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Eugenol-based aluminium packaging: antibacterial performance for cooked ham and cheese preservation and risk assessment for consumer safety

Orlo Elena [a], Nerín Cristina [b,*], Wrona Magdalena [b], Buonocore Giovanna Giuliana [c], Nugnes Roberta [a], Russo Chiara [a], Di Matteo Angela [a], Lavorgna Margherita [a], Isidori Marina [a]

[a] University of Campania "Luigi Vanvitelli", Via Vivaldi 43, 81100, Caserta, Italy
[b] I3A, EINA, Universidad de Zaragoza, Campus Rio Ebro, Maria de Luna 3, 50018, Zaragoza, Spain
[c] Institute for Polymers, Composites and Biomaterials, Italian National Council of Research (IPCB-CNR), Piazzale Enrico Fermi 1, Portici, 80055, Italy

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ABSTRACT

Food contamination and spoilage compromise both safety and commercial value, highlighting the need for innovative preservation technologies. In this study, two vinyl resin coatings were applied to flexible aluminum foil to develop novel antimicrobial packaging materials: one containing free eugenol and the other incorporating eugenol-loaded Santa Barbara Amorphous-15 (SBA15) mesoporous silica particles. Material safety for food contact was assessed through migration tests following European Regulation 10/2011 using 10 % and 95 % ethanol, as food simulants, for 10 days at 20 °C. While overall migration confirmed the compliance of both materials, specific migration analysis demonstrated that only the eugenol-loaded SBA15 coating met regulatory safety limits, making it the only suitable candidate for food packaging.

The antimicrobial performance of this optimized coating was evaluated against *Escherichia coli*, *Staphylococcus aureus*, and mesophilic aerobic flora in Taleggio cheese and cooked ham stored at 5 °C for 10 days. The highest bacterial reduction was observed for *S. aureus*, with 2.77 log CFU/g and 2.13 log CFU/g decreases in Taleggio cheese and cooked ham, respectively. Sensory analysis confirmed that the coating did not significantly alter the organoleptic properties of the cheese.

These findings underscore the potential of eugenol-functionalized SBA15 aluminum packaging as a safe and effective solution for prolonging food shelf life, ensuring microbial safety while maintaining product quality.

1. Introduction

Safety and microbiological quality of food are closely related since the main causes of food safety impairments depend on microbial contamination by pathogens. These contaminations can often occur during food preparation, due to poor hygienic conditions of staff or equipment used, and can cause foodborne illnesses with risk of mortality, especially in vulnerable people including young, elderly, pregnant and immunocompromised people (Lund, 2015).

Nutrients, water activity and pH (food intrinsic parameters), together with humidity and storage temperature (food extrinsic parameters), strongly affect the microbiological quality of foods and their shelf-life. Foods such as soft cheese and meat, are mainly involved in food deterioration because they have a near-neutral pH and a water activity value higher than 0.94 (Hu et al., 2009; Vrdoljak et al., 2016). Packaged meat products, such as cooked ham, have a limited storage time of a few days in chilled storage (Katsaros & Taoukis, 2021) mainly because manipulations such as slicing and packaging may reintroduce bacteria after the cooking process, often resulting in slimy meat juice exudates, souring and package swelling for the production of gas (Vercammen et al., 2011). Similarly, dairy products are strongly perishable and for their richness in nutrients provide a good substrate for the growth and multiplication of many microorganisms (Hassan et al., 2019).

Nowadays, active packaging (AP) strategies are emerging to avoid both foodborne illnesses and food spoilage. AP consists of intentional incorporation of active compounds directly into the packaging material or in its headspace (EU/1935/2004; EU/450/2009). The combination of natural compounds and recyclable packaging materials is of great interest to increase the healthier perception of packaging by consumers and at the same time to reduce its environmental impact. Among natural compounds, eugenol (EG), a component of some essential oils (EOs)

* Corresponding author.
E-mail address: cnerin@unizar.es (N. Cristina).

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such as cinnamon and clove EOs, has been along studied and has proven to be a strong antibacterial agent against several microorganisms (Orlo et al., 2021) with various mechanisms of action (Mourtzinos et al., 2009; Devi et al., 2010; Clemente et al., 2016, 2017).

In general, since EG is a volatile compound, with low stability to light and oxygen, it is preferable to use it in an encapsulated form within carriers. The latter avoid the degradation of active compounds and, in turn, ensure their controlled release (Carpena et al., 2021) from packaging materials into foods, improving food quality and allowing for a longer shelf life (Tescione et al., 2014; Bahrami et al., 2020). Santa Barbara Amorphous15 (SBA15), a mesoporous silica particle, has the ability to encapsulate active molecules within its porous structure, preserving their biological activity while enabling a controlled release. The modulation of this release is influenced by the material's pore architecture, size, and the specific interactions between the encapsulated molecules and the pore walls (Stanzione et al., 2017; Verma et al., 2020). Additionally, SBA15 distinguishes itself from other mesoporous silicas due to its larger pore diameter, which facilitates the incorporation of a greater quantity of molecules (Zhang et al., 2021). Currently, even though many studies in the literature about active packaging materials are carried out on their biological effectiveness (e.g., antimicrobial and antioxidant efficacy), studies concerning their suitability for contact with food are lacking, representing a huge limitation for their commercialization. Indeed, food contact materials (FCM) undergo stringent regulations before being marketed, since their manufacturing process or the intentionally added substances (IAS) used for their production (e.g., plasticizers, antioxidants and so on), may compromise their suitability for contact with food. Migration tests are essential for ensuring consumer safety, as they allow for the verification that packaging materials do not release harmful chemicals into food. These tests replicate real-life conditions of food storage, accurately assessing contamination risks based on the type of food, exposure time, and temperature. Moreover, they are crucial for ensuring compliance with European regulations, which set stringent limits on the migration of chemical substances, thus safeguarding the quality and safety of packaged foods. Since migration can occur without visibly altering the food, migration tests are vital for detecting invisible but potentially harmful contaminations. Finally, for new packaging materials, such as active materials, these tests are indispensable to ensure their safety, preventing unforeseen risks before their commercial release.

