	1156	1159	1748	0.8±0.2	0.5±0.1	0.7±0.1	0.4±0.1	0.6±0.3	0.2±0.0	0.3±0.1	–
19	Terpinen-4-ol	1164	1161	1600	0.4±0.1	0.3±0.1	0.2±0.0	0.2±0.1	0.3±0.1	0.2±0.1	0.2±0.1	–
20	α-Terpineol	1176	1179	1700	0.9±0.2	0.6±0.1	0.6±0.0	0.5±0.1	0.6±0.3	0.4±0.2	0.7±0.3	–
21	Nerol	1210	1212	1799	5.9±0.7	4.8±0.3	1.8±0.1	3.9±1.0	4.3±1.0	2.5±1.9	2.6±0.9	–
22	Neral	1215	1214	1679	5.7±1.5	3.8±0.5	4.5±0.4	2.1±0.1	3.3±1.5	3.6±0.8	4.7±0.6	0.1±0.1
23	Geraniol	1235	1233	1843	6.6±1.3	5.8±0.4	2.1±0.2	5.1±0.9	5.4±0.5	2.7±1.9	2.3±0.6	–
24	Geranial	1244	1245	1710	8.2±2.3	5.1±0.7	6.6±0.6	2.9±0.2	4.2±1.3	4.9±1.0	6.6±1.1	0.1±0.1
25	Neryl acetate	1342	1343	1725	0.3±0.2	0.2±0.1	0.5±0.2	0.3±0.1	0.5±0.2	0.8±0.2	0.6±0.3	–
26	Geranyl acetate	1362	1357	1752	0.4±0.2	0.3±0.1	0.3±0.2	0.2±0.1	0.4±0.1	0.4±0.1	0.5±0.2	tr
27	(*E*)-β-Caryophyllene	1421	1424	1591	0.2±0.1	0.2±0.0	0.2±0.0	0.1±0.1	0.2±0.1	0.1±0.0	0.1±0.0	tr
28	*trans*-α-Bergamotene	1429	1431	1581	0.1±0.0	0.1±0.0	0.2±0.1	0.2±0.1	0.2±0.0	0.2±0.1	0.2±0.0	0.3±0.1
29	Germacrene D	1479	1475	1704	0.1±0.0	0.2±0.1	0.1±0.1	0.1±0.0	0.1±0.1	0.1±0.0	0.1±0.0	0.3±0.2
30	β-Bisabolene	1498	1502	1720	0.2±0.1	0.2±0.0	0.3±0.1	0.2±0.1	0.3±0.1	0.2±0.1	0.2±0.1	0.2±0.2
	Monoterpene hydrocarbons				66.8±5.7	76.1±1.3	79.5±1.5	82.5±1.9	77.0±4.1	80.5±4.4	78.6±2.1	97.9±3.1
	Oxygenated monoterpenes				30.5±4.1	22.2±2.0	18.4±1.2	16.3±1.8	20.3±5.1	16.5±6.1	19.8±1.3	0.2±0.2
	Sesquiterpene hydrocarbons				0.6±0.2	0.7±0.1	0.8±0.2	0.1±0.2	0.8±0.2	0.6±0.2	0.6±0.1	0.8±0.4
	Non-terpenic components				0.2±0.1	0.1±0.1	0.1±0.0	tr	tr	tr	0.2±0.1	–
	Total				98.0±1.2	99.1±0.9	98.8±0.9	99.5±0.2	98.1±1.2	97.6±1.7	99.3±0.9	98.9±0.9

Order of elution is given on apolar column (Rtx-1)
I_l=Retention indices in the literature on the apolar column (*21*)
I_a=Retention indices on the apolar Rtx-1 column
I_p=Retention indices on the polar Rtx-Wax column
*N=9; mean values of three analyses of each three commercial samples obtained for maximum sum of total peak area±standard deviation
tr=trace (<0.05 %)
–=not detected

Table 4. Flavonoid composition of solvent extract and commercial liqueur on dry mass basis from *C. medica* var. *Corsican* peel

