



Selective effects of an industrial produced α -tocopherol active packaging on lipid oxidation pathways in grated Grana Padano cheese

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ABSTRACT

Lipid oxidation is a major factor affecting the quality of grated cheeses, whose high surface area enhances oxidative reactions. Although antioxidant active packaging has been investigated, most studies are limited to laboratory-scale systems and simplified food models, with limited attention to the relationship between antioxidant release and oxidation kinetics under industrially relevant conditions. In this study, an industrially produced α -tocopherol-based active film was characterized and applied to grated Grana Padano cheese during storage at 4 °C and 20 °C.

The incorporation of α -tocopherol induced a moderate plasticizing effect, increasing water vapor permeability by 40%, while oxygen barrier properties remained unchanged. The active compound exhibited rapid release at 25 °C (94% within 30 h) but slower release at 4 °C (50% after 25 days). Despite extensive migration into the cheese matrix, reaching 777 ± 180 mg/kg after 60 days at 4 °C, no significant reduction in peroxide value was observed. Conversely, the active packaging reduced free fatty acid accumulation under accelerated storage (0.043 vs. 0.028 g/g oleic acid after 60 days at 20 °C), limited colour changes (ΔE : 11 vs. 9), and selectively modulated volatile organic compounds associated with secondary oxidation.

These results show that antioxidant migration alone does not ensure control of primary oxidation. Instead, active packaging performance depends on the synchronization between antioxidant release and food oxidation kinetics. The industrially produced active film selectively affected secondary degradation pathways, highlighting the importance of matching antioxidant activity with food degradation kinetics when designing effective antioxidant active packaging systems for industrial applications.

1. introduction

Lipid oxidation is one of the main factors limiting the quality and shelf-life of dairy products, leading to the formation of off-flavours, colour changes, and nutritional losses (Dalsgaard et al., 2010; Kristensen et al., 2001). These processes are initiated by the formation of free radicals and hydroperoxides, which subsequently decompose into volatile compounds responsible for undesirable sensory attributes (Favati et al., 2007). Such phenomena are particularly relevant in grated cheeses, where the increased surface area enhances oxygen exposure and accelerates oxidative reactions (Gumus & Decker, 2021). As a result,

controlling lipid oxidation in these products remains a significant challenge for the food industry.

Active packaging has been proposed as an effective strategy to control oxidative processes in food systems by incorporating functional compounds that can interact with the food matrix and the surrounding environment through the release or absorption of active substances, thereby enhancing food quality and extending shelf-life (Gómez-Estaca et al., 2014; Volpe et al., 2022).

Antioxidant active packaging systems are designed to provide a sustained release of active compounds during storage, maintaining an effective concentration of antioxidants and improving the oxidative stability of

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food products (Gómez-Estaca et al., 2014). A wide range of natural antioxidants, including plant extracts such as rosemary and oregano, have been successfully incorporated into packaging systems, showing promising results in reducing oxidative degradation in different food products (Camo et al., 2011; Sánchez-Escalante et al., 2001).

Among these compounds, α -tocopherol is one of the most extensively studied antioxidants for food applications due to its strong radical scavenging activity and its natural occurrence in lipid-rich matrices such as dairy products (Balestrieri et al., 2002). Previous studies have demonstrated that α -tocopherol can be effectively incorporated into polymeric films and released into the food system, contributing to the control of oxidative processes (Granda-Restrepo et al., 2009; Torrieri et al., 2011). However, the effectiveness of such systems strongly depends on the release behaviour of the active compound and its interaction with the ongoing degradation reactions. Recent studies have also highlighted the importance of evaluating active packaging systems directly in contact with real food matrices, where complex interactions may significantly influence their performance (Fiore et al., 2021). In this context, understanding the relationship between antioxidant release, oxidation kinetics, and food matrix characteristics remains a key challenge for the effective application of active packaging technologies. Consequently, the translation of laboratory-scale results into industrial applications remains largely unexplored.

Despite these advances, most studies on antioxidant active packaging systems are still limited to laboratory-scale production and simplified model systems (Gómez-Estaca et al., 2014; Sanches Silva and Jafari, 2022). In particular, only a few works have addressed the challenges associated with the industrial-scale manufacturing of active films, where processing conditions such as high temperatures, shear stresses, and residence times may significantly affect the stability, retention, and distribution of active compounds within the polymer matrix (Gómez-Estaca et al., 2014; Granda-Restrepo et al., 2009; Tone et al., 2024). Under these conditions, the effectiveness of antioxidant packaging is often evaluated based on the total amount of active compound released, without fully considering the dynamic interaction between release kinetics and the rate of food degradation (Sanches Silva and Jafari, 2022). Consequently, there is still limited experimental evidence demonstrating whether antioxidant active packaging produced under industrial processing conditions retains its functionality once applied to complex commercial food systems.

It is well established that the effectiveness of these systems is governed by the balance between release kinetics, partitioning behaviour, and diffusion processes within the food/packaging system (Gómez-Estaca et al., 2014). If the release of antioxidants is not properly synchronized with the onset and progression of oxidation reactions, their protective effect may be significantly reduced, even in the presence of high levels of migrated compounds. In real food systems, this balance is further complicated by the interaction between multiple factors, including antioxidant diffusion, oxidation pathways, and the evolution of head-space composition. Lipid oxidation in structured food systems is strongly influenced by the physical organization of lipids and their exposure to oxygen, rather than by water activity alone (Gumus & Decker, 2021).

In this context, the aim of the present study was to evaluate the effectiveness of an industrially produced α -tocopherol-based active film obtained by co-extrusion and applied to grated Grana Padano cheese as a real food system. The study was designed to assess the impact of the active packaging on lipid oxidation processes during storage at different temperatures, considering both primary and secondary oxidation phenomena. Particular attention was devoted to the relationship between antioxidant retention during film processing, release behaviour under different storage conditions, and the resulting effectiveness of the active packaging in controlling oxidation under industrial processing conditions. Beyond evaluating packaging performance, the present work aimed to clarify how the synchronization between antioxidant release and oxidation kinetics determines the effectiveness of antioxidant active packaging under industrial conditions.

2. Materials and methods

2.1. Film preparation

In this study, Low-Density Polyethylene (LDPE) and Linear Low-Density Polyethylene (LLDPE) were used to form the polymeric matrix for the development of active films. Both polymers were supplied by Braskem (Braskem, Rotterdam, The Netherlands) (LDPE STS 7600 and LLDPE SLH 218 (1:1)) and are bio-based polyethylenes derived from renewable resources through biorefinery processes.

To confer antioxidant activity, α -tocopherol (TOC, purity 98%), purchased from FluoroChem Ltd (FloroChem, Glossop, UK), was employed as the active compound. The active agent was incorporated into the polymer matrix at 0.03 g/g through a preliminary compounding step.

The films investigated in this work were produced at the industrial facilities of Bluplast Company (Bluplast, Salerno, Italy), by means of a three-layer blown-film co-extrusion process. Although the structure is formally a multilayer system, all three layers share the same formulation, thus resulting in a functional monolayer-equivalent active film. The optimized film selected for subsequent experimental analyses (named Active film) had a total thickness of 100 μ m.

