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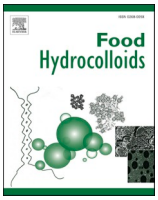

Potential of a smart gelatine hydrogel-based package to extend fresh black truffle (*Tuber melanosporum*) shelf-life preserving its aroma profile

Eva Tejedor-Calvo [a], Víctor Baquero-Aznar [a,c], Sara Vega-Diez [a,c], María Luisa Salvador [c,*], María Ángeles Sanz [b], Sergio Sánchez [a,c], Pedro Marco [a], Sergi García-Barreda [a,c], Jaime González-Buesa [a,c]

[a] Departamento de Ciencia Vegetal, Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA), Instituto Agroalimentario de Aragón - IA2 (CITA-Universidad de Zaragoza), Av. Montañana 930, 50059, Zaragoza, Spain
[b] Laboratories and Technological Assistance, Agrifood Research and Technology Centre of Aragon (CITA), Avda. Montañana, 50059, Zaragoza, Spain
[c] Grupo de Investigación en Alimentos de Origen Vegetal, Instituto Agroalimentario de Aragón-IA2-(Universidad de Zaragoza-CITA), Miguel Servet 177, 50013, Zaragoza, Spain

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ABSTRACT

Many post-harvest technologies have been applied to prolong the shelf life of black truffles; however, most of them negatively modify the genuine fresh truffle aroma. A novel edible gelatine hydrogel-based package (GHP) was tested to extend the shelf life of truffles and trap the aromatic compounds released by fresh truffles. First, the key physicochemical properties and microbiological and sensory attributes of the materials were characterised. The aromatic profile (by gas chromatography and a trained panel) and microbiological populations were monitored for 35 days in truffles stored in GHP and gelatine. Truffle preservation in GHP was compared to that in microperforated modified atmosphere packages (MAP) and that in macroperforated packages (C). The gelatine hydrogel exhibited extraordinarily high oxygen permeability and maintained a low microorganism load during storage (<4 log CFU·g^{-1} after 28 days). Some key volatile organic compounds were detected in the gelatine after seven days. Gelatine from GHP reduced microbial growth in truffles compared to the C and MAP conditions at day 21. Firmness loss at day 28 was an indicator of spoilage behaviour; therefore, 21 days was selected as the shelf-life extension of truffles under GHP, obtaining fresh truffles with high quality and an edible gelatine hydrogel with truffle aroma.

1. Introduction

Truffles are gourmet mushrooms that are appreciated worldwide for their unique aromatic properties. The truffle aromatic profile is a complex mixture composed of more than 300 volatile organic compounds (VOCs) identified in 11 truffle species, approximately 30 of which exhibit aromatic properties (Culleré et al., 2010; Tejedor-Calvo et al., 2023a). However, truffles are extremely perishable due to their high respiration rate, dehydration, development of superficial mycelia, and damage by insect larvae (Rivera, Venturini, Oria, & Blanco, 2011). Given their short shelf life of approximately 7–10 days, several preservation technologies have been studied (Phong, Dykes, & Payne, 2022; Reale et al., 2009; Rivera, Blanco, Salvador, & Venturini, 2010a; Savini et al., 2020; Tejedor-Calvo et al., 2020). Long-term preservation technologies (canning, hot air-drying, freezing, or freeze-drying) make it possible to meet the demand for truffles throughout the year, but their application has a negative impact on the quality of fresh truffles (Campo, Marco, Oria, Blanco, & Venturini, 2017). In contrast, modified atmosphere packaging (MAP) may extend the shelf life of fresh truffles to only 21 and 28 days for *Tuber melanosporum* and *Tuber aestivum*, respectively, but enables good scores for aroma and flavour, maintains a typical hard texture, reduces weight loss, and delays mycelial growth (Rivera et al., 2010a). The application of edible coatings with active ingredients may also extend the shelf life of fresh truffles, as this treatment has a positive impact in halting or postponing the changes of truffle aroma and bacteria community profile during storage (Choo, Bollen, Ravensdale, Dykes, & Coorey, 2021).

Edible coatings are thin layers lower than 25 μm (Priya, Thirunavookarasu, & Chidanand, 2023) which can be applied directly to the surfaces of food products by different technologies (Hanani, Roos, &

* Corresponding author.
E-mail address: mlsalva@unizar.es (M.L. Salvador).

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Kerry, 2014), acting as a protective barrier during the storage of food products (Blancas-Benitez et al., 2022). These coatings should be colourless, odourless, and tasteless to maintain the original appearance and flavour of the fresh product and should provide easy application, good adhesiveness, and fast drying with uniform thickness. Coatings thicker than 50 μm are considered as edible films/sheets (Priya et al., 2023), and could be used over the food as wrapping or between food components for separation (Hanani et al., 2014). Edible packaging is at the forefront of food packaging due to the wide range of materials it can be based on (primarily polysaccharides, proteins or lipids), and its faculty to prolong storage time while maintaining the safety and quality of a wide range of foods (Iversen et al., 2022).

Hydrocolloids have demonstrated their usefulness as matrices of packaging materials or edible coatings (Jiménez, Requena, Vargas, Atarés, & Chiralt, 2018) and their use is increasing year by year (Waraczewski, Muszyński, & Sołowiej, 2022; Yemenicioğlu, Farris, Turkyilmaz, & Gulec, 2020), even though the main use of hydrocolloids is as a thickening and gelling agent in many food formulations (Saha & Bhattacharya, 2010). Among the wide range of hydrocolloids, gelatine has been widely used for preparing edible films and coatings in different products owing to its ability to extend the shelf life and prevent the deterioration of fresh products, such as apples (Mannucci et al., 2017) and strawberries (Temiz & Özdemir, 2021). Gelatine is biodegradable, foaming, emulsifying, and gelling, and has good filmogenic properties (Gómez-Guillén, Giménez, López-Caballero, & Montero, 2011). The different amino acid composition of gelatines from mammalian, poultry, or fish sources and the way in which the collagen polypeptide chains are broken during the different hydrolysis processes (acidic, basic, enzymatic, ultrasonic-assisted, or high-pressure extraction) influence technological properties of gelatines (viscosity, bloom strength and melting temperature, among others), (Rather et al., 2022).

Because the truffle aroma is highly appreciated, there are different strategies to extract and preserve these aromatic compounds. Truffle aroma can be trapped in food matrices such as oil, honey, agar, and gelatine (Tejedor-Calvo et al., 2023a). The presence of disulfide bridges in gelatine may retain key truffle-flavouring compounds, as suggested by Tejedor-Calvo, Marco, Spègel, and Soler-Rivas (2023b). Thus, the creation of a thick layer of gelatine hydrogel around the product could be useful in two ways: (1) to provide an effective barrier limiting gas and vapour exchange, extending the shelf life of the fresh truffle and (2) to retain the aroma compounds of the truffle in the thick edible coating to obtain a new product with potential culinary applications. To the best of our knowledge, the use of hydrocolloids to create a fresh (non-dried) thick coating with the capacity to absorb truffle aroma compounds while concurrently restricting gas and vapour exchange, thereby prolonging the shelf life of fresh truffles, has not yet been explored.

Therefore, the objectives of this study were: i) to verify the potential of a thick edible coating based on gelatine retaining the key aroma compounds of fresh truffles throughout refrigerated storage, creating a new gelatine hydrogel-based package (GHP) with interesting culinary applications; ii) to assess the shelf-life extension of fresh truffle stored in this novel package; and iii) to characterise the GHP material through its gas exchange properties, microbiological aspects and sensory attributes. To achieve this, microbiological and sensory studies were conducted over a 35-day period on fresh black truffles (*T. melanosporum*) stored in GHP and on the gelatine of the GHP. For comparative purposes, truffles stored in microperforated modified atmosphere packages (MAP) and macroperforated packages (C) were also analysed.

2. Materials and methods

2.1. Truffles

Tuber melanosporum ascocarps were harvested from the Moncayo forests (Zaragoza, Spain). Fresh truffles were identified and processed as described by Rivera et al. (2011). For this study, round-shaped truffle ascocarps, weighting around 15 g (average: 14.7 ± 3.5 g) and with no external damage were selected.

2.2. Packages preparation and experimental design

The truffles were packaged under three different conditions. A visual depiction of the packaging options developed in this study is shown in Fig. 1. For GHP packages, porcine gelatine powder with a 180-bloom strength (Instangel, Sosa Ingredients S.L., Barcelona, Spain) was used to prepare a hydrogel from a solution of 144 $g{\cdot}L^{-1}$ of gelatine in distilled water. The mixture was heated up to 75 °C (similar to pasteurisation process) under magnetic stirring for 3 min (RCT basic, IKA, Staufen, Germany). After cooling down to 40 °C, the hydrogel was poured onto 125 mL polypropylene cups until filled, forming a thick coating around each truffle (arranged one per cup). Once they had cooled to 4 °C and the hydrogel had jellified, the packages were closed with a macroperforated lid (10 perforations of 1 mm diameter) for ensuring that no CO_2 accumulation takes place inside the container. The control packages (C) were similar to the GHP packages but without the addition of the gelatine hydrogel (Fig. 1). Finally, for MAP packages, 500 mL capacity TS500 polypropylene trays (Linpac, Pravia, Spain) were used, manually thermosealed (BOV 160, ORA, France) with a microperforated lidding film (PPlus 52LD90, Amcor Flexibles, Cumbria, UK), ensuring the placement of two microperforations (90 × 50-μm) per tray. Three truffles (45 g each) were added to each MAP package (Fig. 1). The density and size of the microperforations were selected based on the amount of truffle to be packaged and previous studies (Tejedor-Calvo et al., 2020). All packaged samples were stored at 4 °C for 35 d.

