Evolution of temporal dynamic of volatile organic compounds (VOCs) and odors of hemp stem during field retting

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Brahim Mazian[1,2] · Stéphane Cariou[1] · Mathilde Chaignaud[3] · Jean-Louis Fanlo[1,3] · Marie-Laure Fauconnier[4] · Anne Bergeret[2] · Luc Malhautier[1]

Abstract

Main conclusion **New non-destructive approach to evaluate the retting process was investigated. Increase of retting duration led to a decrease of VOCs emitted by plants and change of color and plant odor. The variation of VOCs and odor could be used as indicators for the degree of retting.**

Abstract In the hemp industry, retting is an upstream bioprocessing applied to the plants to facilitate the decortication of fibres from the central woody part of the stem. This treatment is currently carried out in an empirical way on the ground which leads to variability in the hemp stems quality, and thus to the hemp fibres quality. Therefore, controlling retting treat-ment is a crucial step for high-performance hemp fibre. In this study, a new approach is used to assess the retting degree by following the evolution of VOCs emitted by plants during different retting durations. Either harvest time or retting induces a change in VOCs released by plants. During plant maturity, volatile compounds emitted decreased with a factor of about 2, in relation to VOCs released at the end of flowering. Regardless of the harvest period, the majority of VOCs and odor concentrations, monitored by olfactometric analysis, decrease gradually until some of them disappear at the end of retting. Likewise, the green plant odor disappears during retting with an increase of dry plants odor and an appearance of fermented odor at the end of retting. Following the evolution of VOCs emitted by plants during retting could be a tool for farmers to improve the retting management.

Keywords Hemp fibres · Field retting · VOCs · Odor · Growth stage

Introduction

The recent consideration of environmental and economic factors in the development of materials pushes manufacturers to develop new materials with less impact on the environment and for which the costs of raw materials and manufacturing are moderate. The use of renewable resources, such as lignocellulosic fibres as reinforcements in polymeric materials forming bio-based composites, provides an answer to these environmental and economic constraints (Joshi et al. 2004). In addition to their availability, these fibres also have advantages directly related to their intrinsic properties such as density (Aziz and Ansell 2004), biodegradability (Stamboulis et al. 2001; Islam et al. 2010), high specific mechanical properties (Bourmaud and Baley 2010; Duval et al. 2011; Liu et al. 2015), and indirectly, the cost of manufacturing bio-based composite materials.

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Plant fibres issued from hemp (*Cannabis sativa L*) are part of these attractive lignocellulosic fibres which could be used in the production of bio-based materials by replacing the conventional fibres (e.g., glass and synthetic fibres). However, any deviation of the quality of these fibres in terms of morphology, physico-chemical properties results in heterogeneous mechanical properties of the fibres. Therefore, this is an important impediment to the use of these fibres in structural application when high consistency and homogeneity are required (Stamboulis et al. 2001).

To extract the hemp fibres form woody core of the stems, the traditional separation and extraction bioprocess called retting is performed in the hemp industry. This treatment is among of the factors that affect the final quality of hemp fibres (Placet et al. 2017; Mazian et al. 2018). In Europe, the field retting (also known as dew-retting) is the most widely used treatment after the prohibition of water-retting that caused water pollution by stem fermentation products. The field retting consists to spread out hemp plants on the ground for a duration ranging from a few days to several weeks. This treatment is currently performed in an empirical way in the field due to its dependence on environmental conditions. Indeed, during field retting, the plants are exposed to stress factors. Biotic stress is caused by living organisms. The microorganisms (fungi and bacteria) attack the plants producing a range of polysaccharide-degrading enzymes, especially pectinolytic which remove the components in the middle lamella region surrounding the fibres (Akin et al. 2007; Pakarinen et al. 2012). Conversely, the abiotic stress is caused by the change in environmental conditions, such as temperature, humidity, salinity, and light. However, the plants have self-defense mechanisms to struggle against these stress and threats provided that its enzymes are functional. Indeed, the plant release odor and volatile organic compounds (VOCs) for plant–plant communication and to reduce biotic and abiotic stress (Takabayashi et al. 1994; Pinto et al. 2007; Monson 2013; Capitani et al. 2009; Copolovici and Niinemets 2010; Filella and Peñuelas 1999; Holopainen and Blande 2012; Kessler 2001; Kessler and Halitschke 2007; Singsaas et al. 1997). The hemp plant (*Cannabis sativa L)* releases many VOCs compounds (Turner et al. 1980; ElSohly and Slade 2005) as terpenes, alcohols, aldehydes. Depending on the plant hemp situation during growth and retting, emitted VOCs would differ. Thus, following these plant characteristics would give some data about the retting-level progress.

Several methods in the literature have been done to evaluate the degree of retting based on biochemical composition, microbial community colonization, stem peeling, and morphology (Djemiel et al. 2017; Liu et al. 2017; Mazian et al. 2018; Réquilé et al. 2018). However, these methods are not rapid and are destructive. Therefore, this paper attempts to use a new non-destructive approach to evaluate the degree of retting treatment based on the odor and VOCs emitted by plants during different retting times for the stems harvested at end of flowering and seed maturity. The VOCs' composition and concentration were identified and measured using gas chromatography–mass spectrometry (GC–MS) analysis and the odor characteristics in term of persistency; acceptability and quality were performed/determined using olfactometric analyses.

Materials and methods

Raw material

Cultivation and sampling

Hemp (*Cannabis sativa L.*, Cultivar 'Santhica 27') was sown at a rate of 30 kg/ha on 10th May, 2017 in the south of France (N 44.130673°, E 4.315895°) by CIVAM Chanvre Gardois (Le Bouquet, France). The hemp plants were harvested manually at the end of flowering (EF) (21th August, 2017), and at the seed maturity (SM) (26th September, 2017) (Fig. 1). The stems' length during EF and SM are 1.56 and 1.58 m, respectively. After each harvest period, the plants were left in the field for retting, and then, a weekly collection of 200 retted stems was performed. Table 1 gives the retting times and corresponding collected samples for each selected growth stage. Plants harvested were turned regularly (once a week) on the ground to homogenize the retting of the stems. To limit the scatter of the results, only the middle part of the stem was considered without presence of residual leaves (Mazian et al. 2018).

Weather conditions

The temperature and the relative humidity were recorded during retting of the stems collected at the end of flowering and the period of seed maturity. This monitoring was performed using hygro-button sensors (Progesplus, Carquefou, France) which are put in contact with the soil. In addition, daily average precipitation data were acquired from Météo France (meteorological station Méjannes-le-Clap, France). Figure 2 highlights the weather conditions during retting of both selected harvest periods.