Elution order	Compound	Solvent extract							Liqueur
		July	August	September	October	November	December	January	
		w(flavonoid)/(mg/g)							
1	Flavanomarein	1.4±0.3	1.3±0.3	1.1±0.3	0.9±0.3	1.0±0.2	1.3±0.3	1.3±0.3	1.1±0.3
2	Hyperosid	1.4±0.1	1.4±0.1	2.0±0.2	2.2±0.5	2.2±0.5	3.1±0.4	3.1±0.2	3.2±0.8
3	Narirutin	0.1±0.1	tr	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.1	0.1±0.1	tr
4	Diosmin	9.9±1.2	6.1±1.1	2.1±0.4	1.0±0.3	0.8±0.3	0.7±0.2	0.5±0.2	0.4±0.1
5	Neohesperidin	1.2±0.1	0.1±0.1	tr	5.4±1.0	6.9±0.8	8.7±0.9	8.7±0.6	4.5±0.5
6	Rutin	10.4±0.9	8.6±1.0	8.0±0.5	8.4±0.6	9.0±1.0	10.3±1.1	11.2±1.0	16.8±1.3
7	Retusin	tr	tr	tr	tr	tr	tr	–	tr
8	Robinetin trimethylether	0.4±0.2	0.2±0.0	0.6±0.2	0.4±0.2	0.2±0.1	0.5±0.1	0.3±0.1	0.3±0.2
9	Luteolin	0.1±0.0	0.1±0.1	tr	0.1±0.1	0.1±0.0	0.1±0.1	tr	tr
10	Sinensetin	tr	–	tr	tr	tr	tr	tr	tr
11	Casticine	1.7±0.4	0.3±0.1	0.1±0.1	0.1±0.1	0.1±0.0	0.2±0.1	0.1±0.0	tr
12	Nobiletin	tr	tr	tr	tr	tr	tr	tr	tr
13	Tangeretin	tr	tr	tr	tr	tr	–	–	tr
	Polymethoxylated flavones	0.1±0.1	0.1±0.1	tr	0.1±0.2	0.1±0.1	0.1±0.1	tr	tr
	Flavones	9.9±1.2	6.1±1.1	2.1±0.4	1.0±0.3	0.8±0.3	0.7±0.2	0.5±0.2	0.4±0.2
	Glycosylated flavanones	2.7±0.5	1.4±0.4	1.1±0.3	6.4±1.2	7.9±1.0	10.1±1.3	10.2±0.8	5.7±1.6
	Polymethoxylated flavonols	2.1±0.5	0.5±0.1	0.6±0.2	0.5±0.2	0.3±0.1	0.7±0.1	0.4±0.1	0.3±0.2
	Glycosylated flavonols	11.8±0.9	10.0±1.0	10.0±0.7	10.6±0.9	11.2±1.4	13.4±1.5	14.3±1.2	20.0±2.1
	Total	26.5±4.3	18.0±3.2	13.8±3.2	18.6±3.9	20.3±3.1	25.0±3.9	25.3±3.7	26.4±3.2

Order of elution corresponds to that in Table 1
*N= 9, mean values of three analyses of three commercial samples±standard deviation
tr=trace

juices are dominated by naringin, neohesperidin, and neoeriocitrin as neohesperidoside forms and by hesperidin, narirutin, and didymin as rutinoside forms (*34*).

Conclusions

Volatile fractions of Corsican citron liqueur were dominated by limonene and γ-terpinene in accordance with the essential oil composition of *C. medica* var. *Corsican* peel. High proportions of rutin and neohesperidin characterized the flavonoid fraction. The essential oil and polyphenolic composition of *C. medica* peel showed quantitative differences depending on fruit maturity. These results can be used for the quality assessment of organoleptic properties of food products (beverage, jam, nougat, and candy) derived from *C. medica* var. *Corsican*. Indeed, the commercial liqueur is traditionally made with mature fruits (harvested from September to November); however, the content of terpenic (γ-terpinene) and polyphenolic (diosmin, neohesperidin, casticin) compounds varied substantially during fruit development.

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