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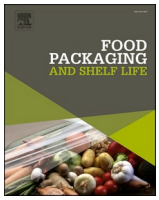

New active packaging based on encapsulated carvacrol, with emphasis on its odour masking strategies

Magdalena Wrona [a], Sofía Manso [a], Filomena Silva [b,c], Leticia Cardoso [d], Jesús Salafranca [a,*], Cristina Nerín [a], María José Alfonso [e], Miguel Ángel Caballero [e]

[a] Instituto de Investigación en Ingeniería de Aragón (I3A), Escuela de Ingeniería y Arquitectura (EINA), Departamento de Química Analítica, Universidad de Zaragoza, María de Luna 3 (Edificio Torres Quevedo), 50018 Zaragoza, Spain
[b] ARAID – Agencia Aragonesa para la Investigación y el Desarrollo, Av. de Ranillas 1-D, planta 2ª, oficina B, 50018 Zaragoza, Spain
[c] Faculty of Veterinary Medicine, University of Zaragoza, Miguel Servet 177, 50013 Zaragoza, Spain
[d] Department of Food Science and Nutrition, School of Food Engineering, University of Campinas (UNICAMP), Rua Monteiro Lobato 80, 13083-862 Campinas, São Paulo, Brazil
[e] Nurel, R+D Department, Polígono Malpica, Ctra. Barcelona km. 329, 50016 Zaragoza, Spain

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ABSTRACT

New active polyethylene packaging with both antioxidant and antimicrobial properties was designed and produced using three different concentrations of encapsulated carvacrol. Films containing 10%, 20% and 30% of active capsules showed strong antioxidant activity as hydroxyl (OH·) radical scavengers, while only films containing 30% capsules showed anti-*Salmonella enterica* activity. Storage of active packaging rolls in sealed aluminium bags maintained both the antioxidant and antimicrobial properties of the films for up to 9 months. Two strategies were used to control or eliminate the strong odour of the developed packaging materials: odour masking spray and multilayer packaging. Migration tests were carried out to assess the safety of packaging. The most successful strategy was the development of two active multilayer packaging materials (polyethylene terephthalate (PET)/adhesive/active low density polyethylene (LDPE) with 10% capsules and aluminium/adhesive/active LDPE with 10% capsules) which reduced the oxidation of packaged crisps stored at 60 °C for 30 days by up to 45%, measured as malonaldehyde using the TBARS assay. In addition, the packaging materials did not affect the organoleptic properties of the stored crisps as determined by sensory analysis. These results show that this novel bifunctional (antioxidant and antimicrobial) active packaging can protect fatty foods from oxidation processes while preserving their organoleptic properties.

1. Introduction

The presence of oxygen in the headspace of packaged foods is one of the most important factors affecting food quality and shelf life. At room temperature, high-fat foods are particularly susceptible to autoxidation due to the formation of free radicals. These radicals can combine with residual oxygen in the packaging to form hydroperoxides, which react to form smaller molecules, mainly aldehydes, alcohols and acids (Chow, 2007). This fact leads to changes in the organoleptic properties of the food, such as colour, odour and taste. In addition, the nutritional value of foods is reduced by oxidation of, among others, vitamins, fatty acids and proteins (Silva et al., 2019). These undesirable processes lead to reduced consumer acceptance of such foods and ultimately result in large economic losses due to the generation of food waste. Moreover, some of the oxidation products, mostly derived from lipid oxidation through free radical reactions (Ahmed et al., 2016), can be toxic to human consumers, as is the case with acrolein, 4-hydroxy-trans-2-nonenal, 4-hydroxy-trans-2-hexenal and malonaldehyde (Vieira et al., 2017). When ingested through the diet, these compounds can be absorbed and bind to proteins, DNA and cell membranes, and have been shown to be implicated in inflammatory diseases, cancer, atherosclerosis, ageing, etc. (Vieira et al., 2017).

For these reasons, the food industry has focused its efforts on strategies to prevent or control free radicals in fatty foods. The main approaches are to reduce the oxygen content within the package and to incorporate antioxidant compounds. Antioxidants were first

* Corresponding author.
E-mail address: fjsl@unizar.es (J. Salafranca).

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incorporated into foods and more recently into packaging materials (Yildirim et al., 2018), leading to the development of active food packaging materials (Wrona, & Nerín, 2019).

Active food packaging is based on the incorporation of, among others, substances with antioxidant, antimicrobial or absorptive properties into the packaging material. These active substances are then able to interact with the atmosphere surrounding the food or with the food product itself to maintain its quality and safety, thereby extending its shelf life.

In the case of antioxidant active packaging, the two main ways of preventing oxidation are through the incorporation of oxygen or free radical scavengers (Vera et al., 2018), also known as primary antioxidants (Silva et al., 2019). These compounds react with oxygen-derived free radicals, which are responsible for initiating oxidation processes in foods. By scavenging the free radicals, primary oxidation of lipids does not occur and further oxidation chain reactions cannot take place (Nerín, Vera, & Canellas, 2017). The current trend is to use natural antioxidants, such as essential oils or extracts, to circumvent the potential harmfulness of synthetic antioxidants to humans (Yildirim et al., 2018; Pokorný, 2007).

The main objective of antimicrobial packaging is to reduce, slow down or even stop microbial growth by interacting with the packaged food (direct contact) or the packaging headspace (indirect contact) (Otoni et al., 2016). By controlling pathogenic and spoilage microbial populations, antimicrobial packaging ensures consumer safety, while extending food shelf life (Silva et al., 2019). Currently, antimicrobial packaging can take several forms, such as sachets or pads containing volatile antimicrobials, polymer films with direct incorporation of antimicrobials (extrusion, casting), and coating or adsorption of antimicrobials onto the polymer surface (Yildirim et al., 2018). Following the same trend as in antioxidant food packaging, the type of compounds used has shifted from synthetic antimicrobials (e.g. organic acids, triclosan, antibiotics, chlorine dioxide, nitrites and ammonium salts) to natural antimicrobials (bacteriocins, enzymes, phages, natural extracts and compounds, essential oils and their constituents) (Becerril et al., 2020).