Gelatine from GHP was characterised as a new packaging material by measuring the pH and oxygen permeability, microbiological analysis, assessment of VOCs, and sensory evaluation. Initially, and every seven days, the truffles stored in the different packages underwent microbiological analysis, assessment of VOCs, and sensory evaluation. Additionally, the weight loss and changes in the headspace gas composition of each package were monitored. Similar analyses were conducted for GHP gelatine, including pH evolution. For this purpose, gelatine was meticulously separated from the truffle using sterilised tweezers and a scalpel.

2.3. Gelatine hydrogel pH

The gelatine pH in the GHP packages was determined using an Orion VersaStar (Thermo Scientific, Indonesia) equipped with a 9107BNMD

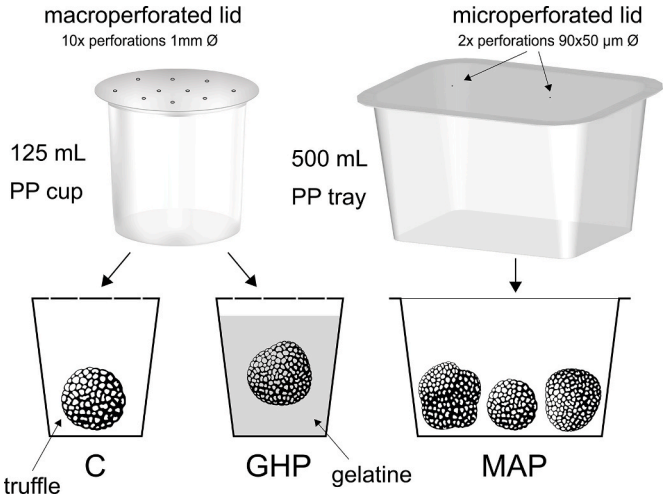

Fig. 1. Visual depiction of the packaging options: control (C), gelatine hydrogel-based package (GHP), and modified atmosphere packaging (MAP). PP: polypropylene.

probe (Thermo Scientific, Indonesia).

2.4. Oxygen permeability of gelatine hydrogel

The oxygen transmission rate (OTR) of the gelatine surrounding the truffle in the GHP packages was measured according to the American Society for Testing Materials (ASTM) method D3985 (ASTM, 2017) using an OX-TRAN 2/22 H (Mocon, Minneapolis, USA) at 10, 15, 20 and 23 °C. The relative humidity (RH) on both sides of the sample was set at 90%. The test was performed on a 3-mm thick sample and conducted in quadruplicate, and the oxygen permeability coefficient (OP) was expressed as $kg{\cdot}m{\cdot}m^{-2}{\cdot}s^{-1}{\cdot}Pa^{-1}$ and obtained as follows:

$$OP = \frac{OTR \bullet l}{\Delta p} \quad (1)$$

Where OTR ($kg{\cdot}m^{-2}{\cdot}s^{-1}$) is the oxygen transmission rate, l (m) is the material thickness, and Δp (Pa) is the differential partial pressure across the material. The OP at 4 °C was estimated considering that the permeability varies with temperature following an Arrhenius-type relationship. The OP of oriented polypropylene, OPP (Envaflex, Utebo, Spain), polylactic acid, PLA (Earthfirst BCFB, Sidaplax, Ghent, Belgium), addition cured food-grade silicone rubber (Dragon Skin 10 Fast, Smooth-On, Lower Macungie, PA, USA), casted pork gelatine films, and compression moulded egg white protein films, EWP, were also measured at 23 °C and 90% RH for comparative purposes.

2.5. Gas composition measurement

The evolution of the O_2 and CO_2 concentrations in the headspace of the MAP, C, and GHP packages was monitored using a gas analyser (CheckMate II, PBI Dansensor, Ringsted, Denmark). The value presented for each sampling day is the average of two different packages.

2.6. Determination of weight loss

Weight loss during storage of both truffles and packages was determined every 7 days for the three packaging conditions (C, MAP, and GHP) using a Sartorius 3716 scale (Sartorius, Göttingen, Germany). The value presented for each sampling day is the average of five different samples. Weight loss is expressed as a percentage of the initial weight.

2.7. Microbiological analysis

Microbial analyses of the truffles stored under three packaging conditions (C, MAP, and GHP) included the quantification of seven microbial groups: aerobic mesophilic microorganisms (MAM), *Pseudomonas* genus, *Enterobacteriaceae* family, actinomycetes, lactic acid bacteria (LAB), and moulds and yeasts. In addition to the truffle from GHP, the gelatine hydrogel was also analysed. Samples were analysed once a week for 35 days. Each sample (one truffle, approximately 15 g) was serially diluted in sterile distilled peptone water 0.1% (Merck, Darmstadt, Germany) and homogenised using a laboratory blender Stomacher 400 Circulator (Seward Laboratory, London, England) for 2 min at 250 rpm, according to ISO Norm 6887-1:2017. Culture media and incubation conditions for each microbial group were: (1) MAM: plate Count Agar (PCA) (Merck) during 72 h at 30 ± 1 °C; (2) *Pseudomonas* genus: *Pseudomonas* agar base (Oxoid, Basingstoke, Hampshire, U.K.) supplemented with cephaloridine-fucidin-cetrimide (CFC) during 48 h at 25 ± 1 °C; (3) *Enterobacteriaceae* family: violet red bile glucose (VRBG) (Oxoid) during 24 h at 30 ± 1 °C; (4) actinomycetes: Starch Casein Agar (SCA) (HIMEDIA) during 5 days at 30 ± 1 °C; (5) LAB: Man, Rogosa and Sharpe agar (MRS) (Merck) during 72 h at 30 ± 1 °C (Oxoid), using anaerobic jars with an atmosphere generation system; and (6) moulds and yeasts: dichloran rose-bengal chloramphenicol agar (DRBC) (Merck), supplemented with 0.1% gentamicin (Carlier, Barcelona, Spain) to avoid *Pseudomonas* spp. growth, during 4 days at 25 ± 1 °C. The value presented for the microbial count on each sampling day was the average of three samples per packaging condition and expressed as log $CFU{\cdot}g^{-1}$.

2.8. VOCs analysis

VOCs analyses were carried out under all packaging conditions (C, MAP, and GHP) and in the gelatine hydrogel before the experiment. Gelatine and truffles were analysed separately in GHP packages.

2.8.1. VOCs extraction by solid-phase microextraction (SPME)

The methodological approach was based on the work of Tejedor-Calvo et al. (2023a), with some modifications. SPME was used to extract aromatic compounds. For that, a fused silica fibre coated with a 50/30 μm layer of divinylbenzene/carboxen/polydimethylsiloxane from Supelco (Barcelona, Spain) was chosen. The samples (2 g of truffle or gelatine) were placed in a 20 mL glass vial closed with a PTFE/silicone septum. After the vial was conditioned at 50 °C for 10 min, the fibre was then exposed to the headspace of the vial for 20 min. All analyses were performed in duplicate.

2.8.2. Gas chromatography-mass spectrometry (GC-MS) analysis

The VOCs profiles of the different samples were analysed by GC-MS using a gas chromatograph Agilent 6890 series coupled with a mass-selective spectrometer detector 5973N series (Agilent Technologies, Santa Clara, CA, USA). This instrument was equipped with a capillary column HP-5MS (Agilent Technologies, California, USA) of 30 m, 0.25 mm internal diameter, 0.25 μm film thickness and a flow of 1 $mL{\cdot}min^{-1}$ with helium as a carrier gas. The oven temperature was 45 °C held for 2 min, 45–246 °C at a rate of 5 $°C{\cdot}min^{-1}$, and finally to 250 °C at 10 $°C{\cdot}min^{-1}$, and held for 4 min. The MS used the electron impact mode with an ionisation potential of 70 eV and an ion source temperature of 230 °C. The interface temperature was 250 °C. MS scanning was performed in the full-scan mode (35–350 m/z). The MSD ChemStation software was used to control the GC–MS system.

2.8.3. Data analysis

Peak identification of the VOCs was achieved by comparison of the mass spectra with mass spectral data from the Wiley275 and NIST MS Search Program 2.0 libraries, and by comparison of previously reported retention indices (RI) with those calculated using an n-alkane series (C6–C20) (purity 99%) (C6, C7 Merck, C8–C20 Supelco, Merck KGaA, Darmstadt, Germany) under the same analysis conditions. The truffle composition was calculated according to the peak area in total ion chromatogram (TIC) mode.

2.9. Sensory analysis

A panel of eight trained tasters (three women and five men) evaluated the aroma of truffles under all packaging conditions (C, MAP, and GHP) and gelatine from GHP samples. Tasters were trained for three sessions of 45 min. Analyses were conducted according to ISO 11035:1994 standards. The following physical aspects of the truffles were evaluated: external appearance, firmness, mycelial growth, bacterial growth, and internal appearance. In addition, sulphurous, mushroom, earthy, butter, black olive, leather animal, blue cheese, nut, and alcohol aromatic parameters were evaluated. An optimal profile of the physical and aromatic aspects of fresh truffles is included in the supplementary material (Table S1). The same aromatic parameters and acidities were evaluated for the gelatine. The physical properties of the gelatine hydrogel were not evaluated by the trained panel because no visible changes were detected. No optimal profile was considered for gelatine with respect to truffle aromatic attributes. Each parameter was assessed using a 9-point rating scale. The values presented for each truffle or gelatine are the averages of two samples tested individually.

