Food Packaging and Shelf Life 52 (2025) 101673

Contents lists available at ScienceDirect

Food Packaging and Shelf Life

journal homepage: www.elsevier.com/locate/fpsl

Microbial safety improvement of date fruits using a *Thymus numidicus* essential oil-based active packaging

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ARTICLE INFO

Keywords:
α-Terpineol
Geraniol
Vapour phase
Antifungal activity
Antimicrobial packaging
Date fruits
Shelf life

ABSTRACT

Volatile profile of *Thymus numidicus* essential oil (EO) contained 51 compounds, with linalool (30.252 ± 0.336 %), α-terpineol (25.167 ± 0.168 %) and geraniol (9.440 ± 0.176 %) being the major ones. Vapour phase antimicrobial assays revealed the antifungal activity of *T. numidicus* EO, α-terpineol, geraniol and a mixture of these two compounds at ratio of 2.5/1 (α-Ter/Ger: 2.5/1) against *Penicillium roqueforti*, *Aspergillus flavus*, *Aspergillus niger* and *Saccharomyces cerevisiae*, with minimum inhibitory concentration (MIC) values ranging from 263 to 526 μL/L. Only α-terpineol and the mixture α-Ter/Ger: 2.5/1 showed antibacterial activity, being able to inhibit *Escherichia coli* growth at 263 and 526 μL/L, respectively. Subsequently, new antimicrobial multilayer films containing a mixture of α--Ter/Ger: 2.5/1 as active agent (6 and 8 %) were prepared and tested for their antimicrobial activity. The active films 6 % and 8 % exhibited antifungal and antibacterial activities that were proportional to the concentration of α--Ter/Ger: 2.5/1 used. A chemical safety assessment of the active films revealed their compliance as food contact materials (FCMs) in accordance with EC N° 10/2011. A proof of concept trial using soft date fruits revealed the antimicrobial potential of the active packaging developed and its ability to extend the shelf life of these fruits. The active packaging developed will not require any changes in packaging material processing at industrial scale and could therefore be suitable for smallholders looking to offer quality natural products free of synthetic preservatives and with larger shelf life.

1. Introduction

In today's globalised food systems, where the distance and time between farm and consumer are increasingly long, the use of packaging has become crucial to prevent contamination, spoilage and guarantee food safety (Chan, 2022). In addition, the rational use of packaging can be advantageous for the environment, as it reduces product losses and avoids the depletion of natural resources (Wikström et al., 2019).

In recent years, the incorporation of food preservatives into packaging systems (active packaging) has boosted packaging technology and proved effective in improving food storage and shelf life. However, this innovation has coincided with severe criticism and concerns about the use of synthetic additives. These worries are mainly due to their harmful effects on health (Pisoschi et al., 2018) and their diminishing effectiveness in the face of the growing health threat associated with antimicrobial resistance (Siddiqui et al., 2023). All these problems have prompted research to replace these synthetic additives with safer, natural bioactive compounds, guaranteeing human safety and environmental protection (Guglielmi et al., 2020).

Many essential oils (EOs) and their compounds have been promoted as a suitable alternative to synthetic preservative. These natural substitutes are known for exhibiting good antioxidant, antibacterial and antifungal properties, without threatening consumer health and the environment (Masyita et al., 2022). Indeed, many of them are classified

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https://doi.org/10.1016/j.fpsl.2025.101673
Received 4 February 2025; Received in revised form 30 September 2025; Accepted 23 November 2025
Available online 29 November 2025

by the FDA as GRAS ingredients (generally recognised as safe) (Hou et al., 2022) and are legally authorised and approved as food flavourings under European Regulation (European Commission, 2024).

Thymus numidicus is a species of thyme from the Lamiaceae family that grows naturally in the wild in North Africa (El Mokni et al., 2023). Like all other thyme species, this plant is highly prized by local populations for its ornamental, culinary and medicinal uses (Elaissi et al., 2020). Thyme EOs are known to contain oxygenated monoterpenes, such as thymol, carvacrol, α-terpineol, linalool and geraniol, as major compounds (Drioiche et al., 2022). In addition to their flavouring properties, oxygenated monoterpenes have an antimicrobial potential against foodborne microorganisms (Paulino et al., 2022), including bacteria such as *Listeria monocytogenes* and *Escherichia coli* (Hussein et al., 2021), and fungi such as *Botrytis cinerea* and *Aspergillus spp*. (Tang et al., 2018; Yu et al., 2015).

When designing an active packaging with EOs, the EO or its active compounds are either incorporated directly into the packaging material or into additional carriers such as sachets or pads (Ribeiro-Santos et al., 2017). Due to their high volatility, they can diffuse and permeate through the carriers or packaging material and reach the food by direct contact or after their diffusion in the packaging atmosphere (headspace) by indirect contact (Simionato et al., 2019).

In addition, to overcome certain limitations of EOs use in active food packaging, such as high volatility, low solubility and strong aroma, strategies based on multilayer packaging systems or EO encapsulation have been developed for more sustained and controlled EO release (Zhang, Jiang, Rhim, Cao, & Jiang, 2022). For instance, it has been reported that a cinnamon EO-based multilayer has successfully extended the shelf life of tomato puree (Gherardi et al., 2016). In another example, low density polyethylene (LDPE) films containing nanocomposites loaded with carvacrol and thymol demonstrated a potential efficiency to control bacteria in *Hummus* (Krepker et al., 2017).

The use of EO-based active packaging for fruit packaging can be more challenging due to their specific characteristics. Some fruits with high post-harvest respiration or ethylene production are more susceptible to perish. Therefore, EOs must be judiciously selected to prevent both their microbial deterioration and their senescence (López-Gómez et al., 2023). Furtherly, fruits are commonly freshly consumed and flavour is key factor in their acceptance by the consumer. The persistence of any strong flavour resulting from EO release may induce undesirable organoleptic changes in fruits, which can hinder their acceptance by consumers (El Khetabi et al., 2022). The selection of suitable EOs which smell is compatible with the aroma and flavour of the food product may reduce the negative impact of EOs on sensory attributes (Perumal et al., 2022). Thus, exploring new combinations between several EOs and/or their active compounds remains an interesting prospect, in particular on targeted fruits with low metabolic activity such as soft dates (*Rutab*) (Kader & Hussein, 2009), which were selected as the food model in the present study.

The soft dates *Rutab* are fruits obtained at early ripening stage from date palm tree (*Phoenix dactylifera* L.), which are appreciated for their soft texture and light golden-brown colour, as well as for their interesting organoleptic and aromatic properties (Hamad et al., 2012). This type of date is marketed as premium dates and accounts for the majority of the international date trade (Cherif et al., 2021). *Rutab* soft dates have a relatively high water content (around 35 %, a_w = 0.80–0.85) and therefore require special conditions to avoid microbial contamination, particularly by yeasts and filamentous fungi, which are the main cause of their spoilage during storage and distribution (Al-Asmari et al., 2017; Belili et al., 2024; Cherif et al., 2021). Soft dates losses at post-harvest stage can reach up to 10 % (El-Ansary et al., 2019), mainly because of mishandling and poor packaging (Suhail et al., 2020). Dates shelf life varies according to storage conditions: they can last up to 12 months at −18 °C, around 6 months at 2–4 °C and around 1 month at 20 °C (Cherif et al., 2021).

Therefore, research into the development of active packaging with natural active agents would be of great interest for reducing soft date losses and providing a high-quality product appropriate for premium label.

In this work, *T. numidicus* EO was analysed by HS-SPME-GC-MS to identify its volatile profile. The antimicrobial activity in vapour phase of the EO and its main compounds was evaluated separately and in mixtures of two or three compounds, to assess potential additive or synergistic effects between compounds. The best antimicrobial combination was selected and incorporated into a multilayer polyethylene terephthalate (PET)/low-density polyethylene (LDPE) film, that was further on assessed in terms of antimicrobial effectiveness against bacteria, yeasts and filamentous fungi to evaluate the potential use of these films as antimicrobial packaging materials. After the successful active packaging design, the active multilayer developed was used to package *Rutab* date fruits while evaluating its preservation efficiency by analysing the packaged date fruit samples during four weeks' storage in terms of microbial load (mesophilic and fungal) and physicochemical parameters (colour and weight loss).

