A study on the fatty acid composition of lipids in truffles selected from Europe and Africa

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Abstract

The gas chromatography of hexane extracts from ascocarps of *Tuber maculatum* (mostly the first report), *T. aestivum/unicantum*, *T. borchii*, *T. melanosporum* and *Tirmania nivea* dominantly showed palmitic, stearic, oleic and linoleic acids followed by traces of polyunsaturated fatty acids. The fatty acid content varied from ca. 8–61 mg g⁻¹, dry-weight-basis with species with highest for *T. maculatum*. Polyunsaturated fatty acids contributions varied from ca. 42–59%. The dominant fatty acid varied with the species. A comparison with existing reports on same species cultivated in different regions showed differences in contributions by saturated, monounsaturated and polyunsaturated fatty acids as well as dominant fatty acids detected. Lesser explored species such as *T. borchii*, *T. maculatum* call for further research. This is a preliminary study that indicates fatty acid composition as a potential tool for distinction like aroma between truffle species and geographies of cultivation. This forms the basis for further studies in different species and regions.

Keywords *Tuber maculatum* · *Tuber melanosporum* · *Tuber aestivum* · *Tuber borchii* · *Tirmania nivea*

Introduction

Truffles are the fruiting bodies of the hypogeous fungi that have been consumed mainly for their specific flavour and aroma profile (Pacioni et al. 2010). Truffles are now researched for their influence on human health as nutraceutical or medicine (Hamza et al. 2016) through various analysis such as in vitro antioxidant activity, cytotoxicity studies, polyphenol profiling and micronutrient content (Beara et al. 2014). Most studies have been conducted on summer truffles (Beara et al. 2014) and desert truffles (Al-Laith 2010; Gouzi, Leboukh, and Bouchouka 2013) cultivated in different regions of the world.

In the current times, there is an increase in curiosity and awareness of the implications of the fatty acid composition of food on the human health. Many novel and existing food sources are being studied for this purpose. Dietary recommendation levels for total fats are at 25–35% of bodily energy intake and those for n-3 polyunsaturated fatty acids at 3 g day⁻¹ (FAO/WHO 2008). Truffles have a low lipid content ranging from 4 to 9% (dry weight basis) (Tang et al. 2011). The study of composition of truffle lipids becomes important when they are also evaluated for their nutraceutical benefits due to the presence of polyphenols (Beara et al. 2014). The fatty acid composition of *Tuber aestivum*, *T. unicanum* and *T. melanosporum* grown in Italy have been compared for effect of chemotaxonomy (Angelini et al. 2015). The *T. aestivum* lipid composition from hexane extracts has been reported to change with maturation (Shah et al. 2020). A study (Hilszczańska et al. 2016) has reported the variation in lipids, proteins, saccharides, polyphenolics, flavonoids, total sterols, ergosterol, volatile flavour and aroma compounds with the geography of cultivation of *T. aestivum*. Such information is vital in case a truffle species from one geography is being cultivated in another for purposes of nutritional value, medicinal value or culinary demands. Also, studies have been widely reported on the distinction of truffle aroma with species and geographies

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of cultivation (Culleré et al. 2013; Díaz et al. 2003; Strojnik, Grebenc and Ogrinc 2020). Other truffle constituents such as fatty acids should be explored to identify potential tools for such distinctions. The five truffles included in this study are native to different countries in Europe and Africa. *T. melanosporum* is a native to the Mediterranean region and is harvested between late November and early March. *T. aestivum* is native of northern Italy and parts of France, *T. borchii* is grown all over Europe (Wang and Marcone 2011). *Tirmania nivea* is a Desert truffle native to the Middle East, Mediterranean basin, northern Africa and parts of Botswana (Sawaya et al. 1985). *T. maculatum* is a wild truffle native of Finland and can be found in other European countries. Unlike, *T. aestivum*, *T. melanosporum* and *T. borchii*, *T. maculatum* is not cultivated.

This work was aimed at studying the lipid composition of five truffles that are native to countries in Europe or Africa. Most of these truffles cultivated in other geographies have been reported for their lipid profile. A relation between the geography of cultivation and lipid composition of truffles is attempted and compared with the existed reports. This will help identify if, like truffle aroma, their fatty acid composition can be a potential tool for distinction.

Materials and methods

Materials

Black Diamond (*Tuber melanosporum*) and *T. borchii* were obtained from Italy. Summer truffle (*T. aestivum/uncinatum*) and Finnish Wild truffle (*T. maculatum*) were obtained from Finland. Desert truffle (*Tirmania nivea*) was obtained from Libya. At least three samples were procured for each truffle type for processing towards the analysis. All samples were mature ascocarps (based on the colour of the gleba and size). The standard fatty acids were purchased from Sigma-Aldrich. The solvents and reagents used were of chromatographic grade.

Sample preparation

Truffles were brushed off any dirt and stored under frozen conditions prior to processing. They were freeze dried (FreeZone 2.0, LabConco) below -40°C under 0.04 mBar vacuum for 24 h and then milled to a powder prior to fatty acid analysis.