2.10. Statistical analysis

Data are presented as the mean ± standard deviation. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test for the comparison of means was performed on the results obtained from the physicochemical, sensory, and microbiological analyses. Differences were evaluated at 95% confidence level ($P < 0.05$). Statistical analyses were performed using GraphPad Prism version 9.3 (GraphPad Software, San Diego, CA, USA). The VOCs data were analysed using principal component analysis (PCA), performed using the statistical software R (R Core Team, 2022) and visualised using XLStat 2009 (Addinsoft, Paris, France) and the R package factoextra (Kassambara & Mundt, 2017).

3. Results and discussion

3.1. Gelatine hydrogel characterization

Physicochemical and sensory characterisations of the gelatine hydrogel as a novel food packaging material were performed. The data obtained will be useful for further implementation of preservation methods in other food products.

3.1.1. pH

The initial pH of the gelatine was 3.33 ± 0.07, a value lower than the isoelectric point (pH 5–10). Consequently, the surfaces of the gelatine molecules become positively charged as various functional groups undergo protonation. The pH increased slightly up to 3.98 (Fig. 2A). This decrease in acidity was also detected by tasters (Table 1) and could be attributed to a combination of several factors, including microbial or fungal enzyme activity (Nadim, Ahmadi, Sarikhani, & Chayjan, 2015), and acid hydrolysis which causes the breakdown of peptide bonds during storage (Baydin, Aarstad, Dille, Hattrem, & Draget, 2022). The absorption of CO_2 generated by the metabolic activity of truffles could counteract these factors. However, it is challenging to establish a direct relationship between acidic pH and its potential inhibitory effect on microbiological growth. Examples of microbial control using both acidic and alkaline gelatine films can be found in the literature (Alemán et al., 2016; Soradech, Nunthanid, Limmatvapirat, & Luangtana-anan, 2017).

3.1.2. Oxygen permeability

The oxygen permeability of the gelatine hydrogel at 23 °C and 90% RH was 1.28 ± 0.20·10^{-15} kg·m·m^{-2}·s^{-1}·Pa^{-1} (Fig. 2B). Compared to the permeability of casted gelatine films (3.04 ± 1.05·10^{-18} kg·m·m^{-2}·s^{-1}·Pa^{-1}), measured at the same temperature and humidity conditions, this value is three orders of magnitude higher. Lower permeability values (3.31–9.93·10^{-20} kg·m·m^{-2}·s^{-1}·Pa^{-1}) have been reported in casted gelatine films obtained from different sources, measured at 23 °C and 55% RH (Avena-Bustillos et al., 2011). These differences can be explained by the fact that at RH values higher than 60%, the oxygen permeability of gelatine films increases exponentially (Lim, Mine, & Tung, 1999). The differences in permeability between fresh and dried gelatine are not surprising, considering that gelatine undergoes changes during the film manufacturing process, making it a very different material (Alipal et al., 2021). Gelatine hydrogel is also much more permeable than other bio-based polymers (2.06 ± 0.23·10^{-17} kg·m·m^{-2}·s^{-1}·Pa^{-1} for EWP and 2.40 ± 0.27·10^{-18} kg·m·m^{-2}·s^{-1}·Pa^{-1} for PLA) or petroleum-based polymers used in food packaging as OPP (0.70 ± 0.01·10^{-17} kg·m·m^{-2}·s^{-1}·Pa^{-1}), but similar to that of silicone hydrogel, 0.87–0.29·10^{-15} kg·m·m^{-2}·s^{-1}·Pa^{-1} (Wu et al., 2021), although in this case, its value strongly depends on the equilibrium water content (Seitz et al., 2017). The oxygen permeability of platinum-cured food-grade silicone rubber was even higher than the gelatine hydrogel (7.11 ± 0.11·10^{-15} kg·m·m^{-2}·s^{-1}·Pa^{-1}). The extremely high permeability of silicone rubber has applications in the post-harvest storage of fresh produce in controlled atmospheres or bulk containers (Gariepy, Raghavan, Theriault, & Munroe, 1988). The oxygen permeabilities of the gelatine hydrogel at 20, 15, and 10 °C were 1.23 ± 0.13·10^{-15}, 1.19 ± 0.13·10^{-15}, and 1.15 ± 0.11·10^{-15} kg·m·m^{-2}·s^{-1}·Pa^{-1}, respectively. Considering an Arrhenius-type dependence on the variation of permeability with temperature, an activation energy of 5.6 kJ·mol^{-1} for the gelatine hydrogel has been obtained. The activation energies of conventional materials as low-density polyethylene, high-density polyethylene or polypropylene range 35.1–55.7 kJ·mol^{-1} (Shi & Chew, 2013) and bio-based materials as PLA range 28.4–41.4 kJ·mol^{-1} (Almenar & Auras, 2010), showing a higher dependence on temperature compared to the gelatine hydrogel. The obtained activation energy of gelatine hydrogel allowed us to determine its oxygen permeability under typical truffle storage conditions (4 °C): 1.09·10^{-15} kg·m·m^{-2}·s^{-1}·Pa^{-1}. Thus, the oxygen permeability of gelatine hydrogels under refrigeration is very high compared to that of other polymers used in food packaging, allowing the entry of oxygen into the product, even in the case of very thick coatings (>1 cm). Because the ratio between CO_2 and O_2 permeability is usually between 3 and 6 (Singh & Singh, 2005), the expected CO_2 permeability of the gelatine hydrogel could be very high, allowing the release of CO_2 produced by the product.

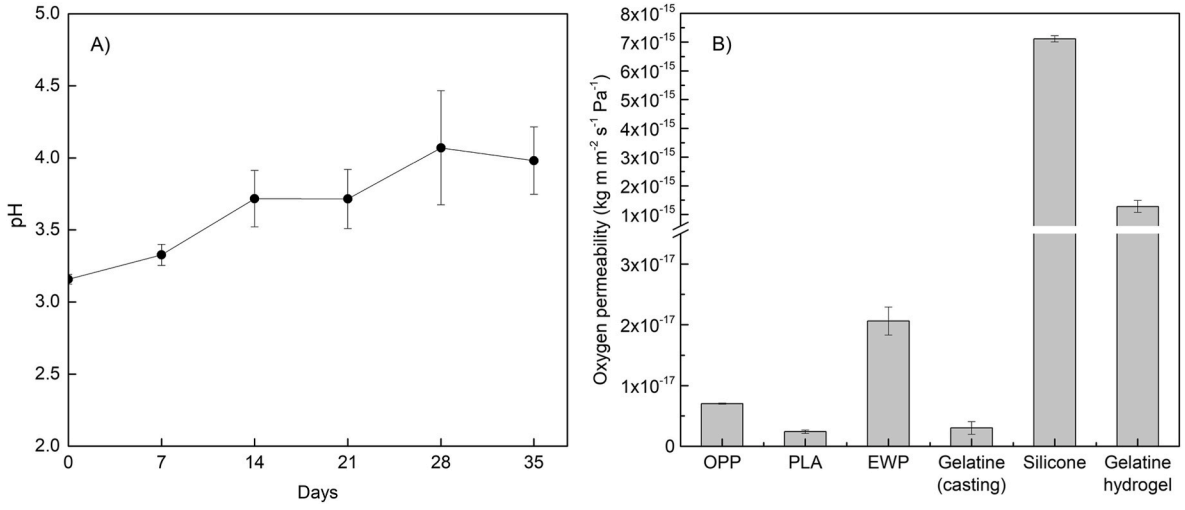

Fig. 2. A) Evolution of gelatine hydrogel-based package pH values; B) Gelatine hydrogel-based package permeability compared with other bio-based and petroleum-based materials (measured at 23 °C and 90% RH): oriented polypropylene (OPP), polylactic acid (PLA), casted pork gelatine film, cured food-grade silicone rubber, and compression moulded egg white protein film (EWP).

Table 1
Scores obtained in the sensory analysis of truffle and gelatine samples from the hydrogel-based package, as evaluated by the trained panel.

