Truffle flavored commercial products veracity and sensory analysis from truffle and non-truffle consumers

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Abstract

The price of the truffle species *Tuber melanosporum* and *Tuber magnatum* can be up to fifty times higher, or even more, than the cheapest edible truffle species due to their appreciated aroma and low production levels. This aroma is seriously affected after the application of treatments for the conservation of food products, usually thermal (freezing or sterilization). Hence, many of the truffled products that are retailed are characterized by the use of truffle species of low economic value and the addition of flavoring substances. Most of the time, the added flavorings do not mimic fresh truffle aroma and do not correspond to the truffle species appearing in the ingredients list and the statement of identity. These products sometimes include pictures of truffles or the term 'white or black truffle' in the label, which might confuse the consumer. To study this practice in the food industry, 51 products were evaluated through instrumental techniques determining truffle species presence by microscopy and molecular techniques, as well as the level of truffle flavorings added by HS-GC-MS analysis and by sensory perception scale. Finally, a sensory analysis of eight products was carried out by consumers distributed into two groups, those who had previously tasted fresh truffles and those who had not. Lower-value truffle species such as *Tuber aestivum* and *Tuber indicum* were frequently found in products in which the labeling did not indicate so. Also, 48% of the products contained high levels of added flavorings. In the sensory analysis, non-consumers of truffles rated flavored products more positively (up to 2 more points in some products) than truffle consumers. Also, this group associated negative attributes (weird and disappointing) to products elaborated with real black truffle, whereas truffle consumers associated positive attributes (truffle flavor, truffle smell and gourmet) to them.

1. Introduction

In recent years, the number of truffled products has increased worldwide, especially in the major truffle-producing countries: Spain, Italy and France (Oliach et al., 2021). By adding truffle, the food industry increases the added value of various products such as cheese, pâté, pasta, pizzas, sauces or oils, giving them truffle attributes as luxury and gourmet (Torregiani et al., 2017; Wernig et al., 2018). Generally, when consumers speak of truffled product, they refer to the species *Tuber melanosporum* (black truffle) or *Tuber magnatum* (white truffle), due to their unique aroma and high economic value (Campo et al., 2018; Khalifa et al., 2019; Lee et al., 2020; Patel et al., 2017). Nevertheless, there is a certain tendency within the food industry to add lower-value truffle species with morphological similarities, such as *Tuber indicum* and *Tuber aestivum* for black-truffled products, or *Tuber borchii* for white-truffled products (Oliach et al., 2021).

Truffle aroma is a complex mixture of many different aromatic volatile compounds (VOCs). Among them sulfur compounds, such as dimethyl disulphide (DMDS) and dimethyl sulphide (DMS), are the most relevant (Costa et al., 2015; Culleré et al., 2010; Culleré et al., 2013; Tejedor-Calvo et al., 2021). However, food processing or preservation technologies can dramatically change or reduce the complexity of this aroma profile (Campo et al., 2017). To compensate the aromatic loss, 2,4-dithiapentane (bis-(methylthio)-methane or BMTM) is commonly used as truffle flavoring (Torregiani et al., 2017). This compound is characteristic of the white truffle aroma, but it is not present in the black truffle (Fiecchi et al.,1967). Other than the BMTM molecule, a mixture of DMS and 2-methyl-butanal (2-MB) is also used in black truffle products as a new formula to replicate black truffle aroma (Talou et al., 2011).

In Europe, all the flavorings to be used in food and food products are regulated by the Regulation (EU) Nº 872/2012, and their labeling is regulated by the Regulation (EC) Nº

1334/2008. According to these, the European Food Safety Authority (EFSA) defines ‘natural flavoring substance’ as one that is obtained by appropriate physical, enzymatic or microbiological processes from materials of vegetable, animal or microbiological origin, and correspond to substances that are naturally present and have been identified in nature. The Food and Drug Administration (FDA, EEUU) applies a very similar definition. Moreover, according to that Regulations, when labeling a flavoring as natural the source of the flavoring should be labeled.

Nowadays there is no international legislation regulating the commercialization of truffles and truffled products, although UNECE has published a non-mandatory standard for the marketing and commercial quality control of truffles (Unece Standard FFV-53, United Nations, 2017). This recommendation only classifies truffles morphologically and by weight, and associates the scientific name of the different truffle species with their common names. The major truffle-producing countries have their own specific regulations (Table 1). France has the most rigorous legislation, being the only country that regulates the term 'Truffle', 'Truffle juice' and 'Aromatized truffle juice' referred to food products and associates scientific names with common names. The legislation of Italy indicates which types of companies can manufacture truffled products and includes a list of species allowed to be processed, with their common names. Finally, Spain has a general legislation for mushrooms that only includes a list of truffle species allowed.

The lack of clear regulations and consensus on the manufacturing and labeling of truffled products allows that nowadays the ‘truffle’/‘truffled’ denomination and the images of highly-prized truffle species can be found in any label despite the truffle species used in the truffled product or the presence of flavoring substances. This ‘regulatory gap’ causes confusion to consumers, depreciates this highly prized product and has a strong negative impact on truffle producers.

Therefore, in this study we examined 51 marketed truffled products to detect and identify which truffle species and flavoring they included, by using four different techniques (microscopy, molecular techniques, VOCs and sensory analysis). We contrasted these results with the information offered in the label in order to detect potential frauds. Besides, 80 consumers evaluated a selection of eight commercial truffled products by sensory analysis with the purpose of determining what perception consumers have about these products.

2. Materials and methods

2.1 Truffled products selection

A total of 51 truffle products were selected from local supermarkets and specialty shops in Spain, many of them fat-based products such as oil, sauce or pâté (Tables 2, 3). Other products, such as condiments and prepared foods, were chosen because of their growing interest for gastronomy professionals and consumers. Truffle products were divided into six different groups: sauces, oils, meat products, condiments, truffles, and ready to eat food (RTE). Before analyzing them, the label information was properly examined and classified.