During retting of the stems harvested at EF, the weather was hot, except for the two last weeks of treatment and maximum temperature can reach 40 °C. As concerns the retting period of the stems harvested at SM, the maximum temperature was less high (up to 30 °C at the beginning of this retting period) that observed during the retting of stems harvested at EF. The rainfall was low during retting for both

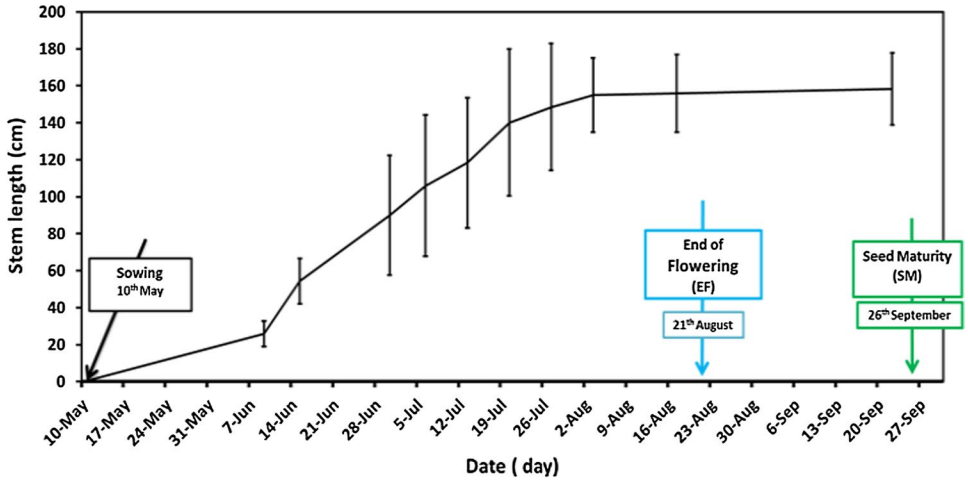

Fig. 1 Hemp plant growth (May 10th, 2017)

Table 1 Name of samples collected during different retting times

Retting time (day)	0	7	14	21	28	35	42	49	63	77
Retting time (week)	0	1	2	3	4	5	6	7	9	11
Samples name	R0EF	R1EF	R2EF	R3EF	R4EF	R5EF	–	R7EF	R9EF	R11EF
	R0SM	R1SM	R2SM	R3SM	R4SM	R5SM	R6SM	–	R9SM	–

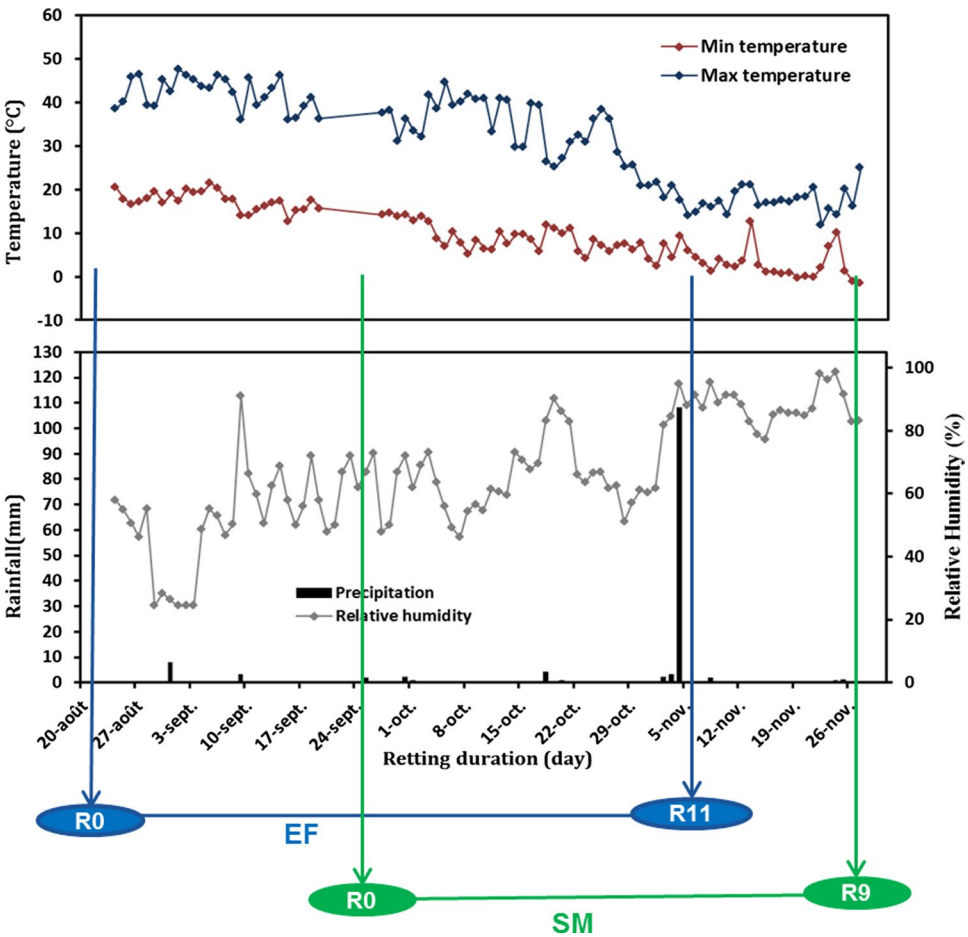

Fig. 2 Daily weather conditions (minimum and maximum temperatures, relative humidity, and precipitation) during retting of stems harvested at EF and SM

harvest periods and the relative humidity is not homogenously distributed during retting periods.

Experimental methods

Color

The color change of the stems collected from different retting durations for each harvest period (end of flowering and seed maturity) was visually evaluated.

TD–GC–MS analyses

Prior to GC–MS analyses, 100 g of stems that were sampled at each retting time were introduced in Nalophan® bags and subsequently the 40L of dry compressed air was used to fill the bags. To reach the VOC equilibrium, the Nalophan® bags were preserved 3 days in clean environment and not exposed to thermal sudden changes before sampling and analyzing air. At the end of the conditioning period, the effluent air from the Nalophan® bags was sampled on a multi-bed sorbent tube containing Carboxen® 569 (100 mg, 20–45 mesh, Supelco) and Tenax® TA (100 mg, 60–80 mesh, Supelco). The air was sampled for 20 min at a 100 NmL/min flow rate with a pump (KNF) coupled with a mass-flow-controller (5850 TR, Brooks). The chromatographic analyses were carried out by a thermodesorption unit (Turbomatrix, Perkin Elmer, USA), coupled with a gas chromatograph (Trace GC, Thermo Scientific, USA) and a mass spectrometer (DSQ2 model, Thermo Scientific, USA). The analytical column was an Optima 5-ms Accent 60 m × 0.25 mm × 1 µm. Helium was used as carrier gas at 1.5 mL/min in constant flow mode. The GC oven temperature program was set as followed: 9 min at 40 °C, a ramp at 15 °C/min until 90 °C, 4 min at 90 °C then a ramp at 10 °C/min until 250 °C, and finally 5 min at 250 °C. The ionization of compounds was made by electronic impact at 70 eV. The full-scan mode was used to analyze fragments from 20 to 250 amu (atomic mass unit). Compounds' identification was led by comparison of our spectra with those referenced in the NIST library. The system was calibrated with toluene (Cariou et al. 2016).

Olfactometric analyses

After each harvesting period, odor concentration of the samples was measured according EN 13725 standard using a dynamic dilution olfactometer ODILE (Odotech Inc., Canada). The tests were carried out inside an odor-free, clean laboratory with selected and 6 trained panelists. Each sample was diluted in the olfactometer several times differing from each other by a factor of 1.58 and presented to the panelist three times. The olfactometer is a computer controlled semi-automatic instrument with six panel member places and computes the odor concentration by means of a special computer program based on the perception response data of panelists. This method employs a "presence or non-presence of odor" technique and determines how many times a sample must be diluted with odor-free air to be at the threshold of detection by 50% of the panel. At this instance, the number of required dilution defines the odor concentration in odor units per cubic meter ($OU_E\ m^{-3}$).