Gas chromatography (GC) analysis

Each sample (50 mg) was weighed into a pre-weighed glass tube. The fatty acid content of the lipids were analyzed by direct methylation method (Suutari, Liukkonen

and Laakso 1990). For hydrolysis and saponification, 1 mL of 3.7 mol L^{-1} NaOH in 49% methanol was added and samples were placed in a boiling water bath for 30 min. This was followed by methylation. The GC (Agilent 7890) was equipped with a FFAP column (Agilent 19091F-105, $50.0\text{ mm} \times 200\text{ }\mu\text{m} \times 0.33\text{ }\mu\text{m}$) and flame ionization detector. Hydrogen, synthetic air and helium gas flow rates were maintained at 30 mL min^{-1} , 300 mL min^{-1} and 24 mL min^{-1} , respectively. The split ratio was maintained at 10:1. The column temperature was raised from 100°C to 230°C at the rate of $5^{\circ}\text{C min}^{-1}$ with a hold time of 30 min.

For the purpose of quantification of total fatty acid content, C17:0 fatty acid ($10\text{ }\mu\text{L}$ of $20.9701\text{ mg mL}^{-1}$) was used as an internal standard and supplemented into the sample before methylation (chromatograms not shown). The concentrations of other fatty acids could be calculated by comparing their peak areas to the standard area of C17:0. For the calculation of relative abundance of fatty acids, samples were run without the internal standard and peak areas were considered to avoid discrepancies in calculations.

Statistical analysis

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

Results and discussion

The Fig. 1 depicts the GC-FID chromatograms of the various truffle lipids. In comparison to the internal standard at 20 mg mL^{-1} , the dominant peaks were observed for palmitic (C16:0), stearic (C18:0), oleic (C18:1) and linoleic (C18:2) acids. These observations are further discussed and compared with reports for lipid composition of truffles collected from other regions. The analysis results with internal standard (chromatograms not reported here) were used for the calculations of fatty acid content to avoid discrepancies as C17:0 was absent in *T. aestivum* and *T. borchii* samples.

The Table 1 enlists the fatty acid content of truffles. To the best of our knowledge, the fatty acid content of *T. maculatum* is reported for the first time and was found to be the highest among the truffles evaluated in this study. The health benefits of the oils in these truffles can be evaluated from the saturated fatty acids (SFA), monounsaturated (MUFA) and polyunsaturated fatty acid (PUFA) content. The comparison of groups of fatty acids in the truffles will be followed by the comparison of the dominant peaks of fatty acids detected. In a study, Italian *T. aestivum* fruiting bodies were found to contain 9, 37 and 54% of saturated, monounsaturated and polyunsaturated chains, respectively (Mannina et al. 2004). The trend remaining similar, only the PUFA content in the Finnish cultivated *T. aestivum* in this study is closer to the

Fig. 1 Chromatograms obtained from the gas chromatography-flame ionization detector assisted analysis of the fatty acids in the dried ascocarps of **a** black diamond (*Tuber melanosporum*) from Italy, **b** summer truffle (*T. aestivum/unicatum*) from Finland, **c** calcium truffle (*T. borchii*) from Italy, **d** desert truffle (*Tirmania nivea*) from Libya, and **e** Finnish wild truffle (*T. maculatum*) from Finland

reported value. This difference in composition may be possibly attributed to location or maturity of *T. aestivum*. Differences in the fatty acid composition between immature and mature *T. aestivum* from Italy have been reported (Shah et al. 2020). Since mature *T. aestivum* ascocarps were selected for the study, the reasoning of changes in location may be possible. Saturated fatty acids are reported to be dominant in the *Tirmania nivea* fruiting bodies from Saudi Arabia, followed by MUFA and then PUFA (Bokhary, Suleiman and Basalah 1989). This trend was opposite in the case of *Tirmania nivea* species from Libya, in our study. The higher PUFA content of truffles can add to their medicinal benefits in treatment of various inflammatory conditions such as cardio-vascular, neurological or dermatological disorders (Rubio-Rodríguez

et al. 2010). The PUFA content of truffle lipids were lower than RDA, however, upon mixing with other PUFA oils, it can add to the health effects along with aesthetic appeal of truffle flavour.

The Fig. 2 represents a comparison of the relative abundance of the fatty acid content of all truffles analysed in this study. Palmitic, stearic, oleic, linoleic acids were the major contributors to fatty acid content. Detectable quantities of α - and γ -linolenic acids were also observed. The absence or non-detection of myristic acid can be due to its decrease on maturity. A study (Sancholle et al. 1988) has reported decrease in myristic acid content on maturation of *T. melanosporum* from Southern France. They have reported linoleic acid to be the major contributor to lipids

Fig. 1 (continued)

followed by oleic, palmitic and stearic acids. Our results for *T. melanosporum* are in lines with this report. Our results are again dissimilar for Italian *T. melanosporum* from its Chinese counterpart (Tang et al. 2011). They detected linoleic to be the major fatty acid followed by oleic, palmitic and then stearic. Thus, indicating that geography or condition for cultivation may or may not influence lipid composition of *T. melanosporum*.