2.2 Truffle species determination

According to Riousset et al. (2001), the fruitbodies of most marketed truffle species can be distinguished by their spore morphology. Besides, specific primers have been developed to unambiguously identify them by molecular techniques (Mello et al., 2006). Furthermore, VOCs analysis is a potential technique to detect target compounds and distinguish between truffle species (Culleré et al., 2010, 2013). Based on these premises, we determined the truffle species present in truffled products following three complementary techniques: microscopy, molecular analysis and VOCs analysis.

2.2.1 Microscopy analysis

Firstly, a sample of each truffled product (0.5 g) was homogenized in 1 mL of distillate water using a pestle, and 1 µl was mounted on slides and observed under a light microscope (Primo Star Zeiss) at different magnifications (100X and 400X). Several images of spores from different fields were captured with a camera (Nikon eclipse E400) connected to a computer. The morphological characteristics of *Tuber* spores were compared with the description of Montecchi & Sarasini (2000) and Riousset et al. (2001).

2.2.2 Molecular analysis

Truffled products samples (0.5 g) were submitted to DNA extraction following REDExtract-N-AmpTM Plant PCR Kit (Sigma, Missouri, USA). Specific primers pairs used were MELF-MELR for *T. melanosporum*, UNCI-UNCII for *T. aestivum* (Mello et al., 2006), ITSB-ITS4LNG for *Tuber brumale* (Paolocci et al., 1999), and ITSCHCH-ITS4LNG for *T. indicum* (Paolocci et al., 1999). The cycling conditions were: 94ºC – 5 min; (94ºC – 30 seconds, 60ºC – 30 seconds, 72ºC – 45 seconds) x 35 cycles; 72ºC – 7 min for *T. melanosporum* and *T. aestivum*, and 94ºC – 5 min; (94ºC – 30 seconds, 62ºC – 30 seconds, 72ºC – 45 seconds) x 35 cycles; 72ºC – 7 min for *T. brumale* and *T. indicum* (Douet et al., 2004; Mello et al., 2006; Paolocci et al., 1999) .

The amplification reaction was prepared according to previous studies with modifications (Douet et al., 2004; Mello et al., 2006; Paolocci et al., 1999). The content for 25 µL as final volume was: 12 µL of sterile double distilled water, 1 µL of each primer, 1 µL of BSA (bovine serum albumin), 2.5 µL of Taq free DNA polymerase (Invitrogen, California, USA), 5 µL of PCR reaction buffer including dNTP and $MgCl_2$ (Invitrogen), and 2.5 µL of template DNA. PCR was performed on MyCycler Thermal Cycler (Bio-Rad, Hercules, CA, USA) using the above-mentioned amplification conditions. Samples were kept at 4 ºC until their revealed by electrophoresis. For that, 1.5% agarose gel was performed with 30 mL of TAE buffer (Buffer Tris, Acetic Acid, EDTA) and 0.8 µL of SYBR Safe DNA gel stain (Invitrogen, USA); 100 mV of current was used from an electrophoresis source BioRad PowerPac HV (BioRad, California, USA). Band revelation was carried out in a transilluminator (Chemidoc XRS + BioRad, USA) with GeneSys software (Syngene, Cambridge, United Kingdom).

2.2.3 VOCs analysis

The HS-GC-MS was carried out following Caboni et al. (2020) methodology. For that, samples (4 g) were placed in 20 mL vials mixed with 1µL of fluorobenzene as internal standard and were hermetically closed. Afterwards, they were heated at 120 ºC for 15 min and 1 min of pressurization time. The injection was carried out for 6 s at 20 psi with an inlet temperature of 220 ºC. Further analysis was carried out on a Clarus 500 GC system coupled to a MS (PerkinElmer, Massachusetts, USA). GC was carried out using a DB-Wax capillary column (60m x 0.25mm i.d.x 0.25 µm film thickness) (Agilent Technologies, California, USA) and a flow of 1 mL/min with helium as a carrier gas. The oven temperature was 45 ºC held for 2 min, 45-200 ºC at a rate of 4 ºC/min, and finally to 225 ºC at 10 ºC/min, and held for 5 min. The MS used the electron impact (EI) mode with an ionization potential of 70 eV and an ion source temperature of 200ºC. The interface temperature was 220ºC. The MS scanning was recorded in full scan mode (35-250 m/z). A TurboMass ver. 5.4.2 software was used for controlling the GC-MS system. Peak identification of BMTM was achieved by comparison of the mass spectra with mass spectral data from the NIST MS Search Program 2.0 library and by comparison of previously reported Retention Index (RI) with those calculated using an n-alkane series (C_6-C_{20}) under the same analysis conditions. Semiquantification was done by integrating the area of one ion characteristic of each compound and normalization by dividing the data with the internal standard.

2.3 Added truffle flavoring evaluation

The truffle flavoring addition was evaluated by two techniques: headspace gas chromatography (HS-GC-MS) (see section 2.2.3) and sensory evaluation by trained experts. These techniques, instrumental and hedonic respectively, are complementary for the determination of flavoring addition. A panel of six truffle experts was previously trained by testing different concentrations of BMTM to evaluate the addition of this molecule. For this purpose, a four-level rating scale were used to evaluate it: 0—no BMTM odor; 1—slight odor; 2—medium odor 3—strong odor.

2.4 Labeling analysis

According to the regulation of food information provided to consumers (Regulation No 1169/2011), the information on the front labeling (images included) and the list of ingredients (species and flavoring) were retrieved to be analyzed (Table 3). The truffle species depicted in the packaging images were identified by the gleba and peridium aspect, establishing that images of smooth and cream-colored peridium and light-colored gleba tried to represent *T. magnatum*; those of rough and black peridium and light-colored gleba, *T. aestivum*; and those of rough and black peridium and black gleba, *T. melanosporum*.