After the determination of odor concentration, the odor acceptability was evaluated using the method developed by Olentica company (Chaignaud et al. 2014): (i) determination of the dilution level for which all members of the panel perceive odor; (ii) evaluation of odor acceptability at this level of dilution, on a closed scale ranging from − 5 to + 5 (− 5 = very unpleasant odor, 0 = neutral odor, 5 = very pleasant odor); (iii) repetition of step 2 in three lower levels of dilution; (iv) calculation of the median acceptability level for each dilution; and (v) plotting of acceptability levels vs dilution levels.

Odor intensity and quality

Both intensity and quality of the odor of the stems sampled at different retting duration were determined using an oriented approach by an expert of Olentica Company (Ales, France).

The odor intensity was determined using the evaluation given by at least 10 jurors of the strength of the odor on a discrete scale from 0 to 5 (Table 2). The results of all the jurors are collected and an average value of the intensity is calculated.

The odor quality was determined after the evaluation of the olfactory profile of each odor. Indeed, at least 10 panelists describe the smell of the stems using their own vocabulary. Each tone perceived by juror is prioritized: 5 points are automatically assigned to the main tone or tones. Secondary tones are rated on a scale of 1–4. After this stage, the olfactory descriptors provided by all the panelists are grouped into odor classes. The odor classes used are chosen according to an odor wheel. Different descriptors of stem odor after

Table 2 Odor intensity assessment

Intensity level	Meaning
0	Odourless/neutral
1	Very low odour
2	Slight odour
3	Medium odour
4	Strong odour
5	Very strong odour

harvest periods and during retting are grouped into these odor classes: dry plants, green plants, pungent, fresh, soft, wooded, fermented, mold, humus, and mushroom.

Results and discussion

Color change

The visual assessment of the color has been performed for the stems harvested at the end of flowering and at the seed maturity and retted at different durations. As can be observed in the photograph (Fig. 3), the color changes with plant development and also during dew-retting. The color of the unretted stems collected at EF was light green, while unretted stems harvested at SM were transformed to yellow. This ranging of color from green to yellow after maturity is presumably due to the degradation of plant pigment due to photodegradation, biodegradation, and microbial colonization. Indeed, the chlorophyll that is responsible of green color was decomposed with the partial retention of carotenoids that emit yellow color (Merzlyak and Gitelson 1995; Matile 2000; Hörtensteiner 2004).

During retting of hemp stems harvested at EF, the color ranges from light green for unretted stems, to yellow for low retted, and to black for highly retted stems. In contrast, for the samples harvested at SM, the color only changes from yellow to a gradual appearance emergence of black spots at retted stems surface. The color change of the stems from yellow to black is attributed to the colonization of microbial communities (Ribeiro et al. 2015; Liu et al. 2017) of the stems surface). Indeed, the appearance of black spots on the stems surface increase is associated with the development of microbial communities (Mazian et al. 2018; Bleuze et al. 2018).

VOCs identification and quantification during plant growth

The evolution of VOCs' composition during growth hemp plants was analyzed. Table 3 gives the list of identified chromatographic peaks and their concentrations. In both sampling periods (EF and SM), the odorous emissions from the stems collected at different plant developments contained several compounds. 75 and 69 VOCs were identified from the stems harvested at the end of flowering and seed maturity, respectively. The measured VOCs were ordered as chemical families such as terpenes (e.g., α-pinene, β-pinene), alcohols (e.g., methanol, hexanol), aldehydes (e.g., acetaldehyde, hexanal), monoaromatics (e.g., benzene, toluene), ketones (e.g., 2-butanone, acetone), esters (e.g., methylacetate), ethers (e.g., 2-ethylfuran), alkanes (e.g., pentane, tetradecane), acids (e.g., isobutyric acid), and alkenes (e.g., 2, 4-dimethylheptene). Many of these compounds were already detected from the emission pattern of the *Cannabis sativa L* plant species (Turner et al. 1980; ElSohly and Slade 2005), and also in many different plant tissues and physiological processes (Kesselmeier and Staudt 1999). Plants are known to release airborne VOCs to attract pollinators and seed dispersers and to prevent herbivores' and pathogens' attacks. In a way, they act as a kind of language for plants (Cookson 1995). For example, it is well known that terpenes act as defense compounds against pathogens and herbivores (Gershenzon and Croteau 1991; Oikawa and Lerdau 2013;

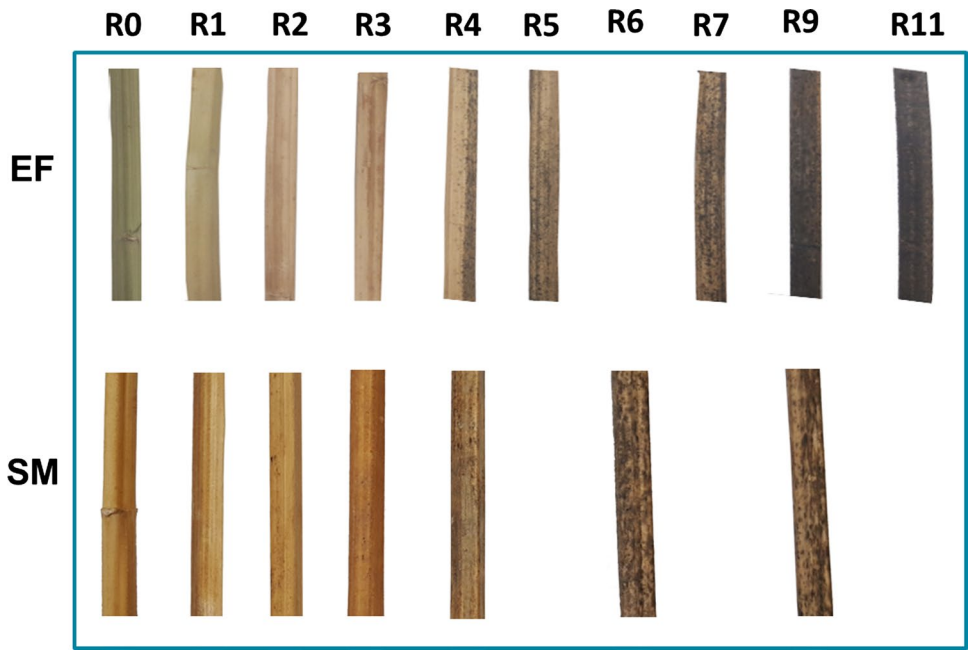

Fig. 3 Photograph of color changes of the stems during retting of hemp harvested at different periods (EF and SM)

Table 3 List of identified compounds and their concentrations (µg toluene equivalent m^{-3})