The processing temperature ranged from 130 to 190 °C. Additional processing details are omitted since they are specific to the industrial manufacturing protocol adopted by the producer. The films used in this study were manufactured under the same optimized processing conditions, ensuring consistent material properties such as adequate printability and sealability, which are essential requirements for effective application in food packaging systems, particularly for grated cheese products.

A film without α -tocopherol was produced as the control.

2.2. Film characterization

Mechanical properties: Mechanical tests were carried out according to ASTM D882 using a Lonos Test1 Universal Testing Machine equipped with a 250 N load cell. Rectangular specimens (15 mm in width and 100 mm in length) were tested at a cross-head speed of 5 mm/min.

Barrier properties: Water vapor permeability were determined using a PermtranW3/31 (Mocon, Neuwied, Germany). Samples with a surface area of 5 cm² were tested at 25 °C. Permeation tests were performed by setting the relative humidity at the downstream and upstream sides of the film to 0% and 50%, respectively. Oxygen permeability was determined using Ox-Tran (Mocon, Model 2/20, Neuwied, Germany). Samples with a surface area of 5 cm² were tested at 23 °C, setting the relative humidity (RH) at the downstream and upstream sides of the film at 50%. Barrier properties were determined from four replicate measurements (two duplicate tests), and the results are expressed as mean $\pm$ standard deviation.

α -tocopherol release tests. Release kinetics of α -tocopherol from the active film into the chosen food simulant (96% ethanol) were determined. Known amounts of active film (about 1.5 g) were placed into contact with 80 mL of 96% ethanol at T = 25 °C. The release kinetics were evaluated by monitoring the α -tocopherol concentration in the surrounding solution as a function of time until a constant value was reached. α -tocopherol concentration in ethanol was determined using a Cary 60 UV-Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA).

2.3. Packaging and quality evaluation

2.3.1. Sample preparation

Grated PDO Grana Padano cheese ripened for 12 months (Zanetti SPA, Italy) was purchased from a local supermarket. The cheese was divided into two groups: the first group was divided into 20 g aliquots and packed under aerobic conditions in thermo-sealed pouches

(16 × 7.5 cm) of low density polyethylene (LDPE) and linear low density polyethylene (LLDPE) film in ratio 50:50 containing 0.03 g/g of α -tocopherol (active package), the second group was divided and packed in the same manner but using an LDPE/LLDPE film without α -tocopherol (control package).

Samples were stored at 4 °C and at 20 °C in a climate chamber for 60 days. Before packaging (time zero) and after 20, 40 and 60 days, samples were subjected to the following analyses: headspace gas composition (O₂%, CO₂%); α -tocopherol content, fat content, triglycerides content (TGs), fatty acid profile (FAs), free fatty acid (FFAs) content, peroxides value (PV), volatile organic compounds (VOCs), and colour. The experiment was performed in triplicate.

2.3.2. Headspace gas composition

O₂ and CO₂ concentrations (mL/L) in the package headspace were monitored by means of a portable Check Mate 9900 O₂/CO₂ (PBI Dansensor A/S; Ringsted, Denmark) analyzer (accuracy ± 0.1%). 3 mL of gas was sampled from the package headspace with a medical-type needle (0.8 mm, 40 mm) (Thermo Europe N.V., 3001 Leuven, Belgium).

2.3.3. Fat extraction from cheese

Fat was extracted from the cheese using the method described in D. M. 1986 based on the Schmid-Bondzynski-Ratzla with modifications. Briefly, 10 g of grated cheese were hydrolyzed with 10 mL of 37% hydrochloric acid and 7 mL of 95% (v/v) ethanol. The cheese suspension was sonicated for 10 min and, subsequently, it was incubated for 30 min at 50 °C in a thermostatic bath. After cooling, the fatty matter was extracted using 10 mL of ethyl ether-petroleum ether (1:1) solution and the suspension was for 10 min at 6037 x g. The organic phase was collected, and the extraction protocol was repeated 3 times.

The three organic extracts were pooled, dried over anhydrous sodium sulfate, filtered through a paper filter, and evaporated under reduced pressure in a rotary evaporator. Fat content was gravimetrically determined and expressed as g/g of cheese. The fat extracted was analyzed for α -tocopherol content, PV, FFAs, and TGs and FAs composition.

2.3.4. Determination of α -tocopherol

The concentration of α -tocopherol in cheese was determined by high-performance liquid chromatography (HPLC) as reported by Aiello et al. (2019), with modifications. The fat extracted from cheese was dissolved in isopropanol and vortexed for 1 min. An aliquot of 20 μ L was then injected into an Agilent 1100 series HPLC instrument equipped with a quaternary pump, a G4225A degasser, DAD G1315B and FLD G1221A detectors and a Spherisorb ODS2 column (5 μ m, 4.6 mm × 250 mm; Agilent Technologies, Santa Clara, CA, USA). The mobile phase used was 100% methanol with a flow rate of 0.8 mL/min under isocratic conditions. The detector wavelength was set at 292 nm. The calibration curve was constructed with standard methanolic solutions. The range of linearity and the square of the correlation coefficient (R^2) were 1–100 ppm and 0.9990, respectively. The results were expressed as mg/kg of cheese.

2.3.5. Lipid oxidation

Peroxide value (PV) was evaluated following the method of Şengül, Erkaya, Dervişoğlu, Aydemir, and Gül (2014), with modifications. Briefly, 500 mg of fat was weighed and dissolved in acetic acid/chloroform (3:2 v/v) solution, then stored in the dark for 5 min after the addition of saturated potassium iodide. Finally, 7.5 mL of deionized water and 1% starch solution were added to mixture and titrated with sodium thiosulfate solution (0.001 N) PV was expressed as meqO₂/kg fat.

Free fatty acids (FFAs) were determined by titration. Briefly, 500 mg of fat was weighed and 5 mL of ethanol/ethyl-ether (1:2) were added. Sodium hydroxide (0.01 N) was used to neutralize the sample acidity, and phenolphthalein was used as indicator. The results were expressed as g of oleic acid per 100 g fat.

Triglyceride (TG) composition was determined by gas chromatography (GC) using an Agilent Technologies 6890N gas-chromatograph coupled

with a flame ionization detector (FID) and a capillary column 65% Diphenyl 35%dimethyl-polisiloxane, 30 m × 0.25 mm ID × 0.1 μ m following the method of Romano et al. (2011), with modifications. An aliquot of the extracted fat dissolved in hexane was injected under the following GC conditions: The oven temperature was initially set at 80 °C and held for 1 min, then increased to 240 °C at a rate of 30 °C/min and held for 10 min. It was subsequently increased to 360 °C at a rate of 4 °C/min and held for 5 min. The injector temperature was initially set at 100 °C for 0.1 min, then increased to 380 °C at a rate of 500 °C/min and held for 5 min. Helium was used as the carrier gas (flow 2 mL/min). Identification of peaks was performed using an external certified standard, BCR-519. The results were expressed as weight percentages (% w/w).