2.5 Sensory analysis

Among the 51 truffled products studied, eight were selected for a CATA (Check that all apply) test. The analyses were conducted according to the ISO 11035:1994 (Sensory analysis – identification and selection of descriptors for establishing a sensory profile by a multidimensional approach). A total of 80 participants contributed to the research by testing 5 products each to avoid feeling overwhelmed. A three-scale hedonic pretest was rated with a nine-point rating scale: (1) I don't like it – I like it (if consumer liked the product in general); (2) artificial product – natural product (if consumer considered that truffled product contain truffle in artificial or natural form; consumers tried to avoid the attributes of food processing, and only evaluated those of the truffle); and (3) without truffle – with truffle (if consumer detected truffle in the samples tested, it could be by sight, smell or flavor). In addition, consumers selected whether or not the product was related to any of the attributes on the list (truffle flavor, truffle aroma, natural, artificial, chemical, weird, astringent, metallic, disappointment, novel, gourmet, tasty, surprising, pleasant and mushroom) previously selected by the panel of six truffle experts. The tasters were previously trained for three sessions of 45 min following the ISO 8586: 2012 (Sensory analysis – General guidelines for the selection, training and monitoring of selected assessors and expert sensory assessors).

2.6 Statistical analysis

For the sensory analysis a Cochran's Q test (Parente, Manzoni, & Ares, 2011) was performed separately on data from each ballot version in order to identify significant differences between samples for each of the terms included in the CATA question. For the statistical analyses of the CATA test, the Consumercheck program (version 2.2.0, University of Life Sciences, Norway) was used. The statistical analyses of VOCs were performed using XLStat 2009 (Addinsoft, Paris, France) and R language.

3. Results and discussion

3.1 Identification of truffle species added as ingredient

The fruitbodies of truffle species can be identified with different techniques (Creydt & Fischer, 2022; Mabru et al., 2004; Schelm et al., 2020; Segelke, Schelm, Ahlers, & Fischer, 2020). Among them morphological spore identification, PCR (Table 3) and VOCs analysis (Fig. 1) were used to cross-check the results and confirm the usefulness of each technique.

Spore determination by microscopy was only effective for half of the products. Among them, *T. aestivum* was detected in 17 samples, whereas *T. indicum* in 4 and *T. melanosporum* in 8. DNA amplification only worked in 22 samples: 8 were identified as *T. aestivum*, 5 as *T. indicum*, and 9 as *T. melanosporum.* In total, only in 12 truffled products (O1, M1, M9, C2, C3, C4, C5, C7, T1, T2, T6 and R5) matched the spore analysis and DNA amplification, and inconclusive results were obtained for sauces and oils, as well as some RTE products.

This could be due to the low amount or absence of truffle content, or because of high degree of grinding to homogenize these products. Moreover, there was no DNA amplification for most of the samples, preventing identification of truffle species, probably due to the stabilization treatments applied to these products to ensure their sanitary suitability and to be stored at room temperature, since high temperature and low pH are the most important factors for DNA breakdown (Gryson, 2010). On the other hand, the presence of truffles was ascertained in all the samples of the condiments group and the truffles group, except for the truffle spherification product. Using microscopy and PCR techniques together, *T. melanosporum*, *T. aestivum* and *T. indicum* were identified, whereas *T. magnatum* was not detected in any product.

Among all these techniques, the DNA extraction is the most frequently used to distinguish truffle species. There are several reports successfully applying molecular techniques for evaluating marketed truffle products, but mostly for non-cooked products (Amicucci, Guidi, Zambonelli, Potenza, & Stocchi, 2002; Mabru et al., 2001). Rizzello et al. (2012) showed the repeatability issues of conventional and quantitative PCR when working with processed butter and cream products, due to the patchy structure. Despite this, with the support of microscopy they were able to detect fraudulent practices in these products.

According to Culleré et al. (2013), the C8 compounds family (octanal, 3-octanol and 1-octen-3-ol) is remarkable in *T. indicum* aromatic profile, whereas sulfur compounds such as DMS and DMDS are key aromatic compounds in *T. melanosporum* and *T. aestivum* (Culleré et al., 2010). Besides, black truffle emits mostly 3-ethyl-5- methylphenol, 5-methyl-2-propylphenol, β-phenylethanol and 3-ethylphenol, whereas summer truffle, methional, 3-methyl-1-butanol, 1-hexen-3-one and 3-ethylphenol (Culleré et al., 2010). Thus, each truffle species has its own VOCs pattern that might be useful for identifying ingredients in truffled products. It must be taken into account that black truffle aroma can be modified by different preservation methods (freeze-drying, hot-air drying, freezing and canning). Some molecules were selected as potential markers for preservation methods such as 2-acetylpyrroline for freeze-drying and hot air drying, and Z-1,5-octadien-3-one for freezing (Campo et al., 2017).

A total of 97 VOCs were detected in truffle products (Fig.1). Among them, propanone, 1-methylpropyl formate, 2,3-butadienone and bis(methylthio)pentane were found in most of the samples in high content. The VOCs analysis in truffled products revealed clear compounds patterns in some products, which showed similar abundance of acids, alcohols, aromatic compounds, esters, heterocyclic compounds and hydrocarbons. The samples S3, O1, M1, C7, T2 and T6 followed a clearly common pattern, with presence of key truffle aromatic compounds (hexanoic acids, 2-mehtyl-propanol, 2-mehyl-1-butanol, methyl-propanal among others). This suggests the presence of a complex aromatic ingredient, as truffle, in these products. While showing fingerprints similar to the other four, the profile of samples O1 and T6 contained a higher number of compounds, suggesting the presence of a different truffle species. Some molecules (3-methyl-1-butanal, 1-propylformate, propanone, 2-butanone and 2,3-butanodione) were present in all samples, indicating that they could be part of the food matrix and could not be used as identification markers of truffle species.

Samples with a clear pattern (S3, O1, M1, C7, T2 and T6) showed high levels of C8 compounds, which were related with *T. indicum* presence (octanal, 3-octanol and 1-octen-3-ol), except for octanol in T6 and 1-octen-3-ol in M1, C7, T2 and T6 products. However, only O1 and T6 samples showed high levels of other molecules such as acetaldehyde, butanal, 2-methyl-1-butanal, hexane, octane, carbon disulfide, methanethiol, dimethyl-sulfide and dimethyl-disulfide, suggesting that these products contained a different truffle species compared to the rest. The microscopy and PCR analysis confirmed the presence of *T. indicum* in O1 and T6 and *T. aestivum* or *T. melanosporum* in the rest of samples with the pattern (S3, M1, C7 and T2).