In Europe, the Regulation 10/2011 (on "plastic materials and articles intended to come into contact with food" is applied to perform the risk assessment of FCM. Specific migration (SM), concerning the migration of distinct and recognizable chemical compounds, and overall migration (OM), referring to the mass of total migrants, must be tested under standardized conditions of time and temperature, representative for the intended use, using specific food simulants and covering the maximum shelf life of packaged food (Vera et al., 2014). According to EU/10/2011, OM ensures the quality of the material and its inertness only if the total concentration of migrants is not higher than 60 mg per kg of food (overall migration limit - OML). The EU Regulation also provides a list of substances and their specific migration limits (SML) which represent the maximum amount of a specific migrant that can be transferred.

In light of the above, this study, which follows two previous researches where the chemical, physical and morphological characteristics, as well as the *in vitro* antibacterial and antioxidant properties of two active aluminium materials were studied (Orlo, Stanzione, et al., 2023, Orlo, Stanzione, et al., 2023), was aimed at studying the two vinyl resin functionalized with eugenol and targeted for cooked ham and Taleggio cheese packaging applications, and at investigating the safety of these materials by performing migration analysis. In detail, the flexible aluminium foils coated with a vinyl-based resin containing either eugenol in free form or eugenol encapsulated in SBA15 particles were studied for their suitability for food contact by overall and specific migration tests in 10 % and 95 % ethanol for 10 days at 20 °C, simulating migration conditions in packaged processed meat products (e.g., bacon, sausages, ham, salami, and others) and natural cheeses with and without edible rind (camembert, gouda and similar) and stringy cheeses (EU/10/2011). Then, the antibacterial activity of the idoneus materials, was assessed against the two most common pathogens responsible for foodborne illnesses, *Escherichia coli* and *Staphylococcus aureus* (EFSA 2014, 2015, 2017; Lee & Yoon, 2021), in cooked ham and Taleggio cheese during different storage times (0, 1, 3, 7 and 10 days) at refrigeration condition (5 °C). Changes of the total viable count in both foods were assessed at the same exposure time and temperature conditions. In addition, to conclude this study a first approach of a sensory analysis was performed.

2. Materials and methods

2.1. Reagents

Eugenol (CAS: 97–53–0, purity ≥99 %), Silica mesoporous Santa Barbara Amorphous-15 (CAS: 7631–86–9), ethanol (CAS: 64–17–5), were supplied by Sigma-Aldrich (Milano, Italy).

Brilliant green bile agar (Oxoid, Milano, Italy), mannitol salt agar (Oxoid, Milano, Italy) and plate count agar (Oxoid, Milano, Italy) were supplied by Microtech (Pozzuoli, Italy). Commercial Taleggio cheese and cooked ham were purchased in a local supermarket.

2.2. Santa Barbara Amorphous15 impregnated with EG

An ethanolic solution containing eugenol was prepared (5 mL/g of EG) and mixed for 15 min, then SBA15 was added and stirred for 30 min. The solution was dried for 5 h under vacuum at 35 °C obtaining a yellow powder. During the filling process, the weight ratio of eugenol to mesoporous silica was equal to 0.71, which was chosen as the optimal value to allow for the maximum amount of eugenol to fill the total pore volume (0.67 cm^3/g) of the hosting SBA15 (Gargiulo et al., 2013). To estimate the real loading capacity after impregnation procedure, thermogravometric analysis was carried out (Orlo, Stanzione, et al., 2023). The percentage of active compound content was equal to 48 % w/w.

2.3. Preparation of antimicrobial coating and deposition onto aluminium foil

A vinyl resin (VIN), conventionally used for food contact applications, was provided by Laminazione Sottile S. p.A. (Caserta). No information is available on the resin due to trade secret protection on the formulation of the material.

Two vinyl-based formulations were prepared by adding i) 5 g EG/100 g of vinyl resin mass, thus with a concentration of eugenol equal to 5 % (the sample was coded as Al/VIN/5 %EG), ii) a combination of 2.5 g EG/100 g of vinyl resin mass and 2.5 g EG loaded in SBA15 per 100 g of vinyl resin mass, with a final concentration of eugenol equal to 5 % (the sample was coded as Al/VIN/5 %EG/SBA15). These two formulations were firstly mixed for 30 min at 1200 rpm and then deposited onto flexible aluminium foils (12 μm thickness) by a bar coater and dried for 20 s at 80 °C, obtaining 10 ± 2 g dry-resin/m^2 aluminium foil. This value was verified by weighting a 100 cm^2 sample before and after the removal of the coating using methyl ethyl ketone. For the sake of comparison, neat resin was deposited using the same procedure to obtain the neat coating to be used as negative control (NC), the sample was coded as Al/VIN.

2.4. Migration assay

2.4.1. Overall migration

Overall migration studies were done in 3 % acetic acid, 10 %, 50 % and 95 % ethanolfor 10 days at 20 °C for packaging meat and cheese,

following the standard procedure of gravimetry, already established. After the exposure the simulants were evaporated to dryness and the residue weighted. Al/VIN, Al/VIN/5 %EG and Al/VIN/5 %EG/SBA15 were simultaneously analyzed.