Although compounds of natural origin have been shown to be effective as free radical scavengers (Borzi et al., 2019; Gherardi et al., 2016; Wrona et al., 2017) and as antimicrobial agents to actively control the growth of microorganisms on the surface of the food (Yildirim et al., 2018), they are usually required in larger quantities than synthetic substances, mainly due to their poor stability and volatility of those substances. In addition, the organoleptic properties of the packaged food or the packaging itself are altered, with particular emphasis on the strong and unpleasant odours released by the packaging material due to the incorporation of the active agent.

To overcome these obstacles, such compounds (essential oils and their main constituents, natural extracts, vitamins, etc.) can be encapsulated in various materials such as aluminosilicates (Wrona et al. 2017), cyclodextrins (Silva et al., 2018), cellulose nanofibres (Silva et al., 2019), nanoclays, or even other polymeric capsules. Porous aluminosilicates have several advantages over other encapsulants due to their inorganic nature, their thermal resistance, their ability to perform in high humidity environments (they do not swell with water) and their use as adsorbents (Novikova & Belchinskaya, 2016). Furthermore, additional measures such as odour masking strategies can be applied to overcome changes in the organoleptic properties of the polymer. These odour masking technologies have long been used by the packaging industry to eliminate or reduce unpleasant odours from packaging materials. The most common odour masking technologies are the addition of odour absorbers (Wyrwa & Barska, 2017) or the addition of fragrances (Gutiérrez et al., 2009).

Taking all this into account, this work describes the development of a new antioxidant active packaging based on the incorporation of an essential oil component encapsulated in aluminosilicate on a polyethylene film and its in vivo application to prevent the oxidation of crisps fried in olive oil. In addition, two different odour masking strategies were tested to enable the commercialisation of the developed active film. For a better understanding, the present research can be summarised in the workflow diagram shown in Fig. 1.

2. Materials and methods

2.1. Chemicals

Carvacrol (98%, CAS 499–75–2), 2-thiobarbituric acid (≥98%, CAS 504–17–6), trichloroacetic acid (≥99.0%, CAS 76–03–9), 1,1,3,3-tetraethoxypropane (≥96.0%, CAS 122–31–6), hydrogen peroxide (30%, CAS 7722–84–1), sodium salicylate (>99.5%, CAS 54–21–7) sodium acetate (≥99%, CAS 127–09–3), acetic acid (≥99.7%, CAS 64–19–7) and sodium chloride (≥99.0%, CAS 7647–14–5) were purchased from Sigma-Aldrich (Madrid, Spain). Ethanol absolute (≥99.9%, CAS 64–17–5), hydrochloric acid (37%, CAS 7647–01–0) and phosphoric acid (85%, CAS 7664–38–2) were from Scharlau (Barcelona, Spain). Methanol (≥99.9%, CAS 67–56–1) was from Honeywell (Madrid, Spain). The ultrapure water was produced by a Wasserlab Ultramatic GR system (Barbatáin, Spain).

2.2. Active capsules and film preparation

The company NUREL (Zaragoza, Spain), belonging to the SAMCA group, carried out the encapsulation of carvacrol in the nanopores of the active capsules (aluminosilicates with particle size of 50 nm to 5 μm with a variable Si/Al ratio) and their incorporation into the polymer, following the processes described in its two patents: EP1564242B1 and EP1923423B1.

Active low-density polyethylene (LDPE) films (0.5 m wide and 10 m long rolls with a thickness of 50 μm) containing 10% (sample M2), 20% (sample M3) and 30% (sample M1) of active aluminosilicates were tested. In addition, LDPE films containing aluminosilicates without carvacrol were used as controls throughout the study.

The stability of the active film rolls was investigated for 9 months under two different storage conditions at room temperature: a) inside a high barrier heat-sealed material with aluminium, and b) open to fresh air. One roll of each type was prepared. Samples taken from each roll for further analysis were prepared in triplicate.

2.3. Antioxidant capacity of the aluminosilicates

The antioxidant capacity of the active aluminosilicates was evaluated using an in situ OH· free radical generator based on the reaction of 8% hydrogen peroxide mist with ultraviolet light (Pezo et al., 2006; Pezo et al., 2008). Briefly, 1 g of active capsules was extracted with 12.5 mL of ethanol at 25 ºC for 30 min in a 40 MHz Branson 3510 ultrasonic bath (Branson Ultrasonics Corporation, Danbury, CT, USA). 10 μL of the extract were placed in a specially shaped glass Pasteur pipette with 0.3 g of glass wool. Pure ethanol was used as blank. The antioxidant capacity of the samples was compared with that of pure carvacrol. The Pasteur pipettes were connected to the OH· radical generator. After passing through the pipettes, the stream of OH· radicals, was collected in impingers containing 2 $\mu g \cdot g^{-1}$ of sodium salicylate solution (pH adjusted to 4.5 with phosphoric acid). Compressed air was used as the carrier gas. After 24 h exposure time, the solutions were analysed by HPLC with a fluorescence detector, since the reaction of OH· radicals with sodium salicylate produces 2,5-dihydroxybenzoic acid, a fluorescent compound, as the main reaction product. Chromatographic separation was performed on a Waters Alliance 2795 HPLC separation module (Milford, MA, USA) coupled to a Waters 474 scanning fluorescence detector ($\lambda_{excitation}$: 324 nm; $\lambda_{emission}$: 448 nm). The chromatographic method was as follows: mobile phase consisted of 10% of methanol and 90% of acetic acid/acetate buffer (0.02 M, pH = 5.9); 1 mL flow rate; isocratic mode; both column and sample temperatures

