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An investigation of change in composition and antioxidant potential of mature and immature summer truffle (*Tuber aestivum*)

Nirali N. Shah^{a*}, Anne Usvalampi^b, Sandeep Chaudhary^a, Tuulikki Seppänen-Laakso^c,
Sandesh J. Marathe^a, Sandip Bankar^b, Rekha S. Singhal^a, Salem Shamekh^d

^aDepartment of Food Engineering and Technology, Institute of Chemical Technology,
Mumbai, India

^bDepartment of Bioproduct and Biosystems, Aalto University School of Chemical
Engineering, Espoo, Finland

^cDivision of Bio- and Chemical Processes, VTT Technical Research Centre of Finland

^dJuvatruf, Truffle Center, Juva, Finland

*Corresponding author E-mail: shahnirali.1989@gmail.com (Nirali N. Shah)

Abstract

Summer truffles (*Tuber aestivum*) collected from Italy were evaluated for changes in chemical composition and hence *in vitro* bioactivity on the basis of their maturity. Truffles were classified as immature and mature based on the colour of glebum. Water and methanol extracts of freeze-dried mature truffles demonstrated higher total phenolics, higher *in vitro* antioxidant activity and lower tannins than immature truffle extracts. Phenolics and flavonoids were detected by Liquid Chromatography- Mass spectroscopy (LC-MS) analysis. Gas chromatography (GC)-MS analysis of hexane extracts showed differences in fatty acid composition. Linoleic acid was highest in both samples, lauric and myristic acids were prominently detected in mature truffle hexane extracts. The aroma profiling of truffles by headspace-GC showed increase in alcohols and decrease in aldehydes. Thus, this study comprehends altered chemical composition and its manifestation in *in vitro* bioactivity on maturation of summer truffles. This would aid development of techniques for identification of maturity of truffles.

Keywords

Summer truffle; maturity; anti-oxidant; polyphenol; aroma profile; fatty acid

1. Introduction

Truffles are the fruiting bodies of the edible fungi belonging to the genus *Tuber*. These are known to possess high economic value among mushrooms mainly for their characteristic aroma and flavour profile [1]. Their flavours are celebrated in many European cuisines [2]. Chiefly, over the past two decades, various species of truffles cultivated in different regions around the globe have been investigated for their nutritional and therapeutic potential [1]. The ever growing human demands for new products, inclination towards natural products and increasing health concerns have driven research in this field. The truffle extracts have been studied for the presence of bioactive constituents and their manifestations of antioxidant, anti-inflammatory and anti-microbial properties in various *in vitro* model systems [3]. These bioactives are present in minute amounts and their concentrations vary with species and location of cultivation [4].

The maturity stage of the truffles, like that of any fresh food commodity is very important for its acceptability. The maturity stage in the life cycle decides the colour of the glebum and more importantly the characteristic aroma of the truffles. The truffle aroma is the most vital component of its culinary allure and consequently decides the monetary return. The changes in aroma profile of truffles during maturation are widely reported for species such as *T. aestivum* [5], *T. melanosporum* [6] and *T. borchii* [7]. These changes are attributed to the continuous biochemical changes. The protein changes during maturation due to formation of enzymes and then subsequent enzymatic activity has been reasoned to be responsible for biochemical changes in composition in *T. melanosporum* [8]. Such biochemical changes may lead to alterations in the composition of various other compounds. More so, this leads to changes in concentrations of compounds with potential bioactivity. The effect of the stage of truffle maturity has been reported on the lipid composition of *T. melanosporum* [9].

The fruiting bodies of some mushrooms have been studied for the effect of maturity on their nutraceutical potential. The (1→3)- β -glucan was reported to increase with maturation of fruiting bodies of *Agaricus brasiliensis* [10]. The *Pleurotus djamo* (Fr.) Boedijn procured from northern Tripura, India showed highest phenol and antioxidant level during the juvenile bud stage [11]. Studies on *Lactarius deliciosus* and *piperatus* mushrooms reveal decrease in bioactive content and anti-microbial activity during maturation [12]. The same can be explored for truffles.