2.4.2. Specific migration analysis

Volatile and non-volatile migrants were subjected to analysis using GC-MS and UPLC-IMS-QTOF MSE, respectively, after being exposed. The NIST library for GC-MS and an in-house library for UPLC-IMS-QTOF facilitated the identification process (Song et al., 2022a, Song et al., 2022b). The analysis of samples containing 10 % ethanol and 3 % acetic acid was carried out using the TI-SPME-GC-MS (total immersion-solid phase microextraction-gas chromatography-mass spectrometry) method (Su et al., 2020). Prior to analysis, samples with 95 % and 50 % ethanol concentrations were diluted with Milli-Q water by factors of 10 and 5, respectively. Each simulant, in an 18 mL volume, was then placed in a glass vial for direct analysis by the TI-SPME-GC-MS method, utilizing a CTC Analytics CombiPal connected to an Agilent Technologies 6890N gas chromatograph with an MS 5975B mass spectrometer (Madrid, Spain), employing an HP-5MS (30 m × 0.25 μm x 250 μm) capillary column. The analysis followed a specific oven program and utilized helium as the carrier gas at a flow rate of 1 mL min^{-1}. The DVB/CAR/PDMS fiber from Supelco (Bellefonte, PA, USA) was used for SPME, with adsorption at 80 °C for 15 min and desorption for 2 min. Injector and MS source/quad temperatures were set at 250 °C, 230 °C, and 150 °C, respectively, with the mass detector operating in SCAN mode across a range of m/z from 50 to 350. Peak mass spectra were compared with the NIST library (2017 version) for identification. Quantitative analysis was performed using external standard calibration with pure standards of each detected compound, prepared in 10 % ethanol. The concentration of quantified compounds was reported in mg/Kg of simulant solution. Blank simulant solutions were analyzed as well. The concentration of detected migrants was calculated and reported in terms of 6 dm^2 of material per 1 kg of food simulant (6:1 ratio), adhering to EU/10/2011 legislation for food contact materials. The examination of non-volatile substances was conducted using an Acquity UPLC system, which includes a binary solvent manager and a flow-through needle injector, linked to a Vion® IMS QTOF mass spectrometer featuring an electrospray interface (ESI), produced by Waters in Manchester, UK. The chromatographic separation was achieved on a UHPLC BEH C18 column with a particle size of 1.7 μm and dimensions of 2.1 mm by 100 mm, maintaining a flow rate of 0.3 mL/min. The separation employed water as phase A and methanol as phase B, each containing 0.1 % formic acid by volume. The elution program began with 5 % of phase B, increasing to 100 % over 13 min, and included subsequent washing and re-equilibration periods lasting 3 min and 2 min, respectively. A sample volume of 5 μL was injected, and the column was kept at 40 °C. Data collection occurred under both positive and negative ionization modes, setting the capillary voltage to 1 kV and the sampling cone voltage to 30 V. Temperature settings for the source and desolvation were 150 °C and 450 °C, respectively, with a desolvation gas flow of 600 L/h and a cone gas flow of 50 L/h. Spectral data were gathered across a mass range of 50–1000 m/z. The method of acquisition was high definition MSE (HDMSE), incorporating a collision energy of 6 eV for low energy scans and a ramp from 20 to 40 eV for high energy scans. UNIFI v1.8 software from Waters Corp was employed for data analysis. Calibration curves constructed using pure standards of each identified compound facilitated quantification. When commercial standards were unavailable, quantification was performed using standards of chemically similar structure.

2.4.2.1. Analytical parameters. The method's analytical characteristics, including its linear range and the thresholds for detection and quantification, were assessed. The detection limit (LOD) and the quantification limit (LOQ) were established through the signal-to-noise ratio approach, with ratios of 3:1 for LOD and 10:1 for LOQ, respectively.

2.4.2.2. Risk assessment. The evaluation of the safety of the newly developed materials involved checking the EU Regulation 10/2011 for each chemical to see if it was listed. When a compound was identified on the positive list, the specific migration limit stipulated by the regulation was applied. For compounds not listed, the risk assessment was carried out using Cramer's classification system (Asensio et al., 2020) through the Toxtree® software, which categorized each compound into one of three oral toxicity classes based on its chemical structure. According to this classification, the maximum permissible migration levels, based on the estimated daily intake threshold that does not induce oral toxicity, were 1.80, 0.54, and 0.09 mg kg^{-1} for Class I, II, and III, respectively.

2.5. Antibacterial activity

2.5.1. Bacterial culture preparation

Escherichia coli (ATCC 13762) and *Staphylococcus aureus* (ATCC 6538) were the two potential foodborne pathogens used. One colony was picked from an agar plate and inoculated at 37 °C in 10 mL of Tryptic Soy Broth for 14–16 h.

2.5.2. Application of antimicrobial material on cooked ham and taleggio cheese

Treatments were carried out according to Suppakul and co-workers (2008), Lim and co-authors (2010) and Arkoun and co-authors (2017). The two samples were cut into 20 g (0.5 cm thickness) cubes or slices, respectively for Taleggio and cooked ham. The latter was decontaminated by its immersion in 70 % ethanol solution for 3 min and then dried completely, while Taleggio due to its texture was exposed to UV light in a laminar flow cabinet for 1 h on each side. The cheese and cooked ham samples were subsequently divided into three sets, two for bacterial contamination with *E. coli* and *S. aureus*, and one for the determination of the total viable count (TVC), and only for the last set of samples the decontamination process was not carried out. Further, each of the three sets was randomly divided to be packaged with the neat material (negative control – NC) and with Al/VIN/5 %EG/SBA15.

Before the packaging step, Taleggio cubes intended to be contaminated were immersed in *E. coli* or *S. aureus* suspension (10^5 CFU/mL) for 30 s, while cooked ham slices were inoculated by spreading the surfaces with 0.5 mL (10^4 CFU/g) of the two pathogens bacteria by a sterile rod, and then drained. Finally, all sets of samples were packaged by wrapping them with the testing material (14 cm × 14 cm), previously sterilized with 70 % ethanol, and stored at 5 °C for 10 days. Microbiological analysis was conducted during storage at 0, 1, 3, 7 and 10 days.

2.5.3. Bacterial count and microbiological analysis

Two replicates of each packaged sample were opened aseptically and 10 g of both Taleggio cheese and cooked ham were moved to Stomacher bags with 90 mL of 0.1 % peptone water for the homogenization (4 min). From each homogenized sample, decimal dilutions were prepared in 0.9 % NaCl and then 1 mL of each dilution was poured in duplicate into Petri dishes containing the agar growth medium specific for each bacterial count. Brilliant green bile agar and mannitol salt agar were used respectively for *E. coli* and *S. aureus*. Plates were incubated at 37 °C for 24–48 h. TVC was performed by using plate count agar left at 22 °C for 5 days. Colonies were counted and results were reported as log CFU/g.

2.6. Sensory analysis

Sensory analysis was carried out according to Joshi et al. (2021) involving 30 consumers (17 females and 13 males), aged between 25 and 65, who only expressed judgments of preference or acceptability. They were recruited at the University of Campania "Luigi Vanvitelli"-Department of Environmental, Biological and Pharmaceutical Sciences

and Technologies.