2. Material and methods

2.1. Chemicals

Geraniol (98 %, CAS 106–24–1), linalool (97 %, CAS 78–70–6) and 2,2-diphenyl-1-picrylhydrazyl (DPPH, CAS 1898–66–4) were purchased from Sigma-Aldrich (Madrid, Spain). α-Terpineol (97 %, CAS 98–55–5) was from Thermo Fisher Scientific (Madrid, Spain). Methanol ($\geq$ 99.9 %, CAS 67–56–1) was provided by Honeywell (Madrid, Spain). Ethanol ($\geq$ 99.9 %, CAS 64–17–5) was supplied by Panreac (Barcelona, Spain). Solid phase microextraction fibers and Tenax® TA 80/100 mesh were obtained from Supelco (Bellefonte, PA, USA). Plastic films (PET 12 μm and LDPE 35 μm) were obtained from Envaflex (Zaragoza, Spain). The ultrapure water was produced by a Milli-Q UltramaticWasserlab GR system (Barbatain, Spain).

2.2. Microbial strains and growth conditions

Antimicrobial susceptibility testing was performed against a Gram-positive bacterium (*Staphylococcus aureus* ATCC 29213), a Gram-negative bacterium (*Escherichia coli* ATCC 25922), a yeast (*Saccharomyces cerevisiae* CECT 1172) and several filamentous fungi (*Penicillium roqueforti* CECT 2905, *Aspergillus flavus* CECT 2687 and *Aspergillus niger* CECT 2088). Bacteria were provided by the American Type of Culture Collection (ATCC) and fungi by the Colección Española de Cultivos Tipo (CECT). All microbial strains were stored in the appropriate culture media supplemented with 30 % (v/v) glycerol at −80 °C until use. Prior to antimicrobial assays, each cryopreserved strain was subcultured in adequate conditions to ensure purity and optimal growth: bacteria on Mueller-Hinton Agar (MHA, Scharlau, Spain) at 37 °C for 24 h, yeast on Sabouraud Dextrose Agar (SDA, Scharlau, Spain) at 30 °C for 48 h and filamentous fungi on SDA slants at 25 °C for 7 days. All microorganisms were incubated in aerobic conditions.

2.3. Thymus numidicus EO extraction and characterization

2.3.1. EO extraction

Plant preparation and EO extraction is briefly described in Supplementary material.

2.3.2. Identification of volatile compounds

The analysis was carried out following previous studies on similar chemical compounds (Asensio et al., 2022). The volatile compounds of *T. numidicus* EO were analysed by headspace solid-phase microextraction (HS-SPME) coupled to gas chromatography-mass spectrometry (GC-MS). A volume of 10 μL of *T. numidicus* EO was deposited at the bottom of a tightly sealed glass vial. The vial was then subjected to

heating and agitation during the extraction (SPME) procedure. The volatile compounds adsorbed on the fibre were then analysed by GC- MS as described underneath.

The equipment included a CTC Analytics CombiPal autosampler interfaced with a GC-MS instrument comprising an Agilent 6890 N gas chromatograph coupled to a MS 5975B mass spectrometer detector. All instruments were from Agilent Technologies (Palo Alto, CA, USA). SPME was conducted using a Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) with a film thickness of 50/30 μm fibre. The fibre was previously conditioned before the first extraction according to the manufacturer specifications. SPME was carried out at 50 °C for 15 min, followed by desorption for 2 min at the inlet. Samples were injected in a capillary HP-5 MS (30 m × 0.25 μm × 250 μm) column from Supelco (Bellefonte, PA, USA), in splitless mode with a helium flow rate of 1 mL/min. The oven program was as follows: held at 50 °C for 5 min, followed by an increase at a rate of 10 °C/min to 300 °C, and held at 300 °C for 2 min. The mass detector was set in scan mode within a mass-to-charge ratio range of 45–400 *m/z*. The constituents of the EO samples were assigned by comparing the retention indexes (RI) for a homologous series of C9-C29 n-alkanes, determined under the same chromatographic conditions as the samples, with the retention indexes of the corresponding spectra in the library database (NIST ChemistryWebBook software). The major compounds were further confirmed by corresponding standards analysed at the same chromatographic conditions as the samples. The content of the compounds in the EO was estimated by calculating the percentage of each compound based on peak area.

2.3.3. Antimicrobial activity of EO and its main compounds

The antibacterial and antifungal activities of *T. numidicus* EO and its main compounds, taken individually or in mixture were performed to determine the minimum inhibitory concentration (MIC) in vapour phase, using the inverted Petri plate assay (Simionato et al., 2019). Combinations of two or three compounds were chosen according to the ratio of the three main compounds, linalool, α-terpineol and geraniol, found in the EO analysed (linalool/α-terpineol/geraniol: 3/2.5/1), as can be seen in Table S1. Firstly, for each bacterial strain, plates containing MHA were inoculated by dropping 20 μL of the corresponding bacterial suspension (1–2 × 10^8 CFU/mL) into the centre of the agar. Bacterial inocula were prepared from colonies from overnight cultures grown on MHA plates at 37 °C. In the case of yeasts and filamentous fungi, the same drop-plate inoculation procedure was used, with an inoculum volume of 10 μL of 1 × 10^6 CFU/mL on SDA. Yeast and filamentous fungus inocula were prepared from 48 h and 7 days cultures on SDA incubated at 30 °C and 25 °C, respectively. Bacteria and yeast inocula were prepared in a 0.9 % (w/v) NaCl solution, while filamentous fungi inocula in water containing 0.1 % Tween 20. Concentrations were estimated by measuring optical density (OD) using a Beckman Coulter DU730 UV-Vis spectrophotometer (TÜV Rheinland North America). For bacteria, an absorbance at 600 nm of 0.1 corresponded to 1–2 × 10^8 CFU/mL, while for yeasts and fungi, the same absorbance value at 625 nm corresponded to 1 × 10^6 CFU/mL. After plate inoculation, a paper disk was placed on top of a coverslip glass on the centre of the inside of the lid of each plate, and loaded with 0 (negative control), 1.25, 2.5, 5 and 10 μL of *T. numidicus* EO or active compounds (linalool, α--terpineol and geraniol), tested separately and in mixtures of two or three compounds. Then, the plates were sealed with Parafilm® and incubated at 37 °C/24 h (bacteria), 30 °C/48 h (yeast) and at 25 °C/7 days (filamentous fungi). The minimum inhibitory concentration (MIC) in the vapour phase was defined as the lowest concentration of the volatile active agent, expressed as μL of added volatile to the paper disk placed on the inside of the Petri plate by the headspace volume, in mL, of the Petri plate, that showed no microbial growth after incubation. At least three independent replicates were performed for each tested active agent. The assays were conducted under aseptic conditions in a level-II biosafety cabinet (Thermo Scientific MSC-Advantage, Germany).

2.4. Active multilayer film

2.4.1. Active multilayer film preparation

The active multilayer was prepared at the laboratory scale, using two different plastic polymer films bonded together using an adhesive into which the natural active agent (α--Ter/Ger: 2.5/1) was incorporated. The chosen adhesive was water-based and suitable for food contact, provided by SAMTACK (Spain). More details about the adhesive were not disclosed due to confidentiality breaching. The plastic films were chosen for their food contact safety, their permeability/impermeability to organic vapours and their technical and economic characteristics (low cost, transparency and heat sealability): *a)* polyethylene terephthalate (PET 12 μm) as an outer layer, with good barrier properties that prevented the loss of the volatile active agent to the outside of the package; *b)* low density polyethylene (LDPE 35 μm) as an inner layer, since it had high permeability to organic vapours and allowed the release of the active agent into the food product and packaging atmosphere. Multilayer films were obtained by spreading the active adhesive uniformly on 20 × 30 cm LDPE sheets using a bar coater with a wet film deposition of 120 μm. After adhesive drying, 20 × 30 cm PET sheets were then laminated onto the LDPE adhesive film. Finally, the prepared multilayer film was pressed at 40 °C and speed 4 using BiO 330 A3 Heavy Duty Laminator (South Korea). A blank was prepared under identical conditions but without active agent. Following this method, two multilayer films with 6 and 8 % (w/w) of the active agent in the wet adhesive were prepared and tested. These active packages were designated as active packaging 6 % and active packaging 8 %, respectively. The multilayer film without EO was taken as blank (control film).