Cas N°	Name	Family	R0EF	R0SM
99-86-5	α-Terpinene	Terpenes	16	N.B.
2867-05-2	α-Thujene	Terpenes	13	0,4
80-56-8	α-Pinene	Terpenes	8445	4924
471-84-1	α-Fenchene	Terpenes	34	22
79-92-5	Camphene	Terpenes	349	169
123-35-3	β-Myrcene	Terpenes	N.B.	116
127-91-3	β-Pinene	Terpenes	1626	874
6874-10-8	*cis*-o-cimene	Terpenes	79	15
138-86-3	Limonene	Terpenes	1303	183
99-85-4	γ-Terpinene	Terpenes	62	11
13474-59-4	α-Bergamotene	Terpenes	47	28
87-44-5	β-caryophyllene	Terpenes	155	580
6753-98-6	α-Humulene	Terpenes	N.B.	117
13466-78-9	delta,3-carene	Terpenes	N.B.	565
555-10-2	β_Phellandrene	Terpenes	N.B.	5
473-13-2	α-selinene	Terpenes	N.B.	15
489-39-4	(+)-Aromadendrene	Terpenes	197	64
75-07-0	Acetaldehyde	Aldehydes	1367	766
107-02-8	2-Propenal (acrolein)	Aldehydes	218	5
123-38-6	Propanal	Aldehydes	39	27
78-84-2	2-Methylpropanal	Aldehydes	248	74
78-85-3	2-Methyl-2-propenal	Aldehydes	6	5
123-72-8	Butanal	Aldehydes	6	3
15798-64-8	(z)-2-Butenal	Aldehydes	N.B.	5
590-86-3	3-Methylbutanal	Aldehydes	81	38
96-17-3	2-Methylbutanal	Aldehydes	92	35
110-62-3	Pentanal	Aldehydes	139	112
66-25-1	Hexanal	Aldehydes	248	113
6728-26-3	2-Hexenal (e)	Aldehydes	24	11
111-71-7	Heptanal	Aldehydes	8	N.B.
124-19-6	Nonanal	Aldehydes	88	N.B.
26882-03-1	α-Campholene aldehyde	Aldehydes	68	11
23727-16-4	Myrtenal	Aldehydes	11	N.B.
67-56-1	Methanol	Alcohols	342	964
64-17-5	Ethanol	Alcohols	2632	492
67-63-0	Isopropanol	Alcohols	26	10
71-23-8	1-Propanol	Alcohols	4	N.B.
78-92-2	2-Butanol	Alcohols	5	12
78-83-1	2-Methylpropanol	Alcohols	10	N.B.
71-36-3	1-Butanol	Alcohols	3	3
616-25-1	1-Pentene-3-ol	Alcohols	8	4
123-51-3	3-Methyl-1-butanol	Alcohols	26	18
137-32-6	2-Methyl-1-butanol	Alcohols	8	3
71-41-0	1-Pentanol	Alcohols	15	3
111-27-3	Hexanol	Alcohols	50	4
111-70-6	1-Heptanol	Alcohols	N.B.	2
15826-82-1	*cis*-4-thuyanol	Alcohols	75	N.B.
17699-16-0	*trans*-4-thuyanol	Alcohols	13	N.B.
22771-44-4	*cis*-p-mentha-2,8-dien-1-ol	Alcohols	12	1
4948-29-2	*cis*-2-pinanol	Alcohols	12	N.B.
547-61-5	Pinocarveol	Alcohols	256	126
562-74-3	Terpinène-4-ol	Alcohols	23	2
78-70-6	Linalool	Alcohols	N.B.	5
515-00-4	(−)-Myrtenol	Alcohols	28	N.B.
67-64-1	Acetone	Ketones	218	148
431-03-8	2,3-Butanedione (diacetyl)	Ketones	95	22
600-14-6	2,3-Pentadione	Ketones	2	1
96-22-0	3-Pentanone	Ketones	3	N.B.
513-86-0	3-Hydroxy-2-butanone	Ketones	96	1
110-43-0	2-Heptanone	Ketones	8	N.B.
110-93-0	6-Methyl-5-hepten-2-one	Ketones	21	3
19890-00-7	*cis*-Pinocarveol	Ketones	92	43
1120-21-4	Undecane	Alkanes	N.B.	2
109-66-0	Pentane	Alkanes	8	13
111-65-9	Octane	Alkanes	N.B.	1
111-84-2	Nonane	Alkanes	7	N.B.
508-32-7	Tricyclene	Alkanes	36	N.B.
124-18-5	Decane	Alkanes	8	11
112-40-3	Dodecane	Alkanes	N.B.	34
629-50-5	Tridecane	Alkanes	14	1
629-59-4	Tetradecane	Alkanes	25	49
930-27-8	3-Methylfuran	Ethers	2	N.B.
534-22-5	2-Methylfuran	Ethers	15	22
497-26-7	2-Methyl-1,3-dioxolane	Ethers	166	N.B.
3208-16-0	2-ethylfuran	Ethers	5	3
470-82-6	Eucalyptol	Ethers	886	168
1139-30-6	β-Caryophyllene oxide	Ethers	26	29
463-58-1	Carbonyl sulfide	Sulfurs	3	N.D.
75-18-3	Dimethylsulfide	Sulfurs	26	48
75-15-0	Carbonedisulfide	Sulfurs	4	N.D.
79-20-9	Methyl acetate	Esters	9	N.B.
141-78-6	Ethyl acetate	Esters	142	1
123-86-4	Butyl acetate	Esters	N.B.	2
19549-87-2	2,4-Dimethylheptene	Alkenes	5	N.B.
64-19-7	Isobutyric acid	Acids	N.B.	136
71-43-2	Benzene	Monoaromatics	N.B.	6
108-88-3	Toluene	Monoaromatics	15	8
535-77-3	m-Cymene	Monoaromatics	N.B.	62

N.B. not identified

Wink 1988), and as well protect plant membrane against high temperatures or the alteration of flowering in nearby plants (Kesselmeier and Staudt 1999; Kessler 2001; Shiojiri et al. 2006; Maffei 2010). Conversely, some of VOCs may act also as a carbon source for microorganisms. Indeed, the microbial community is able to use VOCs as a carbon or energy source (Amaral and Knowles 1997; Paavolainen et al. 1998; Mackie and Wheatley 1999; Owen et al. 2007).

As it can be observed in Fig. 4, the most abundant chemical family in the stems harvested at the end of flowering was terpenes with 60% of total VOCs concentrations, followed by alcohols (17%), aldehydes (13%), ethers (5%), ketones (3%), esters, and alkanes (1%), and less than 1% for other compounds (monoaromatics, alkenes, and acids). Identical compounds were detected in the stems harvested at seed maturity, with similar ordering. Terpenes had also the highest concentration in the stems harvested at seed maturity with 38% of total VOCs' concentrations, and they are followed by alcohols (8%), aldehydes (6%), and 1% for other compounds (ethers, ketones, alkenes, acids, and alkanes). With plant maturity, the sum of concentrations of the detected compounds decreased with a factor of about 2, from 20638 $\mu g_{toluene\ eq}/m^3$ for the unretted stems harvested at the end of flowering (R0EF) to 11368 $\mu g_{toluene\ eq}/m^3$ for the unretted stems harvested at the seed maturity (R0SM). This difference could be linked to the physiological state of plant (annual plant) and biofilm growth on the surface stem. However, only the concentration of some compounds increased significantly with plant maturity, such as β-caryophyllene, α-humulene, δ,3-carene, and methanol. Indeed, it is not surprising, as these compounds are well known to induce a direct resistance to bacterial growth (bacteriostatic effect) and also could prevent pathogen attacks (Huang and Zimmerli 2014). Concerning methanol, the increase of its concentration during plant growth is presumably due to the demethylation of pectins in the primary cell wall during plant growth. The pectin-methylesterase-catalyzed reaction in plant cell wall is a source of methanol production in plants (Fall and Benson 1996; Galbally and Kirstine 2002; Pelloux et al. 2007; Schmidt et al. 2015). Massiot et al. (1997) found that pectin methylerase treatment of apple pectin induced the emission of 65% of the total methanol content of the pectin. Komarova et al. (2014) have reported that the increase of methanol emission from pectin methylerase delayed the growth of the bacterial pathogen *ralstonia solanacearum* in neighboring receiver plants. Indeed, methanol acts most probably as a signal notably in plant-to-plant communication.