Each consumer tasted 5 samples of 1 g of Taleggio: the unpackaged cheese marketed the day of the tasting (Taleggio cheese), the cheese packaged with Al/VIN for 1 (NCt_1) and 10 (NCt_{10}) days, and the cheese packaged with Al/VIN/5 %EG/SBA15 for 1 (EGt_1) and 10 (EGt_{10}) days. The NCt_1, NCt_{10}, EGt_1 and EGt_{10} were stored at 5 °C for the time indicated. Each tasting was conducted in a blind manner, only unpackaged Taleggio marketed on the day of the tasting was specified.

Consumers quantified each sample for sensory characteristics on the basis of sweetness, bitterness, truffle-odor, pungency, and olfactory and gustatory pleasantness.

Sensory characteristics were evaluated using a 3-point hedonic scale, where 1 would be rated the lowest and 3 would be rated the highest (1 = no, 2 = moderately, 3 = strongly). Each consumer was asked to drink water and wait 2 min before proceeding with the next sample and to express an opinion about the samples tasted.

Principal component analysis (PCA) was conducted using the software XLSTAT to analyse sensory characteristics of the samples.

3. Results and discussion

3.1. Migration assay

3.1.1. Overall migration

Overall migration was performed to study the total migration of non-volatile compounds. This is the first screening for measuring the inertness of plastics for avoiding food unacceptable adulteration. The results of OM are reported in Table 1.

The total non-volatile compounds migrated to food simulants under the established exposure conditions were far below the OML of 60 mg/kg of food established by European legislation (10/2011), demonstrating the inertness of the two materials. The negative value recorded by Al/VIN/5 %EG in 10 % ethanol is likely due to the ability of EG in preventing the migration phenomenon. According to Wrona and Nerín (2020), in fact, migration of compounds from food packaging polymer materials can be modified by incorporation of some compounds, like antioxidants. This is also demonstrated by Gavril et al. (2019) who registered a reduction of migration of polylactic acid oligomers when medicinal and aromatic plants powders were used to obtain active packaging materials.

3.1.2. Specific migration of volatile and semi-volatile compounds

Qualitative and quantitative analysis of specific migration of volatile and semi-volatile migrants identified in Al/VIN, Al/VIN/5 %EG and Al/VIN/5 %EG/SBA15, exposed to 10 % ethanol and 95 % ethanol for 10 days at 20 °C were conducted. Most migrants were detected at concentrations below the limit of detection (LOD), therefore only those with concentrations above the LOD are listed in Table 2, while all other migrants are reported in Table S1. No additional compounds were found. According to EU/10/2011 Legislation both 10 % ethanol and 95 % ethanol are used for simulating the behaviour of packaged processed meat products while only 95 % ethanol is used for simulating the behaviour of natural cheeses without rind or with edible rind and stringy cheeses. The mentioned legislation reports that correction coefficient equal to X/3 and X/4 must be applied at 95 % ethanol, respectively for cheese and meat products.

This analysis enabled the detection and identification of a total of 6 migrants in 10 % ethanol and 37 migrants in 95 % ethanol (Table S1). Detection was considered positive when a peak in the chromatogram provided a signal comparable or higher than the detection limit of the identified compound at the same retention time. For this purpose, a very wide library of chemical substances was used. Only the reported compounds in the tables were detected and thus, further identified and quantified. In 10 % ethanol, tributyl acetylcitrate (ATBC) was the only migrant detected at concentrations above the LOD in Al/VIN, Al/VIN/5 %EG, and Al/VIN/5 %EG/SBA15. ATBC is a non-phthalate plasticizer widely used in the production of food packaging, children's toys, and personal care products (Zhang et al., 2023). Since the composition of the vinyl resin used in this study is protected by a patented formulation (this resin is already authorized as FCM), it is not easy to determine if the detected migrant is an intentionally added substance or non-intentionally added substance. Given that ATBC migrated from the pristine resin, and considering its wide use as plasticizer in food packaging, it is reasonable to assume that ATBC was intentionally added to the resin formulation. Currently, tributyl acetylcitrate is regarded as a safe plasticizer, with toxicity concerns only at very high concentrations. As a result, European Union (EU) legislation has approved its use in food-contact plastics without restrictions, as indicated in Regulation EU/10/2011, which classifies ATBC as a compound without a specific migration limit. Migration of ATBC was also observed when the materials were exposed to 95 % ethanol, with detected concentrations one order of magnitude higher than those found in 10 % ethanol. This increase is attributed to the higher extraction properties of 95 % ethanol, which is more effective in solubilizing apolar and semi-polar compounds, determining in many cases higher concentrations of migrants (Asensio et al., 2020; Ajaj et al., 2021). Indeed, in this food simulant, the detected migrants included 1,2,4-trimethylbenzene, 3-methyl--undecane, eugenol, and tetradecane. Eugenol, the antimicrobial compound intentionally added to the resin for obtaining the active material, migrated at different concentration depending on the formulation of the two materials. Specifically, when freely dispersed in the resin, eugenol migrated at higher concentrations compared to the material where it was encapsulated in SBA15, suggesting the potential of SBA15 as a modulator of active compound release (Orlo, Nerín, et al., 2023). Regarding the other detected migrants, they were found only in the modified vinyl resins, indicating that the addition of free eugenol or eugenol-loaded SBA15 may have induced changes, potentially leading to the formation of non-intentionally added substances or altering the migration behavior of the material.

Based on risk assessment, only Al/VIN/5 %EG/SBA15 was found to be suitable for contact with fatty foods (95 % ethanol) when both X/3 and X/4 correction factors were applied. In contrast, when Al/VIN/5 % EG was exposed to 95 % ethanol, 1,2,4-trimethylbenzene migrated at concentrations exceeding 1.8 mg/kg, the threshold established by Class I of Cramer rules.