VOCs analysis allowed to detect different truffle species patterns, similarly to what omics techniques (metabolomics, genomics or proteomics) do. In recent years, omics have been used to distinguish truffle species. The fourier transform near-infrared (FT-NIR) spectroscopy allowed to distinguish 100% of *T. magnatum* and 99% of *T. melanosporum* from the corresponding low-value truffle counterparts, although it only achieved an accuracy of 83% testing Italian vs non-Italian white truffles (Segelke et al., 2020). Recently, a non-targeted lipidomic analysis with mass spectrometry was carried out detecting that only a few marker substances were enough to distinguish both black and white truffle species (Creydt & Fischer, 2022). So far, there are no studies in commercialized truffled products using these techniques, which have already been used to investigate frauds with herbs and spices (Galvin-King, Haughey, & Elliott, 2018), asparagus (Creydt et al., 2022), or beverages (Agrawal et al., 2013).

3.2 Evaluation of aroma addition in truffle products

Truffle products were analyzed by two different methods in order to evaluate the flavorings added. The semiquantification of BMTM, DMS and 2-MB in the samples revealed that in general BMTM levels were higher than levels of the other two compounds studied (Table 2). The added quantities raised up to 10106, 5708 and 968 μg/100g for BMTM, DMS and 2-MB respectively. The highest value of BMTM was for T4 sample, but high values were also detected in all RTE products, sauces (S6), oils (O3, O4, O5) and honey (C4). On the contrary, DMS and 2-MB compounds were in lower levels, indeed only a few products contained levels of these compounds higher than BMTM, *i.e.*: S8, R5. The values in samples with truffle pattern (S3, O1, M1, C7, T2 and T6) raised up 65.7 and 131.5 as maximum in DMS and 2-MB, respectively. Only a few samples (O1, M2 and T1) did not contain BMTM and showed DMS and 2-MB levels beyond these ones.

In general, sauces, oils and RTE were the sample groups with higher doses of flavorings added. These results could be related to aromatic losses in their heat treatments, as expected with sauces, meat products or RTE. In agreement with that, the condiments without heat treatments, showed low BMTM levels in all products studied, except for sample C4. Wernig et al. (2018) reported maximum levels of BMTM in commercial truffle oils around 15000 μg/100g, almost double than our results.

The trained panel evaluated BMTM levels in truffle products (Table 2). Previously, the panelists were trained with a scale of different BTMT dilutions. During these training tests, the judges were not capable to detect BTMT under 0.2 μg/100g, and the minimum concentration detected corresponded to 10 μg/100g (corresponding to 10^{-5} dilution). In agreement with this, the trained panel did not detect the presence of flavorings in samples with 0.1-0.2 μg/100g of BMTM (C1 and C2). In other samples with similar BMTM content (S1, S9) the trained panel evaluated the aroma addition with 1 and 2 scores respectively (Table 2). This difference might be related with the matrix composition and the product humidity, as Whelton & Dietrich (2004) proposed. Their study stablished that these volatile compounds were easily detectable by human nose when the products have high humidity and are warm.

3.3 Evaluation of truffle products labeling

The label information provided in the products (truffle species and flavorings included in the ingredient list, and denomination of the product and photo in the front label) is reported in Table 3. Almost all of them contained the word 'truffle' in the ingredients list, except some oils and meat products. Although the percentage of truffle added was indicated in the label, in many sauces or RTE the truffle fraction is only present as a component of a previous product used as an ingredient. For instance, in sample R4 the labeling indicated: truffle preparation 4.5% (mushrooms, sunflower oil, summer truffle 0.2 % (*Tuber aestivum*), black olives, salt, garlic, parsley, flavor, acidifier: citric acid). In general, the truffle quantity added was low, although some of the products included relatively high amounts (sample S6 with 5% and samples C2, C5, C7, R1 and R6 with approx. 3%). The terms 'with truffle' and 'truffled' were also included, but only in few products the truffle species was properly written.

The aroma addition description in labeling was detected in nearly all the samples. The word 'aroma' could be referred to truffle aroma but also to others usually used in this type of products. Only 5 out 51 products (S1, C1, C4, T3 and R2) listed 'natural aroma' as an ingredient. Despite the legislation related to the term natural flavor described in the introduction section, these products did not disclose the source of flavoring. In no case, "natural flavor" means proceeding directly from the truffle fruitbodies. Other than that, some products included a truffle image even if the labeling did not contain any reference to truffles (*e.g.* sample V8). Most of the images in the label were *T. melanosporum* or *T.*

aestivum, although the images we attributed to the former could also correspond to *T. indicum*, which has a very similar physical aspect.

Our results clearly indicate the existence of a regulatory gap, a lack of a clear regulation that is used by some in the food marketing industry. The actual regulation must be improved to raise transparency for consumers and avoid doubts in truffle products perception. Among the current legislations, the French is the one that more clearly sets out information of truffle requirements in labeling. Anyway, these standards were written a long time ago, and today the food technology and marketing industry for truffled products has grown exponentially.

Nowadays frauds –including substitution, addition, tampering and misrepresentation– in highly value products are mainly related to geographical origin and misleading information, and less frequently to economic reasons. As an example, extra virgin olive oil (Yan, Erasmus, Aguilera Toro, Huang & van Ruth, 2020), beef (Robson, Dean, Brooks, Haughey & Elliott, 2020), milk (Yang et al., 2019), fish (Acutis et al., 2019), or ceviche and sushi (Velez-Zuazo et al., 2021). However, the number of truffled product reports of this practice are very scarce (Rizzello et al., 2012).