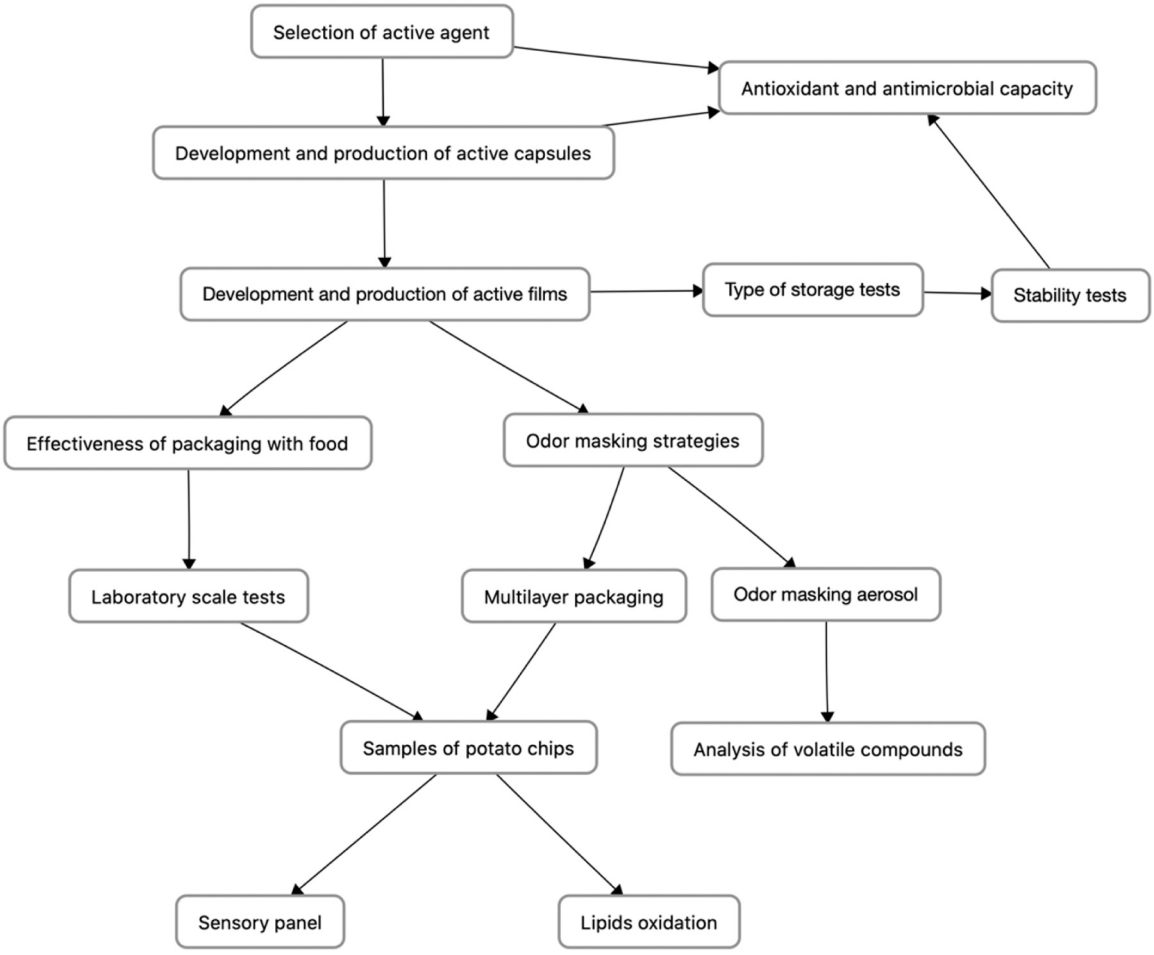


Fig. 1. Workflow diagram of the research carried out.

were 25 °C.

2.4. Antioxidant capacity of the active films

The antioxidant capacity of the active films was also determined using the in situ OH· free radical generator (Pezo et al., 2008) described in the Section 2.3. Measurements were made after 0, 1, 2, 3, 4, 5, 6 and 9 months of storage.

1 dm^2 of each material was placed in specially designed LDPE bags (12 × 12 cm) and heat-sealed. The same LDPE film was used as blank. An eight-compartment holder was used to hold the bags and connect them to the OH· radical generator.

2.5. Antimicrobial activity of active compounds and films

2.5.1. Microbial strains

The antibacterial activity of active aluminosilicates and active films was evaluated against gram-negative bacterial strain *Salmonella enterica* subsp. *enterica* CECT 556. Bacterial growth was performed using different growth media depending on the test performed: Mueller-Hinton Agar (MHA), Mueller Hinton Broth (MHB) and Plate Count Agar (PCA), all purchased from Scharlau. Unless otherwise stated, growth was carried out under aerobic conditions, at 37 °C for 24 h.

2.5.2. Antimicrobial activity of aluminosilicates containing carvacrol

4 mL of sterile MHB were placed in glass tubes. An appropriate amount of active capsules (aluminosilicates) was added to give a final capsule concentration of 1600 $\mu g \cdot g^{-1}$. The tubes were then heated at 60 °C for 7 min to promote homogeneity and inoculated with *S. enterica* to a final concentration of 10^5 $CFU \cdot mL^{-1}$. Uninoculated samples were used as negative control. After incubation at 37 °C for 24 h, serial 1:10 dilutions were made in sterile saline and 20 μL aliquots of the different dilutions were inoculated onto PCA plates. Colony counts were performed after a final incubation at 37 °C for 24 h.