Day	General acceptability	External appearance	Firmness	Mycelium growth	Bacterial growth	Internal appearance	Acidity	Sulphurous	Mushroom	Earthy	Butter	Black olives	Leather animal	Blue cheese	Nuts	Alcohol
Truffle																
0	8.72 ± 0.47^{a}	8.64 ± 0.64^{a}	7.71 ± 0.62^{a}	1.00 ± 0.00^{a}	1.07 ± 0.19^{a}	8.43 ± 0.69^{a}	–	6.57 ± 1.59^{a}	3.36 ± 1.94^{a}	3.00 ± 1.43^{a}	2.14 ± 1.21^{a}	3.43 ± 2.02^{a}	2.57 ± 2.73^{a}	2.07 ± 1.70^{a}	1.64 ± 1.31^{a}	1.64 ± 1.14^{a}
7	8.68 ± 0.35^{a}	8.38 ± 0.75^{a}	7.94 ± 0.76^{a}	1.56 ± 1.40^{a}	1.06 ± 0.18^{a}	7.13 ± 2.35^{ab}	–	6.44 ± 1.54^{a}	3.50 ± 2.50^{a}	2.19 ± 1.61^{a}	2.56 ± 1.55^{a}	3.56 ± 2.38^{a}	2.13 ± 1.62^{a}	2.63 ± 1.43^{a}	1.94 ± 1.36^{a}	2.13 ± 1.39^{a}
14	8.37 ± 0.58^{a}	8.25 ± 0.62^{a}	7.06 ± 1.01^{ab}	1.06 ± 0.18^{a}	1.06 ± 0.18^{a}	6.50 ± 1.76^{ab}	–	4.81 ± 1.68^{a}	2.88 ± 2.08^{a}	2.88 ± 1.71^{a}	1.94 ± 1.17^{a}	3.38 ± 1.71^{a}	3.13 ± 2.32^{a}	2.13 ± 1.58^{a}	2.44 ± 1.46^{a}	1.56 ± 0.82^{a}
21	8.31 ± 0.40^{a}	8.06 ± 0.65^{a}	6.69 ± 1.56^{ab}	1.06 ± 0.18^{a}	1.13 ± 0.35^{a}	7.50 ± 1.03^{ab}	–	4.31 ± 2.12^{a}	2.31 ± 1.15^{a}	2.25 ± 1.21^{a}	2.06 ± 1.47^{a}	2.44 ± 1.47^{a}	3.81 ± 2.19^{a}	2.63 ± 1.90^{a}	1.50 ± 0.79^{a}	1.63 ± 1.06^{a}
28	8.21 ± 0.74^{a}	8.00 ± 0.84^{a}	6.38 ± 1.15^{ab}	1.06 ± 0.18^{a}	1.13 ± 0.35^{a}	6.31 ± 1.16^{ab}	–	5.00 ± 2.07^{a}	2.13 ± 1.55^{a}	2.13 ± 1.18^{a}	2.06 ± 1.04^{a}	2.94 ± 1.97^{a}	3.06 ± 1.51^{a}	2.06 ± 2.05^{a}	1.38 ± 0.63^{a}	3.06 ± 2.18^{a}
35	7.59 ± 0.80^{a}	7.56 ± 0.72^{a}	5.19 ± 1.36^{b}	1.19 ± 0.41^{a}	1.13 ± 0.23^{a}	5.13 ± 1.85^{a}	–	4.50 ± 2.23^{a}	1.94 ± 1.03^{a}	2.75 ± 1.97^{a}	2.00 ± 1.14^{a}	2.31 ± 1.35^{a}	4.81 ± 2.05^{a}	2.19 ± 1.89^{a}	2.00 ± 1.58^{a}	2.00 ± 1.69^{a}
Gelatine																
0		–	–	–	–	–	4.71 ± 1.50^{a}	1.00 ± 0.00^{b}	1.00 ± 0.00^{a}	1.00 ± 0.00^{a}	1.07 ± 0.19^{a}	1.00 ± 0.00^{a}	1.00 ± 0.00^{a}	1.00 ± 0.00^{a}	1.00 ± 0.00^{a}	1.43 ± 0.87^{a}
7		–	–	–	–	–	5.13 ± 1.50^{a}	5.13 ± 1.36^{a}	2.56 ± 1.40^{a}	2.19 ± 1.47^{a}	2.06 ± 1.10^{a}	2.56 ± 1.06^{a}	2.44 ± 1.68^{a}	2.25 ± 1.45^{a}	1.56 ± 0.89^{a}	1.81 ± 1.01^{a}
14		–	–	–	–	–	4.50 ± 1.39^{a}	5.00 ± 1.39^{a}	2.13 ± 1.35^{a}	2.19 ± 1.41^{a}	2.25 ± 1.33^{a}	3.25 ± 1.79^{a}	3.31 ± 2.12^{a}	2.19 ± 1.77^{a}	1.63 ± 0.99^{a}	2.00 ± 1.51^{a}
21		–	–	–	–	–	4.13 ± 1.64^{a}	4.00 ± 1.51^{a}	1.50 ± 0.76^{a}	1.63 ± 1.06^{a}	2.75 ± 2.38^{a}	2.75 ± 1.58^{a}	2.75 ± 1.16^{a}	2.25 ± 1.75^{a}	1.50 ± 0.76^{a}	1.88 ± 1.13^{a}
28		–	–	–	–	–	3.56 ± 1.88^{a}	4.25 ± 1.69^{a}	1.44 ± 0.73^{a}	1.75 ± 1.15^{a}	1.75 ± 1.02^{a}	2.63 ± 1.80^{a}	2.69 ± 1.69^{a}	1.94 ± 1.39^{a}	1.13 ± 0.35^{a}	1.31 ± 0.73^{a}
35		–	–	–	–	–	3.06 ± 1.12^{a}	4.31 ± 2.35^{a}	1.38 ± 0.61^{a}	2.06 ± 1.26^{a}	2.31 ± 1.87^{a}	1.63 ± 1.23^{a}	3.31 ± 1.91^{a}	1.81 ± 1.44^{a}	1.00 ± 0.00^{a}	1.75 ± 1.08^{a}

- not evaluated.
^{ab} letters denote significant differences ($P \leq 0.05$) among different storage days within the same attribute.

3.1.3. Microbiological load

The gelatine hydrogel did not exhibit microbial levels above the detection threshold in the first week in any of the groups studied. At day 14, the *Pseudomonas* group, Actinomycetes, moulds and yeasts counts of gelatine control reached 2 log CFU·g^{-1} (data not shown). The rest of the microbiological group counts did not overcome 4.6 log CFU·g^{-1} after 35 days: MAM (4.6 log CFU·g^{-1}), *Pseudomonas* (3.5 log CFU·g^{-1}), Actinomycetes (4.2 log CFU·g^{-1}), *Enterobacteriaceae* (2.7 log CFU·g^{-1}), moulds (2.7 log CFU·g^{-1}) and yeast (3.6 log CFU·g^{-1}) (data not shown). LAB was not detected in the gelatine. The low counts might be due to several reasons:1) the gelatine hygroscopicity might reduce water availability (Yang et al., 2020); 2) the food matrix structure, in this case the gelatine, might reduce the rate of microbial growth (Antwi, Bernaerts, Van Impe, & Geeraerd, 2007); 3) the proteolytic enzyme activity might be insufficient to degrade the sulfide bridges from the gelatine structure, and therefore might not be able to obtain enough carbon sources to grow (Billinger & Johansson, 2018; Westler & Neal, 1977). Because of the low microbial load of gelatine, the native microbiota of the truffle was not expected to be modified. However, some influence on truffle microbial loads might occur because of O_2, CO_2 and water availability, and the influence of pH on microorganism growth.

3.1.4. VOCs and sensory attributes in gelatine hydrogel

A total of 25 VOCs were detected in the gelatine samples using SPME-GC-MS (Table 2). Among these, 2-propanone (13.7%), dimethyl sulfide (12.3%), 2-methyl-propanal (11.13%), benzaldehyde (11.06%), furfural (8.88%), and methylpropylformate (8.24%) were the major compounds detected. Gelatine is described as odourless (Rather et al., 2022), although in the sensory evaluation, the trained panel detected butter and alcohol attributes in the gelatine samples on day 0 (Table 1). This may be because the samples were analysed by tasting and smelling; therefore, more receptors were involved in the sensory analysis. None of the detected VOCs were exclusive to the gelatine samples, indicating that these small molecules might be common to other food samples, as indicated by Tejedor-Calvo et al. (2023c).

3.2. Truffle weight loss and gas composition

Truffles in the three packaging conditions did not suffer a huge weight loss: up to 8.4% in C samples, 4.2% in MAP samples, and 17.1% in GHP samples, corresponding to days 21, 28, and 35, respectively (Table 3). Comparing all packaging conditions at day 21 (estimated as shelf-life duration by the sensory analysis, see Section 3.5), the weight loss percentages were similar in MAP and GHP samples (3.20 and 2.47% respectively), but higher in C samples (8.40%). Thus, GHP was able to maintain truffle ascocarp weight similar to MAP. Similar weight loss has been observed in black (*T. melanosporum*) and summer (*T. aestivum*) truffles packaged in microperforated MAP (Rivera, et al., 2011). The gelatine in the GHP barely experienced a change in weight. The negative weight loss values could be associated with the incomplete separation between the truffle and gelatine in the GHP samples.

The atmospheric composition inside the packages in MAP samples revealed a O_2 decrease (up to 9.25%) and CO_2 increase (up to 13.06%) during first four days of storage (Table 4). Subsequently, the O_2 and CO_2 levels almost reached gas equilibrium and only slight variations occurred during storage. A similar behaviour was observed in the gas composition of black (*T. melanosporum*) and summer (*T. aestivum*) truffles packaged with microperforated MAP (González-Buesa, Ferrer-Mairal, Oria, & Salvador, 2009; Rivera et al., 2010a). The O_2 and CO_2 levels in the macroperforated packages (C and GHP) matched those in the air.

3.3. Microbiological populations of C, MAP and GHP

Truffles presented an initial average of total microbial load of 7.5 log CFU·g^{-1}, being mainly actinomycetes (7.4 log CFU·g^{-1}), followed by *Pseudomonas* genus (7.3 log CFU·g^{-1}), *Enterobacteriaceae* family (3.2 log CFU·g^{-1}), yeast (2.4 log CFU·g^{-1}), moulds (2.3 log CFU·g^{-1}), and LAB (1.1 log CFU·g^{-1}) (Fig. 3). These values are similar to those of a previous study that analysed truffles ascocarps from the same location:8 log CFU·g^{-1} total microbial load, *Pseudomonas* genus (7.8 log CFU·g^{-1}), *Enterobacteriaceae* family (7.0 log CFU·g^{-1}), LAB (4.3 log CFU·g^{-1}), and yeasts and moulds (3.5 log CFU·g^{-1}) (Tejedor-Calvo et al., 2020). The high microbial population content is because the truffles grow in the soil. According to Rivera, Blanco, Oria, and Venturini (2010b), *Pseudomonas* genus and *Enterobacteriaceae* family constitute the major culturable microbial populations in truffles; however, in our study, actinomycetes showed higher loads. Actinomycetes usually occur in soil or water environments; therefore, they are expected to be found in truffles because they are harvested from the soil (Marozzi et al., 2023).