The analysis of fatty acids (FAs) was performed by using a gas chromatograph (GC) coupled with a flame ionization detector (FID) following the method of Aiello et al. (2019). After trans-esterification with potassium hydroxide (2 N) to hydrolyze fatty acids that were bound to TGs, 2 μ L of a 5% fat solution in hexane was injected into the GC equipped with a programmed temperature vaporizer (PTV) and an SP-2380 fused silica capillary column 50% Cyanopropyl Methyl Silicon (Supelco Inc., Bellefonte, USA) with dimensions of 100 m × 0.25 mm. i.d. and a film thickness of 0.20 μ m. The oven temperature was initially set at 70 °C and held for 1 min, then increased to 140 °C at a rate of 20 °C/min and held for 5 min. It was subsequently increased to 240 °C at a rate of 7 °C/min and held for 10 min. The PTV operating conditions were 70 °C for 0.1 min, increased at 500 °C/min until reaching 260 °C and held for 5 min. The detector temperature was set at 260 °C. Helium was used as the carrier gas (flow 2 mL/min). Identification was performed using a 37 component FAMES mix standard (Supelco, St.Louis, Mo, USA) and the concentration was calculated as weight percentages (% w/w).

2.3.6. Analysis of volatile organic compounds

The extraction and analysis of volatile organic compounds (VOCs) were performed using solid-phase microextraction (SPME) coupled with gas chromatography–mass spectrometry (GC-MS), according to Lee et al. (2013) and Manzo et al. (2019) with modifications. An SPME device equipped with a 50/30- μ m divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber coated with a 2-cm-long stationary phase was used. Then, 1.5 g of frozen grated cheese was transferred into a 10 mL vial, and 3 mL of deionized water and 15 μ L of 2-methyl-3-heptanone were added as internal standard (408 mg/L). Samples were homogenized and heated on a magnetic hotplate stirrer. The SPME fiber was then inserted into the sealed vial containing the sample and exposed to the headspace for 1 h at 50 °C. The SPME was introduced directly into the GC injector where the thermal desorption of the analytes was performed at 250 °C for 10 min. A GC system 6890N equipped with a mass detector 5973 was used. The VOCs were separated on a 30 m × 0.250 mm capillary column coated with a 0.25 μ m film of 5% diphenyl 195% dimethylpolysiloxane. Splitless injection was used for the samples. The column oven temperature was held at 40 °C for 2 min and increased from 40 °C to 160 °C at 6 °C/min and from 160 °C to 210 °C at 10 °C/min, which was held for 10 min. The injection and ion source temperatures were 250 °C and 230 °C, respectively. Helium was used as the carrier gas at a flow rate of 1 mL/min. The ionizing electron energy was 70 eV and the mass range scanned was 40–450 amu in full-scan acquisition mode. The compounds were identified using the NIST Atomic Spectra Database version 1.6 and verified by the retention indices. VOC concentrations were calculated using the internal standard method and expressed as mg/kg of cheese.

2.3.7. Colorimetric measurement

The colour of the grated cheese was measured with a tristimulus colorimeter Minolta Chroma Meter, model CR-300 (Konica Minolta, New York, USA) with a circular measurement area (D = 8 mm). The colorimeter was calibrated using a white standard plate (L = 100). The L*, a* and b* values were measured on the product at five different positions. The total colour difference (ΔE) was calculated with respect to the control sample at the initial time (t_0) according to the following equation:

$$\Delta E = \sqrt{(L_t^* - L_{t0}^*)^2 + (a_t^* - a_{t0}^*)^2 + (b_t^* - b_{t0}^*)^2}$$
 (1)

where L^* , a^* , and b^* represent the colour coordinates in the CIELAB space. The subscript (t_0) refers to the initial values of the control sample, while the subscript (t) refers to the values measured at a specific storage time.

2.3.7.1. Statistical analysis. The study included three technological replicates for each experimental condition, and all subsequent analytical measurements were performed in triplicate. The results are expressed as mean $\pm$ standard deviation. Statistical analyses were performed using XLSTAT software (Addinsoft, New York, USA). Data were analyzed by one-way analysis of variance (ANOVA). When significant differences were detected ($p < 0.05$), Tukey's honestly significant difference (HSD) post hoc test was applied for pairwise multiple comparisons among group means. Different superscript letters in the tables indicate statistically significant differences according to Tukey's test ($p < 0.05$).

3. Results and discussion

3.1. Film characterization

Mechanical properties (Table 1) showed a clear anisotropic behaviour between the machine direction (MD) and transverse direction (TD), reflecting the molecular orientation induced during film processing. The higher yield stress observed along the MD confirms the preferential alignment of polymer chains along the bubble growth direction, while the slightly higher elastic modulus in the TD is consistent with the presence of anisotropic domains within the polymer matrix. The incorporation of α -tocopherol (3 wt%) induced a plasticizing effect on the polymer structure, leading to a general reduction in mechanical performance in both directions. This behaviour can be attributed to increased chain mobility and free volume, as the additive does not chemically interact with the polyethylene matrix, and is consistent with previous studies reporting similar plasticizing effects of α -tocopherol in extruded polyolefin films (Azevedo et al., 2024). Notably, despite this plasticizing effect, the mechanical integrity of the films was maintained after processing, indicating that the incorporation of α -tocopherol does not compromise the structural stability of the material under industrial processing conditions, which is a critical requirement for the practical application of active packaging systems.

Barrier properties (Table 1) provide further insight into the effect of α -tocopherol on mass transfer phenomena within the polymer matrix. The incorporation of the active compound led to a significant increase in water vapor permeability (WVP) from 4.9×10^{-13} to 7.0×10^{-13} g/(m·s·Pa) (approximately +40%), which can be attributed to its plasticizing effect, resulting in enhanced chain mobility and free volume. This behaviour is consistent with the diffusion-driven transport of small polar molecules in polymer systems containing low-molecular-weight additives (Gómez-Estaca et al., 2014).

In contrast, oxygen permeability was not significantly affected by the presence of α -tocopherol (from 4.5×10^{-6} to 4.9×10^{-6} cm³/(m·s·atm), indicating that the structural modifications induced by the additive do not alter the transport of non-polar gases through the film. This aspect is particularly relevant in the context of lipid oxidation, as oxygen availability is one of the primary drivers of oxidative reactions.

Table 1

Mechanical and barrier properties of control and active film: (Elastic Modulus (EM); MaximumTensile strength (TS); Strain at Break (ϵ)); Water vapor permeability (WVP); oxygen permeability (OP).

Mechanical properties				Barrier properties	
Film Sample	EM (MPa)	TS (MPa)	ϵ (%)	WVP g/(m s Pa)	OP cm ³ /(m s atm)
Control	303 $\pm$ 21 (MD)	22 $\pm$ 1.8 (MD)	480 $\pm$ 60 (MD)	4.9 $\pm$ 0.7 10 ⁻¹³	4.5 $\pm$ 0.2 10 ⁻⁶
	400 $\pm$ 28 (TD)	30 $\pm$ 2.4 (TD)	660 $\pm$ 85 (TD)		
Active	280 $\pm$ 18 (MD)	20 $\pm$ 2.1 (MD)	580 $\pm$ 45 (MD)	7.0 $\pm$ 0.3 10 ⁻¹³	4.9 $\pm$ 0.1 10 ⁻⁶
	370 $\pm$ 23 (TD)	27 $\pm$ 1.6 (TD)	700 $\pm$ 58 (TD)		

The preservation of oxygen barrier properties suggests that any differences observed in oxidation behaviour cannot be attributed to variations in oxygen transmission through the packaging material. Instead, these effects are more likely governed by the release kinetics of α -tocopherol and its interaction with the oxidation pathways in the food system.