2.4.2. Antimicrobial activity of multilayer active films

After *in vitro* assays, the natural agent with the best antimicrobial activity was selected to prepare the active packaging. The antimicrobial activity of the active multilayer films was also investigated in vapour phase (Wrona et al., 2023). Briefly, MHA and SDA plates were inoculated by streaking a cotton swab previously soaked with bacterial inocula of 1 × 10^6 CFU/mL or yeast/filamentous fungi inocula of 1 × 10^5 CFU/mL. Microbial suspensions were prepared as described in Section 2.3.3, with a dilution factor of 1:100 for bacteria and 1:10 for yeasts and filamentous fungi. Then, the inoculated plates were covered with a 12 × 12 cm^2 of the prepared films. The films were held firmly in place with a nylon tie around the outer rim of the plate. Plates were visually inspected for growth, after incubation at 37 °C/24 h (bacteria), 30 °C/48 h (yeast) and 25 °C/5 days (filamentous fungi) in aerobic conditions. Three independent replicates were performed for these assays.

2.4.3. Migration assays

Migration assays were carried out according to Regulation (EC) N° 10/2011 (European Commission, 2011) to verify any release of undesirable compounds from the active multilayer film to the food. Since the active multilayer film was intended to be used for dried fruits packaging, Tenax® (small granules of poly(2,6-diphenyl-*p*-phenylene oxide) was the food simulant used for the migration assays. Tenax® is a porous polymer with a high adsorption potential (specific surface area of 35 m^2/g). The experimental conditions for the migration assays were previously optimised by Canellas et al. (2015). Briefly, 5 × 5 cm cut-outs of each prepared multilayer film were placed in Petri dishes, with the inner side facing upwards. One gram of Tenax® was spread evenly over each piece of film to maintain a ratio of 4 g of Tenax®/dm^2 of film, in accordance with UNE-EN 14338 (AENOR et al., 2004). The entire system was then put in an oven set at 40 °C for 10 days. In the next step, Tenax® simulant from each sample was collected and extracted with 3 mL of ethanol for one hour in an ultrasonic bath. Each sample was extracted three consecutive times and the three extracts obtained were combined and concentrated to 0.5 mL under a constant flow of nitrogen (Vera et al., 2024). Three replicates of the experiment were performed

for each multilayer. Volatile and non-volatile migrants present in the ethanol extract were analysed using the chromatographic methods described underneath:

2.4.3.1. Volatile compounds. Volatile migrants were identified using direct injection gas chromatography coupled to mass spectroscopy (DI-GC-MS). One microliter of the ethanolic extract recovered from the food simulant was injected in splitless mode. The chromatographic conditions were the same as those reported previously in Section 2.3.2.

2.4.3.2. Non-volatile compounds. The analysis of non-volatile compounds was done by ultra-high performance liquid chromatography coupled to quadruple time-of-flight with MSE technology (UPLC-ESI-Q-TOF-MSE). Chromatography was performed with an ACQUITY UPLC® I-Class system comprising an UPLC BEH C18 column (1.7 μm particle size, 2.1 mm × 100 mm), both from Waters (Milford, MA, USA). Column flow rate and temperature were 0.3 mL/min and 35 °C, respectively. The mobile phase was water with 0.1 % formic acid (phase A) and methanol with 0.1 % formic acid (phase B). The gradient was 95 % A/5 % B (initial time), 5 % A/95 % B (6 min), 5 % A/95 % B (10 min), 95 % A/5 % B (13 min), 95 % A/5 % B (15 min). The injection volume was 10 μL. The detector consisted of an electrospray ionization (ESI) interface coupled to a mass spectrometer comprising a hexapole, a quadrupole, a collision cell and a time-of-flight analyzer (Xevo G2) from Waters (Milford, MA, USA). The electrospray probe was set in positive mode (ESI+). Sensitivity mode was used to operate the mass spectrometer with a capillary voltage of 2.6 kV and a sampling cone voltage of 40 V. Nitrogen was used as the desolvation gas: the flow rate was 600 L/h at 450 °C. The cone gas flow rate was 50 L/h. Source temperature was set at 130 °C. MSE mode was selected for the acquisition and a collision energy ramp from 15 to 30 eV was used. The mass range considered was 50–1200 Da. Centroid mode was used for data collection. The accuracy and reproducibility of all the analyses were guaranteed by use of a LockSpray™. To check the retention time shift, mass accuracy and reproducibility reference, a standard at a concentration of 2 ng/mL in water-acetonitrile with 0.1 % formic acid was used. The software MassLynx(version 4.1, Waters) was used for data acquisition and processing.

2.5. Active packaging application

2.5.1. Food sample

The active multilayer film developed was tested on soft date fruits (*Phoenix dactylifera*). The samples were obtained from a local supermarket in Spain and stored refrigerated in the same way as they were marketed until their use. The date variety chosen was *Deglet-Noor*, originally from Algeria.

2.5.2. Food packaging trial conditions

The trial was based on a completely randomised design (CRD). Three groups of packages were defined: control packaging, active packaging 6 % and active packaging 8 %. Each group was represented by at least three replicates for each time point. Each replicate consisted of five date fruits packed in 14 × 14 cm pouches (Figure S1), prepared by heat sealing. Control and active pouches with dates were stored at 25 °C and sampling was performed each week for a period of a month to ensure that this active packaging would be effective under normal dates storage conditions (1 month at 20 °C) (Kader & Hussein, 2009). At each sampling, date fruit quality parameters (weight loss, colour and microbial growth) in the different groups were assessed.

2.5.3. Quality characteristic changes during the active packaging trial

2.5.3.1. Weight loss. The weight loss of packaged date fruit samples was determined by weighing each sample (consisting of 5 dates) at the start of the trial and after 1, 2, 3 and 4 weeks. The percent weight loss (WL%) was calculated following the Eq. (1). Each sample was taken in three replicates.

$$WL\% = \frac{W_i - W_f}{W_i} \times 100 \quad (1)$$

where W_i and W_f were the initial and final weights, respectively, of the date fruit samples for each time interval considered (1, 2, 3, 4 weeks).

2.5.3.2. Colour change. The colour was measured according to the CIE $L^* a^* b^*$ (International Commission on Illumination) coordinate system using a Chroma Meter CR-400 colorimeter from Konica Minolta (Tokyo, Japan) featuring a D65 as a light source. Before measurement the equipment was calibrated with white chroma-meter standard plate. The colour coordinates: L^* (lightness), a^* (redness or greenness) and b^* (blueness or yellowness) were measured at eight different points on the surface of each date sample (5 fruits). Measurements were performed at the beginning and after each one-week interval of the trial period (4 weeks). Three replicates were measured for each sample.

2.5.3.3. Microbial analyses. The antimicrobial capacity of the active packaging was tested by assessing the evolution of the total aerobic mesophilic flora (TMF) and microbial fungal flora (MFF) load of the date fruits during trial storage at 25 °C. TMF and MFF were estimated at each sampling interval using plate count method in accordance with ISO 4833–1: 2013 (ISO, 2013) and ISO 21527–2: 2008 (ISO, 2008) standards with slight alterations, respectively. In brief, 4 g of each sample was mixed with 36 mL of sterile buffered peptone water in Stomacher bag and homogenized using a Stomacher apparatus (Seward Stomacher® 400, UK) at 200 rpm for 10 min. After homogenization, the sample suspension was diluted in saline solution (NaCl 0.9 %) and plated on the appropriate culture media, i.e, plate count agar (PCA) for TMF, and Sabouraud dextrose agar (SDA) for MFF and incubated at 30 °C for 3 days (TMF) and 25 °C for 5 days (MFF). After incubation, the colonies developed on the agar surface were counted and subsequently, the total number of colony-forming units per gram of dates was calculated, taking into account the dilution factor for each sample. At least three replicates were analysed for these parameters.