T. aestivum is cultivated widely across various countries in Europe and occurs in habitats over broad ecological breadth [13]. *T. aestivum* cultivated in different geographies,

due to changes in genetic diversity, are reported to possess different aroma profiles [5]. For example, it is reported that *T. aestivum* cultivated in Hungary, Switzerland and Serbia are reported for their aroma profiles and nutraceutical potential [3], [14]. The effect of storage conditions on the antioxidant activity and volatile profile of the truffle *T. melanosporum* have also been studied [15]. Mature *T. magnatum* from Italy have been reported for influence of environmental condition on biochemical properties of the fruiting body [16]. To the best of our knowledge, there are no studies reporting the effect of maturity or differentiation between mature and immature truffles on the nutraceutical potential of *T. aestivum*. This area needs to be explored to promote the medicinal appeal of truffles like their culinary appeal. There are separate studies evaluating aroma compounds and fatty acids of various truffle spp. but not on comparing aroma, fatty acids, phenolics of one or more truffle species of a particular geographical location in one single study. Since the reported separate studies involve using truffles from different regions and species, a lateral comparison becomes difficult. It is known that truffles of same spp. but different geographical origin have different composition. This study attempts a holistic differentiation between the composition of mature and immature *T. aestivum* from Italy with respect to nutraceutical potential, aroma and lipids.

The present study aimed at investigating the differences between immature and mature truffles from a nutraceutical point of view by selectively studying chemical composition. The water and methanol extracts of truffles were evaluated for changes in bioactive constituents, *in vitro* anti-oxidant activity and phenolic profile. The hexane extracts were evaluated for fatty acid profile. The aroma profile were also studied to fathom the reason for the characteristic aroma of mature black truffles.

2. Materials and Methods

2.1. Materials

Summer truffles (*T. aestivum*) were purchased from Italian market and shipped to Juva, Finland in frozen and vacuum packaged conditions. The standard phenolic compounds (vanillin, gallic acid, quinic acid, catechin, quercetin, myricetin and rutin) for Liquid chromatography- mass spectroscopy (LC-MS) were purchased from Sigma-Aldrich Ltd. The solvents and reagents used for all chromatographic techniques were of HPLC grade. All other chemicals and reagents used were of analytical grade.

2.2. Methods

2.2.1. Differentiation among truffles and their solvent extraction

The mature and immature truffles were thawed and then classified as two groups from the lot on the basis of subjective analysis of colour and aroma. The truffles were cleaned for removal of surface soil, and then sliced. These were then subjected to lyophilisation (FreeZone 4.5 litre -50 °C benchtop freeze dryer, LabConco, Kansas City, USA) for 24 h under 0.04 m Bar vacuum at -50 °C. The dried truffle slices were ground to a fine powder and stored in LDPE bags in dark prior to the analysis or extraction. Ten gram of truffle powder was extracted with 100 mL of methanol or water under shaking conditions at 27±2 °C for 72 h.

2.2.2. Bioactive content determination

The total phenols content, total flavonoids and tannins for the methanol and water extracts of freeze-dried ascocarps of immature and mature *T. aestivum* were determined as per reported methods[3], [17]. The total phenols were estimated in terms of gallic acid equivalents from standard graph ($y = 0.0056x - 0.0318$; $R^2 = 0.99$), total flavonoids in terms of quercetin from standard graph ($y = 0.0062x$; $R^2 = 0.99$) and tannins in terms of catechin from standard graph ($y = 0.0003x$; $R^2 = 0.97$).