These results indicate that industrial processing successfully preserved the functional characteristics required for practical application of the active film.

3.2. Release kinetics of α -tocopherol

The release behaviour of α -tocopherol from the active film (Fig. 1) showed a strong dependence on temperature, highlighting the key role of diffusion-driven processes in controlling the availability of the active compound. At 25 °C, the release was rapid, reaching approximately 94% of the initial content within 30 h, indicating a high α -tocopherol mobility within the polymer matrix and an efficient mass transfer toward the surrounding medium. This finding is consistent with the thermogravimetric analysis (TGA), which showed negligible mass loss up to approximately 350–360 °C, followed by the main degradation step at higher temperatures. These results confirm that the extrusion temperatures adopted during film processing (130–190 °C) were well below the onset of thermal degradation, indicating that the active formulation remained thermally stable during film manufacture. The asymptotic release value ($\approx$ 94% of the nominal content) was reached after approximately 30 h, indicating a sustained release behaviour and effective availability of the active compound.

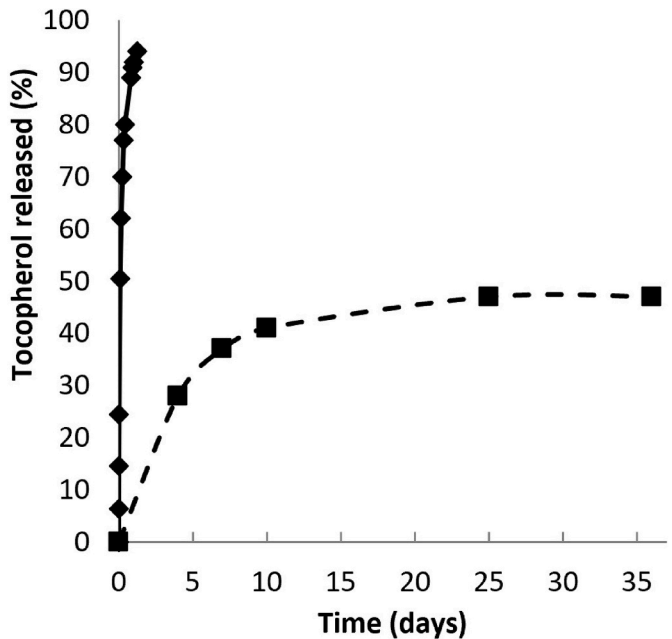


Fig. 1. Percentage of α -tocopherol released (with respect to nominal loaded amount) over time in 96% ethanol at 25 °C (■) e 4 °C (●).

In contrast, at 4 °C the release kinetics were significantly slower, with only about 50% of the total amount released after 25 days. This behaviour can be attributed to the reduced chain mobility of the polymer and the lower diffusion coefficient of the active compound at lower temperatures, which limits its migration from the film to the surrounding environment.

These results are particularly relevant when considering the application of the active film to real food systems stored under refrigerated conditions. Under these conditions, the release of α -tocopherol occurs over a timescale comparable to, or even slower than, the progression of oxidation reactions in the food matrix.

This mismatch between release kinetics and oxidation kinetics represents a critical factor affecting the effectiveness of antioxidant active packaging systems. While the total amount of α -tocopherol available may be sufficient, its delayed release limits its ability to interact with the

early stages of lipid oxidation, particularly the formation of primary oxidation products (Gómez-Estaca et al., 2014).

As a consequence, the antioxidant activity of the film is expected to be more effective in influencing secondary oxidation pathways, which occur at later stages of storage, rather than in preventing the onset of oxidation. This behaviour is consistent with the experimental results observed in the food system, where no significant differences in primary oxidation were detected despite measurable migration of α -tocopherol, as discussed in the following sections.

3.3. Headspace gas composition

The evolution of headspace gas composition during storage is shown in Fig. 2. After 60 days of storage at 4 °C, the O_2 concentration decreased from an initial value of 0.21 mL/mL to 0.01 ± 0.01 mL/mL, while CO_2 increased

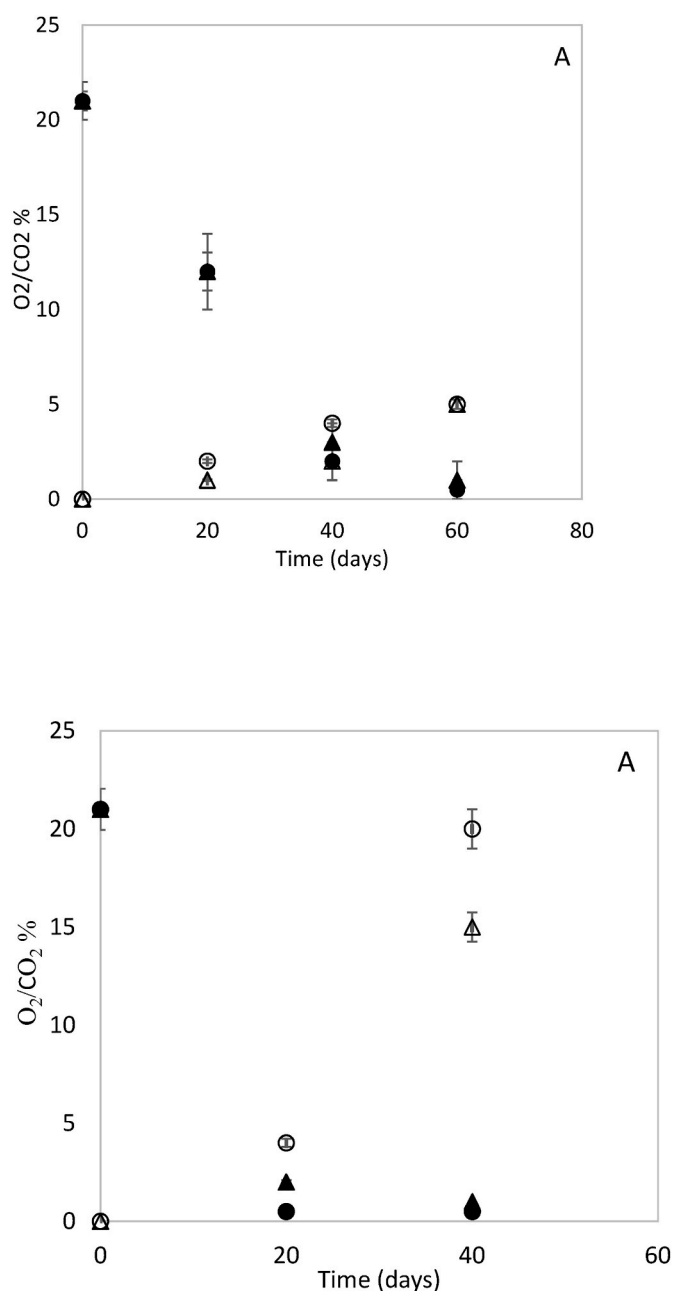


Fig. 2. Headspace gas composition (% O₂, % CO₂) of samples packed with control and active film and stored at 4 °C and 20 °C for 60 days. Control (●;▲), active (○;△).