2.6. Statistical analysis

Results were expressed as mean values and standard deviations (SD). Differences between treatments were analysed using a one- and/or two-way ANOVA statistical test, considering $p < 0.05$. The Tukey test was then applied to identify specific differences between the groups.

3. Results and discussion

3.1. Identification of volatile compounds

T. numidicus EO was characterised by HS-SPME-GC-MS. The chromatograms (Figure S2) were analysed on the basis of retention index and mass spectra. The abundance of each compound was estimated semi-quantitatively by calculating the percentage of the surface area of each peak in relation to the total surface area. The results revealed the presence of 51 volatile compounds, listed in order of elution in Table 1. Oxygenated monoterpenes were the dominant constituents (70 %), of which three belonging to the monoterpene alcohol group accounted for around 65 %, namely: linalool (30.252 ± 0.336 %), α-terpineol (25.167 ± 0.168 %) and geraniol (9.440 ± 0.176 %). The hydrocarbon monoterpenes group came in second place with a proportion of around 17 %. Remarkably, thymol, a major constituent of most thyme EO (Pandey et al., 2021), was not detected in the samples. The chemical composition of the present study differs from that of other origins in North Africa, where the phenolic monoterpene, thymol, prevailed in *T. numidicus* (Elaissi et al., 2020; Hadef et al., 2007; Kabouche et al., 2005; Laouer

Table 1
List of volatile compounds identified in the EO of *T. numidicus* by HS-SPME-GC-MS. Main components (Area percentage higher to 2 %) are highlighted in bold.

N°	Compound	Chemical class	Molecular Formula	CAS number	Retention Index	Area (%) Mean ± SD
1	Hashishene	Bicyclic alkene	C10H16	16626–39–4	921	0.053 ± 0.005
2	*α*-Thujene	Bicyclic alkene	C10H16	2867–05–2	929	0.296 ± 0.010
3	**α-Pinene**	**Bicyclic monoterpene**	**C10H16**	**80–56–8**	**937**	**2.225 ± 0.042**
4	Camphene	Bicyclic monoterpene	C10H16	79–92–5	952	0.108 ± 0.012
5	Sabinene	Bicyclic monoterpene	C10H16	3387–41–5	974	1.085 ± 0.041
6	*β*-Pinene	Bicyclic monoterpene	C10H16	127–91–3	979	0.618 ± 0.044
7	**β-Myrcene**	**Acyclic monoterpene**	**C10H16**	**123–35–3**	**991**	**5.938 ± 0.150**
8	*α*-Phellandrene	Monocyclic monoterpene	C10H16	99–83–2	1005	0.279 ± 0.022
9	*α*-Terpinene	Monocyclic monoterpene	C10H16	99–86–5	1017	0.701 ± 0.016
10	*p*-Cymene	Monocyclic monoterpene	C10H14	99–87–6	1025	0.328 ± 0.005
11	**D-Limonene**	**Monocyclic monoterpene**	**C10H16**	**5989–27–5**	**1031**	**2.515 ± 0.027**
12	*β-cis*-Ocimene	Acyclic monoterpene	C10H16	3338–55–4	1038	0.481 ± 0.022
13	*β*-Ocimene	Acyclic monoterpene	C10H16	13877–91–3	1037	0.872 ± 0.016
14	*γ*-Terpinene	Acyclic monoterpene	C10H16	99–85–4	1060	1.369 ± 0.011
15	*trans*-Sabinene hydrate	Bicyclic monoterpenoid	C10H18O	17699–16–0	1070	0.083 ± 0.008
16	Terpinolene	Monocyclic monoterpene	C10H16	586–62–9	1088	0.734 ± 0.018
17	**Linalool**	**Acyclic monoterpenoid**	**C10H18O**	**78–70–6**	**1099**	**30.252 ± 0.336**
18	endo-Borneol	Bicyclic monoterpenoid	C10H18O	507–70–0	1167	0.058 ± 0.005
19	Terpinen-4-ol	Monocyclic monoterpenoid	C10H18O	562–74–3	1177	0.816 ± 0.036
20	**α-Terpineol**	**Monocyclic monoterpenoid**	**C10H18O**	**98–55–5**	**1189**	**25.167 ± 0.168**
21	*cis*-Geraniol	Acyclic monoterpenoid	C10H18O	106–25–2	1228	0.496 ± 0.030
22	Neral	Acyclic monoterpenoid	C10H16O	106–26–3	1240	0.547 ± 0.012
23	**Geraniol**	**Acyclic monoterpenoid**	**C10H18O**	**106–24–1**	**1255**	**9.440 ± 0.176**
24	*α*-Citral	Acyclic monoterpenoid	C10H16O	141–27–5	1270	0.184 ± 0.032
25	**Anethole**	**Monocyclic monoterpenoid**	**C10H12O**	**104–46–1**	**1287**	**2.108 ± 0.108**
26	Carvacrol	Monocyclic monoterpenoid	C10H14O	499–75–2	1299	0.052 ± 0.004
27	Geranyl formate	Monoterpene ester	C11H18O2	105–86–2	1301	0.036 ± 0.000
28	*α*-Cubebene	Tricyclic sesquiterpene	C15H24	17699–14–8	1351	0.130 ± 0.005
29	Eugenol	Monocyclic monoterpenoid	C10H12O2	97–53–0	1358	0.061 ± 0.002
30	Geranyl acetate	Acyclic monoterpenoid	C12H20O2	105–87–3	1382	0.529 ± 0.014
31	*β*-Bourbonene	Tricyclic sesquiterpene	C15H24	5208–59–3	1384	1.345 ± 0.010
32	*α*-Gurjunene	Tricyclic sesquiterpene	C15H24	489–40–7	1409	0.151 ± 0.006
33	**Caryophyllene**	**Bicyclic sesquiterpene**	**C15H24**	**87–44–5**	**1419**	**4.610 ± 0.032**
34	*β*-cis-Copaene	Tricyclic sesquiterpene	C15H24	18252–44–3	1432	0.265 ± 0.006
35	Cadina-3,5-diene	Bicyclic sesquiterpene	C15H24	267665–20–3	1458	0.239 ± 0.028
36	Humulene	Macrocyclic sesquiterpene	C15H24	6753–98–6	1454	0.216 ± 0.004
37	*cis*-Muurola-4(15),5-diene	Bicyclic sesquiterpene	C15H24	157477–72–0	1463	0.314 ± 0.007
38	*γ*-Muurolene	Bicyclic sesquiterpene	C15H24	30021–74–0	1477	0.474 ± 0.035
39	Germacrene D	Macrocyclic sesquiterpene	C15H24	23986–74–5	1481	1.928 ± 0.089
40	*γ*-Amorphene	Bicyclic sesquiterpene	C15H24	6980–46–7	1496	0.274 ± 0.020
41	*α*-Muurolene	Bicyclic sesquiterpene	C15H24	10208–80–7	1499	0.269 ± 0.007
42	*β*-Bisabolene	Monocyclic sesquiterpene	C15H24	495–61–4	1509	0.361 ± 0.021
43	*γ*-Cadinene	Bicyclic sesquiterpene	C15H24	39029–41–9	1513	0.403 ± 0.019
44	*δ*-Cadinene	Bicyclic sesquiterpene	C15H24	483–76–1	1524	0.940 ± 0.038
45	Cubenene	Bicyclic sesquiterpene	C15H24	29837–12–5	1532	0.087 ± 0.006
46	*α*-Cadinene	Bicyclic sesquiterpene	C15H24	24406–05–1	1538	0.140 ± 0.006
47	*α*-Calacorene	Bicyclic sesquiterpene	C15H20	21391–99–1	1542	0.058 ± 0.006
48	Nerolidol	Acyclic sesquiterpenoid	C15H26O	40716–66–3	1564	0.129 ± 0.014
49	Caryophyllene oxide	Bicyclic sesquiterpenoid	C15H24O	1139–30–6	1581	0.126 ± 0.016
50	Caryophylla-4(12),8(13)-dien-5beta-ol	Bicyclic sesquiterpenoid	C15H24O	19431–80–2	1644	0.069 ± 0.005
51	*α*-Cadinol	Bicyclic sesquiterpenoid	C15H26O	481–34–5	1653	0.041 ± 0.006
	Grouped compounds [%]					
	Monoterpene hydrocarbons					17.25
	Oxygenated monoterpenes					69.83
	Sesquiterpene hydrocarbons					12.20
	Oxygenated sesquiterpenes					0,37
	Others					0,35