2.2.3. Phenolic profiling of extracts using LC-MS

The phenolic profiling of water and methanol extracts of immature and mature black summer truffles was performed as per a reported technique [3] with minor modifications. Seven different standard phenolic compounds (vanillin, gallic acid, quinic acid, catechin, quercetin, myricetin and rutin) were dissolved in a mixture of 0.5 % formic acid and methanol in 1:1 ratio, in 10 µg/mL concentration. Samples were prepared from extracts, which were dissolved in a 1:1 mixture of 0.5 % formic acid and methanol in a concentration of 600 µg/mL. The solution was vortexed and centrifuged to remove insoluble solids. All standards and samples were filtered through 0.2 µm filter units. An HPLC (1200 Infinity, Agilent, Santa Clara, CA, USA) equipped with Zorbax Extend-C18 (2.1 x 50 mm with 1-8 µm particle size), Agilent, USA was used. Buffers were A) 0.05 % formic acid in Milli-Q H₂O and B) methanol. The gradient used was 3 min with 70 % A, then 70 % to 30 % A in 6 min and 30 % to 0 % A in 3 min. The column was washed with 100 % B for 3 min and equilibrated with starting conditions for 5 minutes. Injection volumes between 0.25 µL and 25 µL were used for the samples and 0.25 µL and 5 µL for the standards. The sugars were detected using Q-TOF MS (6530, Agilent, Santa Clara, CA, USA) in the negative ion mode with following settings: Source gas temperature 350 °C, drying gas 9 L/min, nebulizer 40

psig, capillary voltage 4000 V. MassHunter software version 7.0 (Agilent, Santa Clara, CA, USA) was used for all data processing.

2.2.4. *In vitro* anti-oxidant activity estimation of the solvent extracts

The EC₅₀ values for neutralization of DPPH radical and scavenging of ABTS radical were determined for methanol and water extracts as per reported techniques[18], [19]. These values were compared to BHA and L-ascorbic acid which were used as positive controls for comparison purpose. The anti-oxidant activity in terms of ferric ion reducing power (FRAP value) was also determined for the extracts and reported in terms of BHA and L-ascorbic acid equivalents referring a reported protocol[20].

2.2.5. Lipid profiling of the hexane extracts using Gas chromatography- mass spectroscopy (GC-MS)

The hexane extracted lipids were methylated as follows; approximately, 100 mg of extracted lipids were dissolved in 1 mL of *n*-hexane (HPLC grade) and vortex for 2 min, followed by addition of 400 µL of methanolic potassium hydroxide (2 M) and vortexing for 5 min at room temperature (20 °C). The solution was allowed to stand for phase separation. Further, the upper layer of *n*-hexane containing the fatty acid methyl esters was pipetted cautiously and dried on anhydrous sodium sulphate and subjected to GC-MS analysis.

The fatty acids were analyzed by GC-MS, using a TRACE 1300 ISQ gas chromatograph. The fused HP-5MS capillary column was coupled to a HP 5972A mass-selective detector (Hewlett–Packard). The oven temperature was programed at 50 °C for 1 min, then 7 °C/min to 250 °C, and then left at 250 °C for 5 min. The injection port temperature was 250 °C and that of the detector was 280 °C (split ratio: 1/100). The carrier gas was helium (99.995% purity) with a flow rate of 1.2 mL/min, and the analyzed sample volume was 1 µL. The mass spectrometer conditions were as follows: ionization voltage 70 eV; ion source temperature, 220 °C; electron ionization mass spectra were acquired over the mass range of 50–550 m/z.

2.2.6. Aroma profiling of truffles using Headspace-GC

The aromatic profile of immature and mature truffles was performed as per reported technique[21]. The truffle samples were analysed for the compounds contributing to flavour by using a Chrompack column on a Headspace-GC.

2.3. Statistical analysis

The results were analysed by IBM®SPSS® version 20 at a significance level of 95%.

3. Results and Discussion

3.1. Classification of immature and mature *T. aestivum* ascocarps

The distinction between mature and immature truffles was based on the colour of the glebum (Fig. 1a and b). This corroborates with another study which reported that the size of the fruiting body is not an appropriate judge of *T. aestivum* maturity[14]. They classified the maturity on the basis of glebum colour. Our results support their study of size-independency of ascocarps, as evident from the figure. A darker glebum is an indicator of mature *T. aestivum*. This also gives the name ‘Burgundy truffle’ to *T. aestivum* fruiting bodies[14].