Table 2

Concentration of α -tocopherol and in PDO Grana Padano cheese packed with active and control packaging and stored at 4 °C and 20 °C. The results are expressed such as mean $\pm$ standard deviation of three replications.

		Time of storage (days)	α -tocopherol (mg/kg of cheese)
Control packaging	4 °C	0	3.7 $\pm$ 0.1 ^b
		60	3.6 $\pm$ 0.1 ^b
	20 °C	0	2.5 $\pm$ 0.1 ^b
		60	777 $\pm$ 180 ^a
Active packaging	4 °C	60	623 $\pm$ 12 ^a
	20 °C	60	

Different letters in the same column indicate statistically significant differences ($p < 0.05$).

to 0.05 $\pm$ 0.01 mL/mL. At 20 °C, oxygen was completely depleted after 40 days, whereas CO₂ reached values up to 15% after 60 days.

The faster depletion of oxygen observed at 20 °C can be attributed to the acceleration of both oxidative reactions and microbial activity at the higher temperature. This progressive reduction of oxygen concentration highlights the dynamic nature of the system and its potential impact on the kinetics of lipid oxidation.

Notably, no significant differences were observed between active and control samples in terms of headspace gas composition. This indicates that the presence of α -tocopherol in the packaging material does not influence oxygen availability within the system.

Therefore, any differences observed in oxidation behaviour cannot be attributed to variations in oxygen concentration, but rather to the interplay between antioxidant release and oxidation kinetics.

3.4. α -tocopherol concentration

The concentration of α -tocopherol in cheese after storage is shown in Table 2. In control samples, α -tocopherol levels remained low and comparable to the initial value ($\approx$ 3.7 mg/kg), indicating no significant variation during storage.

In contrast, samples packaged with the active film showed a substantial increase in α -tocopherol concentration, reaching values of 777 $\pm$ 180 mg/kg at 4 °C and 623 $\pm$ 12 mg/kg at 20 °C after 60 days. These results clearly demonstrate the effective migration of the active compound from the packaging material into the cheese matrix under both storage conditions. Intermediate measurements indicated that the α -tocopherol concentrations in cheese after 15 days of storage were 92 mg/kg at 4 °C and 228 mg/kg at 20 °C. These data were obtained from samples packaged with a different batch of the active film and were therefore considered preliminary. Nevertheless, they provide useful information on the migration trend.

Notably, the slightly higher α -tocopherol content observed at 4 °C compared to 20 °C suggests that, although release kinetics are slower at lower temperatures, the cumulative migration over time remains significant.

Table 3

Fat content, peroxide value (PV) and free fatty acids (FFAs) of PDO Grana Padano cheese packed with active and control packaging and stored at 4 °C and 20 °C for 60 days. The results are expressed such as mean $\pm$ standard deviation of three replications.

		Time (days)	Fat (g/g cheese)	PV (meqO ₂ /kg fat)	FFAs (g oleic acid/g fat)
Control packaging	4 °C	0	0.270 $\pm$ 0.007 ^a	1.3 $\pm$ 0.1 ^f	0.022 $\pm$ 0.001 ^{b,c}
		20	0.267 $\pm$ 0.003 ^a	3.5 $\pm$ 0.1 ^e	0.029 $\pm$ 0.001 ^{b,c}
		40	0.275 $\pm$ 0.007 ^a	2.6 $\pm$ 0.1 ^{e,f}	0.025 $\pm$ 0.001 ^{b,c}
		60	0.270 $\pm$ 0.014 ^a	10.8 $\pm$ 1.3 ^{a,b}	0.022 $\pm$ 0.001 ^{b,c}
	20 °C	20	0.265 $\pm$ 0.014 ^a	2.5 $\pm$ 0.1 ^{e,f}	0.021 $\pm$ 0.001 ^{b,c}
		40	0.266 $\pm$ 0.017 ^a	6.2 $\pm$ 0.1 ^d	0.029 $\pm$ 0.007 ^{b,c}
		60	0.269 $\pm$ 0.013 ^a	9.8 $\pm$ 0.2 ^b	0.043 $\pm$ 0.002 ^a
		60	0.279 $\pm$ 0.001 ^a	2.6 $\pm$ 0.1 ^{e,f}	0.022 $\pm$ 0.001 ^{b,c}
Active packaging	4 °C	20	0.269 $\pm$ 0.006 ^a	2.9 $\pm$ 0.1 ^e	0.023 $\pm$ 0.001 ^{b,c}
		40	0.274 $\pm$ 0.001 ^a	11.7 $\pm$ 0.2 ^a	0.021 $\pm$ 0.002 ^{b,c}
		60	0.268 $\pm$ 0.014 ^a	2.4 $\pm$ 0.3 ^{e,f}	0.024 $\pm$ 0.003 ^{b,c}
		60	0.275 $\pm$ 0.007 ^a	8.3 $\pm$ 0.1 ^c	0.032 $\pm$ 0.006 ^b
	20 °C	20	0.272 $\pm$ 0.006 ^a	11.4 $\pm$ 0.2 ^a	0.028 $\pm$ 0.001 ^{b,c}
		40			
		60			
		60			

Different letters in the same column indicate statistically significant differences ($p < 0.05$).

Despite the high concentration of α -tocopherol detected in the cheese matrix, no corresponding inhibition of primary oxidation was observed (see section 3.5). This finding demonstrates that antioxidant availability alone is not a reliable predictor of oxidative stability. Similar behaviour has been reported in previous studies, where the presence of α -tocopherol in polymeric films did not always result in effective control of oxidation processes in food systems (Granda-Restrepo et al., 2009; Wessling et al., 1998).

These results provide strong evidence that the effectiveness of the active packaging is governed by the relationship between release kinetics and oxidation kinetics. Although α -tocopherol becomes available in the food system and reaches high concentrations in the cheese matrix, it does not effectively interfere with the formation of primary oxidation products. These findings suggest that the observed antioxidant release kinetics were not optimally synchronized with the evolution of oxidation in the cheese matrix (see section 3.6).

3.5. Lipid oxidation

The evolution of lipid oxidation during storage was evaluated through the analysis of primary and secondary oxidation indicators, providing insight into the mechanisms governing the effectiveness of the active packaging system. Fat content did not differ significantly among samples ($p > 0.05$), with an average value of 0.271 g/g of cheese, indicating that storage conditions and packaging did not affect the overall lipid content.

Primary oxidation, assessed by peroxide value (PV), showed a progressive increase during storage under both temperature conditions (Table 3). PV increased from an initial value of 1.3 meqO₂/kg fat to approximately 10–11 meqO₂/kg fat after 60 days, with a faster increase observed at 20 °C compared to 4 °C. However, no significant differences were detected between samples packaged with active and control films at any storage time. This result indicates that the presence of α -tocopherol in the packaging material does not significantly affect the formation of primary oxidation products. The lack of effect on PV, despite the measurable migration of α -tocopherol into the cheese matrix, suggests that the presence of the antioxidant is not sufficient to inhibit primary oxidation. This behaviour cannot be solely explained by delayed release, as rapid migration is observed at higher temperatures. Instead, these findings suggest that antioxidant availability was not sufficiently synchronized with the progression of primary oxidation to effectively interfere with hydroperoxide formation.