According to EU legislation, for materials requiring testing with multiple food simulants, compliance is achieved only when migration limits meet the regulatory thresholds for all simulants tested. In this case, Al/VIN/5 %EG was suitable for contact with aqueous foods (10 % ethanol) but not with fatty foods (95 % ethanol), making it unsuitable for applications involving lipid-rich products. Consequently, Al/VIN/5 %EG/SBA15 was identified as the only viable food packaging candidate, and further analyses were conducted exclusively on this material. Similar situation was found in other cases, where the active material was

Table 1
Overall migration ($mg \cdot Kg^{-1}$) results obtained from Al/VIN, Al/VIN/5 %EG and Al/VIN/5 %EG/SBA15.

Exposure condition	Food simulant	Overall migration ($mg \cdot kg^{-1}$)			OML ($mg \cdot kg^{-1}$)
		Al/VIN	Al/VIN/5 %EG	Al/VIN/5 %EG/SBA15	
10 days at 20 °C	10 % ethanol	1.06 ± 0.34	−0.75 ± 0.11	0.94 ± 0.26	60.00
	95 % ethanol (X/3)	1.25 ± 0.02	1.11 ± 0.42	1.58 ± 0.32	60.00
	95 % ethanol (X/4)	0.94 ± 0.01	0.84 ± 0.32	1.19 ± 0.24	60.00

Table 2
Detected and quantified migrants in 10 % and 95 % ethanol with correction coefficient X/3 (for cheese, EU 10/2011) and X/4 (for meat, EU 10/2011) after 10 days at 20 °C.

t_{Ret} (min)	Compound detected	Standard for quantification	Concentration 6:1			LOD ($mg \cdot kg^{-1}$)	SML ($mg \cdot kg^{-1}$)	Cramer class
			Al/VIN	Al/VIN/5 %EG	Al/VIN/5 %EG/ SBA15			
10 % ethanol								
26.14	Tributyl acetylcitrate	Tributyl acetylcitrate	0.0548 ± 0.0032	0.1463 ± 0.1376	1.1685 ± 0.0720	0.0022	a	
95 % ethanol - (X/3)								
10.64	1,2,4-Trimethylbenzene	Mesitylene	–	2.8925 ± 0.8175	–	0.0001	–	I
14.55	3-Methyl-undecane	Undecane	–	0.0164 ± 0.0001	0.0162 ± 0.0000	0.0020	–	I
16.50	Eugenol	Eugenol	–	0.6579 ± 0.2672	0.4572 ± 0.3960	0.0131	a	
17.02	Tetradecane	Tetradecane	–	0.0490 ± 0.0001	0.0487 ± 0.0001	0.0001	–	I
26.19	Tributyl acetylcitrate	Tributyl acetylcitrate	0.2472 ± 0.1178	0.8764 ± 0.2098	3.6683 ± 0.8933	0.0022	a	
95 % ethanol - (X/4)								
10.64	1,2,4-Trimethylbenzene	Mesitylene	–	2.1694 ± 0.6131	–	0.0001	–	I
14.55	3-Methyl-undecane	Undecane	–	0.0123 ± 0.0001	0.0122 ± 0.0000	0.0020	–	I
16.50	Eugenol	Eugenol	–	0.4934 ± 0.2004	0.3429 ± 0.2970	0.0131	a	
17.02	Tetradecane	Tetradecane	–	0.0367 ± 0.0001	0.0366 ± 0.0001	0.0001	–	I
26.19	Tributyl acetylcitrate	Tributyl acetylcitrate	0.1854 ± 0.0884	0.6573 ± 0.1574	2.7513 ± 0.6700	0.0022	a	

[a] This compound appears on the positive list of EU No 10/2011 without specific migration limit.

not appropriate for any kind of food (Nerin, 2012; Barbosa et al., 2023; Borzi et al., 2019; Nerin et al., 2016; Sharma et al., 2021; Wrona & Nerin, 2019).

3.1.3. Specific migration of non-volatile compounds

UPLC-IMS-QTOF-MS^E was used for the migration analysis of non-volatile compounds. This analysis was performed only on Al/VIN/5 % EG/SBA15 as it was the only one with a migration below the SML and below the limits imposed by the Cramer rules (for volatile compounds). The results are reported in Table 3.

Two non-volatile compounds migrated in 10 % ethanol and three compounds in 95 % ethanol after 10 days of exposure at 20 °C. However, these compounds were also detected as semivolatiles and the migration values were much lower than the SML or the OML, when no SML was established according to the EU/10/2011.

3.2. Antibacterial activity

Antibacterial efficacy of the active material Al/VIN/5 %EG/SBA15 was assessed in real food systems under refrigeration conditions to verify its capability to extend the shelf life of Taleggio cheese and cooked ham. The results are reported in Fig. 1.

It can be clearly seen that the CFU log number per gram of sample was lower in the food packaged with the eugenol-based material than in those packaged with the neat one. In detail, the highest reduction was observed in *S. aureus* viable count (Fig. 1a) starting from the third day of storage with a maximum microbial inhibition after 10 days of storage especially in Taleggio cheese (2.77 log reduction). A lower reduction of *E. coli* CFU/g was achieved in Taleggio over the entire storage period (Fig. 1b). The effectiveness of the active material in reducing the growth of this microorganism in cooked ham was lower than that recorded in Taleggio, however after 10 days of storage a similar logarithmic reduction (1.76 log) of *E. coli* was achieved in both foods wrapped with the active material.

Total viable count (Fig. 1c) in Taleggio cheese and cooked ham was also determined over the entire storage period. After 10 days of exposure, both foods packaged with the neat material reach a microbial count higher than 9 log. Whereas, the use of the active packaging Al/VIN/5 %EG/SBA15 leads to a reduction of the total viable count by approximately 2 log in Taleggio and by 1.55 log in cooked ham, demonstrating its significant effectiveness in extending the Taleggio shelf-life.