2.5.3. Antimicrobial activity of the active films

The vapour phase activity against *Salmonella enterica* subsp. *enterica* CECT 556 was determined at time 0 and after 9 months of storage. A bacterial suspension of *S. enterica* was prepared in sterile 0.8% NaCl to give an absorbance at 625 nm of 0.08–0.12 (10^8 $CFU \cdot mL^{-1}$). A 1:100 dilution of the above suspension was then prepared to give a final cell density of approximately 10^6 $CFU \cdot mL^{-1}$ in sterile 0.8% NaCl. 100 μL of the 10^6 $CFU \cdot mL^{-1}$ suspension was pipetted into the centre of an MHA plate and spread with a sterile L-shaped cell spreader until the entire surface of the plate was covered. All plates were allowed to dry at room temperature for 15 min. The inoculated plates were covered with a 12 × 12 cm square of the film material. The films on top of the plate were held firmly in place by a nylon tie around the outer rim of the inoculated plate. Plates were incubated for 24 h at 37 °C and visually inspected for growth inhibition.

2.6. Food samples

The developed active packaging was tested in laboratory scale trials using crisps fried in olive oil. Bags of crisps were purchased from a local supermarket. The samples were stored in a dry place at 25 °C prior to

use. After crushing in a mortar to increase the surface area, the crisps were stored in the multilayer active packaging in a dry place at 25 °C with ambient light to simulate real supermarket packaging.

2.7. Odour masking strategies

2.7.1. Odour masking spray

Active films were coated with the commercial odour masking product Sanytol Textile Disinfectant Deodorizer from Grupo AC Marca (Barcelona, Spain), which contains dimethyldidecylammonium chloride (CAS 7173–51–5) and benzalkonium chloride (CAS 68424–85–1) as the main components. They were then dried in fresh air for 24 h. The composition of the volatile compounds of the odour masking spray and their influence on the carvacrol-containing active films were analysed by solid phase microextraction gas chromatography coupled to mass spectrometry (SPME-GC-MS).

For the analysis, 1 dm^2 of LDPE with 10 μL of odour masking spray added by pipette was placed in 18 mL glass vials and analysed directly by SPME coupled to a 6890 N gas chromatograph and MS 5975B mass spectrometer from Agilent Technologies (Madrid, Spain). The SPME holder was managed by a CTC Analytics CombiPal (Agilent Technologies, Spain). Chromatographic separation was performed on a BP20 column (30 m × 0.25 μm × 250 μm) from Trajan Scientific and Medical (Milton Keynes, UK). The following temperature programme was used: 40 °C (2 min), at a rate of 10 °C·min^{-1} up to 200 °C (2 min). Helium (1 mL·min^{-1}) was used as the carrier gas. A divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR, 30 μm; PDMS 50 μm) fibre from Supelco (Bellefonte, PA, USA) was used for SPME with adsorption at 80 °C for 15 min and analyte desorption for 2 min in the injection port set at 250 °C. Screening of volatile compounds was performed in the *m/z* range of 45–400 in SCAN mode. Samples of the active film without odour masking spray were also analysed for comparison.

2.7.2. Multilayer packaging

The active films developed were incorporated into two different multilayer packaging in an attempt to eliminate the unpleasant odour of carvacrol. The first one consisted of a combination of 16 μm thick polyethylene terephthalate (PET) film from Samtack (Barcelona, Spain) and the active LDPE film bonded with a water-based adhesive (PET/adhesive/active LDPE), while in the second the PET was replaced by 13 μm thick aluminium (Al/adhesive/active LDPE). The water-based adhesive for food contact applications was purchased from Samtack. The active LDPE film containing 10% of aluminosilicates was always placed on the inside of the packaging as the food contact side. Fig. 2 shows the finished packaging.

2.8. Quality of crisp samples

2.8.1. Oxidation of lipids

10 g of crushed crisps were placed in both multilayer packaging bags and stored at 60 °C for 30 days to accelerate lipid oxidation. The thiobarbituric acid reactive substances (TBARS) test was used to assess lipid oxidation by extracting 10 g of crushed crisps with 60 mL of 10% trichloroacetic acid (TCA). The samples were mixed for 10 min and the extract was filtered through a paper filter. Then, 2 mL of the extract were added to 2 mL of 0.02 M thiobarbituric acid (TBA) and heated at 97 °C for 20 min. The TBARS index was determined by measuring the absorbance at 532 nm against a blank (mixed solution of TBA with TCA). Malondialdehyde (MDA) was quantified from the calibration curve of 1,1,3,3-tetraethoxypropane in 1 M HCl in the range of 0.1–1.0 μg·g^{-1}.

2.8.2. Sensory panel

Bags of multilayer materials PET/adhesive/active LDPE and Al/adhesive/active LDPE with 10% aluminosilicates loaded in the LDPE layer were prepared and filled with 10 g of crushed crisps. The bags were