The microbial load increased slightly during storage under all packaging conditions. In MAM counts, the C sample showed 8.4 log CFU·g^{-1} after seven days of storage time, as much as MAP after 21 days (8.5 log CFU·g^{-1}). The truffle from GHP showed lower microbial counts after 35 days (7.6 log CFU·g^{-1}), although in the gelatine material the MAM increased up to 6.1 log CFU·g^{-1} (Fig. 3). Regarding the actinomycetes group, a load increase was observed in all the packaging conditions, but the presence of these microorganisms was significantly higher in C and MAP (8.8 and 9.0 log CFU·g^{-1} at day 21 and 28, respectively) than in GHP truffles and gelatine (7.2 and 4.6 log CFU·g^{-1} at day 28 and 35 respectively, Fig. 3). This bacterial group may grow on the truffle surface in contact with gelatine, using it as a protein source for their metabolism. The *Enterobacteriaceae* family counts showed significantly higher levels in C and MAP (4.7 and 4.9 log CFU·g^{-1}, respectively) than in the GHP truffle (3.4 log CFU·g^{-1}) at day 21 (Fig. 3). Perhaps these bacteria have problems growing in protein-based media due to microorganism mobility, water availability in the media, or a lack of proteases. Indeed, the growth observed in the GHP gelatine was minimal (1.4 log CFU·g^{-1} at day 28, Fig. 3). Similarly to actinomycetes group, the *Pseudomonas* genus counts showed approx. 1 log CFU·g^{-1} more in C and MAP than in GHP samples (Fig. 3). CO_2 showed a bacteriostatic effect on MAP, slowing down *Pseudomonas* development, whereas the truffles from GHP showed a standing bacteriostatic effect, with no significant difference from days 7 to 35. The CO_2 also affected to the gelatine, which apparently showed a bactericidal effect since it showed 5 log CFU·g^{-1} the first week, and a reduction of 2.9, 3.0 and 1.7 log CFU·g^{-1} the following weeks (in comparison with data from day 7). Along with low pH, these conditions could create a synergistic effect on gelatine. Although high levels of CO_2 facilitate LAB growth, no significant differences were found among the three packaging conditions up to day 21 of storage, and LAB growth in the GHP gelatine was not relevant (up to 2 log CFU·g^{-1}). The number of moulds and yeasts increased during the storage period, particularly in the GHP truffle and gelatine samples. Despite the fungicidal effects of high CO_2 concentrations, they did not negatively affect any of the microbiological groups. Up to day 21, the GHP mould counts were statistically lower than those of C and MAP. However, from that moment, the counts were not significantly different despite the fact that GHP gelatine might control O_2 availability (Fig. 3). This might be due to the loss of hygroscopic properties in the gelatine and the subsequent increase in water availability or because some compounds used by mould metabolism are released by the truffle's own degradation. In some ways, gelatine can act as a microorganism encapsulation agent, avoiding motility and, therefore, their development (Patarroyo et al., 2020).

3.4. VOCs of truffles packaged in C, MAP and GHP

The main truffle VOCs detected in fresh truffle samples were 2-methyl-butanal (36%), propanal-2-methyl (13%), 2-methyl-1-butanol (7.9%), 3-methyl-1-butanal (6%), 3-methyl-1-butanol (6%), and dimethyl-disulfide (5.6%) (Table 2; Fig. 4). These compounds have been reported as key VOCs in *T. melanosporum* by other authors (Culleré et al.,

Table 2
List of volatile organic compounds identified by SMPE-GC-MS. Relative percentage of area values (%) obtained for the fresh truffles and gelatine samples.

Nº	Name	Odour descriptor[a]	CAS nº	RT	RI_{exp}	RI_{lit}	Mass (*m/z*)			Truffle	Gelatine
1	Ethanol	sweet	64-17-5	1.330	<500	427	45	46	43	1.02	4.86
2	2-propanone	Caramellic, burnt	67-64-1	1.378	<500	500	43	58	42	2.83	13.79
3	Dimethyl sulphide	Cabbage, sulphur,gasoline	75-18-3	1.474	521	521	62	61	47	1.45	12.33
4	Methylene chloride	–	75-09-2	1.503	532	531	49	86	84	0.00	4.81
5	1-propanol	Alcohol, pungent	71-23-8	1.568	555	548	31	29	42	0.00	0.00
6	Propanal-2-methyl	pungent, malt, green	78-84-2	1.575	558	560	43	72	41	12.94	11.13
7	2,3-butanedione	butter	431-03-8	1.626	577	587	43	87	86	0.00	0.00
8	Isopropyl formate	Cocoa, tropical, fruity	625-55-8	1.640	582	–	45	73	42	0.00	1.14
9	Butanal	Pungent, green	123-72-8	1.676	595	598	44	72	57	0.00	0.00
10	3-methyl-2-butanone	camphor	563-80-4	1.683	597	–	43	86	41	0.00	0.74
11	2-butanone	ether	78-93-3	1.719	603	602	43	72	57	1.39	0.00
12	Hexane	–	110-54-3	1.726	604	–	57	86	56	0.00	0.00
13	Ethyl acetate	pineapple	141-78-6	1.770	609	607	43	80	70	0.03	7.08
14	2-methyl-1-propanol	Solvent, bitter	78-83-1	1.878	622	626	43	42	41	7.18	1.31
15	Acetaldehyde	Pungent, ether	75-07-0	1.899	624	–	44	43	42	0.00	0.00
16	3-methyl-butanal	malt	590-86-3	2.080	645	646	44	71	58	5.92	3.21
17	2-methyl-butanal	Cocoa, almond	96-17-3	2.123	653	653	57	86	58	35.93	3.00
18	2-propanone-1-hydroxy	Caramellic, sweet	116-09-6	2.224	662	–	43	74	42	0.00	0.00
19	1-penten-3ol	Butter, pungent	616-25-1	2.230	663	680	57	58	55	0.00	0.00
20	Metylpropylformate	–	589-40-2	2.310	672	–	45	73	59	2.04	8.24
21	2-pentanone	Ether, fruit	107-87-9	2.368	679	687	43	86	71	0.14	0.00
22	2,3-pentadione	Cream, butter	600-14-6	2.454	689	696	43	100	57	0.22	0.00
23	Pentanal	almond, malt, pungent	110-62-3	2.469	691	704	44	58	57	0.10	0.37
24	2-pentanol	green	6032-29-7	2.483	692	700	45	73	55	0.23	0.00
25	2-butanone,3-hidroxy	butter, cream	513-86-0	2.613	704	707	45	88	55	0.00	0.00
26	3-methyl-1-butanol	Whiskey	123-51-3	2.966	723	737	55	70	57	5.96	0.40
27	2-methyl-1-butanol	Wine, onion	137-32-6	3.045	728	743	57	70	56	7.90	1.21
28	Dimethyl-disulphide	onion, cabbage, putrid	624-92-0	3.103	731	733	94	79	61	5.60	0.66
29	2-methyl-pentanal	Green, fruity	123-15-9	3.180	735	–	43	58	41	0.00	0.00
30	Propanoic-ac-2methyl-esther	sweet, rubber	97-62-1	3.434	775	760	43	29	71	0.07	0.00
31	Isobutylacetate	fruit, apple, banana	110-19-0	3.737	783	770	43	56	41	0.03	0.00
32	Propanoic-ac-2methyl	rancid, butter, cheese	79-31-2	3.989	735	753	43	88	73	0.00	0.00
33	1,3-butanediol	fruit, onion	513-85-9	4.300	785	803	45	27	43	0.04	0.00
34	Octane	–	111-65-9	4.342	800	800	43	85	71	0.00	0.00
35	Hexanal	grass	66-25-1	4.378	802	801	44	57	56	1.47	1.16
36	ethylbutanoate	apple	105-54-4	4.443	803	803	71	43	29	0.00	0.00
37	Furfural	bread, almond, sweet	98-01-1	5.394	830	830	96	95	67	0.06	8.89
38	2-methylthio-ethanol	Sulphurous, meaty	5271-38-5	5.639	873	–	61	92	47	0.00	0.00
39	ethyl-2-methylbutanoate	apple	7452-79-1	6.086	849	853	102	85	74	0.05	0.00
40	ethyl-3-methylbutanoate	fruit	108-64-5	6.208	853	851	88	85	60	0.02	0.00
41	4-pentenal	roasted	2100-17-6	6.392	858	–	55	29	41	0.04	0.00
42	Hexanol	resin, flower, green	111-27-3	6.750	868	867	56	69	55	0.06	0.00
43	2-methyl-butyl-acetate	fruit	624-41-9	7.167	880	880	43	70	55	0.04	0.00
44	2-heptanone	soap	110-43-0	7.570	890	889	43	71	59	0.00	0.00
45	Heptanal	fat, citrus, rancid	111-71-7	7.945	902	894	70	57	55	0.22	0.00
46	Methional	cooked potato	3268-49-3	8.111	906	908	48	104	47	0.02	0.00
47	Anisole	Anise	100-66-3	8.485	916	918	108	93	78	0.94	0.00
48	isobutyl isobutyrate	Pineaple, banana	97-85-8	8.550	917	914	71	89	57	0.00	0.00
49	dimethyl-sulfone	sulphur, burnt	67-71-0	8.795	924	924	79	15	94	0.00	0.00
50	isobutyl butyrate	Pineapple	539-90-2	10.085	957	961	71	56	43	0.15	0.00
51	Benzaldheyde	Almond, burnt sugar	100-52-7	10.102	957	961	77	106	105	0.14	11.06
52	1-heptanol	green	111-70-6	10.617	971	967	70	69	56	0.00	0.00
53	1-octen-3-ol	mushroom	3391-86-4	10.970	980	978	57	72	55	0.25	0.57
54	3-octanone	mushroom	106-68-3	11.245	987	988	43	57	29	0.10	0.98
55	2-octen-4-ona	yeast	4643-27-0	11.490	993	–	69	41	84	0.26	0.00
56	hexanoic acid, ethyl ester	apple peel, fruit	123-66-0	11.757	1000	998	88	29	27	0.00	0.00
57	Butyric acid	Fruity, sweet	2445-67-2	11.800	1002	1002	57	85	103	0.00	0.00
58	Octanal	Green, citrus	124-13-0	11.822	1002	1003	43	84	56	0.44	0.00
59	3-methyl-acid butanoic		589-59-3	12.016	1003	1004	85	57	41	0.05	0.00
60	3-methylanisole		100-84-5	12.384	1018	1028	122	107	92	2.63	0.00
61	Benzeneacetaldehyde	hawthorne, honey, sweet	122-78-1	13.277	1043	1047	91	120	92	0.10	0.59
62	E−2-octenal	green, nut, fat	2548-87-0	13.803	1058	1059	41	83	70	0.04	0.00
63	1-octanol	chemical, metal, burnt	111-87-5	14.286	1071	1067	56	84	70	0.01	0.73
64	3-methyl-phenol	fecal, plastic	108-39-4	14.502	1088	1083	118	107	79	0.02	0.00
65	linalool oxide trans	flower	34995-77-2	14.890	1088	1090	59	94	55	0.00	0.00
66	2-nonanone	hot milk, soap, green	821-55-6	15.028	1091	1090	43	58	41	0.01	0.58
67	isoamyl-2methylbutyrate		27625-35-0	15.331	1101	1103	70	103	85	0.00	0.00
68	Nonanal	fat, citrus, green	124-19-6	15.424	1103	1106	57	98	70	0.08	1.16
69	2-methyl-butanoic acid		2445-78-5	15.453	1104	1105	70	57	85	0.12	0.00
70	Benzeneethanol	honey, spice, rose, lilac	60-12-8	15.720	1113	1113	91	122	65	0.88	0.00
71	Benzene, 1,2-dimethoxy-		91-16-7	16.880	1147	–	138	95	77	0.41	0.00
72	Benzene, 1,3-dimethoxy-		151-10-0	17.507	1167	–	138	109	95	0.09	0.00
73	ethyl caprylate	fruit, fat	106-32-1	18.516	1198	1196	88	101	57	0.01	0.00
74	2,4-nonadienal	fat, wax, green	5910-87-2	18.997	1213	1214	81	41	67	0.04	0.00
75	2-undecanone	orange, fresh, green	112-12-9	21.412	1293	1296	68	43	59	0.00	0.00