The results highlighted that the antimicrobial efficacy of Al/VIN/5 % EG/SBA15 is evident from the early stages of storage and persists over

Table 3
Characterisation of compounds determined in migration assays by UPLC-IMS-Q-TOF-MS^E in positive.

t_{Ret} (min)	Compound	CAS	m/z	Adduct	Fragments	CCS ($Å^2$)	Drift (ms)
10 % ethanol							
7.17	Tributyl acetylcitrate	77-90-7	425.2147	$[M+Na]^+$	207.0629; 281.1360; 365.1937	209.22	6.29
7.54	1-Propene-1,2,3-tricarboxylic acid tributyl ester	7568-58-3	365.1936	$[M+Na]^+$	214.9177 233.0704; 333.1366	207.42	6.35
95 % ethanol							
6.90	Butyl citrate	77-94-1	383.2046	$[M+Na]^+$	158.9639; 284.3312 327.1418;	204.47	6.13
7.17	Tributyl acetylcitrate	77-90-7	425.2147	$[M+Na]^+$	207.0629; 281.1360; 365.1937	209.22	6.29
7.54	1-Propene-1,2,3-tricarboxylic acid tributyl ester	7568-58-3	365.1936	$[M+Na]^+$	214.9177 233.0704; 333.1366	207.42	6.35

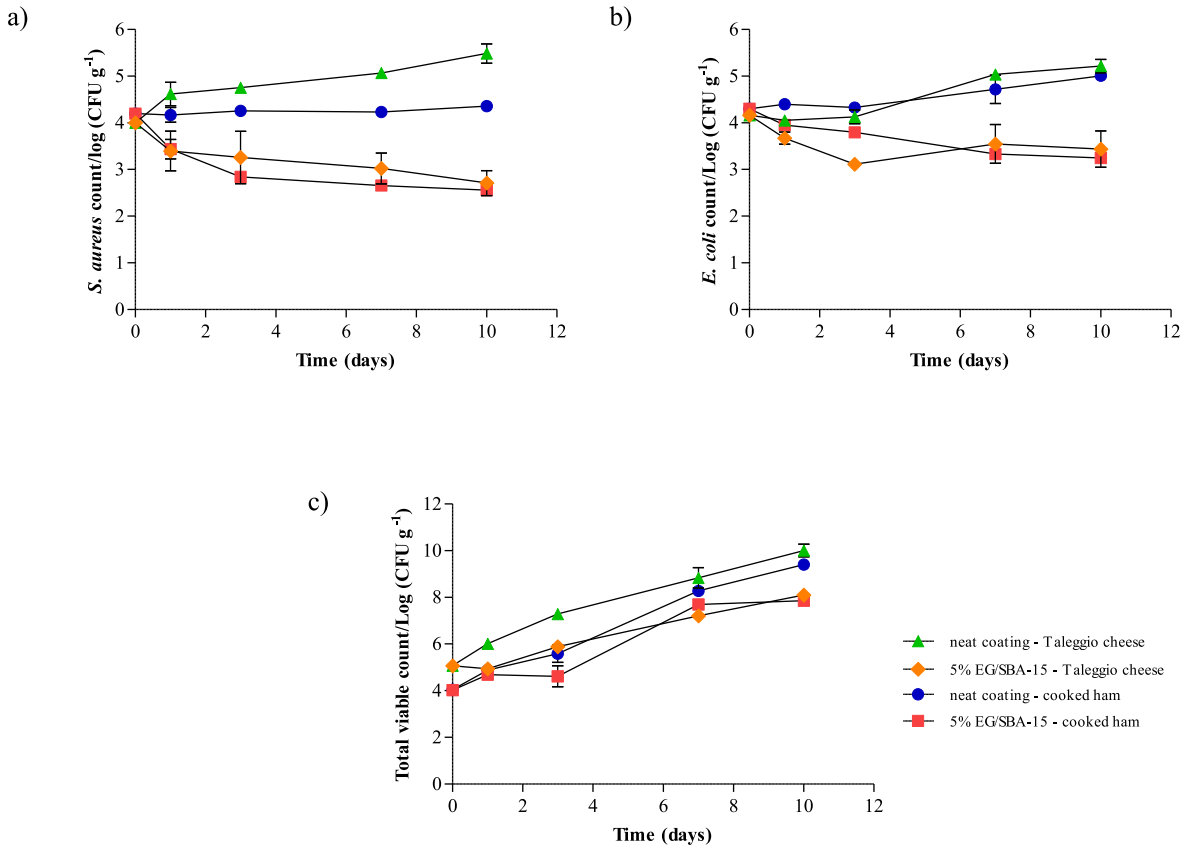

Fig. 1. Changes in *S. aureus* (a), *E. coli* (b) and total viable count (c) in Taleggio cheese and cooked ham after 0, 1, 3, 7 and 10 days of storage at 5 °C in Al/VIN and Al/VIN/5 %EG/SBA15. Each point is the mean of two samples taken from two independent experiments. Error bars show standard deviation.

time, suggesting a gradual and prolonged release of the active compound, with higher effectiveness in the preservation of Taleggio than of ham.

This can be likely due to the fact that eugenol is a lipophilic phytochemical and, as such, it is more prone to diffuse onto surfaces of fatty foods such as Taleggio. This could also explain the higher antimicrobial activity of Al/VIN/5 %EG/SBA15 in reducing *S. aureus* and *E. coli* viability. Suppakul and co-workers (2008) studied the antimicrobial activity of low density Polyethylene film with methylchavicol or linalool against *Listeria innocua.* In line with the present study, the authors affirmed that these lipophilic compounds could diffuse well onto the surface of fat-based food like cheese, explaining the inhibitory effect on *L. innocua* growth. This aspect also emerges from the study performed by Carpena and collaborators (2021), who affirmed that foods with a higher lipid content have a greater ability to extract fat-soluble active compounds from the packaging, *vice versa* foods rich in water (for example, vegetables and fruit) have a greater ability to extract active compounds with a water-soluble character. Despite the evidence reported in the cited studies, a correlation between the food matrix and the antibacterial activity of the functionalized material is not always observed. In fact, Requena et al. (2019) investigated the antibacterial activity of PHBV films enriched with various antimicrobial compounds, including eugenol, on different food matrices (cheese, chicken breast, pumpkin, and melon). Their findings revealed that the highest antibacterial effect was observed in cheese and pumpkin, emphasizing the role of multiple compositional factors in modulating the availability of the active compound and its ability to exert antibacterial action within a specific food system. In the same study by Requena et al. a discrepancy between the antibacterial activity results obtained from *in vivo* and *in vitro* experiments was also highlighted, with *in vivo* tests showing less pronounced effects compared to *in vitro* assays. This observation aligns with the findings of the present study and our previous study in which the antibacterial activity of the Al/VIN/5 %EG/SBA15 material was evaluated *in vitro* (Orlo, Stanzione, et al., 2023). Specifically, the log reduction of *E. coli* and *S. aureus* was 2.41 and 3.62, respectively, after 24 h. In contrast, the *in vivo* experiments conducted in the present study showed a significantly lower reduction for both microorganisms after one day of storage. This suggests that food matrices significantly influence the antibacterial activity of a material and highlights the importance of *in vivo* studies to understand the actual antibacterial performance of a developed material. Another aspect to take into consideration is the increase in the antibacterial effectiveness of the material as the contact time with the packaged food increases. The material studied in this work is designed by embedding the active compound into the carrier SBA15 with the aim to slow down eugenol kinetic release (Orlo, Nerín, et al., 2023), making it functional in extending the shelf-life of the packaged foods in prolonged time. In the context of food packaging, the use of carriers is regarded as a valuable strategy to prolong the release rate of active compounds while simultaneously serving as an efficient delivery system, enhancing the bioavailability of bioactive compounds to target cells over time. (Pabast et al., 2018). He and collaborators (2020) demonstrated that silica-based nanomaterials can disrupt bacterial membranes, reducing viability in food matrices. In this context, SBA15 proved to be a carrier of active compounds throughout the bacterial wall (Gámez et al., 2020; Wu et al., 2019). Indeed, as reported in these cited studies, SBA15 embedded with natural compounds leads to an improvement in their antibacterial efficacy against pathogens.