et al., 2009). However, it was also reported, in a previous study with *T. numidicus*, that *α*-terpineol was the main compound (34.4 %), followed by thymol (15.00 %) (Hazzit et al., 2006). Generally, the absence of thymol in thyme is very rare and has only been observed in a few studies on certain species such as *Thymus algeriensis* (Souiy et al., 2023), *Thymus webbianus* (Asensio-Casas et al., 2022) and *Thymus citriodorus* (Villanueva Bermejo et al., 2015). A previous study also reported the variation in thymol content in *T. numidicus* during the biological cycle. The study showed that thymol increases at the flowering stage when its precursor, *p*-cymene, decreases, and vice versa (Hadef et al., 2007). In the present study, *p*-cymene content (0.328 ± 0.005 %) was very low and thymol was also not detected. These results suggest the identification of a new chemotype of *T. numidicus* which has not yet been reported. It was also noted that linalool often appeared in *T. numidicus* and was considered as characteristic compound for this species (El Mokni et al., 2023). The difference in chemical composition can be attributed to a number of factors, such as origin, stage of growth, environmental conditions and genetic material (Liu et al., 2023). According to the literature, plant species rich in linalool (Mączka et al., 2022), *α*-terpineol (Sales et al., 2020) or geraniol (Maczka et al., 2020) exhibit potential antimicrobial and antioxidant properties. These compounds are also known for being safe and less corrosive and irritating than their homologues containing a phenolic function, such as thymol or carvacrol (Pinto et al., 2014). In essence, these beneficial properties make these compounds suitable candidates to be used as natural preservatives.

3.2. In vitro screening of antimicrobial activity

The antimicrobial potential of EOs against food microorganisms through direct contact has been widely documented, but their vapour phase activity, inherent to their volatility have not been thoroughly explored (Reyes-Jurado et al., 2020). In the present study, the *in vitro* antimicrobial activity of *T. numidicus* EO and its main volatiles compounds (linalool, α-terpineol and geraniol) was assessed in vapour phase against different groups of microorganisms including bacteria (Gram--positive and Gram-negative), yeast and filamentous fungi. Main *T. numidicus* EO volatile compounds were tested individually or in combination. After a preliminary antimicrobial susceptibility screening (Table S1), only the three compounds (α--terpineol, geraniol and *T. numidicus* EO) and one combination (α--Ter/Ger: 2.5/1) that showed the best antimicrobial activity results, were selected for further examination and determination of their MICs (expressed as microlitres of the volatile active agent per litre of headspace) against the considered microorganisms (Table 2). Despite being the main compound present in this *T. numidicus* EO, linalool and its combinations with other compounds were sidelined due to their relatively low antimicrobial activities against the tested microorganisms. This may be due to the fact that some EO compounds are less effective in vapour phase probably due to their ability to volatilize.

After assuming that all the volatile active agents tested were completely volatilized in the headspace of the Petri dish, MIC values recorded for the four tested active compounds against filamentous fungi and yeast ranged from 132 to 526 μL/L, with *S. cerevisiae* being the most sensitive species, followed by *P. roqueforti, A. niger* and then *A. flavus*. On the other hand, bacteria showed greater antimicrobial resistance to the compounds tested, since the four active volatile substances were unable to inhibit the growth of *S. aureus* at 526 μL/L, while only α-terpineol and the combination α-Ter/Ger: 2.5/1 were able to inhibit *E. coli* growth at 263 and 526 μL/L, respectively. Overall, volatile compounds appeared to be more effective against fungi than bacteria, which is consistent with previous studies (Mukurumbira et al., 2023). It has been suggested that the vapour phase of EOs has a particular impact on filamentous fungi due to their superficial growth, rendering them more sensitive to the vapour phase action of volatile compounds (Reyes-Jurado et al., 2020).

Unlike the most common literature findings, in this study, *E. coli* (Gram-negative) was more sensitive than *S. aureus* (Gram-positive) to the active compounds tested, which is nevertheless consistent with some previous studies, on the same strains using both the agar diffusion (Adrar et al., 2016) and disc diffusion methods (Tagnaout et al., 2022). This could be explained by the fact that some EO compounds have difficulty penetrating the cell wall of Gram-positive bacteria, which have a thicker cell wall with a higher peptidoglycan content and a greater number of special teichoic acid structures, unlike Gram-negative bacteria (Wang et al., 2017).

Table 2
Minimal Inhibitory Concentration (MIC) values of selected volatile active compounds in vapour phase against tested fungal and bacterial strains. Values presented are the modal values (n = 3) of at least three independent replicates.

	MIC (μL/L)			
Microbial strains	α-Terpineol	Geraniol	*T. numidicus* EO	[a]α-Ter/Ger: 2.5/1
P. roqueforti CECT 2905	263	263	263	263
A. flavus CECT 2687	526	526	526	526
A. niger CECT 2088	263	263	526	263
S. cerevisiae CECT 1172	526	132	263	132
E. coli ATCC 25922	263	> 526	> 526	526
S. aureus ATCC 29213	> 526	> 526	> 526	> 526

[a] α-Ter/Ger: α-terpineol/geraniol mixture.

In terms of comparison, due to the lack of studies on the same volatile active substances tested in the vapour phase against the same microorganisms, our results were compared with more closely related ones in terms of compounds and/or test methods. For instance, in previous study, *Thymus vulgaris* EO in vapour phase was found to inhibit the growth of both *S. cerevisiae* and *A. niger* at a MIC value of 19.79 μL/L, as well as *E. coli* and *S. aureus* at 39.58 and 9.89 μL/L, respectively (Mukurumbira et al., 2023), which is much lower when compared to MICs of *T. numidicus* EO in the present study (263–526 μL/L). These differences can be ascribed to the different compositional profile of the EO, as in our study, *T. numidicus* oil obtained lacked more active phenolic compound, as thymol. On the other hand, in another study with two *T. numidicus* EOs, Elaissi et al. (2020) reported a MIC of 400 μg/mL against *A. flavus*, in liquid phase, which is closer to the results of the present study. In fact, in several studies, the antimicrobial activity of EO is more effective in the liquid phase than in the vapour phase (Lin et al., 2022; Mukurumbira et al., 2023). However, with the lack of standardization in antimicrobial susceptibility testing for food-related purposes, it becomes very difficult to compare and interpret results among all the studies using the same EOs or extracts.