(a)

(b)

Fig. 1. Cross-section of ascocarps of (a) immature and (b) mature *Tuber aestivum*

3.2. Bioactive content determination

The presence of polyphenols, flavonoids and tannins in fungi, aids in quenching free radicals and peroxide radicals and hence helps demonstrate anti-oxidant, anti-inflammatory, anti-cancer, chemo-protective and anti-microbial properties in *in vitro* model systems [3], [22].

Table 1: Bioactive composition of methanol and water extracts of mature and immature *T. aestivum*

Extracts	<i>T. aestivum</i>	Total phenolic content (mg GAE/ g dried extract)	Tannins (mg Catechin/ g extract)	Total Flavonoids (mg Quercetin/ g dried extract)
Methanol	Immature	10.95±0.016 ^a	57.23±2.02 ^a	0.42±0.08 ^a
	Mature	12.84±0.83 ^b	10.77±0.61 ^b	0.45±0.02 ^a
Water	Immature	7.63±0.49 ^c	11.46±0.00 ^{bc}	0.71±0.07 ^b
	Mature	4.52±0.69 ^d	38.4±2.26 ^d	0.58±0.01 ^c

All values are mean ± SD of three or more determinations; Means in the same column with the same superscript do not vary significantly (p<0.05).

The total phenolic and tannin content of methanol extracts of *T. aestivum* was observed to be higher than water extracts (Table 1). This indicates moderately polar nature of these molecules along with better solubility and diffusion in methanol. The flavonoid extraction was higher in water than methanol. This indicates polar profile of the flavonoids. Similar observations have been reported for bioactive composition in methanol and water extracts of *T.aestivum* and *Terferzia boudieri* [3], [17]. The total polyphenols, tannins and flavonoids increased with maturation of the truffles. A decreasing trend or unchanged content of total phenols are reported during the maturation of mushrooms [12], [23]. The increased content of total phenols may be due to the development of darker glebum colour[13]. The variation in content of extracted tannins may be due to the formation of polar tannins during maturation. This may be a part of development of darker pigments[24]. There are other studies comparing total phenol, tannin and flavonoid content among Tuber spp.[25]. To the best of our knowledge there are no studies comparing between immature and mature forms of a spp. Hence, we do not have other results to compare with.

3.3. Phenolic profiling of methanol and water extracts

The methanolic extracts of mature *T. aestivum* showed highest total polyphenol content. The phenolics used as standards (vanillin, gallic acid, quinic acid, catechin, quercetin, myricetin and rutin) could not be detected in the truffle extracts studied. However, some other phenolics could be detected (Table 2). In the water extracts of both mature and immature truffles, small amounts of C₉H₈O₂, likely cinnamic acid could be detected. In both methanol extracts, C₁₀H₁₀O₄ corresponding to ferulic acid was detected along with traces of C₁₁H₁₂O₄ corresponding to 3, 4-dimethoxycinnamic acid and C₁₅H₁₀O₆ corresponding to either kaempferol or luteolin. These phenolics and flavonoids are responsible for the

antioxidant activities demonstrated[22]. Kaempferol, *p*-hydroxybenzoic acid, procatechuic acid and amentoflavone are reported in methanol and water extracts; epicatechin and catechin are reported in methanol extracts of *T. aestivum*[3]. These researchers could however not detect cinnamic, 3, 4-dimethoxycinnamic and ferulic acids. The observed variations in results can be attributed to the difference in phenol extraction techniques and also to the geographical location of cultivation of the truffles.