Montmorillonite, a hydrated alumina-silicate layered clay, was incorporated with eugenol to prolong its release time and develop an active LDPE film (Tornuk et al., 2015). The storage of fresh beef in this polymer resulted in a TVC reduction of 0.26 log CFU/g after 10 days,

indicating a relatively lower antibacterial efficacy compared to the material developed in the present study. Indeed, Al/VIN/5 %EG/SBA15 determined a 2 log reduction in Taleggio cheese and a 1.55 log reduction in cooked ham, demonstrating a significantly enhanced antimicrobial performance.

To date, active packaging has been designed using not only silica-based nanomaterials but also alternative biopolymer-based approaches. Lin et al. (2023) developed an edible film enriched with sodium caseinate and trimethyl chitosan loaded with eugenol for extending chicken shelf-life. Their study reported a 1.83 log reduction in *S. aureus* count in packaged chicken after five days of storage. However, this effect was achieved with the application of 80 % of the eugenol-nanoparticle complex, indicating a significantly higher eugenol concentration compared to the formulation used in the present study (5 %EG/SBA15). In contrast, Zeng et al. (2023) examined the antibacterial efficacy of carboxymethyl chitosan/pullulan composite film incorporated with eugenol at 5 %. Their results demonstrated that this percentage was effective in inhibiting microbial growth over time, achieving a 2 log reduction in aerobic count after six days of chilled meat storage. These findings suggest that the antibacterial potential of eugenol-based films strongly depends not only on the concentration of the active compound but also on the physicochemical properties of the polymeric matrix in which it is incorporated.

In addition to chitosan- and pullulan-based films, eugenol has also been utilized to functionalize biodegradable materials derived from yam starch (Cheng et al., 2019). The study demonstrated the material's efficacy in inhibiting the growth of *E. coli*, *S. aureus*, and *L. monocytogenes*, with a dose-dependent increase in antimicrobial activity. The highest efficacy was observed at a 5 % eugenol concentration, particularly against *E. coli*. However, the application of this biodegradable material for fresh pork packaging revealed significant limitations. The polysaccharides present in starch promoted bacterial growth, resulting in more severe spoilage of the packaged pork compared to the unpackaged control after four days of storage. Additionally, after seven days of exposure to pork, the mechanical properties of the starch-based material deteriorated significantly, further highlighting its limitations for long-term food storage applications.

In contrast, aluminum-based materials exhibit outstanding mechanical and barrier properties, which are essential for food preservation and the maintenance of organoleptic qualities. While they lack biodegradability, their high recyclability rate makes them a sustainable alternative, aligning with the principles of eco-friendly packaging and offering a viable solution for overcoming the limitations associated with biodegradable materials.

3.3. Sensory analysis

The sensory analysis was performed only on taleggio cheese wrapped with materials containing eugenol because this film recorded the best efficacy on *S. aureus*. PCA analysis demonstrated a good statistical significance with the principal component PC1 accounting for 54.86 % of the variance and PC2 accounting for 29.31 % (Fig. 2). This means that the two components together collect a substantial proportion of the dataset variability (84.17 %). The Kaiser-Meyer-Olkin value (KMO) indicated a sampling adequacy equal to 0.552, suitable to perform a PCA analysis.

PC1 positively correlated with most of the variables, sweetness, truffle odor and pungent odor and the variables related to the pleasantness of the samples (olfactory and gustatory pleasantness) both for females and males. Bitterness for males and females clustered in the negative side of PC1 in the second (positive side of PC2) and third (negative side of PC2) quadrants, respectively.

The sensory analysis was conducted to certify whether eugenol added to the film to improve the shelf life of taleggio cheese with its bitter taste and pungent aroma interfered with the sweet taste of the cheese or less either after a film storage of a day or after 10 days compared to the respective controls without eugenol.