A report (Angelini et al. 2015) on fatty acid profile comparison of *T. aestivum/unicantum* from Italy shows dominance of methyl linoleate and linoleic acid. They however report almost 1/6–1/7th of the methyl linoleate values of other fatty acid methyl esters. The decreasing order of fatty acid content in those samples were: palmitic > oleic > stearic

acids. This order is different for the Finnish cultivated *T. aestivum* that we report. The palmitic, stearic and oleic acid content are lower in the samples we analysed but not as low as those reported for *T. aestivum* from Italy. Thus, *T. aestivum* and *T. melanosporum* results indicate that geography or condition for cultivation may influence lipid composition and call for further studies for validation.

Unlike *T. melanosporum* and *T. aestivum*, the lipid composition of *T. borchii* is dominated by oleic acid. This is different from the lipid composition reported for the fruiting bodies of *T. borchii* from China (Tang et al. 2011). They report the dominance of linoleic acid followed by oleic, palmitic, and stearic acids. *Tirmania nivea* from Riyadh has been reported to have maximum oleic acid content followed

Fig. 1 (continued)

Table 1 Fatty acid content and relative percentage composition of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) of determined by gas chromatography in truffles selected from Europe and Africa

Truffle	Fatty acid content (mg/g, dwb)	Relative composition*		
		SFA (%)	MUFA (%)	PUFA (%)
<i>Tuber melanosporum</i>	8.19 ± 0.50 ^a	25.35 ± 0.20 ^a	25.10 ± 0.42 ^a	49.55 ± 0.62 ^a
<i>T. aestivum</i>	11.87 ± 1.33 ^b	15.14 ± 0.70 ^b	26.32 ± 10.33 ^a	58.54 ± 11.03 ^b
<i>T. borchii</i>	37.43 ± 2.48 ^c	12.69 ± 0.89 ^c	44.81 ± 0.58 ^b	42.50 ± 1.48 ^c
<i>T. maculatum</i>	61.31 ± 19.12 ^d	31.02 ± 0.04 ^d	11.12 ± 0.53 ^c	57.85 ± 0.50 ^b
<i>Tirmania nivea</i>	33.84 ± 6.72 ^c	11.62 ± 3.67 ^c	38.29 ± 5.12 ^d	49.45 ± 1.44 ^a

The content of fatty acids detected were calculated from addition of internal standard of known concentration. All values are mean ± SD of two or more determinations; Means in same columns with same superscripts do not differ significantly ($p < 0.05$); *-detected. Does not include all known fatty acids in the group dwb dry weight basis

Fig. 2 Relative abundance of the fatty acids analysed by gas chromatography-flame ionization detector in black diamond (*Tuber melanosporum*) from Italy, summer truffle (*T. aestivum/unicatum*) from Finland, calcium truffle (*T. borchii*) from Italy, desert truffle (*Tirmania nivea*) from Libya, and Finnish wild truffle (*T. maculatum*) from Finland

by palmitic, stearic, linoleic and linolenic acids (Bokhary et al. 1989). *Tirmania pinoyi* from Libya is also reported to contain oleic acid as the dominant fatty acid (Stojković et al. 2013). This may be a peculiarity of *Tirmania* species for dominant oleic acid content. Since we do not have more reports to compare, we cannot state the possibility of the influence of geography on the lipid profile of *T. borchii* and *Tirmania nivea*. In *T. maculatum*, the linoleic and oleic acid contents are statistically similar ($p < 0.05$). Also, as observed for the other truffles, the stearic acid content is the lowest. *T. maculatum* is a species similar to *T. borchii*. However, both these species show differences in macroscopic characters, taste, smell, and neotyping (Mello et al. 2000). To the best of our knowledge, there are no other reports on the fatty acid profile of *T. maculatum* found in the same or another region and hence we cannot comment on the influence of the geography.

Conclusions

T. melanosporum and *T. aestivum/unicatum* are widely studied truffles and hence a comparison of their fatty acid composition with reported data was plausible. The fatty acids in *T. maculatum* were reported for the first time and hence require more studies for discussion. Further studies on the other truffles cultivated in other regions is necessary to draw any conclusion. The biochemical pathways involved in the maturation of truffles and their environmental conditions influence the chemical composition. This is a preliminary study highlighting the potential of fatty acid composition in identification of the geography of cultivation of particular truffle species. Finally, a deeper study compiling the same becomes necessary if truffles are to be looked above their culinary appeal for their health benefits.

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Author contributions NNS: Data analysis, manuscript drafting; SH: conducting experiment in the laboratory; OP: Study planning; AE: Expert help in manuscript drafting; SS: Study planning.

Compliance with ethical standards

Conflict of interest On behalf of all the authors, the corresponding author declares that they have no conflict of interest in the publication.

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