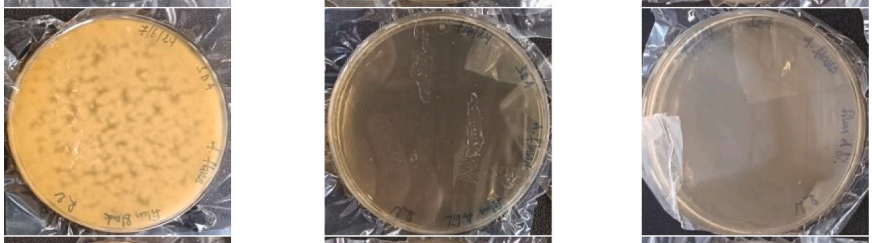
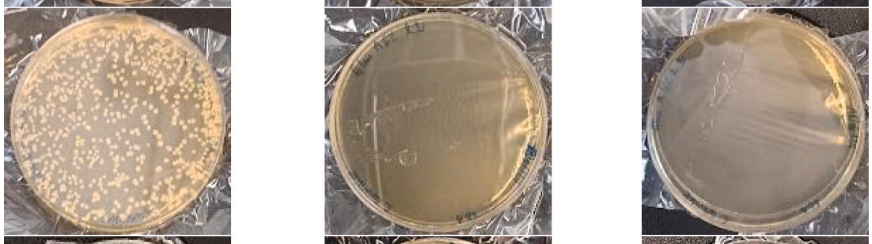
In the present work, the antimicrobial activity of the individual monoterpene alcohols α-terpineol and geraniol was quite similar. Both were able to inhibit *P. roqueforti* and *A. niger* at 263 μL/mL and *A. flavus* at 526 μL/L. Geraniol was more effective against *S. cerevisiae* (132 μL/mL) than α--terpineol (526 μL/L), but geraniol was ineffective against the two bacteria (> 526 μL/L), while α-terpineol was active only against *E. coli* at 263 μL/mL. The results obtained are in agreement with the ones obtained by other authors. For instance, Tang et al. (2018) reported that geraniol inhibited *A. flavus* by 98.44 % at a concentration of 0.4 μL/mL. Meanwhile, Pinto et al. (2014) showed that α--terpineol also exhibited antifungal activity against 19 strains of yeasts and filamentous fungi, including *A. flavus* and *A. niger*, with MICs ranging from 0.64 to 1.25 μL/mL. For *S. cerevisiae,* Scariot et al. (2021) registered a lower MIC value for geraniol (6.25 mM) than for α-terpineol (25 mM). Regarding the antibacterial activity, El Atki et al. (2020) observed that *E. coli* and *S. aureus* were resistant to geraniol (> 5 mg/mL) and Hussein et al. (2021) found that *E. coli* was more sensitive to α-terpineol than *S. aureus*, with MICs of 0.125 μL/mL and 0.250 μL/mL, respectively.

3.3. Antimicrobial potential of multilayer active films

After *in vitro* testing, a combination of α-terpineol and geraniol, at the same ratio they were present in *T. numidicus* EO (α-Ter/Ger: 2.5/1), was selected as the active agent due to its higher antimicrobial activity when compared to *T. numidicus* EO and the other compounds tested individually. This active agent was subsequently incorporated into the multilayer film at concentrations of 6 and 8 % (w/w) of the active agent in the wet adhesive. The prepared active films were then tested once more against all the bacteria and fungi studied using vapour phase assays. The results of the test are depicted in the Table 3. The antimicrobial activity of the active films was proportionally correlated with active agent concentration. Multilayer films containing 8 % of the active agent completely inhibited the growth of filamentous fungi and yeast (*P. roqueforti, A. flavus, A. niger* and *S. cerevisiae*), while the film with 6 % was relatively less effective against *A. flavus*. This finding highlights the antifungal potential of the prepared active films against filamentous fungi. Similarly to what was obtained in the *in vitro* vapour phase assays, neither 6 % nor 8 % active films were able to completely prevent the growth of bacteria (*E. coli* and *S. aureus*), although a visual reduction in growth was observed, which was proportional to active agent concentration. This suggested that the antibacterial effect in the vapour phase is concentration-dependent, and in the case of bacteria, higher concentrations would be required to completely inhibit growth.

The antimicrobial activity of the films suggests the release and diffusion of the active agent (α-Ter/Ger: 2.5/1) through the LDPE layer, as control films (without active agent) did not yield any antimicrobial

Table 3
Vapour phase antimicrobial activity of active multilayer films.

Microbial strains	Multilayer films		
	Control film 0%	Active film 6%	Active film 8%
P. roqueforti CECT 2905			
A. flavus CECT 2687			
A. niger CECT 2088			
S. cerevisiae CECT 1172			
E. coli ATCC 25922			
S. aureus ATCC 29213			

activity. The effective release of the active agents is also supported by the migration assays that showed the presence of both compounds in the food simulant used. This is in agreement with previous studies showing the permeation of various volatile organic compounds through LDPE films (Leelaphiwat et al., 2018; Leelaphiwat et al., 2016). The antimicrobial activity of volatile active compounds is determined by their release kinetics from packaging materials. A large number of studies have shown that the transfer of active compounds between the

packaging material and the food can be modelled using Fick's laws, which depend on various factors such as the diffusion coefficient of the compound, the diffusion distance (or thickness) of the medium and the concentration gradient (Kuai et al., 2021; Wang et al., 2022). Previous research has revealed that different volatile compounds exhibit different release behaviours depending on their physical-chemical characteristics (Sivalingam et al., 2024; Tonyali et al., 2020). In the case of EOs and their volatile compounds, some studies have reported that oregano (Yanela, Martínez-Tenorio Nelly, María Teresa, Aurelio, & Emma, 2024), thyme (Solano & de Gante, 2012), savoury (Abedi et al., 2016), cinnamon (Dong et al., 2018), garlic (Dong et al., 2019), rosemary (Dong et al., 2018) and cypress (Kim et al., 2020) EOs, as well as some of these EOs volatile compounds such as linalool (Sun et al., 2019), thymol (Giannakas et al., 2024; Rojas et al., 2017), carvacrol (Shemesh et al., 2015), carvone (Goñi et al., 2018), 4-allylanisole (Swilem et al., 2019) and D-limonene (Sun et al., 2019) incorporated either in LDPE composite film (Yanela et al., 2024) or multilayer films with LDPE (Dong et al., 2019) were able to diffuse successfully through the LDPE film layer and be released into the food packaging atmosphere. According to the current literature review, there are no reports on fungal inhibition by a combination of α-terpineol and geraniol incorporated into a packaging material. However, some studies have demonstrated the antibacterial effect of active packaging incorporated with these two natural active compounds (α--terpineol and geraniol) used separately. For instance, it is reported that ethylene–vinyl alcohol copolymer (EVOH) films containing 6 % geraniol and 6 % α-terpineol slowed *E. coli* growth by 2.02 $\pm$ 0.01 and 1.42 $\pm$ 0.05 log units, respectively (Wang et al., 2017). Similar results were obtained in another study, where a controlled release sachet made from EVOH coated paper containing 10 % of geraniol encapsulated in poly(butylene succinate) (PBS) showed inhibition of *S. aureus* and *E. coli* over a diameter of 3–4 cm (Petchwattana et al., 2021).

In summary, the film developed showed a good antimicrobial activity with potent antifungal potential, enabling it to be promoted as a good candidate for an active packaging material for intermediate moisture food products susceptible to fungal contamination, such as soft dates.

3.4. Active film migration test and safety assessment

Migration tests are carried out to ensure safety and compliance with regulatory requirements (EC) N° 10/2011 (European Commission, 2011). The active multilayer film developed was prepared with two food grade polymer films, PET 12 μm and LDPE 35 μm, which are known for their relative chemical resistance. In addition, the adhesive used is water-based and approved to be employed in food contact materials (FCMs). However, in some cases, the added active agent can compromise the integrity of the materials and lead to the release of toxic and undesirable compounds that can reach foodstuffs and threaten the health and safety of consumers. In the present work, the two active agents incorporated, α-terpineol and geraniol, are monoterpene alcohols which, in theory, are not corrosive and should not affect the integrity of the polymers used (PET and LDPE), and this was confirmed by performing the corresponding migration assays in the appropriate food simulant to dried fruits (Tenax).