RT = retention time.
RI exp = Retention Index experimental.
RI lit = Retention Index Literature database NIST.
[a] Flavornet and thegoodscentcompany websites.

Table 3
Weight loss (%) in truffle packaging and truffle samples under different storage conditions: control (C), modified atmosphere packaging (MAP), and gelatine-based packaging (GHP). All the samples were stored at 4 °C for 35 days. Some data are lacking in the final control and MAP times because truffles were completely degraded. Data expressed as a mean ± standard deviation (SD) of three samples. $^{A-C}$ Different letters above the columns for packaging material indicate statistical difference at $P \leq 0.05$. $^{a-d}$ Different letters above the columns for different days indicate statistical difference at $P \leq 0.05$.

Day	Truffle			Gelatine
	C	MAP	GHP	GHP
0	$0.00 \pm 0.00^{A,c}$	$0.00 \pm 0.00^{A,d}$	$0.00 \pm 0.00^{A,bc}$	0.00 ± 0.00^{d}
7	$4.32 \pm 1.41^{A,b}$	$2.11 \pm 0.19^{B,c}$	$-1.53 \pm 0.57^{C,c}$	0.57 ± 0.17^{ab}
14	$6.09 \pm 0.75^{A,ab}$	$2.20 \pm 0.18^{B,c}$	$0.04 \pm 1.69^{C,bc}$	0.78 ± 0.25^{a}
21	$8.40 \pm 1.18^{A,a}$	$3.20 \pm 0.25^{B,b}$	$2.47 \pm 1.19^{B,b}$	0.65 ± 0.28^{ab}
28	–	$4.16 \pm 0.57^{B,a}$	$11.01 \pm 4.22^{A,a}$	-0.59 ± 1.06^{b}
35	–	–	17.14 ± 2.67^{a}	0.62 ± 0.38^{b}

Table 4
Evolution of O_2 and CO_2 levels in truffle samples stored under MAP during 35 days at 4 °C. Data expressed as a mean ± standard deviation (SD) of three samples. ^{a,b} Different letters above the columns for the same gas indicate statistical difference at $P \leq 0.05$.

Days	O_2 (%)	CO_2 (%)
0	20.78 ± 0.00^{a}	0.00 ± 0.00^{c}
2	12.36 ± 1.53^{b}	8.40 ± 1.60^{b}
4	9.25 ± 2.88^{bc}	13.06 ± 3.92^{ab}
6	9.07 ± 3.23^{bc}	13.94 ± 5.16^{ab}
9	9.05 ± 3.48^{bc}	14.04 ± 6.29^{ab}
14	9.79 ± 1.26^{bc}	10.96 ± 1.36^{ab}
21	10.75 ± 0.07^{b}	9.45 ± 0.21^{b}
28	9.20 ± 1.40^{c}	12.30 ± 1.10^{a}

2010; Phong, Gibberd, Payne, Dykes, & Coorey, 2022; Tejedor-Calvo et al., 2023a). Aromatic profiles evolved throughout the storage period under the three packaging conditions studied. Some molecules, such as hexanal (grass odour), benzaldehyde (almond odour), 1-octen-3-ol (mushroom odour), and octanal (fatty odour), increased with storage time under the three packaging conditions. Apart from the key truffle VOCs, truffles from packaging C showed high levels of 3-methylanisole (narcissus odour), acetaldehyde (ether odour), and 2,5-dimethoxytoluene (no odour described); truffles from MAP showed hexanal and 3-methylanisol; and truffles from GHP showed hexanal and octanal on the first day and 3-methylanisol and benzaldehyde at the end of the storage period. These aroma changes during storage can be attributed to several factors, including spoilage damage and microbiological growth, as suggested by other authors (Culleré, Ferreira, Chevret, Venturini, & Sánchez-Gimeno, 2012; Splivallo et al., 2015; Splivallo, Ottonello, Mello, & Karlovsky, 2011).

A recent study reported that some truffle-flavouring compounds could be trapped in fresh gelatine (Tejedor-Calvo, et al., 2023b) and egg whites (Tejedor-Calvo, et al., 2023a). In the first week, compounds such as 2-methyl-propanol (solvent, bitter), 3-methyl-1-butanol (whiskey), 2-methyl-1-butanol (wine, onion), and dimethyl-sulfide (cabbage, sulphur, gasoline) were detected in the gelatine. These compounds have been reported to be key black truffle compounds (Tejedor-Calvo et al., 2023a). In the following weeks, the truffles stored in GHP presented an aromatic profile similar to that on day 0. However, at the end of the experiment, increases in 3-methyl-2-butanone (camphor), 2-butanone (ether), 2-methyl-1-propanol (solvent, bitter) and 2-methyl-butanal (cocoa, almond) were observed. The same behaviour was observed in gelatine from GHP samples, except for some molecules such as hexanal (grass), which was only slightly detected in gelatine samples compared to truffles packaged in GHP. These differences in the VOCs profiles indicate that truffles in GHP have more green aromatic notes than those in gelatine. Nevertheless, this study clearly demonstrates the transfer of key truffle aromatic compounds from the truffle into the fresh gelatine of the GHP. This product could be preserved and used as a naturally truffle-flavoured product in cuisine. According to recent studies (Phong, Dykes, & Payne, 2022; Tejedor-Calvo et al., 2023d), no natural aroma has been obtained from truffle ascocarps on the market. This technology provides a natural truffle product with a genuine aroma.