In contrast, parameters associated with secondary oxidation and quality degradation showed a different behaviour. Free fatty acids (FFAs) remained relatively stable during storage at 4 °C, with average values around 0.022–0.023 g/g oleic acid and no significant differences between samples. At 20 °C, a marked increase was observed in control samples, reaching 0.043 g/g after 60 days, whereas lower values were detected in samples packaged with the active film (0.028 g/g) (Table 3).

Table 4

Concentration of volatile organic compounds (VOCs) (mg/kg of cheese) in PDO Grana Padano cheese packed with active and control packaging and stored at 4 °C and 20 °C. The results are expressed such as mean $\pm$ standard deviation of three replications. Different letters in the same row indicate statistically significant differences ($p < 0.05$).

		Control packaging						Active packaging						
		4 °C			20 °C			4 °C			20 °C			
Time (days)	0	20	40	60	20	40	60	20	40	60	20	40	60	
Esters														
Ethyl butanoate	0.4 ± 0.0	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
Ethyl hexanoate	2.2 ± 0.2 ^a	1.2 ± 0.1 ^b	nd	nd	nd	nd	nd	1.0 ± 0.0 ^b	nd	nd	nd	nd	nd	
Isopropyl caproate	nd	nd	nd	nd	0.5 ± 0.0 ^a	nd	nd	nd	nd	nd	nd	nd	0.2 ± 0.1 ^b	
Methyl octanoate	nd	nd	nd	nd	0.7 ± 0.1 ^{a,b}	1.0 ± 0.3 ^a	0.4 ± 0.3 ^{b,c}	nd	nd	nd	0.3 ± 0.0 ^c	0.7 ± 0.0 ^{a,b}	0.3 ± 0.1 ^{b,c}	
Ethyl octanoate	0.5 ± 0.1 ^a	0.6 ± 0.2 ^a	0.4 ± 0.0 ^a	nd	0.5 ± 0.3 ^a	nd	0.2 ± 0.0 ^a	0.5 ± 0.0 ^a	nd	nd	nd	0.5 ± 0.0 ^a	0.3 ± 0.0 ^a	
Isopropyl octanoate	nd	nd	nd	nd	0.5 ± 0.0 ^b	4.0 ± 0.5 ^a	nd	nd	nd	nd	nd	0.5 ± 0.1 ^b	0.1 ± 0.0 ^b	
Methyl-dece-4-enoato	nd	nd	nd	nd	2.0 ± 0.6 ^a	1.2 ± 0.0 ^b	0.4 ± 0.2 ^c	nd	nd	nd	0.3 ± 0.0 ^b	nd	nd	
Methyl decanoate	nd	nd	nd	0.1 ± 0.0 ^b	0.6 ± 0.0 ^b	nd	3.6 ± 0.3 ^a	nd	0.3 ± 0.1 ^b	0.7 ± 0.2 ^b	nd	nd	2.3 ± 0.0 ^a	
Ethyl decanoate	0.7 ± 0.2 ^b	0.5 ± 0.2 ^b	0.4 ± 0.0 ^b	nd	nd	4.6 ± 0.3 ^a	0.4 ± 0.0 ^b	0.6 ± 0.1 ^b	0.2 ± 0.0 ^b	nd	nd	0.1 ± 0.1 ^b	0.6 ± 0.4 ^b	
Total esters	3.7 ± 0.5 ^{b,c}	2.2 ± 0.5 ^c	0.7 ± 0.1 ^{c,d}	0.1 ± 0.0 ^d	4.7 ± 1.2 ^b	10.8 ± 1.1 ^a	5.0 ± 0.8 ^b	2.1 ± 0.1 ^c	0.5 ± 0.1 ^d	0.7 ± 0.2 ^d	0.6 ± 0.1 ^d	1.8 ± 0.2 ^d	3.9 ± 1.0 ^b	
Alcohols														
2-heptanol	nd	nd	1.3 ± 0.5 ^b	nd	2.9 ± 0.5 ^{a,b}	5.6 ± 2.4 ^a	0.7 ± 0.5 ^c	nd	2.7 ± 0.4 ^{a,b}	1.5 ± 0.4 ^b	3.0 ± 0.9 ^{a,b}	2.4 ± 1.4 ^{a,b}	2.1 ± 0.6 ^{a,b}	
2-nonanol	nd	nd	1.0 ± 0.1 ^e	1.1 ± 0.0 ^e	10.4 ± 0.7 ^{b,c}	12.7 ± 0.9 ^{a,b}	4.3 ± 0.4 ^{d,e}	nd	12.6 ± 0.4 ^{a,b}	17.0 ± 2.1 ^a	12.1 ± 1.4 ^b	12.2 ± 3.0 ^b	5.9 ± 0.9 ^{c,d}	
Benzeneethanol	nd	nd	1.4 ± 0.6 ^a	1.7 ± 0.1 ^a	nd	0.7 ± 0.2 ^b	nd	nd	1.0 ± 0.1 ^{a,b}	nd	nd	1.4 ± 0.3 ^a	1.0 ± 0.6 ^{a,b}	
Total alcohols	nd	nd	3.6 ± 1.2 ^{c,d,e}	2.8 ± 0.1 ^{d,e}	13.3 ± 1.2 ^{b,c}	19.1 ± 3.5 ^a	5.0 ± 0.9 ^c	nd	16.4 ± 1.0 ^{a,b}	18.5 ± 2.5 ^a	15.1 ± 2.3 ^{a,b}	15.9 ± 4.6 ^{a,b}	9.0 ± 2.0 ^{b,c}	
Aldehydes														
trans-2-butenal	1.0 ± 0.23 ^a	0.9 ± 0.0 ^a	nd	nd	nd	nd	nd	0.8 ± 0.0 ^a	nd	nd	nd	nd	nd	
Benzaldehyde	0.1 ± 0.0 ^b	nd	0.5 ± 0.1 ^{d,e}	0.3 ± 0.1 ^b	nd	nd	nd	nd	0.6 ± 0.1 ^a	0.2 ± 0.0 ^{b,c}	nd	0.3 ± 0.1 ^b	0.3 ± 0.0 ^b	