Table 2: Phenolics detected in methanol and water extracts of immature and mature *T. aestivum*

	Immature <i>T. aestivum</i>		Mature <i>T. aestivum</i>	
	Methanol extract	Water extract	Methanol extract	Water extract
Ferulic or hydroxycinnamic acid	✓	-	✓	-
3,4-dimethoxycinnamic acid	✓	-	✓	-
Kaempferol or luteolin	✓	-	✓	-
Cinnamic acid	-	✓	-	✓

✓: indicates detection of compound; -: indicates non-detection of compound

3.4. *In vitro* anti-oxidant activity

Significant changes ($p < 0.05$) in bioactive content were detected in *T. aestivum* ascocarps during its maturation. These compounds are known to manifest antioxidant properties[22]. The dietary antioxidants are known to reduce the incidence of inflammatory biomarkers, blood pressure, onset of new type 2 diabetes mellitus, obesity and overall mortality[26].

The *in vitro* antioxidant activity (Table 3) manifested by the extracts corroborate with the concentration of the total phenols, flavonoids and tannins in methanol and water extracts. The scavenging of DPPH radical and BHA and ascorbic acid equivalents of ferric reducing power are best for mature *T. aestivum* methanol extracts. Similar results of lower EC₅₀ values for DPPH and ABTS due to higher concentrations of total phenols, flavonoids and tannins in extracts/solvent fractions of *T. indicum*[27], *T. aestivum*[3] and *Terfezia boudieri*[17] have been reported. The DPPH radical scavenging action of phenols is attributed to the presence of hydroxyl groups at ortho position in aromatic ring structure[4]. The phenols detected in the

extracts (detected in LC-MS analysis) possess such chemical structure and would have thus contributed to the manifested activity.

Table 3: The DPPH and ABTS activities of the truffle extracts in comparison to standard anti-oxidants BHA and AA.

Sample		EC ₅₀ value (mg/mL)	
		DPPH	ABTS
Methanol extract	Mature <i>T. aestivum</i>	11.66±0.12 ^a	0.45±0.02 ^a
	Immature <i>T. aestivum</i>	17.68±1.34 ^b	0.42±0.01 ^a
Water extract	Mature <i>T. aestivum</i>	8.16±0.09 ^c	0.40±0.03 ^a
	Immature <i>T. aestivum</i>	13.99±1.05 ^d	0.62±0.04 ^b
Butylated hydroxyanisole		0.04±0.002 ^e	0.0012±0.000 ^c
L-Ascorbic acid		0.03±0.001 ^e	0.0011±0.000 ^c

All values are mean ± SD of three or more determinations; Means in the same column with the same superscript do not vary significantly (p<0.05).

Flavonoids such as kaempferol demonstrate antioxidant and antiradical activity by acting as transient metal chelaters, radical scavengers, and involvement in electron and hydrogen atom transfer [4]. Thus, the better ferric reducing ability of immature *T. aestivum* extracts (Table 4) may be due to the higher amounts of flavonoids in it.

Table 4: Ferric reducing anti-oxidant power of the extracts expressed as BHA and AA equivalents

		FRAP in equivalents of (μmoles/ g dried extract)	
		BHA	L-ascorbic acid
Methanol extract	Mature <i>T. aestivum</i>	18.78±1.02 ^a	11.14±0.78 ^a
	Immature <i>T. aestivum</i>	14.29±0.99 ^b	8.38±0.09 ^b
Water extract	Mature <i>T. aestivum</i>	33.77±2.11 ^c	20.38±1.98 ^c
	Immature <i>T. aestivum</i>	18.36±1.32 ^a	10.88±1.17 ^a

All values are mean ± SD of three or more determinations; Means in the same column with the same superscript do not vary significantly (p<0.05).

Truffles, owing to the presence of phenols, tannins and flavonoids, are reported to demonstrate *in vitro* antioxidant activity by scavenging radicals such as DPPH, superoxide anion, nitric oxide and lipid peroxides and reducing ferric ions [3]. *T. aestivum* has lower culinary/olfactory appeal than *T. melanosporum*. However, a report indicates *T. aestivum* possesses better phenol content than *T. melanosporum* and *T. indicum*[25] and better bioactive content, antioxidant and anti-inflammatory activities than *T. magnatum* [3]. The aroma profiles during maturation are widely studied but there are no reports on the antioxidant activity with maturation of *T. aestivum*. Hence, this study becomes important to improve the selling potential of *T. aestivum* for nutraceutical or medicinal purpose.