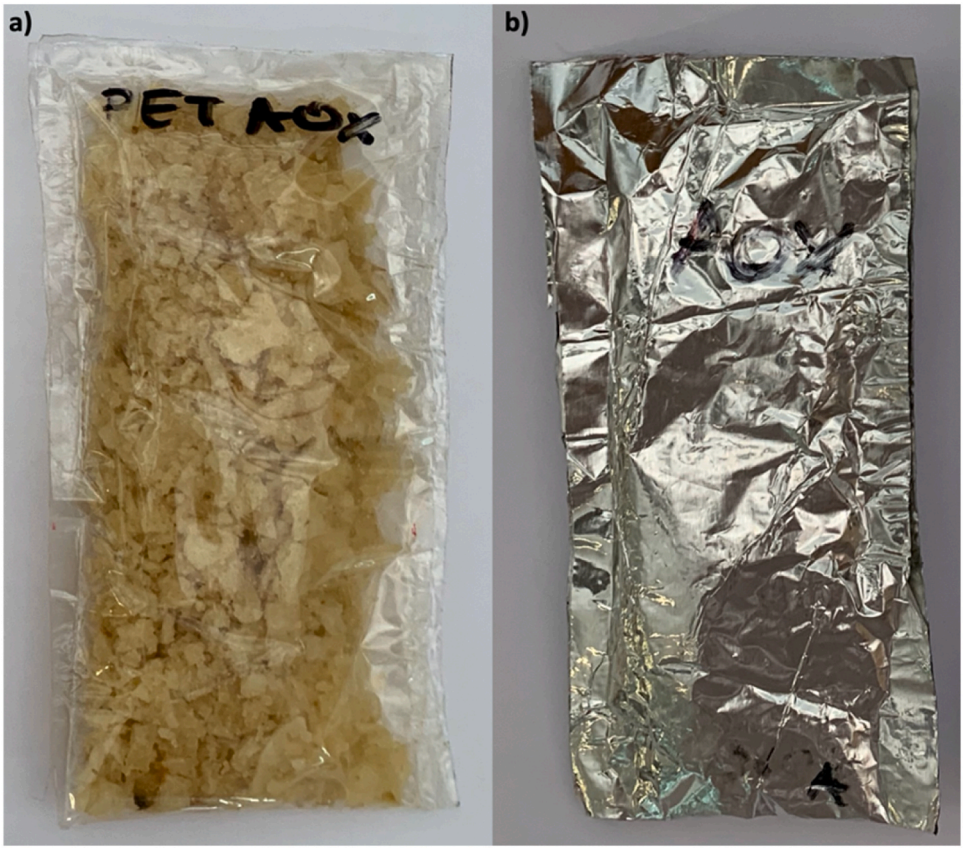


Fig. 2. Multilayer packaging used in the study of crisps, where: a) PET/adhesive/active LDPE and b) Al/adhesive/active LDPE.

stored at room temperature for 30 days to simulate the real market situation. After this period, eight trained tasters (28–40 years old, 6 women and 2 men) assessed the smell of the crisps directly in the bags according to ISO 6658 (ISO, 2017). The selected panellists underwent rigorous training sessions that included familiarisation with a range of odours, with a particular focus on carvacrol and its associated odour profile. Training tools included standard odour kits and samples of carvacrol at various concentrations. Panellists were also prepared to identify a range of odours associated with crisps at different stages of storage. They were provided with samples of crisps at known stages of freshness and deterioration as reference standards. Sensory testing and training were conducted at room temperature in a standardised tasting room at the pilot plant for Food Science and Technology at University of Zaragoza.

The following ethical principles, in line with established guidelines (IFST, 2021) were strictly adhered to during this study to ensure the protection and welfare of all testers involved. A detailed overview of the purpose, procedures, potential risks and benefits of the research was provided to each participant prior to their participation. It was also ensured that the research procedures would not cause harm or distress to the participants. All samples used for sensory evaluation were safe to smell as all materials used in the packaging and crisps were food grade and approved for food contact.

A triangular test (ISO, 2021, 2003) was used, where the tasters had to choose the best from three samples presented: two blank samples (multilayer packaging without carvacrol) and one sample of the active packaging. In addition, the smell of the sample of the blank and active packaging samples was rated on a scale of 1–5, with 1 being a very unpleasant smell and 5 being a pleasant, fresh smell. Two tests were performed: one with the PET multilayer packaging and one with the high barrier aluminium packaging (ISO, 2003).

2.9. Migration tests

One-sided migration of the developed materials was performed in different food simulants (3% acetic acid, 10% ethanol and 95% ethanol) for 2 h at 70 °C (Commission Regulation (EU No 10/2011), 2011). Pure simulants were also tested as blanks. All samples were analysed in triplicate. The determination of chemical compounds in the food simulants was performed using analytical techniques described in Section 2.7.1. 18 mL of 3% acetic acid and 10% ethanol were analysed directly by SPME-GC-MS, while 95% ethanol was diluted 10 times before analysis. Quantitative analysis was performed in all cases using external calibration curves plotted against pure standards.

2.10. Statistics

All samples were analysed in triplicate. The data presented were calculated as the mean values of the results obtained, while the error was estimated as the standard deviation. Statistically significant differences between samples were assessed by two-tailed t-test analysis ($P < 0.05$) for TBARS results.

With regard to the statistical analysis applied to the sensory analysis results, the number of correct identifications made by the panel was compared with a critical number derived from the binomial distribution, which depended on the number of panelists and the chosen significance level ($P < 0.05$). If the number of correct identifications was equal to or greater than this critical number, it was possible to conclude that there was a detectable difference at the chosen level of confidence (95%). Conversely, if the number of correct identifications was less than the critical number, we would conclude that there was no discernible difference.

3. Results and discussion

3.1. Antioxidant capacity of encapsulated carvacrol

The antioxidant capacity obtained when samples were exposed to the free radical generator was expressed as the percentage of hydroxylation (H%) of the sample compared to the blank, with lower values of H% corresponding to a higher antioxidant capacity. In terms of the results obtained, both the ethanolic extract of encapsulated carvacrol and pure carvacrol gave 0% hydroxylation. This corresponded to chromatograms in which the peak of the 2,5-dihydroxybenzoic acid (2,5-DHB), the product of the hydroxylation reaction, was not detected (Fig. 3), indicating that the active samples tested were able to scavenge all OH· radicals. This result also shows that the encapsulation of carvacrol in the aluminosilicates did not affect its antioxidant properties, as the chromatograms obtained for encapsulated and pure carvacrol were equivalent.

3.2. Antimicrobial activity of encapsulated carvacrol

As shown in Table 1, the active capsules exhibited antibacterial activity against *S. enterica*, as no colony growth was detected in inoculated samples containing carvacrol capsules, indicating a more than 9-fold reduction in bacterial growth. These results indicate that encapsulated carvacrol is able to maintain its antimicrobial activity as it is released from the capsules into the aqueous medium, thus exerting its antibacterial activity through direct contact with the bacterial cells of *S. enterica*. These results are in agreement with those obtained by other authors regarding the anti-*S. enterica* activity of carvacrol (Trevisan et al., 2018; Knowles et al., 2005), although a different serovar (*Typhimurium*) of the same species was used.

Growth is expressed in colony forming units per millilitre ($CFU{\cdot}mL^{-1}$). The mean ± standard deviation of three independent replicates is given. No growth indicates that no colonies were detected in the PCA plate (limit of detection = $2{\cdot}10^{2}$ $CFU{\cdot}mL^{-1}$).