The analysis of the chromatograms obtained after injection of the diluted pure active agent (α-Ter/Ger: 2.5/1) and the extracts recovered from the blank simulant, the control multilayer (0 %) and the active multilayers (6 % and 8 %) in GC-MS (volatile compounds) (Figure S3) and UPLC-ESI-Q-TOF-MSE (non-volatiles compounds) (Figure S4) revealed the absence of any peak corresponding to a foreign volatile or non-volatile compound. The peaks detected for the 6 % and 8 % multilayer films correspond to the incorporated active agent. Detection of the active agent indicates that it has been successfully released from the adhesive and has diffused through the LDPE film to reach the food simulant. Unlike certain adhesives containing isocyanates, which previous study has shown to prevent the release of the antimicrobial agent and therefore its diffusion through polyethylene (PE) film (Gherardi et al., 2016). These results confirm that the materials of the passive part of the packaging (PE and PET films) have preserved their integrity and that the adhesive components have been retained behind the LDPE layer. These findings are in line with a previous study which noted the absence of undesirable migrants (non-volatile compounds) through the LDPE layer of an active multilayer film based on ethanolic extracts of Sage leaves (Oudjedi et al., 2019).

Therefore, the active film developed here complies with the European legislation with regard to Regulation N° 450/2009 (European Commission, 2009) and Regulation N° 1935/2004 (European Commission, 2004), which requires the absence of migration of toxic and undesirable molecules from the material in contact with foodstuffs to the foodstuffs. As for α--terpineol and geraniol, they are classified as flavouring substances in the EU food Regulation and are considered safe when used in accordance with good manufacturing practices (European Commision, 2008; European Commission, 2024). This confirms the absence of migration of any toxic or undesirable IAS (Intentionally Added Substances) or NIAS (Non-Intentionally Added Substances) into the packaged product during long-term storage, and ensures safety for the consumer.

3.5. Effect of active packaging on date fruits during storage

3.5.1. Weight loss

The effect of the studied active packaging on the weight loss of date samples during 4 weeks of storage at 25 °C was examined and results were presented in Table S2. The weight loss varied between 0.56 % and 2.57 %. The lowest value was recorded in week 1, while the highest was in week 4. Statistical analysis revealed that the concentration of the active agent in the packaging had no effect on date weight loss, whereas the storage length was positively correlated with date weight loss ($p < 0.001$). In a previous study on the effect of a coating based on *Aloe vera* gel and lemongrass EO on date fruits (*Tamer* stage) storage during 4 weeks at 25 °C, the weight loss was merely non-significant for coated samples (~0.1 %) (Alkaabi et al., 2022). Conversely, in another study involving a different date variety (*Barhi*) sampled at the first edible maturity stage (*Khalal*) and coated with coatings based on gelatin, chitosan, guar gum and their combinations, the weight loss after 4 weeks of storage in polyethylene bags at 6 °C ranged from 9.10 % to 11.35 %, and after 8 weeks it reached a value around 15 % (Abu-Shama et al., 2020). These large variations could be explained by the difference in variety and maturity stage of the date samples, the initial moisture content of fruits, the storage conditions during the trial (temperature and relative humidity), as well as the nature and composition of the packaging.

3.5.2. Colour change

Colour is an important quality indicator that influences the appeal and acceptance of soft date fruits. In the present study, the colour coordinates of date samples stored in active packaging for 4 weeks at 25 °C were determined at each one-week interval and the results obtained are summarised in Fig. 1. Statistical analysis revealed that the differences in the colour coordinate values (L^*, a^* and b^*) registered for date samples in the different packages were not significant, meaning that the active packaging had no effect on colour change of the dates during storage. The present results are in agreement with those reported by Alkaabi et al. (2022) who observed that the different active coatings tested had no significant effect on the colour change of date fruits.

3.5.3. Microbial analyses

The effect of the active packaging on the microbial growth of date fruits was evaluated by determining the growth of TMF and MFF each week during the trial. The results are summarised in Fig. 2 and show that the highest TMF and MFF growths were recorded in dates from the control packaging, while the lowest growths were observed in dates

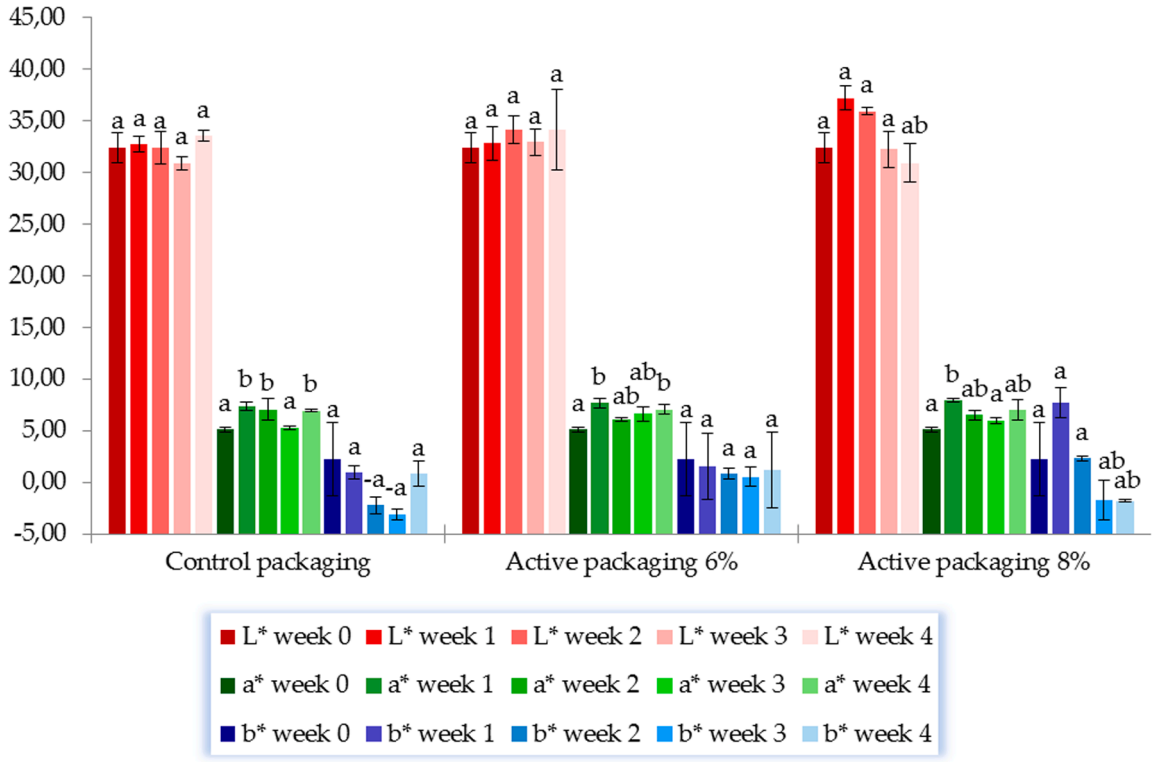


Fig. 1. Colour coordinates (L^*, a^* and b^*) of date samples during trial storage in control and active packages. Results are expressed as mean and standard deviation (error bars) values of three replicate measurements. Means with different superscript letters in each packaging group for a specific colour parameter indicate differences in mean values ($p \leq 0.05$) between storage times.