The results of the PCA analysis indicate that Taleggio packaged with

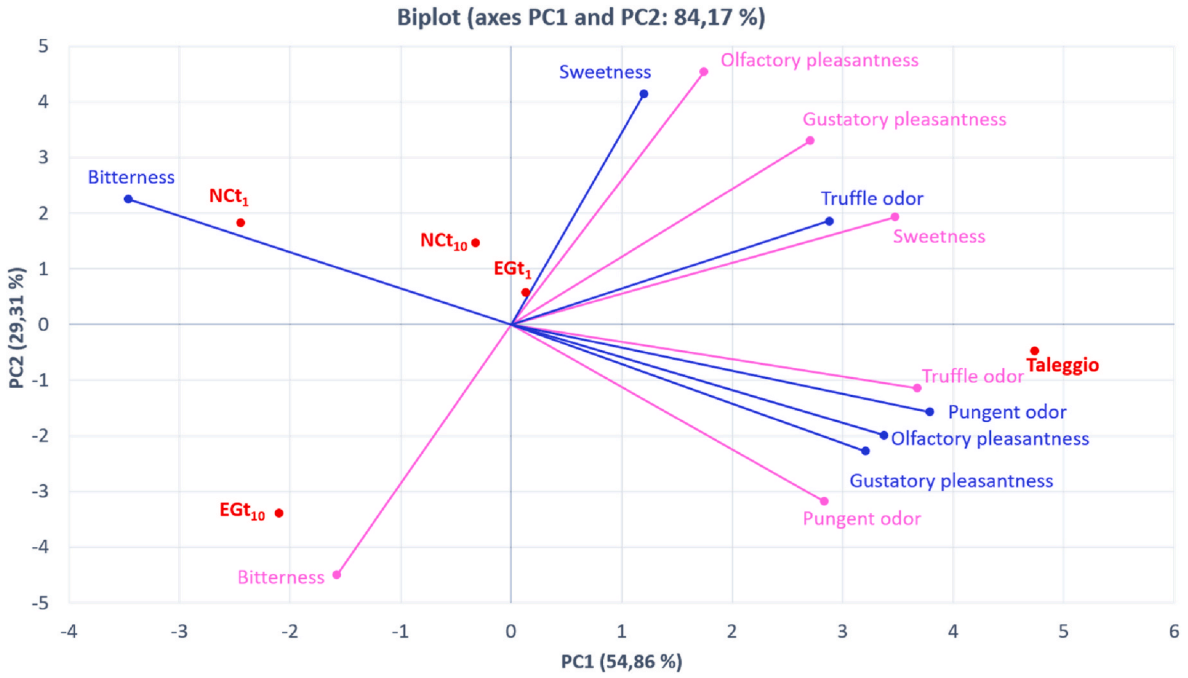

Fig. 2. Principal Component Analysis (PCA) of sensory characteristics of 5 samples of Taleggio cheese. Impact on sensory characteristics (sweetness, bitterness, truffle odor, pungent odor, gustatory and olfactory pleasantness) were rated based on a 3-point hedonic scale with 1-no impact and 3-highest impact at all. Data depicted is an average response of 30 consumers for each sample. Taleggio: unpackaged cheese marketed the day of the tasting; NCt1: cheese packaged with Al/VIN for 1 day, NCt10: cheese packaged with Al/VIN for 10 days, EGt1: cheese packaged with Al/VIN/5 %EG/SBA15 for 1 day, EGt10: cheese packaged with Al/VIN/5 % EG/SBA15 for 10 days. Blue and pink vectors were for male and female consumers, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Al/VIN/5 %EG/SBA15 for 1 day (EGt_1) was perceived sweeter for males than females and the two variables have Pearson Coefficient of 0.396. Taleggio packed with Al/VIN/5 %EG/SBA15 for 10 day (EGt_{10}) is perceived bitter only by females. For the EGt_{10} there are no other perceptions in its characteristics. PC2 was positively correlated with the negative controls NCt_1 and NCt_{10} and the only perception was a higher bitterness raised for CNt_1, exclusively for males. Taleggio was very appreciated for its pleasantness and the pungency is felt especially by males, while the females tasted sweetness and smelled truffle odor more than males.

In conclusion, the PCA analysis highlighted that the consumers involved in the sensory analysis perceived Taleggio and EGt_1 without particular sensory differences. This means that eugenol does not alter the taste of the Taleggio and it may be considered for its inclusion in flexible aluminium foil coatings for obtaining food packaging materials to extend the shelf life of this perishable food. This is a very positive finding, as the high volatility and strong flavour of eugenol often limit its use in food packaging, leading to the failure of previous attempts in which it was directly incorporated as an active ingredient (Silva et al., 2024). The encapsulation of essential oils or their components has proven to be an effective strategy for preserving the sensory properties of food during storage (Ribeiro-Santos et al., 2017; Buendia-Moreno et al., 2019; Carpena et al., 2021). This technique effectively prevents the organoleptic changes that could arise due to the interaction of essential oils or other lipid-based compounds with the food matrix, ensuring broader applicability in active food packaging systems.

4. Conclusions

This study investigates, for the first time, the efficacy in extending the shelf life and food contact compliance of an active packaging system prepared by depositing on a thin aluminium film a vinyl-based coating containing eugenol incorporated into the mesoporous silica SBA15. The newly developed active material was tested for its ability to extend the shelf life of two different foods: Taleggio cheese and cooked ham. The findings indicate that the eugenol- and SBA15-functionalized material represents a promising strategy for active food packaging, effectively limiting the growth of pathogenic and spoilage microorganisms during storage. The different antimicrobial efficacy observed in the two tested foods suggests that intrinsic food matrix factors may modulate the material's action. This underscores the need for further investigations to optimize its performance based on the specific characteristics of the products intended for packaging.

Migration studies revealed that when eugenol is not encapsulated and is freely dispersed in the vinyl-based coating, the detected migrants into suitable food simulants (10 % and 95 % ethanol) exceed the limit established by Cramer's rules. Conversely, all migrants from Al/VIN/5 %EG/SBA15 were within the safety thresholds defined by EU regulations (10/2011/EU and 1935/2004/EEC) and Cramer's rules. These results broaden the commercial interest of this antimicrobial packaging system, suggesting its potential use in food packaging applications without compromising consumer safety.

CRediT authorship contribution statement

Orlo Elena: Writing – original draft, Methodology, Investigation, Formal analysis. **Nerín Cristina:** Writing – review & editing, Resources, Methodology, Funding acquisition, Conceptualization. **Wrona Magdalena:** Supervision, Investigation, Data curation. **Buonocore Giovanna Giuliana:** Validation, Data curation, Conceptualization. **Nugnes Roberta:** Investigation, Formal analysis. **Russo Chiara:** Visualization, Investigation. **Di Matteo Angela:** Methodology, Investigation. **Lavorgna Margherita:** Validation, Supervision, Conceptualization. **Isidori Marina:** Writing – review & editing, Validation, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2025.111416.

Data availability

No data was used for the research described in the article.

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