3.3. Antioxidant stability of active films

A very high antioxidant capacity was observed for active films M1 (30% capsules), M2 (10% capsules) and M3 (20% capsules) stored in sealed aluminium bags at room temperature, as indicated by the total absence of the 2,5-DHB peak after 0, 1, 2, 3, 4, 5, 6 and 9 months of storage. The chromatograms obtained were the same as those of the ethanolic extract of encapsulated carvacrol and pure carvacrol (Fig. 3). On the other hand, the antioxidant capacity of the active films M1, M2 and M3 stored at ambient air and room temperature was lost after 3 months of storage, as shown by the similar results obtained for the 2,5-DHB peak for these samples and the blank.

Considering that all three active films M1, M2 and M3 stored in aluminium bags maintained their high antioxidant activity during the 9 months of storage, the film with the lowest concentration of capsules was chosen, as the most economical. Other authors have also reported that active films stored in ventilated chambers had lost almost all of their active volatile agent (n-hexanal) after 35 days of storage (Monedero et al., 2010). Although the addition of nanoclays and other inorganic compounds has been reported to increase the tortuosity of the material and thus reduce the release of volatile active agents from the packaging material (Tunç & Duman, 2011), the aluminosilicate capsules used in this study were probably able to slow down the release of carvacrol, but not to a great extent.

3.4. Antimicrobial stability of active films

The evaluation of the vapour phase antimicrobial activity of carvacrol active films against *Salmonella enterica* immediately after manufacture (day 0) and after 9 months of storage (day 270) in an aluminium

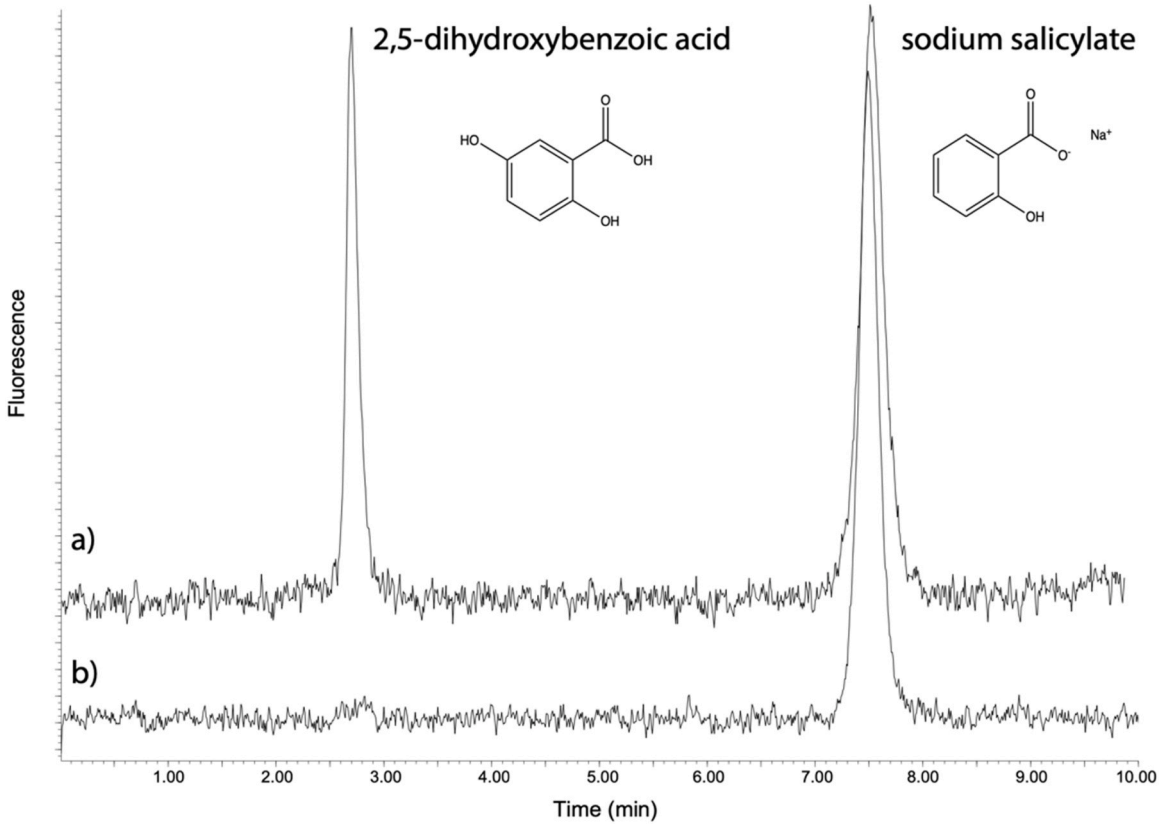


Fig. 3. Chromatogram of 2,5-dihydroxybenzoic acid and sodium salicylate of a) blank sample and b) pure carvacrol obtained by HPLC-fluorescence.

Table 1
S. enterica counts at 1600 μg·g^{-1} of the different capsules.

Sample	Capsule concentration (μg·g^{-1})	Bacterial growth (CFU·mL^{-1})
Inoculated control (positive control)	0 (no capsules)	$> 10^9$
Inoculated carvacrol capsules	1659.74 ± 17.62	No growth
Non-inoculated carvacrol capsules (negative control)	1672.20 ± 16.25	No growth

bag are presented in Table 2. No bacterial growth inhibition was obtained for samples M2 and M3, whereas a partial bacterial growth inhibition was obtained for the replicates of sample M1 at time 0 and after 9 months of storage in sealed aluminium bags. This partial growth inhibition in the vapour phase is in agreement with the results obtained by other authors using polypropylene (PP) films and carvacrol contained in nanoclays against another Gram-negative bacteria (*E. coli*) (Krepker et al., 2018), suggesting that plastic-based carvacrol films have limited antimicrobial activity. Nevertheless, the antimicrobial activity of carvacrol appears to be higher in the vapour phase (indirect contact) than in direct contact (Krepker et al., 2018; Ramos et al., 2012). These results suggest that higher concentrations of active capsules are required to achieve adequate antimicrobial activity.

