

Searching for naturally generated volatiles from *Tuber Melanosporum* as authenticity markers for black truffle infused vegetable oils

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Abstract

The secret of the great popularity of truffles and its derivatives resides mainly in its volatile aromatic fraction, which contributes to their unique aroma. Some culinary preparations are made with this fungus, such as truffle-infused oils. The adulteration of these products must be controlled and prevented due to the high economic cost of natural black truffles.

In this preliminary work the volatile composition of truffles and some commercial truffle-infused oil samples were determined by headspace solid-phase micro-extraction (HS-SPME) combined with gas chromatography-mass spectrometry (GC-MS) in order to confirm the authenticity of the infused oils. Complementary, a descriptive sensory analysis was also performed with the same purpose.

Principal component analysis (PCA) was applied to the data obtained and different groups were established according to the sensory profiles and the variation among samples.

Introduction

Truffles are hypogeous fungi that live in symbiosis with the roots of several host trees. These fungi are widely appreciated for their organoleptic properties. As it is well-known, the culinary and commercial value of truffles is mainly due to their sensorial properties such as their aroma [1, 2] the quality of which clearly provides the economic value of this edible fungus.

The aim of this preliminary study was to characterize sensory and analytically the organic volatile compound composition of commercial truffle oils by headspace-solid phase microextraction gas chromatography-mass spectrometry (HS-SPME-GC-MS) coming from the truffles and not from the oil in order to characterize its natural origin or to detect the presence of added flavourings.

Experimental

Materials

Two fresh *Tuber melanosporum* black truffle samples in its optimum maturation level purchased in a local market were analysed as a reference for the generic volatile compound profile. Six samples from different geographical origins of commercially available vegetable oils infused and/or aromatized with truffle were evaluated. Samples

were purchased in local (Spain: S1, S2, S3) and foreign markets (France: F1, Italy: I1, I2) August- November 2013. All samples were labelled as artificially aromatized except S3. S1 and S2 were a mixture of naturally infused and aromatized oil. One more sample (ES) was studied: a truffle-infused oil sample prepared in our laboratory with 5 g of minced *Tuber melanosporum* in 250 ml of olive oil in order to have a positive authenticity control sample. The infusion was made at ambient temperature and darkness during 2 weeks. The oil infused samples were stored at 4°C and the analysis was done maximum 24-48h after each bottle was opened.

Sensory evaluation

A group of thirteen trained panellists (7 women and 6 men, between 25 and 65 years old) participated in the evaluation of the aroma of truffle oils. First, the descriptors or sensory terms for describing the odour sensations perceived from truffle were established. After that, the panellists were trained in the identification of descriptors and the use of continuous scales for evaluating the intensity of each descriptor. Finally, the trained panellists evaluated the seven truffle oil samples and the aroma profile of each sample was obtained. For each sample, panellists scored the perceived intensity in duplicate using an unstructured 10 cm line with anchors “weak” and “strong”. Panel performance was studied using Panelcheck software. For each sensory attribute, a 2-way ANOVA (sample and panellist) with interaction was applied to the data obtained. To study the significance of the sample effect a mixed model ANOVA, considering panellists as random factor and the sample as fixed factor was performed for each attribute. In order to study the sensory differences among samples, taking into account all sensory attributes, a principal component analysis (PCA) was performed. A one-factor analysis of variance (ANOVA) was used in order to study differences between samples on aroma compounds of truffle-infused oil samples. Significance of differences among means was established using Tukey's Test ($\alpha \leq 0.05$). Principal component analysis was used to evaluate relationships among selected aroma volatile compounds obtained by GC-MS data and samples. A one-factor analysis of variance (ANOVA) was used to study differences between samples on aroma sensory attributes. Principal component analysis was used to evaluate relationships among selected aroma attributes and samples. Partial Least Squares Regression (PLSR) was applied to model the relation among the variance of sensory attributes among samples (Y variables) and the variance in volatile compounds obtained by GC (X variables). All the analysis were carried out with XLSTAT Pro software version 2013 (Addinsoft, France).

Analysis of the volatile compounds

Extraction of organic volatile compounds was performed with static headspace solid phase microextraction (HS-SPME) using 2 g of sample. At least two replicates of each sample were prepared and analysed and the final results are the average of all samples analysed. For fresh *Tuber Melanosporum* black truffle samples two fibres from Supelco were used: 50/30 μm DVB-CAR-PDMS and 100 μm PDMS Truffle samples were sliced, incubated for 5 min at 50°C and extracted for 10 min at the same temperature. For truffle-infused oil samples only the triple phase fibre was used due to its better results, obtained in previous studies. A direct 30 min extraction of 2 g sample at 50°C was made to avoid oil oxidation. After extraction, the volatiles were thermally desorbed for 10 minutes at 250°C in splitless mode. Volatiles were separated on two different columns: a polar column and an apolar one. Detection was carried out in a single quadrupole mass spectrometer.