3.4 Evaluation of commercial truffled product in non-truffle and truffle consumers

In order to evaluate consumer perception, a selection of eight products was made among the samples studied. Two of them contained black truffle (3 % in C7-jam and 2 % in C3-honey), whereas the rest only contained flavorings. Three contained BMTM (S8- vinegar (61.9 μg/100g), M10-Turkey (2.7 μg/100g), R9-rice cake (172.3 μg/100g), R13-cheese (101.3 μg/100g)) and the other three contained a mixture of DMS and 2-MB (M2-pate (DMS: 25.5 μg/100g; 2-MB: 226.7 μg/100g), R10-chips (DMS: 8.3 μg/100g; 2-MB: 4.2 μg/100g)). Participants ranged in age between 18 and 65 years old and the sex ratio was balanced, with 50.9% females and 49.1% males. The analysis of these products was carried out by consumers distributed into two groups, those who had previously tasted fresh black truffles (61.7%) and those who had not (38.3%). Among them, 3.7% of fresh truffle consumers had never tasted truffled products before, whereas 28.7% of non-consumers of fresh truffles never had. As a result, 13.2 % of the participants in the study had never tasted truffled products before, which is difficult nowadays due to the huge offer of truffled products in the retail market. The truffled products that the remaining participants had tasted before were oil, meat products, cheese and pasta with sauce (around 20% each one), followed by snacks, eggs and honey in lower proportion.

The hedonic results obtained in the CATA test showed slight differences among samples and between consumers groups (Fig. 2). Non-consumers of fresh truffle made a more positive evaluation, up to 2 more points in some products (cheese, chips and rice cake) (Fig. 2-A); this suggests than non-consumers preferred truffles products than fresh truffle. Regarding the rating as artificial or natural (Figure 2-B), the honey and the jam were the most highly rated products by truffle consumers, and the cheese by non-consumers. The high rating of truffled products by non-truffle consumers could be due to the absence of fresh truffle aroma. The truffle consumers pointed the jam as the product with more truffle, however non-consumers of truffles pointed to the chips and the cheese (Figure 2-C).

The consumers selected different attributes, previously picked by a trained panel, and associated them with the products. Afterwards, the attributes were analyzed by Cochran test and those with less percentage score were discarded, such as ‘astringent’, ‘metallic’ and ‘mushroom’. The ‘truffle flavor’, ‘truffle aroma’ and ‘tasty’ attributes obtained the highest percentages, indicating they were representative of the selected products (Table S1). A Correspondence Analysis (CA) was used to explore the possible correlations of the consumers attributes with the preference of the products (liking score) by the two different consumer groups: truffle consumers and non-consumers of truffles (Fig. 3). The CA analysis of truffle consumers explained 75% of the data variability with the two first components. The attribute that showed the more positive loading with the first CA component was 'artificial' whereas those showing the more negative loading was 'natural'. However, the second CA component showed 'tasty' and 'disappointment' as the attributes with the more positive and negative loadings (Fig. 3-A).

The CA analysis for non-consumers of truffles explained 73% of the data variability, however their axes were not as clearly defined as for the CA of truffle consumers. 'Truffle flavor' and 'truffle aroma' versus 'artificial' were the attributes showing the more positives and negative loadings with the first CA component. In the second CA axis, 'chemical' depicts the more positive loadings and 'natural' the most negative loadings (Fig. 3-B). The CA revealed that non-consumers of truffles associated negative attributes ('weird' and 'disappointing') with products containing black truffle, whereas truffle consumers associated positive attributes ('truffle flavor', 'truffle smell' and 'gourmet') to them. This suggests that non-consumers of truffles are familiarized with BMTM as the main odor or truffles products and valued it as the positive one.

Vulnerability to food fraud increases when consumers lack information about the food chain stages. According to Soon, Krzyzaniak, Shuttlewood, Smith, & Jack (2019), one-third of food manufacturers surveyed were victims of food fraud. The agri-food industry needs to be constantly vigilant to protect the integrity of the food supply chain. To date, research has tended to focus on analytical methods to detect food fraud, but control measures such as legislation and powerful food safety management are needed to reduce or avoid this global problem.

4. Conclusions

There is a major conflict in the marketing of truffled products because only 20% were correctly labeled. The main problem detected in truffled products was the truffle species terminology, either it was wrong written or was not same species that the product contained. According to labeling, 73% of the products contained BMTM, however this molecule was detected in 81% of them; this means that there are products with added flavoring that do not disclose it in the labeling. Besides, 22% of the products analyzed used lower-value truffles (*T. aestivum* and *T. indicum*) while their labeling referred to 'truffles' or '*T. melanosporum'*. In general, the techniques used can be useful in order to detect fraud but should be carried out together as supplementary detection methods. The sensory analysis indicated that non-consumers negatively rated the use of fresh black truffle in these products. Therefore, it is necessary to educate and raise consumer awareness, and improve the actual legislation to raise transparency for consumers and avoid doubts in truffle products perception.

Declaration of competing interest

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CRediT authorship contribution statement

Eva Tejedor-Calvo: Conceptualization, Investigation, Methodology, Writing - original draft. **Sergi García-Barreda:** Data curation, Software, Writing - original draft**. María Felices:** Formal analysis, Methodology**. Domingo Blanco:** Supervision, Validation, Visualization. **Sergio Sánchez:** Funding acquisition, Supervision**. Pedro Marco:** Supervision, Validation, Visualization, Writing - review & editing.

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Tables

Table 1. General and specific legal norms referencing truffled products.