3.5. Sensory analysis of truffles packaged in C, MAP and GHP

Truffles stored in C packages showed rapid spoilage, with some attributes exceeding the optimal values (Table S2) after 14 days. For example, the mycelial and microbial growth reached 3.3 and 1.8 at day 14, but 5.2 and 4.6 at day 21. This growth usually involves texture loss, as observed with the firmness value (5.7 on day 21. In addition, the general acceptability and external appearance values at day 14 (5.9 and 5.7%, respectively) were slightly below the optimal attribute values (Tables S1 and S2). Also, it was observed a sulphur aroma reduction (from 6.6 to 4.8) and leather-animal increase (from 2.6 to 4.8) at day 14, and of course it was even more noticeable at day 21 (Table S2). Therefore, the shelf-life extension of the C samples was considered up to 7 days. The scores obtained for the MAP truffles were similar during the first two weeks of storage (Table S2). Some attribute scores reduced on day 21, including general acceptability (6.5), external appearance (6.6), firmness (5.8), and internal appearance (5.8); however, they were not below the spoilage value limits (Table S1). At day 28, these attributes exceeded the spoilage threshold as well as mycelial and microbial growth; therefore, the shelf-life extension of the MAP samples was considered to be up to 21 days. Furthermore, none of the sensory attributes were out of range, except for alcohol, which reached 5.1 score at the end of storage. In the MAP packages, the truffles could produce alcohol as a stress response to MAP gas conditions on day 28 (low O_2 available). Different authors have reported similar shelf life expectations for truffles stored in MAP (Rivera et al., 2010a, 2011; Tejedor-Calvo et al., 2019). The physical evaluation of the GHP truffles was similar for the initial 28 days, except for firmness (6.4), which was below the spoilage value limits (Table 1, Table S1). Because of this value, the shelf life of the truffle in GHP was extended by up to 21 days, as in MAP. In GHP truffles, mycelial and bacterial growth were almost unnoticeable compared to MAP truffles, and alcohol odour scores were not excessive (Table S1). This might indicate that gelatine maintains a better gas atmosphere for truffle respiration and metabolic rates. The aromatic profile of the truffles under the three packaging conditions showed similar behaviour during storage (Fig. S1). For example, the sulphur aroma reduced with time, but the reduction was exponential in the C samples and slight in the MAP and GHP samples. In general, MAP and GHP maintained better aromatic attributes during storage, particularly in the black olives. Nevertheless, increases in alcohol and leather-animal odour attributes in MAP and GHP, respectively, were remarkably negative.

The GHP gelatine showed the ability to trap some VOCs (see Section 3.4), and these results were supported by the trained panel that detected a sulphurous aroma from the first week (5.3 score) (Table 1). Other odour attributes such as mushroom, earthy, butter, blue cheese, nuts, and alcohol were detected (scores below 3). However, in the second week, black olive (3.3) and leather animal (3.3) attributes were more

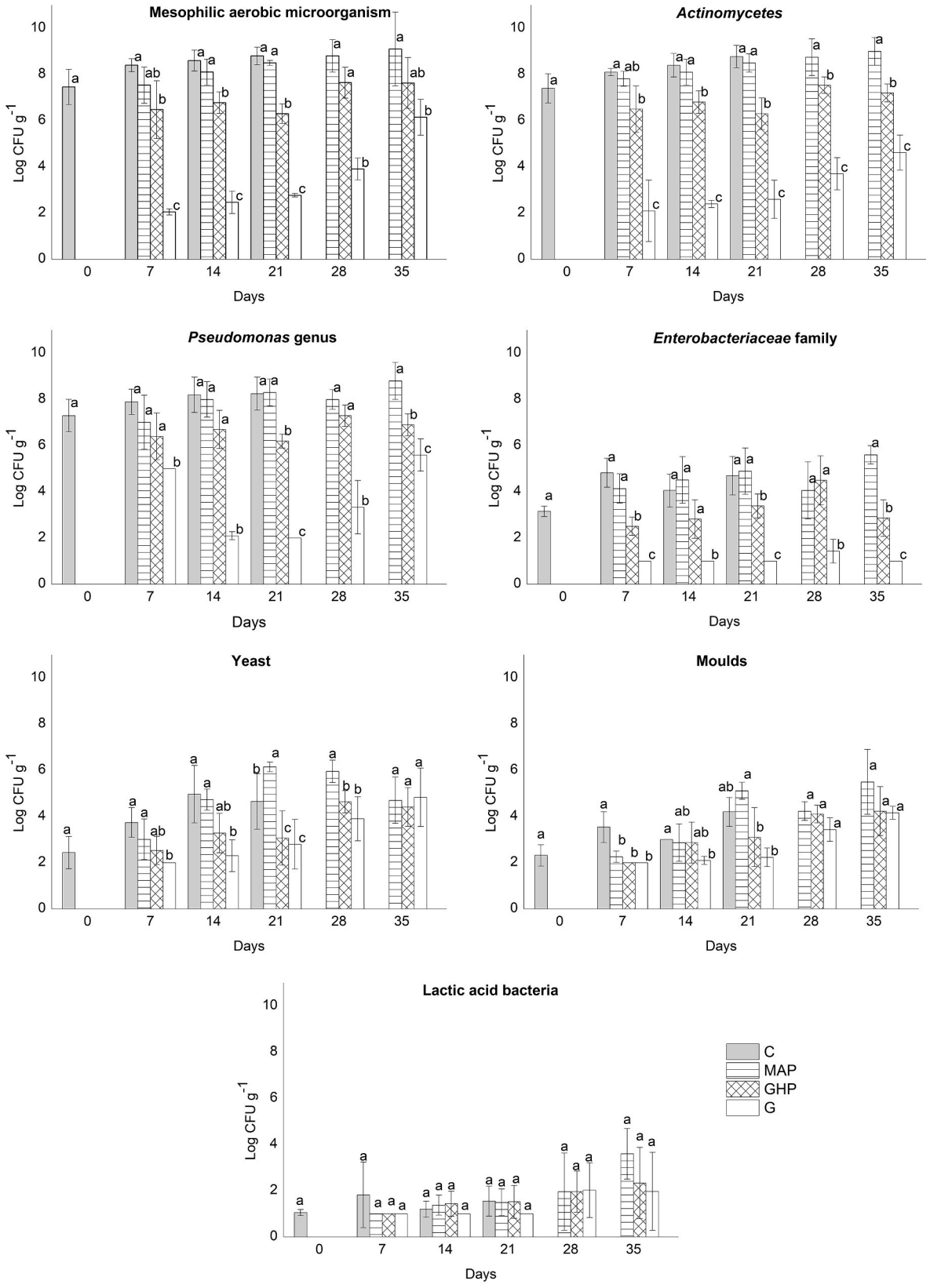

Fig. 3. Time trend of mesophilic aerobic microorganisms, *Pseudomonas* genus, *Enterobacteriaceae* family, lactic acid bacteria, actinomycetes, moulds, and yeasts populations for fresh truffle packaged and stored under different conditions: ▮ control (C) packaging, ▣ modified atmosphere packaging (MAP), ▩ gelatine hydrogel-based package (GHP), and □ 1 fresh gelatine sample from GHP packaging condition (G). Data expressed as mean ± standard deviation (SD) of three samples. [a, b, c] Different superscript letters within the same day of storage indicate statistically significant differences at $P \leq 0.05$.

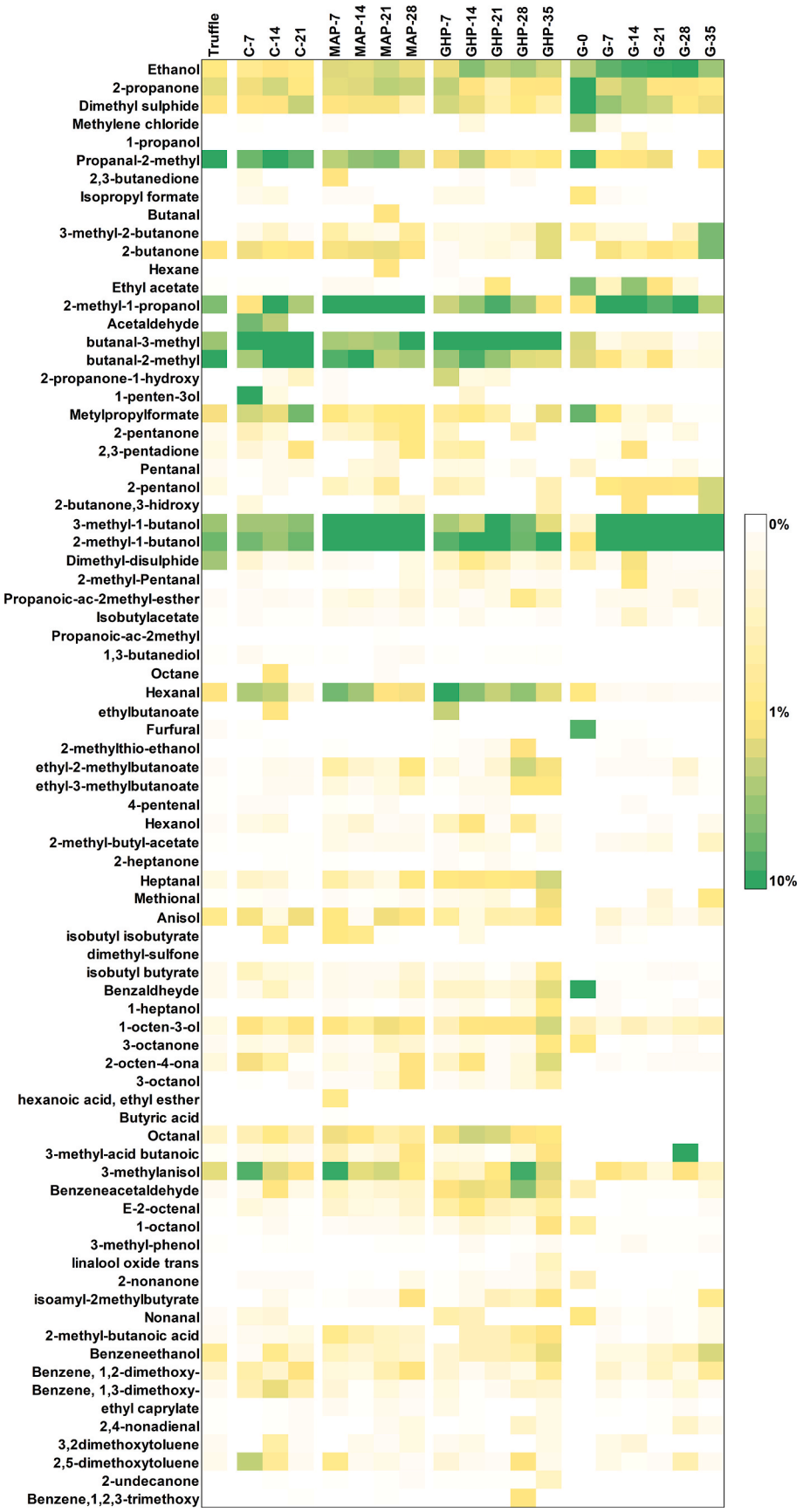

Fig. 4. Heatmap of the volatile profile of truffle and gelatine samples according to the relative percentage of area values detected using SPME-GC-MS. Samples were fresh truffle (truffle), truffles with control packaging (C), truffles packaged in modified atmosphere (MAP), truffles in gelatine hydrogel-based package (GHP), and gelatine from GHP samples (G). The numbers (7, 14, 21, 28 and 35) correspond to the different storage days of the experiment.