3.5. Odour masking strategies

3.5.1. Odour masking spray

Table 3 shows the compounds identified with and without the masking spray. Up to 26 different volatile compounds were detected in the odour masking spray. The application of the spray on the surface of the developed active packaging effectively changed its unpleasant, strong odour due to its absorbing properties. It was shown that the addition of volatile compounds from the odour masking spray did not affect the presence of carvacrol, which was successfully detected in all three different active films. Furthermore, the peak area of carvacrol in active films with or without spray was identical. Nine compounds from the spray were not detected in the active films after treatment with the odour masking strategy. This may indicate that they interacted with the polymer structure and inhibited the odour of an active agent. Finally, after evaluating the active films with the odour masking spray, it was concluded that the unpleasant smell was no longer perceived.

Unfortunately, the compounds detected in the odour masking spray are not suitable for food contact applications as the migration tests performed in all simulants (10% ethanol, 95% ethanol and 3% acetic acid) exceeded the migration limits set by EU 10/2011 (Commission Regulation (EU No 10/2011), 2011). Therefore, this approach was not considered further.

3.5.2. Multilayer packaging

The evaluation of the smell of an active film incorporated in a multilayer packaging showed that the unpleasant odour of carvacrol was not perceived through the outer layer of the packaging not in contact with the food (PET and aluminium, respectively). This could be due to the increased barrier properties of these materials to gases and volatile compounds, as has already been demonstrated for PET films compared to polylactide (PLA), LDPE and PP films (Leelaphiwat et al., 2016).

3.6. Quality of crisps samples

Given the good results obtained in vitro for the antioxidant capacity of active films with carvacrol in vitro, we further investigated their antioxidant activity in vivo by testing their ability to prevent the oxidation of fatty foods (crisps).

Table 2
Results of vapour phase antimicrobial activity of active films with encapsulated carvacrol immediately after manufacture and after 9 months.

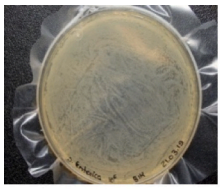
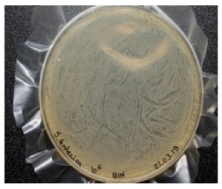
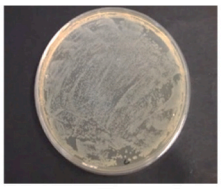
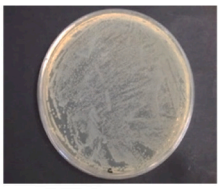
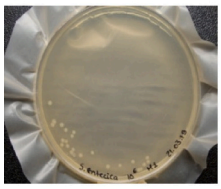
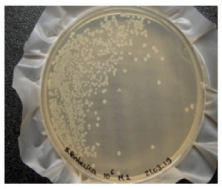
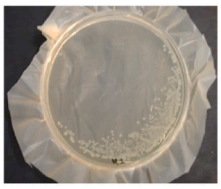
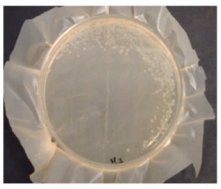
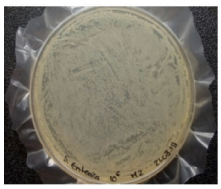
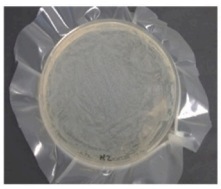
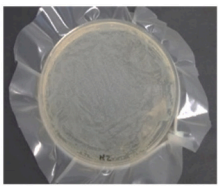
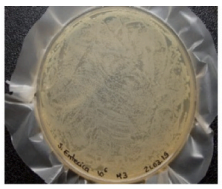
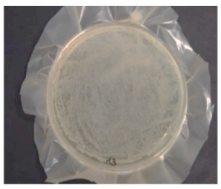
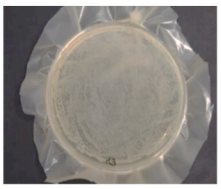
Sample	Immediately after manufacture		After 9 months	
	1st replicate	2nd replicate	1st replicate	2nd replicate
Control (with and without film)				
M1				
M2				
M3				

Table 3
Qualitative analysis of volatile compounds by SPME-GC-MS.

No.	Ret. time (min)	Compound	CAS	Odour masking spray	Active films M1-M2-M3 + odour masking spray
1	14.309	decanal	112–31–2	•	•
2	14.509	benzaldehyde	100–52–7	•	•
3	14.847	linalool	78–70–6	•	•
4	15.198	cyclododecane	294–62–2	•	
5	15.599	N-dimethyl-1-dodecanamine	112–18–5	•	
6	16.050	4-tert-butylcyclohexyl acetate	32210–23–4	•	•
7	17.085	benzyl acetate	140–11–4	•	•
8	17.461	citronellol	106–22–9	•	•
9	18.050	1′-oxybis-2-propanol	110–98–5	•	•
10	18.647	2-(2-hydroxypropoxy)− 1-propanol	106–62–7	•	•
11	19.407	diethoxy-methane	462–95–3	•	•
12	19.707	nonyl-cyclopropane	74663–85–7	•	•
13	20.200	4-methoxy-benzaldehyde	123–11–5	•	•
14	20.563	N-decyl-N-methyl-1-decanamine	7396–58–9	•	
15	21.194	1-chloro-hexadecane	4860–03–1	•	
16	21.423	butylhydrazone propanal	20607–75–4	•	
17	21.436	5-hexyldihydro-2(3 H)-furanone	706–14–9	•	•
18	21.828	gamma-dehydro-himachalene	51766–65–5	•	
19	21.991	3-methyl-4-isopropylphenol	3228–02–2	•	
20	22.055	carvacrol	499–75–2		•
21	22.162	piperonal	↱120–57–0	•	
22	22.789	methyl (3-oxo-2-pentyl-cyclopentyl) acetate	24851–98–7	•	•
23	23.223	galoxolide	↱1222–05–5	•	•
24	23.553	2-(phenylmethylene)-octanal	101–86–0	•	•
25	23.953	tonalide	21145–77–7	•	•
26	24.282	decyl decanoate	1654–86–0	•	•
27	24.793	N-benzyl-N-ethyl-p-isopropylbenzamide	15089–22–2	•	

"•" means that the compound has been detected in the sample.