Country	Norm	Specifications
Spain	Royal Decree nº 30/2009 establishing the sanitary conditions for the marketing of mushrooms for food use.	Regulates the sanitary quality of truffles intended for human consumption. Includes the list of allowed species that can be marketed fresh and canned: *T. aestivum*, *T. borchii*, *T. brumale*, *T. indicum*, *T. magnatum*, *T. melanosporum*.
France	Decree nº 2012-129 on the marketing of truffles and foodstuffs containing them.	Only allows the word 'Truffle' in products with minimum 3% of *T. melanosporum*, *T. brumale* or *T. magnatum*. Include genus and species in products with more than 1% of other species 'Truffle juice' and 'Aromatized truffle juice' in products with minimum 3% of *T. melanosporum* and *T. brumale*. Includes a list of common names: *T. melanosporum* (Black truffle, Perigord truffle, Perigord black truffle), *T. brumale* (Brumale truffle), and *T. magnatum (*Alba white truffle, Piedmont white truffle).
Italy	Law 752/85 Framework legislation on the collection, cultivation and trade of fresh or preserved truffles for consumption.	Indicates which types of companies can manufacture truffled products and includes a list of species allowed to be processed (with corresponding common name): *T. magnatum* (white truffle), *T. melanosporum* (black truffle), *T. brumale* var. moschatum (muscat truffle), *T. brumale* (black winter truffle or black trifola), *T. aestivum* (summer truffle), *T. aestivum* var. uncinatum (truffle uncinate), *T. borchii* (bianchetto or maruolo), *Tuber macrosporum* (smooth black truffle), *Tuber mesentericum* (ordinary black truffle).
United Nations	Unece Standard FFV-53 concerning the marketing and commercial quality control of Truffles 2017 Edition.	Defines the quality requirements for truffles after preparation and packaging. Classify truffles in three categories, Extra, First and Second, and define the tolerances allowed for each category. *T. melanosporum* (Black Truffle, Périgord Truffle, French Truffle, Périgord Black Truffle), *T. brumale* (Winter Truffle), *T. brumale* var. moschatum (Musky Truffle), *T. indicum*, (Asian Black Truffle), *T. aestivum* (Summer Truffle), *T. mesentericum* (Bagnoli Truffle), *T. aestivum* var. uncinatum (Burgundy Truffle), *T. magnatum* (White Piedmont Truffle), *T. borchii* (Whitish Truffle, Bianchetto Truffle), *T. macrosporum* (Smooth Black Truffle), and *Tuber gibbosum* (Oregon White Truffle).

Table 2. Analysis of flavoring addition in the truffled products with HS-GC-MS.

Code	Product denomination	BMTM (µg/100g)	DMS (µg/100g)	2-MB (µg/100g)	Ratio (DMS/2-MB)	Flavoring detection*
Sauces						
S1	Mayonnaise	0.1	121.1	-	-	1
S2	Sauce	33.8	3.9	11.0	0.4	2
S3	Sauce	-	-	-	-	0
S4	Cream	137.8	2.4	90.6	0.1	3
S5	Sauce	21.6	1.8	6.4	0.3	2
S6	Sauce	8395.7	528.8	23.0	23.0	3
S7	Balsamic vinegar	43.9	0.7	-	-	3
S8	Balsamic vinegar	61.9	5631.7	22.3	252.7	3
S9	Balsamic vinegar	0.1	120.3	-	-	2
Oils						
O1	Olive oil	-	23.0	129.2	0.2	2
O2	Olive oil	-	-	-	-	0
O3	Olive oil	2406.1	1852.0	0.6	3031.7	3
O4	Olive oil	411.7	0.4	7.5	0.1	3
O5	Olive oil	3134.6	46.1	-	-	3
Meat products						
M1	Pork pâté	0.1	1.1	29.4	0.1	0
M2	Duck pâté	-	25.5	226.7	0.1	0
M3	Foie gras	-	0.6	66.0	0.1	0
M4	Foie gras	2.1	5.1	62.1	0.1	2
M5	Duck pâté	-	43.7	58.6	0.7	0
M6	Foie gras	-	10.3	15.4	0.7	0
M7	Turkey	0.6	-	-	-	1
M8	Turkey	0.4	0.7	4.4	0.2	1
M9	Turkey	-	1.5	5.7	0.3	0
M10	Turkey	2.7	-	-	-	1
M11	Meatballs	199.3	5.6	10.9	0.5	2
Condiments and other foods						
C1	Salt	0.1	-	2.3		0
C2	Salt	0.2	-	-	-	0
C3	Honey	-	0.4	7.7	0.1	0
C4	Honey	878.2	109.4	1.0	110.4	3
C5	Honey	1.0	0.6	10.6	0.1	2
C6	Chocolate	-	0.6	18.9	0.1	0
C7	Jam	-	-	2.3	-	0
Truffles						
T1	Truffle in brandy	-	1055.9	120.7	8.7	0
T2	Truffle slices in oil	36.8	-	-	-	2
T3	Truffle spherification	-	10.3	15.4	0.7	0
T4	Canned truffle	10106.7	53.2	956.2	0.1	3
T5	Canned truffle	0.4	46.1	103.5	0.4	1
T6	Canned truffle	-	65.7	131.5	0.5	1

RTE						
R1	Pasta	221.9	15.5	40.5	0.4	2
R2	Fresh pasta	34.4	0.7	31.0	0.1	2
R3	Fresh pasta	-	6.2	29.6	0.2	0
R4	Fresh pasta	7.0	2.2	15.1	0.1	2
R5	Rice	818.0	5708.2	968.9	5.9	3
R6	Croquettes	57.0	1.8	0.1	1860.0	3
R7	Croquettes	151.9	114.5	2.7	42.3	3
R8	Omelette	95.0	98.5	23.9	4.1	3
R9	Rice cakes	172.3	3.1	76.1	0.1	1
R10	Chips	-	8.3	4.2	2.0	0
R11	Cheese	13.8	0.3	0.5	0.6	1
R12	Cheese	9.0	0.1	14.6	0.1	1
R13	Cheese	101.3	1.3	-	-	2

*The presence of added aroma was sensory evaluated by a trained truffle sensory panel.

Aroma addition was punctuated between 0-3 by their intensity.

Table 3. Species determination by spore microscopic analysis and identification by PCR test and label analysis of truffled products.