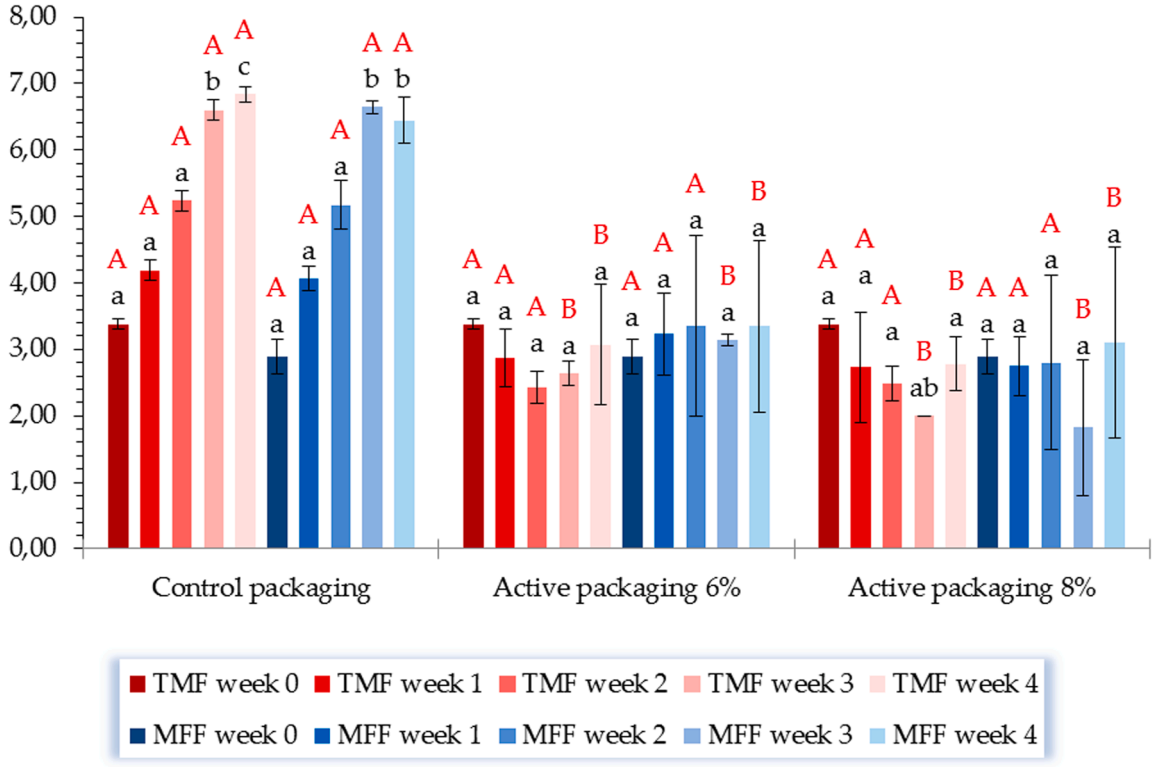


Fig. 2. Total aerobic mesophilic flora (TMF) and microbial fungal flora (MFF) of date samples during trial storage in control and active packages. Results are expressed as mean and standard deviation (error bars) values of three replicate measurements. Means with different superscript letters differ significantly ($p \leq 0.05$). Capital letters are used to indicate differences in mean values between packaging types at each sampling time, while lower case letters are used to indicate differences in mean values between storage times in a specific packaging group.

from the active packaging with 8 % of α--Ter/Ger: 2.5/1. In control packaging, the microbial load increased gradually, with TMF and MFF loads increasing by around 3.46 log and 3.56 log CFU/g respectively after four weeks. Whereas, in the active packagings (6 % and 8 %), the TMF and MFF loads in date fruits were almost stable during the storage period, suggesting the bacteriostatic power of the two active packaging materials developed. The sustained activity of the films throughout the storage period could be explained by the long-lasting diffusion of the active agent (α-Ter/Ger: 2.5/1) through the LDPE layer due to its relatively high thickness (35 μm) (Cava et al., 2005). Other authors have also reported that active multilayer films prepared with LDPE with a thickness of 50 μm retained their antimicrobial properties for up to 9 months (Wrona et al., 2023). The film thickness is inversely proportional to the release rate (Wang et al., 2022). Seo et al. (2012) found that the vapour release of the volatile antimicrobial allyl isothiocyanate (AIT) in an active packaging system increased significantly with decreased LDPE thickness, recording release rates from AIT beads of 27.9 % and 55.1 % in 150 μm and 20 μm thick LDPE sachets, respectively. Since LDPE has a multidirectional release, to ensure the unidirectional release of volatile active compounds towards the food packaging atmosphere, the LDPE layer is usually combined in a multilayer material with another layer with good volatile organic barrier properties such as PET or PP (polypropylene). Statistical analysis revealed a significant difference ($p < 0.001$) in TMF and MFF growth between the dates of the different packagings. The active packaging exhibited a dose-dependent antimicrobial effect and the time of storage was positively correlated with microbial growth in control packaging. As a result, the active packaging developed with the mixture (α--Ter/Ger: 2.5/1) can be used to extend the shelf life of soft date fruits. Up to date, active packaging studies on date fruits have focused on the development of active edible coatings using EOs, and no reports were found on an active packaging films for date fruits. The present results are in agreement with a previous study, which reported that lemongrass EO coatings applied to date fruits (*Khalas* variety) demonstrated antimicrobial potential over one month of storage (Alkaabi et al., 2022). In another study on the effect of thyme EO coating on the microbial properties of soft date fruits (*Deglet-Noor* variety), the results showed that the coating had a significant inhibition of fungal growth and a non-significant effect on bacterial growth during the first four months of storage (Belili et al., 2024). It was also reported that EVOH film incorporated with 6 % of geraniol or α--terpineol exerted a strong antibacterial effect and extended the shelf life of snakeheads slices by 4–5 days during cold storage (4 °C) (Wang et al., 2017). In another study, a poly (butylene adipate terephthalate) and poly(lactic acid) (PBAT/PLA) films containing 6 % α-terpineol inhibited quality deterioration of Pacific white shrimp, including microbial growth, for 9 days (Laorenza & Harnkarnsujarit, 2021).

Finally, it is worth noting that in this study, the sensory qualities of date fruits should be less affected due to the application of multilayer films that have shown to allow a slower and more sustained release of EOs (Zhang et al., 2022). Additionally, the use of EOs in the vapour phase and not in direct contact with the food product has generally less impact on the sensory qualities of foods (Laird & Phillips, 2012). Nevertheless, slight changes in flavour or aroma may persist. These effects can be further minimised by redesigning the packaging in combination with encapsulation technologies to allow an even more controlled release of the EOs into the packaging atmosphere, thereby minimising their accumulation and diffusion into the food product (Wrona et al., 2023), or by employing masking strategies through the addition of aromas (Gutiérrez et al., 2009).

4. Conclusions

In the present study, a new chemotype of Algerian *T. numidicus* EO was reported with linalool being the major compound (30 %) followed by α-terpineol (25 %) and geraniol (9 %). In addition, assessment of the antimicrobial activity in the vapour phase showed that *T. numidicus* EO, α-terpineol, geraniol and the combination α--Ter/Ger: 2.5/1 had strong antifungal potential against *P. roqueforti*, *A. flavus*, *A. niger* and *S. cerevisiae*. Moreover, a new antimicrobial multilayer film incorporating 8 % of the combination of α-terpineol and geraniol in 2.5/1 ratio demonstrated a strong antifungal activity in vapour phase against the above cited microorganisms and compliance as food contact material for dried fruits according to the European legislation. Finally, the use of the developed active films as pouches for soft date fruits revealed their antimicrobial potential and their capacity to extend the shelf life of soft date fruits. As a result, the active packaging developed has the advantage of not using synthetic additives for food preservation and of not requiring direct contact of the active packaging with the food product. Furthermore, since the preparation process for these new active multilayer films is compatible with the standard processing techniques used by commercially available multilayer film production machines, they can easily be produced on an industrial scale. Additionally, these active LDPE-PET multi-layer films are commonly used in Modified Atmosphere Packaging (MAP), which could even potentiate the films' antimicrobial activity.

Funding sources

This work was funded by MCIN/AEI/ 10.13039/501100011033 and by the European Union NextGenerationEU/PRTR [grants TED2021–129138B-C21], by the Gobierno de Aragón [T53_23R: GUIA]. It was also supported by the Algerian Ministry of Higher Education and Scientific Research (MESRS) with scholarship for author Azem Belasli [129/PNE/2022–2023/Doctorant/Espagne].

CRediT authorship contribution statement

Elena Canellas: Conceptualization. **Marinelly Quintero:** Investigation. **Raquel Becerril:** Conceptualization. **Lidia Ait Ouahioune:** Conceptualization. **Laura Aguerri:** Investigation. **Azem Belasli:** Writing – original draft, Investigation. **Filomena Silva:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Cristina Nerín:** Supervision, Resources. **Djamal Djenane:** Supervision. **Agustín Ariño:** Supervision, Resources.

Declaration of Competing Interest

The authors have no conflict of interest to declare.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fpsl.2025.101673.

Data availability

Data will be made available on request.

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