Benzeneacetaldehyde	0.2 ± 0.0 ^c	0.4 ± 0.2 ^{d,e}	0.5 ± 0.1 ^{d,e}	2.9 ± 0.5 ^a	2.0 ± 0.0 ^{a,b,c}	2.1 ± 0.5 ^{a,b,c}	0.1 ± 0.0 ^c	0.3 ± 0.0 ^c	1.4 ± 0.2 ^{b,c,d}	0.7 ± 0.1 ^{d,e}	2.4 ± 0.3 ^{a,b}	1.1 ± 0.1 ^{c,d,e}	0.5 ± 0.1 ^{d,e}	
Nonanal	0.2 ± 0.0 ^c	0.4 ± 0.1 ^a	nd	nd	nd	nd	nd	0.3 ± 0.1 ^b	nd	nd	nd	nd	nd	
Total aldehydes	1.5 ± 0.3 ^{b,c}	1.6 ± 0.2 ^{b,c}	0.5 ± 0.1 ^{c,d}	3.2 ± 0.6 ^a	2.0 ± 0.1 ^{a,b,c}	2.1 ± 0.7 ^{b,c}	0.1 ± 0.0 ^d	1.3 ± 0.1 ^{b,c}	2.0 ± 0.3 ^{a,b,c}	1.0 ± 0.1 ^{b,c,d}	2.4 ± 0.3 ^{a,b}	1.3 ± 0.2 ^{b,c}	0.8 ± 0.1 ^{a,b}	
Ketones														
Acetone	nd	nd	nd	1.6 ± 0.0 ^b	nd	nd	0.5 ± 0.0 ^d	nd	nd	1.1 ± 0.2 ^c	nd	nd	2.5 ± 0.4 ^a	
2-pentanone	nd	2.8 ± 1.5 ^{b,c,d}	15.1 ± 6.4 ^b	nd	14.7 ± 1.2 ^{b,c}	17.6 ± 7.0 ^b	0.4 ± 0.0 ^{c,d}	16.3 ± 3.6 ^b	12.5 ± 4.7 ^{b,c,d}	nd	8.5 ± 0.2 ^{b,c,d}	34.2 ± 0.5 ^a	8.6 ± 2.6 ^{b,c,d}	
2-heptanone	1.1 ± 0.2 ^b	3.8 ± 0.2 ^b	25.3 ± 8.8 ^a	2.6 ± 0.2 ^b	27.6 ± 0.2 ^a	24.9 ± 2.0 ^a	3.4 ± 1.2 ^b	18.6 ± 4.2 ^{a,b}	23.5 ± 5.7 ^a	4.0 ± 2.3 ^b	15.9 ± 4.0 ^{a,b}	13.2 ± 2.1 ^{a,b}	9.7 ± 3.1 ^{a,b}	
2-ottanone	nd	nd	2.1 ± 0.2 ^a	nd	1.5 ± 0.5 ^{a,b}	1.2 ± 0.1 ^{a,b}	0.3 ± 0.1 ^b	0.8 ± 0.1 ^{a,b}	2.1 ± 0.3 ^a	nd	0.9 ± 0.2 ^b	0.7 ± 0.0 ^{a,b}	0.5 ± 0.1 ^b	
8-nonen-2-one	0.3 ± 0.1 ^c	1.5 ± 0.8 ^c	11.7 ± 2.2 ^{a,b,c}	6.4 ± 0.1 ^{c,d,e}	17.2 ± 2.9 ^a	13.1 ± 3.5 ^{a,b}	2.6 ± 0.9 ^c	4.9 ± 1.2 ^{d,e}	17.9 ± 1.0 ^a	5.7 ± 0.8 ^{c,d,e}	9.8 ± 1.1 ^{b,c,d}	6.1 ± 1.0 ^{c,d,e}	4.9 ± 1.0 ^{d,e}	
2-nonanone	2.1 ± 0.3 ^f	7.0 ± 2.9 ^e	61.7 ± 6.7 ^c	38.0 ± 1.4 ^{c,d}	101.2 ± 21.3 ^{a,b}	88.8 ± 6.5 ^b	28.4 ± 8.6 ^{d,e}	26.2 ± 6.6 ^{d,e,f}	114.9 ± 7.2 ^a	41.7 ± 5.1 ^{c,d}	6.5 ± 0.6 ^e	45.2 ± 3.4 ^{c,d}	41.8 ± 8.2 ^{c,d}	
2-decanone	nd	nd	0.5 ± 0.1 ^c	0.5 ± 0.1 ^c	2.4 ± 0.3 ^a	2.1 ± 0.5 ^a	0.7 ± 0.3 ^{b,c}	nd	2.7 ± 0.0 ^a	1.1 ± 0.1 ^{b,c}	1.3 ± 0.0 ^b	1-0±0.1 ^{b,c}	0.8 ± 0.2 ^{b,c}	
2-undecanone	0.5 ± 0.1 ^d	0.7 ± 0.3 ^d	4.9 ± 1.6 ^c	6.6 ± 1.3 ^c	50.1 ± 7.2 ^a	58.1 ± 8.5 ^a	14.7 ± 2.9 ^c	2.6 ± 0.7 ^c	37.1 ± 2.2 ^{a,b}	13.7 ± 1.7 ^c	15.9 ± 0.5 ^{b,c}	17.1 ± 2.3 ^{b,c}	13.5 ± 1.4 ^c	
2-tridecanone	nd	nd	nd	nd	nd	nd	0.4 ± 0.3 ^b	nd	nd	nd	nd	2.9 ± 0.2 ^a	0.2 ± 0.0 ^b	
Total ketones	4.1 ± 0.1 ^f	15.7 ± 4.8 ^e	121.3 ± 26.1 ^{b,c}	55.6 ± 3.0 ^{d,e}	214.6 ± 33.5 ^{a,b}	205.8 ± 28.1 ^a	51.5 ± 14.3 ^{d,e}	69.4 ± 16.4 ^{c,d,e}	210.7 ± 21.1 ^a	67.3 ± 10.2 ^{c,d,e}	58.7 ± 6.5 ^{d,e}	120.4 ± 9.5 ^{b,c,d}	82.6 ± 16.9 ^{c,d}	
Acids														
Acetic acid	0.7 ± 0.0 ^b	2.4 ± 0.0 ^a	nd	nd	nd	nd	nd	0.8 ± 0.0 ^b	0.2 ± 0.0 ^b	nd	nd	nd	nd	
Butanoic acid	2.3 ± 0.1 ^b	0.9 ± 0.3 ^c	nd	nd	3.8 ± 0.1 ^a	nd	nd	1.05 ± 0.23 ^c	nd	nd	nd	nd	nd	
Hexanoic acid	12.6 ± 0.8 ^a	6.6 ± 1.8 ^b	nd	nd	5.4 ± 0.4 ^{b,c}	4.6 ± 0.4 ^{b,c}	nd	6.3 ± 0.4 ^b	1.9 ± 0.8 ^c	nd	nd	0.6 ± 0.2 ^c	nd	
Octanoic acid	7.0 ± 0.7 ^a	5.2 ± 0.7 ^{a,b}	0.7 ± 0.4 ^b	nd	4.6 ± 0.4 ^{a,b}	6.7 ± 1.1 ^a	nd	9.6 ± 0.5 ^a	nd	nd	nd	0.9 ± 0.0 ^b	nd	
Decanoic acid	6.5 ± 1.2 ^a	2.2 ± 1.1 ^b	0.5 ± 0.1 ^b	nd	nd	7.1 ± 1.5 ^a	nd	7.0 ± 0.23 ^a	nd	nd	nd	0.9 ± 0.0 ^b	nd	
Dodecanoic acid	0.6 ± 0.1 ^b	0.6 ± 0.3 ^b	nd	nd	nd	1.1 ± 0.1 ^b	nd	1.9 ± 0.8 ^a	nd	nd	nd	nd	nd	
Total acids	29.6 ± 2.9 ^a	17.8 ± 4.1 ^{a,b}	1.3 ± 0.5 ^c	nd	13.8 ± 0.9 ^b	19.4 ± 3.0 ^{a,b}	nd	26.6 ± 2.1 ^a	2.1 ± 0.8 ^c	nd	nd	2.4 ± 0.3 ^c	nd	
Limonene	0.2 ± 0.1 ^b	14.9 ± 0.7 ^a	nd	1.1 ± 0.1 ^b	nd	nd	nd	11.0 ± 0.3 ^a	26.8 ± 6.1 ^a	nd	nd	nd	nd	

nd: not detected.