The influence of the growth stage on VOCs' emission by the plant has been already reported (Kesselmeier and Staudt 1999; Ohta 1986). Biogenic VOCs are emitted by various organs (seeds, flowers, leaves, stems, and roots) (Gfeller et al. 2013). Staudt et al. (1997) have shown that VOCs emissions of umbrella pine (Pinus pinea) change by a factor of 20 during plant development. Therefore, these results indicate clearly that the physico-chemical composition of hemp plants changes during plant development (from end of flowering to seed maturity) which is a result of complex interactions between the organism and its environment. This is not surprising, since the stem color changes, as well, ranging from green to yellow during plant growth.

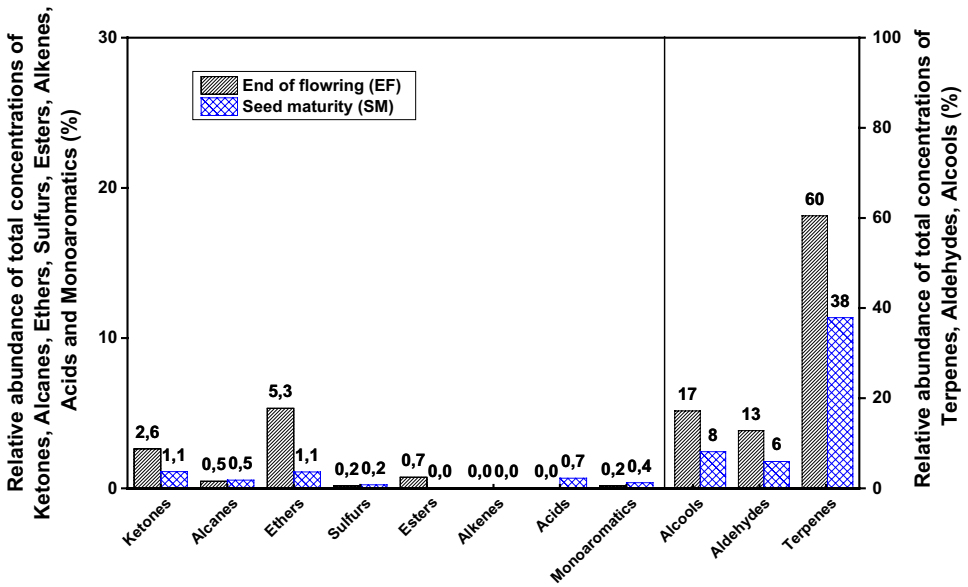

Fig. 4 Most abundant chemical families emitted by stems harvested at EF and SM

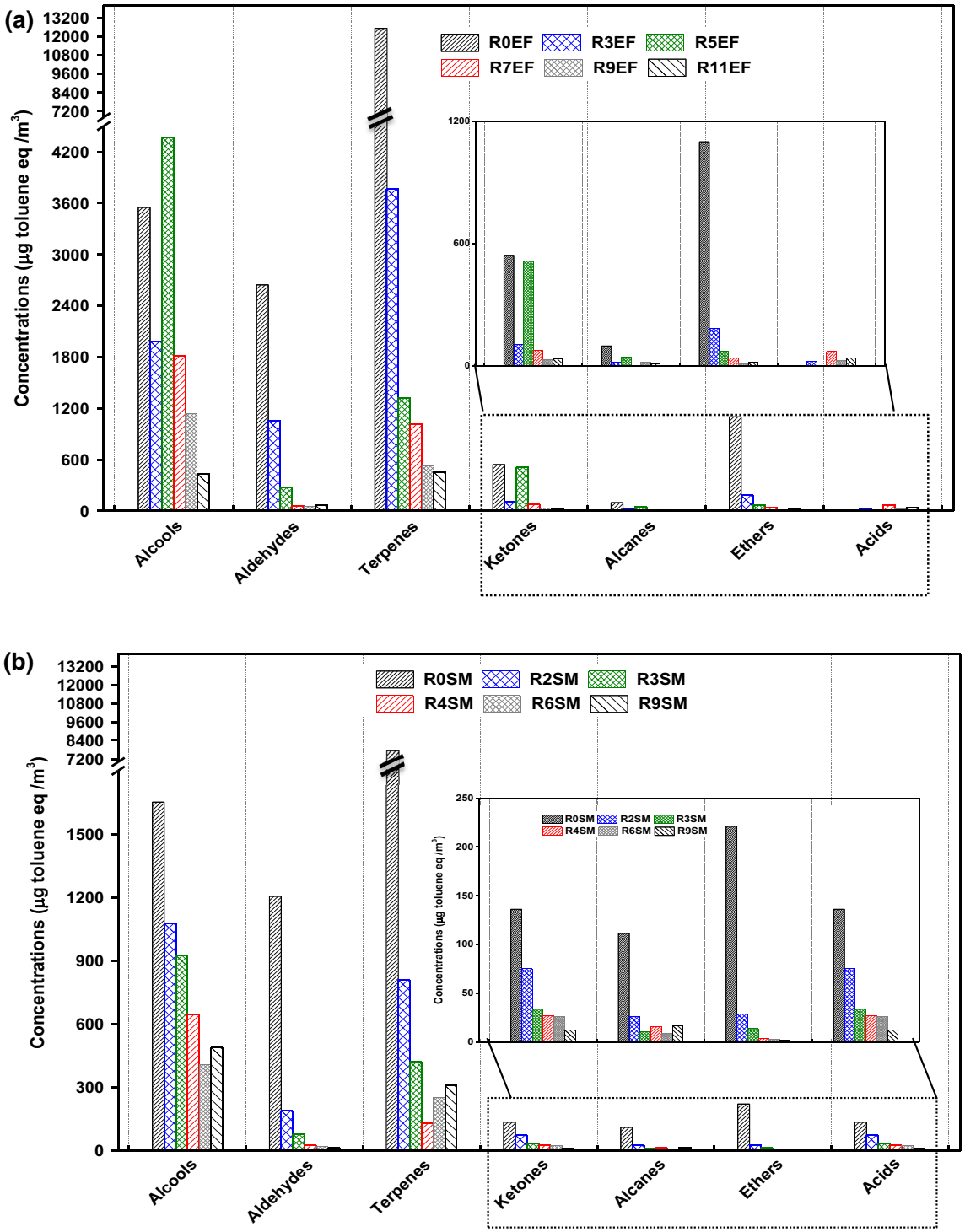

Fig. 5 Temporal dynamic of VOCs of hemp stems during field retting; **a** stems harvested at EF, **b** stems harvested at SM