3.5. Lipid profiling of hexane extracts

The GC-MS chromatograms of the hexane extracts of mature and immature truffles can be observed in Fig. 2. The chain-length as well as degree of unsaturation of the lipids changed on maturation (Table 5). The fungi growing in colder environments are known to have higher unsaturated fatty acid composition to modulate the fluidity of their cell membranes in the harsh climate[28]. This is commonly observed among truffles as well[29].

(a)

(b)

Fig. 2. Chromatograms of GC-MS analysis of lipids extracted from (a) immature and (b) mature *Tuber aestivum*

Linoleic acid dominated the lipid profile. The truffle lipid fatty acid composition is reported to change over the maturity of the ascocarps of *T. melanosporum*[9]. The lipid composition of the fruiting bodies of mature truffle is reported to be dominated by linoleic acid[29]. Angelini et al.[30] have reported the comparison of fatty acid profile of *T. aestivum/unicatum* collected from Italy for the chemotaxonomic value. They also reported the dominance of methyl linoleate and linoleic acid. Linoleic acid is utilized for the generation of the volatile organic compound (VOC) 1-octen-3-ol in fungi[31]. This can be the plausible reason for decrease in relative abundance of linoleic acid in mature truffles.

Table 5: Relative amounts of identified fatty acids in the hexane extracts and decreasing order of relative amounts of compounds contributing to the aroma of ascocarps of immature and mature *T. aestivum*

Immature <i>T. aestivum</i>		Mature <i>T. aestivum</i>	
Compound detected	Relative amount (%)	Compound detected	Relative amount (%)
Fatty acid analysis [#]			
C12:0	0	C12:0	0.38
C14:0	0	C14:0	0.27
C16:0	11.11	C16:0	12.39
C18:2	65.01	C18:2	53.37
C18:1	18.93	C18:1	28.41
C18:0	4.95	C18:0	5.19
Aroma analysis			
Hexanal	16.21	1-Octen-3-ol	41.51
2-Butanone	12.75	2-Butanone	11.05
Ethanol	9.63	3-Octanone/	7.33
Acetaldehyde	9.22	Acetaldehyde	5.14
Dimethyl sulfide	7.36	Hexanal	4.30
Isopropyl alcohol	6.96	Dimethyl sulfide	3.71
Butanal, 3-methyl-	5.91	1-Butanol, 2-methyl-	3.54
2-Octenal	5.38	2-Octen-1-ol	3.46
2-Butenal	5.13	1-Hexanol	3.06
2-Butanol	4.11	Ethanol	2.48
Benzaldehyde	2.41	2-Octenal	2.18
Heptanal	1.85	2-Butanol	2.10
1-Butanol, 2-methyl-	1.77	1-Propanol, 2-methyl-	1.36
1-Octen-3-ol	1.72	Butanal	1.36
1-Propanol, 2-methyl-	1.54	Octanal	1.33
2-Heptenal	1.32	3-Octanol	0.93
Benzeneacetaldehyde	1.28	1-Octen-3-one	0.82
3-Octanone	0.88	1-Butanol	0.73
5-Decanol, 4-methyl	0.83	1-Octanol	0.59
3-Octanol	0.59	Benzaldehyde	0.50

2-Butenal, 2-methyl	0.55	2,4-Nonadienal	0.39
Phenylethyl alcohol	0.50	2-Undecanone	0.36
2,4-Nonadienal	0.49	Heptanal	0.35
Hexanoic acid	0.45	2-Butenal	0.31
2-Undecanone	0.37	2-Heptenal	0.27
2,4-Decadienal	0.25	Hexanoic acid	0.26
1-Octen-3-one	0.25	Benzeneacetaldehyde	0.22
2-Octanone	0.17	2,4-Decadienal	0.18
2-Butenal, 3-phenyl-	0.13	Phenylethyl alcohol	0.18

