Application of supercritical CO_2 to truffle species: aromatic and flavoring extracts

Eva Tejedor-Calvo[a,b,*], Pedro Marco[a], Peter Spègel[c], Cristina Soler-Rivas[b]

[a] *Agrifood Research and Technology Centre of Aragon (CITA), Zaragoza, 50059, Spain*

[b] *Institute of Food Science Research (CIAL), Madrid, 28049, Spain*

[c] *Centre for Analysis and Synthesis (CAS), Lund, 22362, Sweden*

* etejdorc@aragon.es

1. Introduction

Truffles are a well-known worldwide product mainly appreciated for their unique aroma. In the recent years, popularity of truffle products has increased in restaurants and supermarkets. Usually artificial aroma containing bis(methylthio)methane is added to enhance truffle aroma in those products however this molecule is not present in the black truffle[1]. Nowadays, the aromatic profile that evokes the real smell of black truffles is still undefined and therefore, no extracts or supplements are available to be used as food flavoring. Besides that, the current truffle categorization (UNECE Standard FFV-53)[2], only consider physical aspects in truffle quality evaluation. Therefore, damaged truffles or those with small size are considered as low quality units and achieve a lower prize. So, they could be a potential source of flavoring and aromatic compounds that can be extracted (revalorizing these other truffles) and used to design natural extracts to improve the aroma and the quality of truffled products acting as flavor enhancers.

2. Materials and Methods

A new methodology to obtain aromatic compounds from truffles using supercritical CO_2 was recently proposed[2]. In this study, parameters such as time, pressure and flow rate were optimized and using HS-GC-MS (head space – gas chromatography) and GC-O (olfactometry) to study aromatic compounds from the extracts. However, some of the compounds were not extracted or were lost in the depressurization time. For that, grapeseed oil was added into the separators to test it as trapping matrix for volatiles and aromatic molecules. Some key truffle aromatic compounds such as 2,3-butanodione, 2-methyl-1-butanol, octanal and dimethyl disulphide were detected in oil samples. Some modifications on the instrument were needed to enhance trapping and avoid volatile compounds losses.

Figure 1. Bubbling process to trap flavoring compounds into food trapping material

For that, new experiments were carried out using surface response methodology. In that, the effect of extraction time (15-90) and flow rate (1-3 mL/min) were selected as independent factors, and the extraction yield as variable response was examined. Pressure and temperature were kept at 300 bar and 40 ºC. The design was adjusted to maximize the extraction yield, considering flow rate and time equally important. After that, bubbling the CO_2 into food matrices (oil, gelatin, water and agar-agar) (Figure 1) was tested with different amount of volume 1, 3 and 5 mL.

The aim was to improve the extraction yield and extract lipid compounds such as fatty acids and sterols, that are also related to truffle aroma and flavour. For that, an electrospray ionization quadruple time-of-flight mass spectrometry (UHPSFC/ESI-QTOF-MS) was used to detected sterols and fatty acids compounds.

3. Results and discussion

The optimal extraction conditions producing the highest extraction yield (1.44%) from truffles were 2.3 mL/min CO2 flow rate and 82.5 min. Time, more than flow rate, was positively influencing the extraction yields (Figure 2). Totally, 32 lipids, belonging to classes such as, fatty acids (saturated, unsaturated), and sterols (ergosterol and derivates), were identified. Among them, ergosterol, brassicasterol, ergosta-7,22-dienol, oleic and linoleic acid their major constituents. Also, the highest extraction yield was obtained using gelatin (1.42 %) followed by oil (1.16 %), with water and agar-agar showing much lower yields.

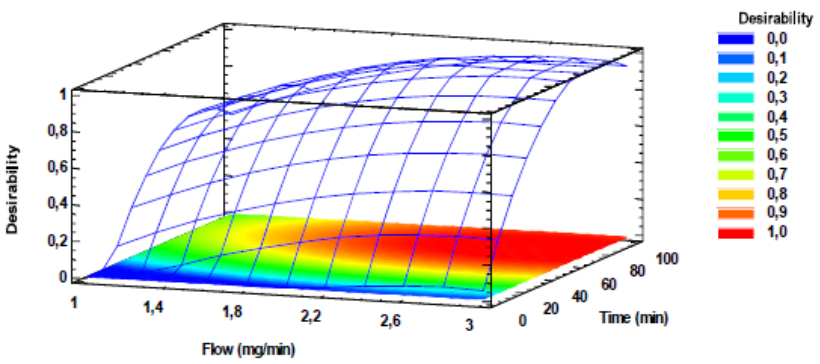

Figure 2. Response Surface 3D plots (desirability function) of SFE extractions from *T. melanosporum*.

The use of oil as trapping matix allowed capturing of higher sterols levels in truffle extracts than the other matrices. The gelatin and oil extracts revealed higher variability and extraction yield as compared to the rest of trapping materials. Independent of the trapping material used, oleic, linoleic and hexadecenoic acid remained the most abundant fatty acids. The difference of compounds compositions can be related with the chemistry of the trapping material, *i.e.* gelatin is only protein-based matrix whereas oil is fatty-based.

Later on, this last method was applied to different truffles species (*Tuber aestium, Tuber indicum and Terfezia claveryi*) with positive results. Ergone was the main sterol in *T. aestivum*, whereas cholesterol and fecosterol were detected in major quantities in *T. claveyi* and *T. indicum*, respectively. Stigmasterol and ergone levels where higher in *Terfezia*, while brassicasterol, ergosta-7,22-dienol, fecosterol, and ergosterol were higher in *Tuber* truffles. In addition, the *T. indicum* extracts were characterized by higher levels of lignoceric, vaccenic and behenic acid. *T. aestivum* and *T. claveryi* showed a similar fatty acid profile, except for linoleic and vaccenic acid, levels of which were higher in *Terfezia* truffle. And the oil extracts were enriched in DHA, behenic acid, hexadecenoic and 14-Z-eicosenoic.

4. Conclusions

The use of supercritical fluids methodology allows for an efficient enrichment of molecules underlying the aromaticity and flavor of different truffle species. The use of trapping material (1mL) in SFE enhanced some key truffle molecules capture, indicating a new methodology to obtain naturally truffle products. Similar tendency was shown in the rest of truffle species, in that oleic and linoleic, compounds related with aroma pathways, were found in high levels. In conclusion, we found a potential method to naturally extract flavoring molecules from truffles.

References

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