Code	Spore microcroscopic analysis	Truffle Species identifiction by PCR test	Truffle name reference in the packaging	Truffle species labeled as ingredient	Aroma mention included as ingredient	Truffle Picture included in front label
Sauces						
S1	-	-	With truffle	*T. aestivum* (1%)	Natural aroma	*T. aestivum*
S2	-	-	With truffle	*T. aestivum* (0.003%)*	Aroma	*T. aestivum*
S3	-	*T. aestivum*	With black truffle	*T. aestivum* (1%); *T. melanosporum* (0.1%)	Aroma	*T. melanosporum*
S4	-	*T. aestivum*	With white truffle	*T. magnatum* (1.5%)	Aroma	*T. magnatum*
S5	*T. aestivum*	-	With truffle preparation	*T. aestivum* (0.003%)*	Aroma	*T. aestivum*
S6	*T. aestivum*	-	With truffle	*T. aestivum* (5%)	Aroma	*T. aestivum*
S7	*T. aestivum*	-	Tartufo	Truffle (1%)	Aroma	-
S8	-	-	With black truffle	-	Aroma	*T. aestivum*
S9	-	-	With black truffle aroma	-	Aroma	-
Oils						
O1	*T. indicum*	*T. indicum*	With black truffle oil	*T. indicum*	Black truffle aroma	*T. melanosporum*
O2	-	-	Black truffle aroma	-	Black truffle aroma	*T. melanosporum*
O3	*T. aestivum*	-	With white truffle	-	White truffle aroma	-
O4	-	-	With black truffle (*Tuber melanosporum*)	-	Black truffle aroma	-
O5	-	-	With White truffle aroma (*Tuber magnatum*)	-	White truffle aroma	*T. magnatum*
Meat						
M1	*T. indicum*	*T. indicum*	Truffled	*T. indicum* (1.2%)	Black truffle aroma	-
M2	-	-	With truffled oil	-	Truffle aroma	-
M3	*T. aestivum*	-	With truffle	Truffle	-	-
M4	-	-	With truffle	-	Truffle aroma	-
M5	-	-	With truffle	*T. indicum* (0.7%)	Black truffle aroma	-
M6	T. melanosporum	-	Truffled	*T. melanosporum*	-	-
M7	-	-	Truffled	-	Aroma	-
M8	-	*T. melanosporum*	Truffled	-	Aroma	-
M9	*T. melanosporum*	*T. melanosporum*	Truffled	-	Aroma	-
M10	*T. indicum*	-	With truffle	Truffle (0.5%)	Aroma	-
M11	*T. aestivum*	-	With truffle	Truffle (0.005%)*	Aroma	Unidentified
Condiments and other foods						

C1	*T. melanosporum*	*T. indicum*	With truffle	Truffle powder (0.6%)	Truffle aroma naturel	*T. melanosporum*
C2	*T. melanosporum*	*T. melanosporum*	With black truffle	*T. melanosporum* (3%)	-	-
C3	*T. melanosporum*	*T. melanosporum*	With black truffle	*T. melanosporum* (2%)	-	-
C4	*T. aestivum*	*T. aestivum*	With truffle	*T. aestivum* (0.5%)	Truffle aroma naturel	-
C5	*T. melanosporum*	*T. melanosporum*	Truffled	Truffle (3%)	Aroma	-
C6	*T. aestivum*	*T. melanosporum*	With black truffle	*T. melanosporum*	-	-
C7	*T. melanosporum*	*T. melanosporum*	With truffle	*T. melanosporum* (3%)	-	-
Truffles						
T1	*T. aestivum*	*T. aestivum*	Truffle	*T. melanosporum* (>50%)	-	-
T2	*T. aestivum*	*T. aestivum*	Truffle	*T. aestivum* (>50%)	-	-
T3	-	-	Black truffle (*T. melanosporum*)	*T. melanosporum* (50%)	Truffle aroma naturel	-
T4	-	*T. aestivum*	Truffle	*T. aestivum* (>50%)	-	*T. aestivum*
T5	-	*T. indicum*	Black truffle	*T. indicum* (>50%)	-	Unidentified
T6	*T. indicum*	*T. indicum*	Truffle	*T. aestivum* (>50%)	-	*T. melanosporum*
RTE						
R1	-	*T. aestivum*	With truffle	*T. aestivum* (3%)	Aroma	*T. melanosporum*
R2	*T. aestivum*	-	With truffle	*T. aestivum* (0.06%)	Aroma naturel	*T. aestivum*
R3	*T. aestivum*	-	With truffle preparation	*T. aestivum* (0.01%)*	Aroma	*T. aestivum*
R4	-	-	With truffle	*T. aestivum* (0.01%)*	Aroma	*T. aestivum*
R5	*T. melanosporum*	*T. melanosporum*	With black truffle (*T. melanosporum*)	*T. melanosporum*	-	-
R6	*T. aestivum*	*T. aestivum*	With tuflle	*T. aestivum* (3.2%)	Aroma	*T. melanosporum*
R7	-	-	With tuflle	*T. aestivum* + *T. indicum* (1%)	-	*T. melanosporum*
R8	-	-	With tuflle	*T. aestivum* (0.021%)*	Aroma	*T. melanosporum*
R9	-	-	With truffle	Truffle	Truffle aroma	*T. melanosporum*
R10	-	-	With black truffle flavor	Truffle (0.1%)	Black truffle aroma	*T. melanosporum*
R11	*T. aestivum*	-	With truffle	*T. aestivum* (0.0007%)*	Aroma	*T. aestivum*
R12	*T. aestivum*	-	With truffle	Truffle	-	*T. aestivum*
R13	*T. aestivum*	*T. melanosporum*	-	Truffles (1.6%)	Aroma	-

* The percentage of truffle added was calculate with in those products with truffle fraction included belong to a previous product used as an ingredient

Figures

Figure 1. Heat map of VOCs detected in truffled products by HS-GC-MS. Product names correspond to codes reported in Table 3. Colours ranged from white to green (up to 0.1 mg/100g) and green to orange (up to 1 mg/100g). The compounds are grouped in functional groups as indicated in the right side of the figure.

Figure 2. Hedonic-CATA test results of truffle consumers (red) and non-truffle consumers (black). Answers correspond to A) I don’t like it (1) - I like it (9), B) artificial product (1) - natural product (9), C) without truffle (1)- with truffle (9). The scores range from 1 to 9.

Figure 3. Bi-plot from CA of significant attributes for 8 truffled product samples. Black circles correspond to sensory attributes and red triangles to truffled products samples. The blue circle marked the close sensory attributes to those products with fresh truffle (honey and jam).