VOCs' identification and quantification during field retting

The temporal dynamic of VOCs of hemp stems during field retting has been studied. As it can be seen in Fig. 5a, b, retting has an impact on emitted VOCs by the stems. The VOCs' emission behavior is similar during retting, irrespective of the harvest period. The concentration of the most abundant groups emitted by the stems harvested at EF, i.e., terpenes, aldehydes, ethers, and ketones decreased,

Table 4 Olfactometric analyses and intensity evaluation of the stems harvested at the end of flowering

Retting duration (week)	Odor concentration ($OU_E m^{-3}$)	Intensity	Acceptability level
R0EF	1779	3.5	−4.9
R1EF	1572	3.3	−3.9
R3EF	1253	3.0	−5.6
R5EF	898	3.0	−6.6
R7EF	571	2.3	−6.6
R9EF	401	2.9	−5.9
R11EF	333	2.7	−3.8

Table 5 Olfactometric analyses and intensity evaluation of the stems harvested at the seed maturity

Retting duration (week)	Odor concentration ($OU_E m^{-3}$)	Intensity	Acceptability level
R0SM	1469	3.3	−4.3
R1SM	770	2.7	−3.6
R2SM	358	2.4	−4.1
R3SM	564	2.8	−3.8
R4SM	253	2.3	−4.4
R6SM	396	2.2	−5.1
R9SM	86	1.7	–

respectively, from 12481, 2643, 1100, and 542 $\mu g_{toluene\ eq}/m^3$, for unretted stems (R0EF) to 1324, 272, 71, and 513 $\mu g_{toluene\ eq}/m^3$ for 5 weeks retted stems (R5EF) and then decreased significantly to 455, 65, 16, and 33 $\mu g_{toluene\ eq}/m^3$, respectively, after 11 weeks of retting (R11EF). The concentration of alcohols emitted by the stems harvested at EF increased at first step of retting from 2654 $\mu g_{toluene\ eq}/m^3$ for unretted stems (R0EF) to 4371 $\mu g_{toluene\ eq}/m^3$ for 5 weeks retted stems (R5EF) and then decreased to 434 $\mu g_{toluene\ eq}/m^3$ after a prolonged field retting (R11EF). For the stems harvested at SM, terpenes, alcohols, aldehydes, ethers, and ketones decreased, respectively, from 7690, 1651, 1206, 221 and 226 $\mu g_{toluene\ eq}/m^3$, for the unretted stems (R0SM) to 421, 926, 79, 14, and 89 $\mu g_{toluene\ eq}/m^3$ for 5 weeks retted stems (R5SM) and then decreased to 310, 490, 14, 2, and 11 $\mu g_{toluene\ eq}/m^3$, respectively, after 9 weeks of retting (R9SM).

The concentration of the most abundant VOCs before and after retting for the stems harvested at EF and SM is presented in the online appendices S1, S2, S3, S4, S5, and S6. The number of terpenes emitted decreased from 13 for unretted stems (R0EF) to 8 for 11 week retted stems (R11EF). Similar trend is observed during retting of stems harvested at SM. The number of VOCs emitted decreased from 15 for unretted stems of seed maturity (R0SM) to 6 for 9 week retted stems (R9SM). The α-pinene, β-pinene, and limonene release (relative abundance of 93% of total terpenes concentration decreased from 8445, 1626, and 1303 $\mu g_{toluene\ eq}/m^3$ to 893, 139, and 45 $\mu g_{toluene\ eq}/m^3$, for 5 week retted stems (R5EF) and then to 354, 46, and 16 $\mu g_{toluene\ eq}/m^3$ after 11 weeks of retting (R11EF), respectively. A gradual decrease of the major terpenes emitted by the stems harvested at SM is also observed. The α-pinene, β-pinene, and β-caryophyllene concentration decreased, respectively, from 4924, 874, and 580 $\mu g_{toluene\ eq}/m^3$ for unretted stems (R0SM) to 253, 35, and 2 $\mu g_{toluene\ eq}/m^3$ for 9 week retted stems (R9SM). This phenomenon could be related to both (i) damage to the cells of the plant after cutting the stems for retting; indeed, the enzymes leading to biosynthesis will no longer be functional with increasing of retting duration and VOCs will no longer be bio-synthesized and (ii) microbial activity during retting. The terpenes are considered to be recalcitrant to biodegradation (Cookson 1995). However, it has been reported that different bacteria and fungi populations can degrade such compounds (α-pinene, β-pinene, and limonene) under aerobic and anaerobic conditions (Misra et al. 1996; Paavolainen et al. 1998; Van Groenestijn and Liu 2002; Ramirez et al. 2010). Likewise, the structures of the terpenes are rationalized as derivatives of isoprene, and this latter is known to be degraded by soil microorganisms including members of the *Arthrobacter* (Ramirez et al. 2010), *Nocardia* (Ginkel et al. 1987) and *Rhodococcus* genera (Van Hylckama Vlieg et al. 2000).

As concern alcohols components, it can be observed in the online appendices S3 and S4 that the number of individual components in these groups decreased either during retting of the stems harvested at the end of flowering or seed maturity. The number of the compounds emitted by unretted stems harvested at EF and SM is 19 and 15, respectively, and then decreased to 7 and 3, after a retting period of 11 weeks of the stems harvested at the end of flowering (R11EF) and 9 weeks of retting of the seed maturity stems (R9SM), respectively. Moreover, the concentration of these compounds decreased as well, except for the methanol concentration that increases at early stage. Indeed, methanol emission increased from 342 $\mu g_{toluene\ eq}/m^3$ for R0EF and 964 $\mu g_{toluene\ eq}/m^3$ for R0SM to 3913 $\mu g_{toluene\ eq}/m^3$ after 5 weeks of retting of the stems harvested at EF (R5EF) and 1017 $\mu g_{toluene\ eq}/m^3$ for 2 weeks of retting of the stems harvested at SM (R2SM). The increase of methanol at early stage of retting could be due to, the pectin demethylation process by the pectin methylesterase, and the demethylation of lignin during fungal decomposition (Kirk and Farrell 1987). Indeed, during retting, the attack of stems by the microbial community by producing polysaccharide-degrading enzymes, especially pectinolytic, results in the removal of components in the middle lamella (pectic substances) and allows the cortex fibres to be progressively separated from the plant (Henriksson et al. 1997; Akin et al. 2007; Mazian