prevalent. This indicates that the truffle in GHP constantly produced an aroma, and the VOCs accumulated inside the gelatine. However, more time was required for some aromas to be detected by the trained panel. The acidity of gelatine was slightly reduced after 35 days from 4.7 to 3.0, probably due to microbial or fungal enzyme activity and acid hydrolysis.

3.6. Multivariable analysis of truffles packaged in C, MAP and GHP

The possible correlations of different packaging conditions and storage times with the relative abundance of VOCs detected by SPME-GC–MS were explored by PCA (Fig. 5). The first two PCA components explained only 32.2% of the total variability, thus indicating the complexity of the relationships. The first component (explaining 18.4% of the variability) showed a clear correlation with the storage time of the packaged truffles. The C, MAP, and GHP samples from days 7–21 clustered together, whereas the samples from days 28–35 showed a positive correlation with the first component. The most positive loadings of the former corresponded to heptanal (C45; fat, citrus, and rancid), benzene ethanol (C70; honey, spice, rose, and lilac), ethyl-3-methylbutanoate (C40; fruit), 1-octen-3-ol (C53; mushroom), and 3-octanone (C54; mushroom). The second component (13.8% of variability) was associated with the transfer of truffle VOCs into the GHP gelatine, with the fresh gelatine on day 0 showing a positive correlation with the second component. More positive loadings of the second PCA corresponded to isopropyl formate (C8; cocoa, tropical, fruity), methylene chloride (C4; odourless), furfural (C37; bread, almond, sweet), methylpropylformate (C20; odourless), and benzaldehyde (C51; almond, burnt sugar). The compounds that showed more negative loadings of the second component, and thus a higher association with the gelatine of GHP from days 7–35, were 3-methyl-1-butanol (C26; whiskey), 2-methyl-1-butanol (C27; wine, onion), 2-methyl-1-propanol (C14; solvent, bitter), and 2-pentanol (C24; green). These results objectively revealed that gelatine was aromatised with key truffle aroma compounds, thus confirming the sensory analysis of the trained panel. In a recent study on oil and honey aromatised with fresh truffles, some key truffle VOCs, such as 3-methyl-1-butanol and 2- methyl-1-butanol were detected in both matrices after 24 h of aromatisation (Tejedor-Calvo et al., 2023a), as detected in the gelatine of the GHP. This study also identified 2-methyl-1-propanol as a component of the fresh truffle aroma.

This study adds to the numerous existing ones that reveal the great potential of gelatine in food packaging (Lu et al., 2022; Said, Howell, & Sarbon, 2023). The smart gelatine hydrogel-based packaging proposed is innovative, and it could serve as a basis for subsequent improvements in aroma absorption capacity, gel stability, and consistency. Additionally, the protein formulation and crosslinking could be adjusted to extend the shelf life of the product, and bioactive compounds could be incorporated to the hydrogel matrix evolving toward an active packaging. The extrapolation of the results obtained to other circumstances should be done with caution since the influence of the source and bloom strength of the gelatine in permeability and aroma retention capacity has not been analysed, nor the implications on the results (microbiological populations, product weight loss, VOCs, sensorial attributes) if a different type of truffle (*Tuber aestivum*) were to be packaged.

4. Conclusions

In the present study, a novel gelatine hydrogel-based package (GHP) was used to extend the shelf life of truffles by up to 21 days, with the loss of firmness being the main factor limiting shelf-life extension. Compared to the macroperforated packages (C) and microperforated modified atmosphere packages (MAP), GHP showed lower microbiological loads and similar weight loss during the experiment. The key aromatic compounds from the truffles were trapped in GHP gelatine from day 7. In addition, sensory analysis showed positive results for the physical and aromatic properties of truffles stored in GHP and gelatine. Therefore, with this new packaging strategy, two products were obtained: truffled gelatine for culinary uses and fresh truffles preserved with high quality for three weeks. This novel packaging concept has great potential for black truffle distribution and marketing, especially for producing countries that export a significant proportion of their truffle products, such as Spain, France, Italy, and Australia.

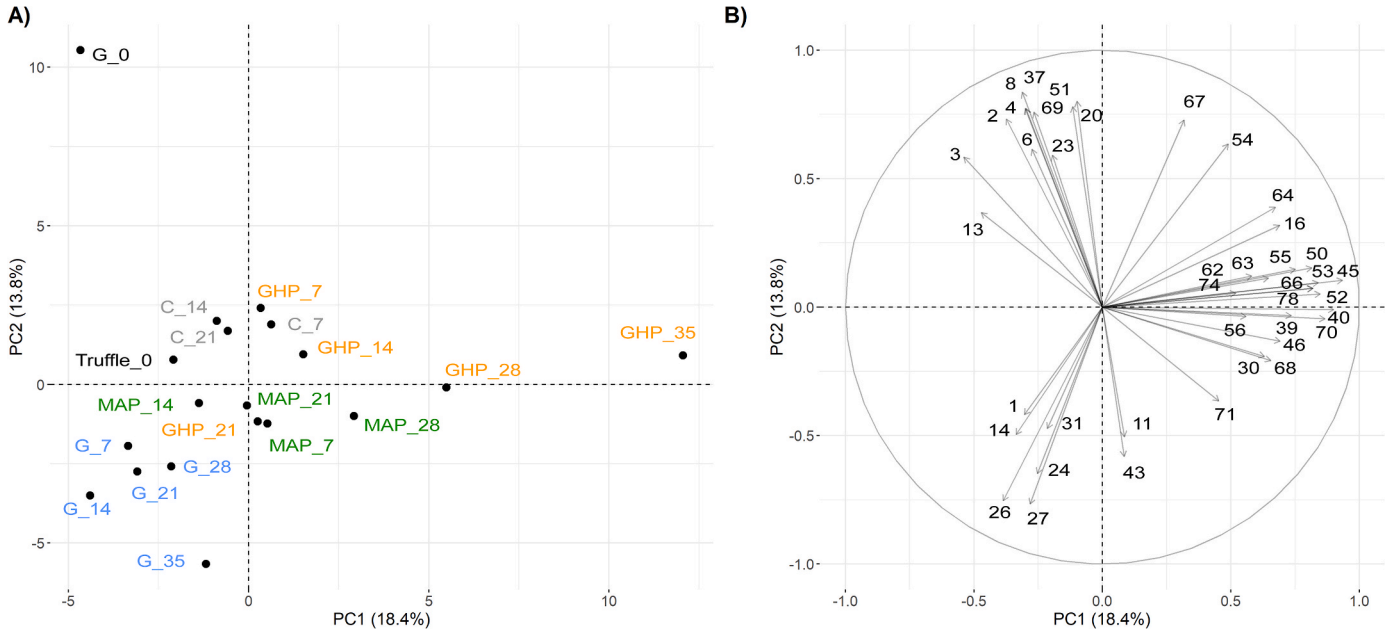

Fig. 5. PCA results for truffles packaged in three different conditions: (A) score plot for VOC profile variation among samples, and (B) loading plot for the VOCs detected using SPME-GC–MS. In (A), the sample name truffle-0 refers to fresh-truffle without packaging, C refers to truffle samples in control packaging, GHP to truffle samples in gelatine packaging, MAP to truffle samples in modified atmosphere packaging, and G to gelatine used in GHP samples. The numbers (7, 14, 21, 28, and 35) correspond to the different storage days of the experiment. In (B), compounds are identified with numbers corresponding to those in Table 2. For clarity purposes, only the compounds with a significant ($P < 0.05$) Pearson correlation with either PC1 or PC2 are shown.

CRediT authorship contribution statement

Eva Tejedor-Calvo: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Víctor Baquero-Aznar:** Investigation, Formal analysis, Data curation. **Sara Vega-Diez:** Investigation, Formal analysis, Data curation. **María Luisa Salvador:** Writing – review & editing, Supervision, Conceptualization. **María Ángeles Sanz:** Resources, Data curation. **Sergio Sánchez:** Writing – review & editing, Resources. **Pedro Marco:** Writing – review & editing, Methodology, Conceptualization. **Sergi García-Barreda:** Visualization, Validation, Software. **Jaime González-Buesa:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: J. González-Buesa, V. Baquero, S. Sánchez, P. Marco, S. García-Barreda, the research team of J. González-Buesa, and Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA) have a financial conflict of interest resulting from a patent application for smart thick coatings.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodhyd.2024.109874.

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