Acetic acid
Propanoic acid
2-Methylpropanoic acid
Butanoic acid
4-Hydroxybutanoic acid
2-Methylbutanoic acid
Hexanoic acid
Ethanol
2-Butanol
1-Propanol
2-Methylpropanol
2-Pentanol
1-Butanol
1-Penten-3-ol
2-Methyl-1-butanol
3-Methyl-1-butanol
2-Pentylfuran
2-Hexanol
1-Pentanol
1-Hexanol
1-Octanol
1-Octen-3-ol
3-Octanol
3-Methylhexanol
1-Heptenol
1-Dodecanol
Acetaldehyde
Propanal
Methyl propanal
Butanal
2-Methyl-1-butanal
3-Methyl-1-butanal
Pentanal
Hexanal
Heptanal
Octanal
(E)-2-Heptenal
Nonanal
2-(E)-Octenal
2-Nonenal
(E)-2-Nonenal
2,4-Decadienal
Tetradecanal
Anisol
3-Methylanisole
Furfural
Benzaldheyde
4-(2-Butyl)phenol
Benzeneacetaldehyde
3-Methoxyanisole
Benzeneacetic acid, methyl ester
3,4-Dimethoxytoluene
3,4,5-Trimethoxytoluene
Methyl isobutyrate
1-Metylpropyl formate
Ethyl 2-methylbutanoate
Ethyl 3-methylbutanoate
Isobutyl isobutyrate
Isoamyl isobutanoate
sobutyl 2-methylbutanoate
Methyl caproate
Methyl 2-hydroxypropanoate
Methyl 3-hydroxybutanoate
2-Ethylfurane
(E)-2-Pentenylfuran
2-Methylpyrazine
2-Hexylfuran
2-Furanmethanol
Pentane
Hexane
Heptane
Octane
2,3-Dimeyhl, 2-butene
4-Methyl-1-pentene
2,3,6-Trimethyl-4- octene
2,6,11-Trimethyldodecane
Propanone
2-Butanone
2,3-Butanedione
2-Pentanone
2,3-Pentanedione
3-Penten-2-one
2-Heptanone
2-Octanone
2-Hydroxy-3-butanone
Hydroxypropanone
2-Nonen-4-one
2-Octen-4-one
2-Decanone
1-Propen-2-ol acetate
Methyl acetate
Carbon disulfide
Methanethiol
Dimethyl sulfide
Dimethyl disulfide
Bis(methylthio)mentane
Acids
Alcohol
Aldehyde
Aromatic compounds
Ester
Heterocyclic
Hydrocarbon
Ketone
Salt
Sulfur-containing
0
0.1
1

Figure 1

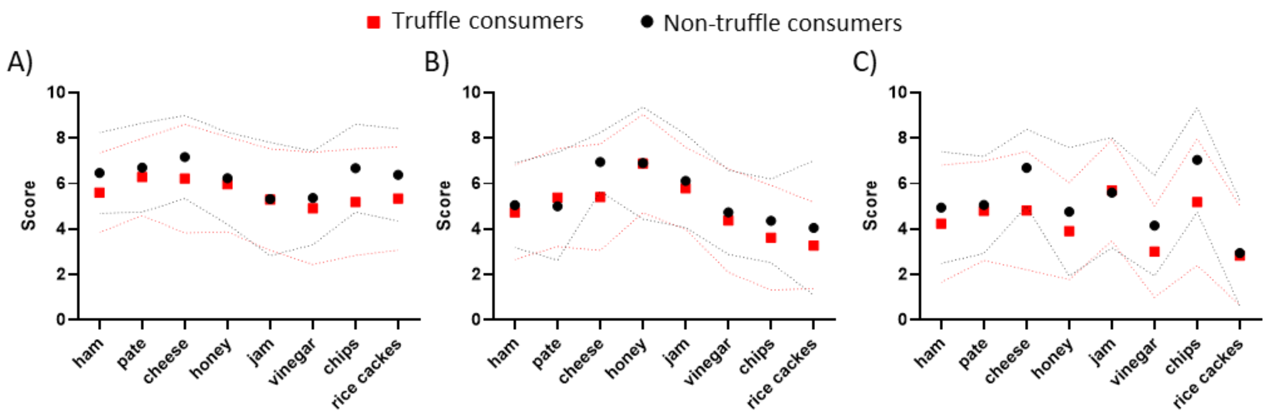

Figure 2

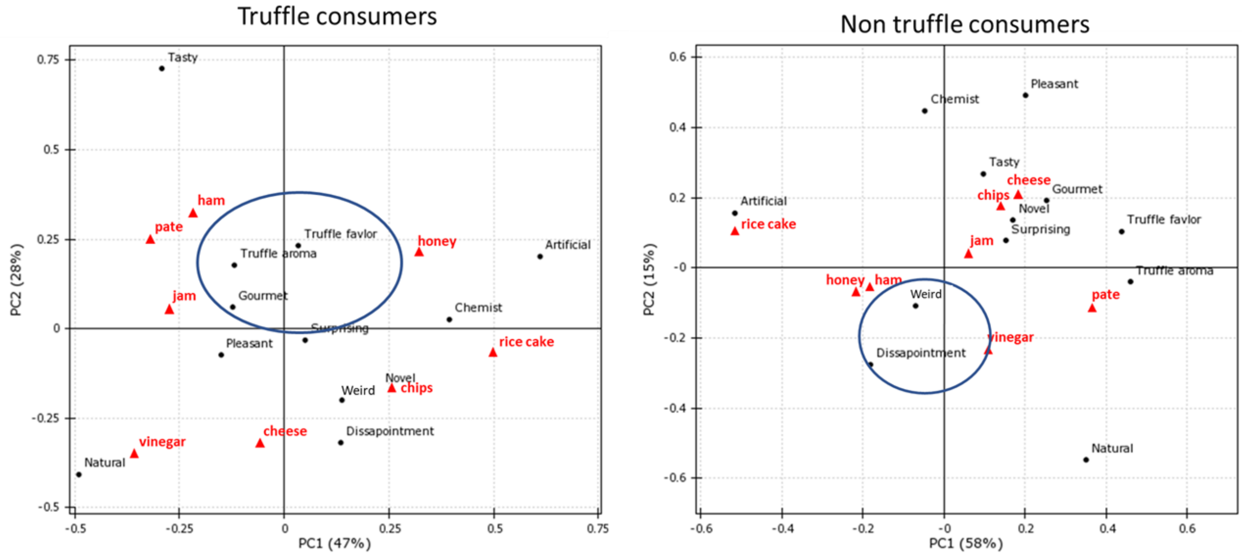

Figure 3