Results and discussion

107 volatile components were identified in fresh black truffles. Only 43 of those components were also found in an olive oil sample infused with the same type of truffles in our laboratory. From those 43 products some alcohols like ethanol, isobutanol, 2-methyl-1-butanol, 2-butanol, 2-pentanol, 2,3-butanediol, 2-methylthioethanol and 3-methylthiopropanol, were higher in the authentic infused sample than in the flavoured samples. The high quantity of ethanol found could be due to truffle fermentation processes in the oil at ambient temperature and in this case it cannot be considered a true marker. On the other hand, 1-octen-3-ol, a typical mushroom component and 2,4-dithiapentane, a typical white truffle component, were only present in trace quantities in our analysis made to fresh black truffles. Both have been found in huge quantities in flavoured samples.

Looking to the sensory analysis a total of eleven odour attributes were found to be useful for describing the odour of truffle oils: fungus, fermented, cockle, moist soil, rancid, hazelnut, faecal, boiled cabbage, garlicky gas, potato and carob. Fifteen panellists initially evaluated the intensity of the odour attributes of the seven oil samples. Data from two panellists that showed low concordance with the rest of panel were not considered in further analysis. The results of a mixed model ANOVA showed significant differences ($\alpha=0.05$) among samples for all attributes, even in those (faecal, garlicky gas and potato) for which the effect of panellists' x sample interaction had been found significant. The mean values of the perceived intensity for each attribute in the oil samples were obtained and the sensory profile of each sample is presented in spider web plots (Figure 4)

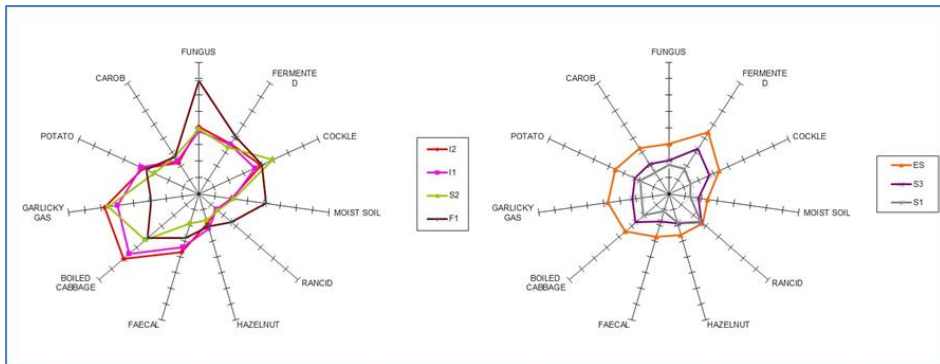


Figure 1: Mean values of the intensity perceived for each attribute in the oil samples

For samples infused with truffle the odour intensity was low (S1 and S3) or high (ES) but it was equilibrated among the different attributes. However, oil samples aromatized with truffle flavours presented high intensity of only certain attributes, such as: fungus, cockle, garlicky gas and boiled cabbage.

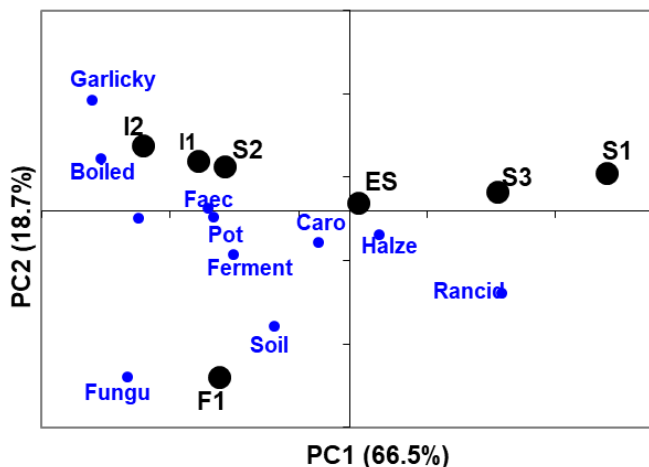


Figure 2: Sensory attributes PCA Analysis of truffle infused oils

The PCA (principal component analysis) of the data (Figure 5) showed that the first two dimensions accounted for 85.2% of the variability in the odour of truffle oil. The first dimension clearly separated on the right side the oil samples infused naturally with truffle and on the left side the oil samples aromatized with truffle flavourings. The second dimension separated the aromatized oil samples S2, I1 and I2 (upper side) with more intense garlicky gas and boiled cabbage odours and aromatized oil sample F1 (bottom side) with more intense fungus and soil odours.

Conclusions

Analytical and sensory differences were clearly seen between oil samples naturally infused with *Tuber Melanosporum* fresh black truffles and artificially aromatized oil samples. Some volatile components frequently present in other mushroom flavours but not present in black truffles were found in aromatized samples. Some alcohols present in the flavour of fresh black truffles and also present in naturally infused oils were found in higher quantities in those samples than in artificially flavoured oil samples.

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