#Values are expressed as percent of total identified fatty acids

The palmitic and oleic acid contents decreased on maturity. Moreover, myristic and lauric acids were found in detectable amounts in hexane extracts of mature truffle. Similar results are reported for fatty acids of *T. melanosporum* Vittad during their maturation stages. This change in lipid composition is attributed to the structural and organisational role of lipids causing alterations in the ascocarps during maturation [6]. Along with, the improvement in bioactive content, the changes in lipid composition (increased oleic acid content) also enhance the functionality of mature *T. aestivum* as a nutraceutical.

3.6. Aroma profiling by headspace-GC

The evaluation of changes in aroma profile aids in indirect assessment of the biochemical changes occurring during the maturation of *T. aestivum*. The truffle aroma is generally described as earthy, musky and pungent. Many researchers have attempted analysis of truffle aroma as a means to differentiate among the various species. The difference in the aroma perceived among various species is due to the variation in composition of the contributing compounds [1]. As there is variation in aroma among truffle species, so is there a variation in aroma with maturity. These changes in aroma profile are considered to be odorant cues for their detection by mammals and insects[32].

Table 5 enlists the relative amounts of compounds contributing to the aroma of *T. aestivum* based on its maturity. The C8-VOCs dominate are the key molecules responsible for the aroma[33]. Apart from 1-octen-3-ol whose concentration increased drastically on maturity, the qualitative and quantitative abundance of other C8-VOCs also increased from 7.27% to 16.64%. There is a decrease in dimethyl sulphide on maturity. 2-methyl-1-butanol is considered as one of the important aroma compounds in *T. aestivum*. Its absence in our

samples can be attributed to the difference in geographical location of cultivation of the *T. aestivum*, also reported [34][35] for similar observations in their study. Thus, along with maturity, the geographical origin also has an influence on the composition of the aroma contributing compounds. The microbiome associated with the truffles such as *T. borchii*, *T. melanosporum*, *T. aestivum* and *T. magnatum* are also reported to contribute to the aroma development [36].

There is an increase in alcoholic compounds and decrease in aldehyde compounds upon maturity (Fig. 3). Qualitative as well as quantitative changes in compounds contributing to truffle aroma are observed. Researchers have reported minor or lesser effects of maturity on the aroma profile of *T. aestivum* [5]. We have however perceived profound variation in aroma through sensory as well as GC analysis. There is an increased amount of 1-octen-3-ol in mature truffle aroma and this has led to drastic changes in the relative amounts of molecules contributing to aroma on maturity. Changes in volatile organic acids and sulphur compounds have been reported for *T. borchii* on maturity [37]. Thus VOC is proposed to be a marker molecule for identification of *T. borchii* maturity by them but has been ruled out for *T. unicanum* and *T. borchii* by other studies [7]. Our results for *T. aestivum* maturity were in lines with the former study, supporting its use as a marker molecule. A sensor has been developed for detecting the aroma of truffles [38]. Identification of marker compounds may help in using such sensors to identify the stage of maturity of *T. aestivum* in a non-invasive manner.

4. Conclusion

The difference in lipid, phenolic and aroma profile of the *T. aestivum* ascocarps from Italy have also implicated in the enhancement of nutraceutical potential. Thus, the maturation of *T. aestivum* not only decides its culinary appeal but also medicinal/nutraceutical importance. The increased bioactive content during maturation of *T. aestivum* can help towards research in species with higher bioactives and hence higher medicinal properties. This study can also be built to set-up marker compounds for identification of maturation stage of *T. aestivum*. The prominently detected 1-octen-3-ol in mature *T. aestivum* aroma profile may become a marker compound. On similar lines, research can be pursued on evaluating the difference of lipid, phenolics and aroma profile between immature and mature truffles from other species from diverse geographical locations. This will facilitate in selecting the truffles at the appropriate stage for desired use, either culinary or medicinal.

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Conflict of interest

The authors have no conflicts of interest.

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