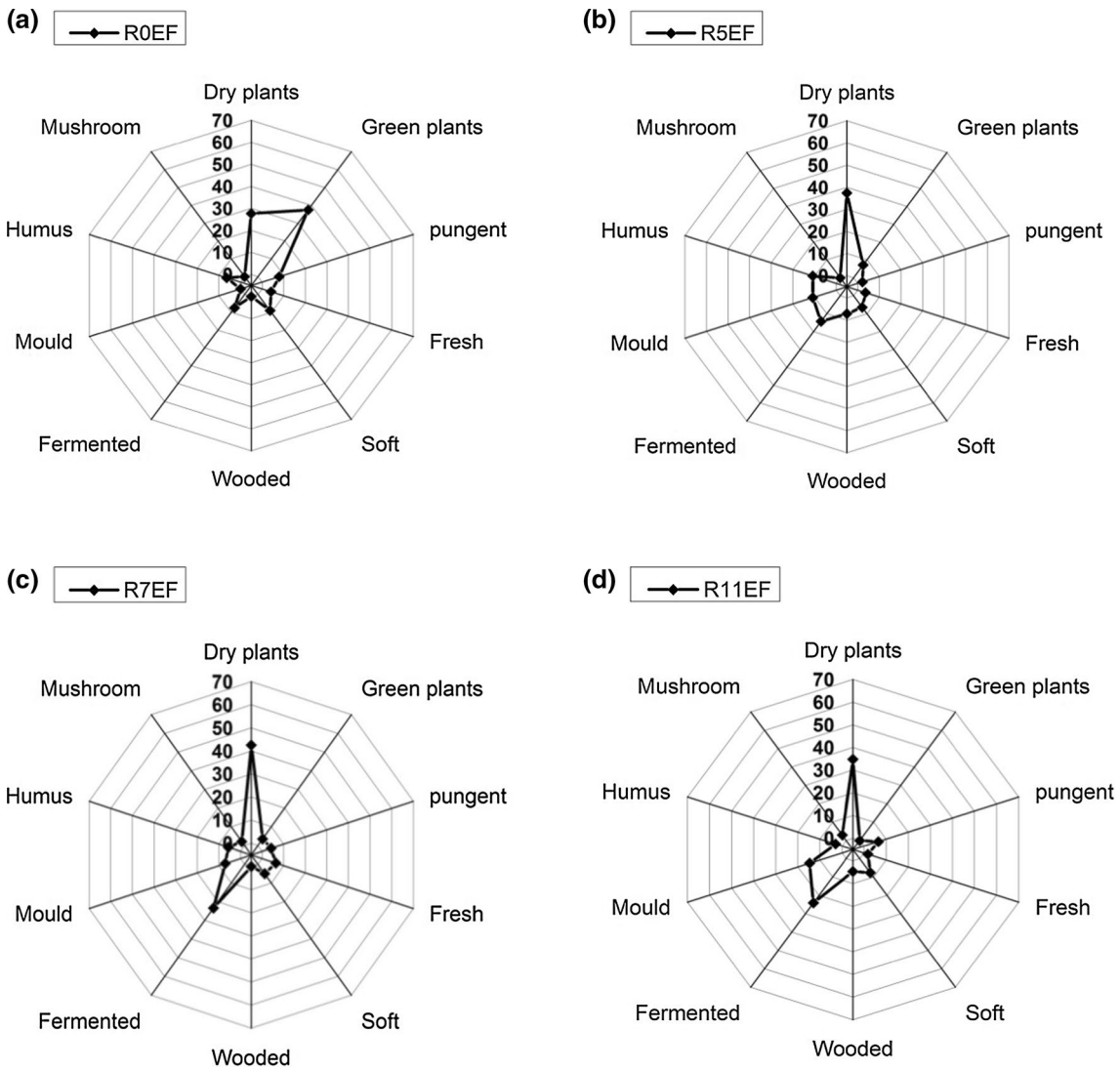

Fig. 6 Odor quality of retted stems previously harvested at EF. **a** R0EF, **b** R5EF, **c** R7EF et **d** R11EF

et al. 2018). Therefore, methanol is considered as a major microbially end product of pectin removal. However, it is probable that other microorganisms growing on by-products could develop on the stems such as methylotrophs (Schink and Zeikus 1982; Galbally and Kirstine 2002). Moreover, this phenomenon can be interpreted as the expression of species coexistence which may arise from functional complementarity, through resource partitioning or positive interactions among different species (Cabrol and Malhautier 2011).

In addition, the number and the concentration of individual components within aldehydes (Online Appendices S5 and S6) decreased as well during retting of the stems harvested at the end of flowering and seed maturity. The number of these compounds emitted by unretted stems harvested at the end flowering (R0EF) decreased from 15, 9, 6, and 2, respectively, to 4, 4, 1, and 1, respectively, after 11 weeks of retting (R11EF). Identical trend is observed when the stems are harvested at SM. A decrease of the number of aldehydes, ketones, and ethers emitted from 13, 5, and 4 for R0SM to 5, 1, and 1 for R9SM. The predominant aldehyde is acetaldehyde with a concentration of 1367 $\mu g_{toluene\ eq}/m^3$ for R0EF and 766 $\mu g_{toluene\ eq}/m^3$ for R0SM. This compound decreased rapidly after 3 weeks of retting either for EF or SM. The concentrations of ketones, ethers, and esters compounds are lower compared to other VOC groups, but the same pattern is observed.

The obtained results in this work also highlight that the field retting leads to a gradual decrease of VOCs' emission by stems. Different studies reported that during field retting, the biofilm development on the stems surface leads to a change of stems morphology and composition (Liu et al. 2017; Placet et al. 2017; Mazian et al. 2018). This VOCs