3.6.1. Oxidation of lipids

The high concentration of MDA in the TBARS test confirms lipid oxidation. Fig. 4 shows the TBARS results of crisps stored in active packaging M2. Carvacrol incorporated in aluminosilicate capsules prevented lipid the oxidation in crisps stored at 60 °C for 30 days. Concentrations of MDA were found to be 45% and 35% lower in the M2/PET and M2/Aluminium active materials than in the blank. This is consistent with the fact that the antioxidant activity of carvacrol-containing films depends on the fat content of the food product, with greater efficacy being obtained for fatty foods (Wang et al., 2020).

3.6.2. Sensory panel

The minimum number of correct responses required to conclude that there are perceptible differences, based on a triangular test with eight tasters, is six.

According to the results of the triangular test presented in Table 4, eight out of eight tasters chose crisps from antioxidant bags as the best sample in the case of aluminium multilayer packaging. However, the results for the active PET multilayer packaging showed that the smell of the crisps was lost and only one out of eight tasters chose crisps from antioxidant bags as the best sample.

According to the results of the scaling test, the tasters rated the sample from the active aluminium multilayer packaging as better than the sample from the blank packaging. It also shows that the multilayer has fulfilled its function as an odour masking strategy.

In short, crisps stored in active PET multilayer packaging lose their volatile compounds. This could be due to faster oxidation of the samples as a result of exposure to light.

4. Conclusion

New active LDPE packaging with three different concentrations of aluminosilicates loaded with carvacrol (encapsulated carvacrol) was designed and produced to meet the defined objective of this study. Firstly, the antioxidant and antimicrobial capacities of the active capsules and then of the active films were tested and demonstrated.

The storage conditions of the active films clearly influenced their antimicrobial and antioxidant activity, and it was concluded that that the best method of storage was to place the active film rolls in high barrier aluminium bags where they could maintain their activity for up to 9 months. The partial *S. enterica* growth inhibition of sample M1 (30% of active capsules) was also maintained during the 9 months of storage.

In order to avoid the strong odour of the developed packaging, due to the volatile nature of the active agent, odour masking strategies were applied. Although effective in reducing odour, the application of a spray-masking substance into the film could not be used for food contact applications according to the results of migration tests performed. However, the second approach where the active film was incorporated into multilayer packaging materials showed that the unpleasant odour of carvacrol could no longer be perceived.

According to the TBARS test results, encapsulated carvacrol incorporated in the film prevented the oxidation of fats in crisps stored at 60 °C for 30 days. Tasters on the sensory panel (both triangular and scaling tests) selected the crisps stored in the antioxidant multilayer bags containing the encapsulated carvacrol and aluminium as the best sample.

Overall, the developed active films were able to improve the shelf life of crisps in the laboratory, which means that with the appropriate odour control strategy, these films can be applied to a wide range of foods prone to oxidation.

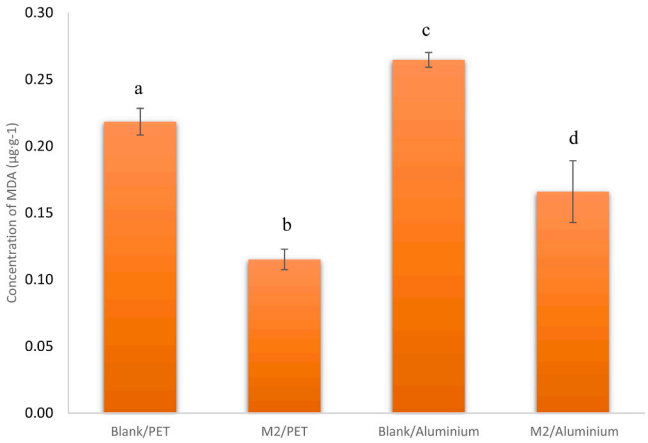


Fig. 4. TBARS results of crisps stored in active packaging containing 10% of active capsules (sample M2). Mean ± SD of three independent replicates are shown. Different letters indicate significant differences (P = 0.05) between samples.

Table 4
Results of sensory panel (smell) for crisps based on triangular and scale tests.

Triangular test		
Sample	Number of tasters who have chosen sample from antioxidant packaging as good	Maximum number of tasters
PET/Adhesive/ active LDPE	1	8
Al/Adhesive/ active LDPE	8	8
Scale test		
Sample	Average rating assigned by all tasters / Maximum rating	
	Blank	Antioxidant
PET/Adhesive/ active LDPE	3.37/5	1.87/5
Al/Adhesive/ active LDPE	1.50/5	4.12/5

CRediT authorship contribution statement

Magdalena Wrona: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Sofía Manso**: Methodology, Investigation, Visualization. **Filomena Silva**: Conceptualization, Methodology, Validation, Writing – original draft, Visualization, Supervision. **Leticia Cardoso**: Investigation, Validation. **Jesús Salafranca**: Conceptualization, Validation, Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Cristina Nerín**: Conceptualization, Methodology, Resources, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition. **María José Alfonso**: Conceptualization, Resources, Writing – original draft, Supervision, Project administration, Funding acquisition. **Miguel Ángel Caballero**: Resources, Writing – original draft, Supervision, Project administration, Funding acquisition.

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.

Data availability

Data will be made available on request.

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