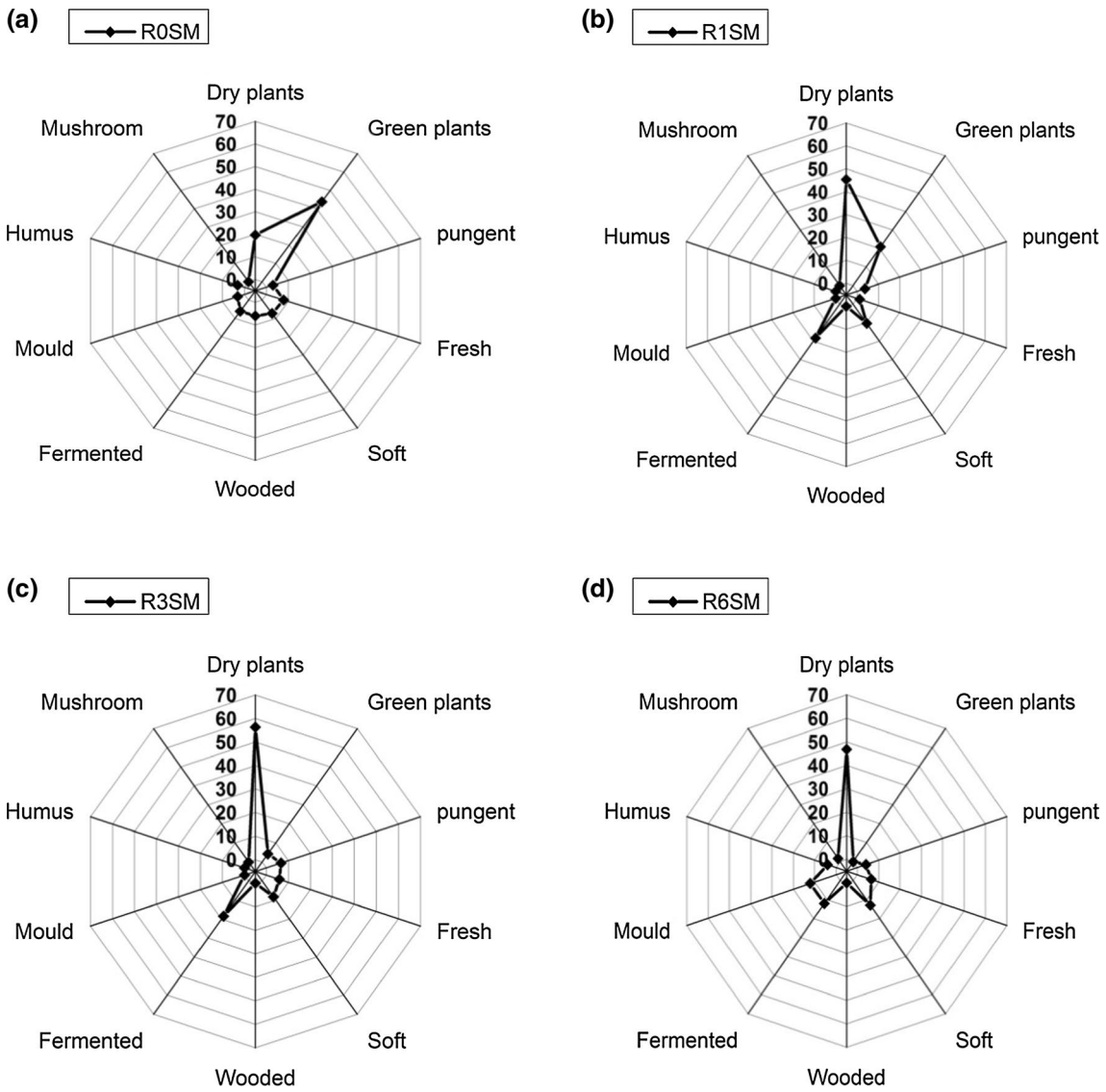

Fig. 7 Odor quality of retted stems previously harvested at SM. **a** R0SM, **b** R1SM, **c** R3SM et d-R6SM

decrease could then be linked to the removal of some fibre constituents (pectins, wax,…) and stems' layers such as epidermis, considered as among of VOCs' source by the plant (Kesselmeier and Staudt 1999; Hartikainen et al. 2009).

Odor characteristics evaluation

The results of olfactometric analyses of the stems harvested at end of flowering and seed maturity, and retted at different durations are given in Tables 4 and 5. First, it can be observed that the odor concentrations and intensity during plant development decreased, respectively, from 1779 $OU_E m^{-3}$ and 3.5, for the unretted stems harvested at EF (R0EF) to 1469 $OU_E m^{-3}$ and 3.3 for the unretted stems harvested at seed maturity (R0SM). This is consistent with the results of the chemical analysis obtained with GC–MS. This decrease of odor concentration and intensity during plant ageing could presumably due to breakdown and decomposition of some compounds. On the other hand, regarding the odor acceptability evaluation, no difference could be noticed between the stems harvested at EF and SM. However, the obtained values of the odor acceptability indicate that the odor is considered as an unpleasant odor.

Second, it can also be observed that the retting has also an impact on the odor concentrations and the intensity. Indeed, during retting of the stems harvested at EF, the odor concentration and the intensity decreased gradually from 1779 $OU_E m^{-3}$ and 3.5, respectively, for unretted stems (R0EF), to

1253 $OU_E m^{-3}$ and 3, respectively, for 5 weeks retted stems (R5EF) and then to 898 $OU_E m^{-3}$ and 2.7, respectively, after 11 weeks of retting (R11EF). Similar trend of odor concentration is observed during retting of the stems harvested at SM. The odor concentration and the intensity decreased rapidly and gradually from 1469 $OU_E m^{-3}$ and 3.3, respectively, for unretted stems (R0SM) to 396 $OU_E m^{-3}$ and 2.2, respectively, for 6 weeks retted stems (R6SM) and then to 86 $OU_E m^{-3}$ and 1.7, respectively, for 9 weeks retted stems (R9SM).

The odor acceptability of stems retted at different durations either at the end of flowering or at the seed maturity varied between −3.5 and −6 which represent unpleasant characteristics.

In addition, the odors description given by panels during retting is green plants, dry plants, spicy, fresh, soft, woody, fermented, moldiness, humus, and mushroom odors (Figs. 6, 7). The green plants' descriptor is predominant when the stems are harvested at the end of flowering (R0EF) and seed maturity (R0SM) with a percentage of 37% and 44%, respectively. This high green plants odor of the unretted stems of EF and SM could be related to the high presence of aldehydes, alcohols, and terpenes, as it has been observed from GC–MS analysis ("TD-GC–MS analyses"). Hatanaka (1996) associated the fresh green odor emitted by plants to C6-aldehydes and C6-alcohols, which are used by plants to attract or repel insects and also to kill some bacteria. Högnadóttir and Rouseff (2003) reported also that the aldehydes and some terpenes such as α-pinene, β-pinene, limonene, β-myrcene, and β-phellandrene contribute to the green aroma.

During field retting of the stems harvested at EF and SM, the "green plant" odor decreased significantly to 8% for R3EF and 4% for R3SM, and subsequently disappeared at the end of the retting. This decrease of "green plant" tonality could be related to the disappearance of green color during retting. Likewise, according to VOCs concentration results, the C6-aldehydes and C6-alcohols emissions that seem to be linked to the green odor were reduced with retting. In contrast, the "dry plant" odor increased up to a maximum value and then decreased with a prolonged field retting. The fermented, moldiness odor emitted by stems increased as well during field retting. Mushroom odor starts to appear at the end of field retting. This observation is not surprising, since the microbial communities (bacteria and fungi), which develop at the fully surface of the stems, lead to the appearance of black color. Regardless of the initial state of the stem after harvest period, the odor evolution is identical during retting like the evolution of VOCs' concertation.

Conclusion

The influence of field retting treatment on VOCs emitted by the stems harvested at the end of flowering and seed maturity was investigated in this study. Many compounds were emitted by stems, including α-pinene, β-pinene, methanol, hexanol, and acetaldehyde, irrespective of the harvest period. The most abundant chemical family is terpenes, followed by alcohols and aldehydes groups. However, during plant maturity, the VOCs' compounds emitted by stems decreased with a factor of about 2, from 20638 $\mu g_{toluene\ eq}/m^3$ for the stems harvested at the end of flowering (R0EF) to 11,368 $\mu g_{toluene\ eq}/m^3$ for the stems harvested at the seed maturity (R0SM).

During field retting process, the majority of VOCs concentration decreased until some of them disappeared at the end of retting. This phenomenon is mainly due to a biotic factor, especially the development of the biofilm at the surface of the stems. In contrast, methanol increased owing to the demethylation of pectins in the primary cell wall. In addition, this behavior is confirmed by the olfactometric analyses. Indeed, the odor concentration decreased gradually during retting either for the stems harvested at the end of flowering and seed maturity. Likewise, the "green plant" tonality disappears during retting with an increase of "dry plant" odor and an appearance of "fermented odor" at the end of retting. The combination of color change and variation of VOCs and odor could be used as a good predictor to follow the retting degree. Nevertheless, a thorough work is required to establish some correlations between VOCs and odor emission and the mechanical properties of the fibers. This supplementary work would lead to define potential retting degree indicators that could be used by farmers.

Author contribution statement LM, AB and JLF designed the research. BM performed the research, analyzed the data and wrote the manuscript. MC and SC contributed to the odor and VOCs parts, respectively. MLF participated in discussion about biogenic VOCs emissions. All authors read the manuscript and approved it.

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