This result suggests a partial inhibitory effect of the active packaging on degradation processes occurring at later stages of storage.

No significant differences were observed in triglyceride (TG) and fatty acid (FA) profile between samples packaged with active and control films, nor between storage temperatures (Tables S1 and S2).

The absence of significant changes in triglyceride and fatty acid composition is an important finding, as it indicates that neither storage conditions nor active packaging altered the overall lipid profile of the cheese. Consequently, the differences observed in free fatty acids, volatile compounds, and colour cannot be attributed to modifications of the bulk lipid composition but rather to selective effects on oxidative degradation pathways. This observation further supports the hypothesis that the active packaging influences lipid oxidation processes without affecting the native lipid composition of the product.

The behaviour observed in this study is consistent with previous findings obtained using similar α -tocopherol-based active films produced at laboratory scale (Torrieri et al., 2011). In that work, the antioxidant effect of the film became evident only when oxidation kinetics were slowed by modified atmosphere packaging, highlighting the importance of the balance between oxidation and diffusion processes.

In contrast, in the present study, the application of the same active system under industrial-scale production and in a real food matrix resulted in a substantial migration of α -tocopherol without a corresponding inhibition of primary oxidation. This difference can be attributed to the more complex and dynamic nature of the food system, in which oxidation processes are not sufficiently delayed to allow effective interaction with the released antioxidant.

Taken together, these findings indicate that the amount of antioxidant released should not be considered the sole criterion for evaluating active packaging performance. Rather, the temporal synchronization between antioxidant availability and oxidation pathways determines whether oxidative deterioration can be effectively controlled.

3.6. Volatile organic compounds (VOCs)

The evolution of volatile organic compounds (VOCs) during storage is reported in Table 4. A marked increase in total VOCs was observed at 20 °C compared to 4 °C, confirming the strong effect of temperature on oxidative and biochemical processes.

At 20 °C, control samples showed a significant accumulation of esters during storage, with total ester content increasing from 4.7 ± 1.2 mg/kg at 20 days to 10.8 ± 1.1 mg/kg at 40 days, followed by a decrease at longer storage times. In contrast, samples packaged with the active film showed consistently lower ester concentrations at the same time points (e.g., 0.6 ± 0.1 mg/kg at 20 days and 1.8 ± 0.2 mg/kg at 40 days), indicating a reduced formation of these compounds in the presence of α -tocopherol.

A similar trend was observed for ketones, which were the most abundant class of VOCs. In control samples stored at 20 °C, total ketone

concentration reached 214.6 ± 33.5 mg/kg at 20 days, whereas significantly lower values were detected in active samples at the same time (58.7 ± 6.5 mg/kg). However, these differences were not consistently maintained during storage, suggesting a time-dependent effect of the active compound.

In contrast, aldehydes showed a more complex behaviour. At 4 °C, lower concentrations were observed in active samples than in controls at the end of storage, whereas at 20 °C, higher values were detected in active samples at the same time point, indicating that the presence of α -tocopherol does not uniformly inhibit all oxidation pathways.

Overall, the presence of the active film does not result in a uniform reduction of VOCs but rather in variations in the concentration of some compounds, particularly during the early and intermediate stages of storage. Given the complexity of the biochemical mechanisms underlying the formation and degradation of volatile organic compounds, as well as the influence of additional factors such as storage time and temperature, these results might suggest that α -tocopherol selectively affects secondary oxidation pathways.

The observed differences, together with the absence of significant effects on primary oxidation, indicate that the effectiveness of the active packaging is governed by the interplay between release kinetics and the progression of oxidation. The observed release behaviour of α -tocopherol may limit its interaction with early oxidation intermediates, while allowing it to influence reactions occurring at later stages of storage. The selective modulation of VOC formation further supports the hypothesis that antioxidant action depends on the stage of the oxidation process rather than simply on the amount of antioxidant released.

3.7. Colour measurement

Changes in colour parameters during storage are reported in Table 5. A progressive decrease in L* value was observed under all conditions, indicating a loss of brightness over time, which was more pronounced at 20 °C than at 4 °C. In particular, after 60 days at 20 °C, L* decreased to 70 ± 2 in control samples and to 73 ± 2 in samples packaged with the active film.

In contrast, no significant differences were observed for a* and b* parameters between active and control samples, indicating that the main colour changes are associated with a reduction in lightness rather than chromatic variations (Table 5).

The overall colour variation (ΔE) increased during storage, with a stronger effect at higher temperature. At 4 °C, ΔE reached values of approximately 6 ± 1 in control samples and 4 ± 1 in active ones, whereas at 20 °C values increased to 11 ± 1 and 9 ± 1 , respectively, after 60 days.

According to previous studies on food colour evaluation, ΔE values greater than approximately 5 are generally considered visually perceptible under practical observation conditions (Mokrzycki & Tatol, 2011; Pathare et al., 2013). Accordingly, the lower ΔE values observed in samples packaged with the active film indicate that the packaging reduced visually

Table 5

Colour parameters of PDO Grana Padano cheese packed with active and control packaging and stored at 4 °C and 20 °C for 60 days.

		Time (days)	L*	a*	b*	delta E
Control packaging	4 °C	0	81±4a	−2.4 ± 0.3a	15±1a	0a
		20	82±1a	−3.5 ± 0.2c	15.9 ± 0.4a	1.33b
		40	85±2a	−3±2b	15.6 ± 0.4a	3.92c
		60	81±1a	−2.8 ± 0.3b	15±3a	6.17d
	20 °C	20	79±2a	−1.5 ± 0.3b	16.5 ± 0.2b	2.73b
		40	73±2b	−1.5 ± 0.3b	15.5 ± 0.6a	8.4d
		60	70±2b	−0.5 ± 0.3c	16.9 ± 0.2b	11.24e
		20	83±1a	−3.8 ± 0.2c	15.3 ± 0.4a	1.92b
Active packaging	4 °C	40	81±2a	−3.5 ± 0.1c	14.8 ± 0.4a	1.53b
		60	77±2b	−2.1 ± 0.7a	15.7 ± 0.1a	4.3c
	20 °C	20	80±2a	−2.4 ± 0.4a	15.9 ± 0.4a	1.31b
		40	77±1b	−2.4 ± 0.4a	16.3 ± 0.4b	4.08c
		60	73±2c	−1.5 ± 0.4b	16.7 ± 0.4b	8.74d

Different letters in the same column indicate statistically significant differences ($p < 0.05$).

perceptible colour changes during storage. This behaviour is consistent with the modulation of secondary oxidation processes observed in the VOC analysis, confirming that α -tocopherol is more effective in limiting later stages of oxidation rather than preventing its onset.

Although the present results consistently support the proposed interpretation, some limitations should be acknowledged. The study evaluated a single industrial film formulation containing one α -tocopherol concentration in a single food matrix (grated PDO Grana Padano cheese). Therefore, the proposed relationship between antioxidant release and oxidation kinetics should be further validated using different packaging formulations, antioxidant loadings and food systems characterized by different oxidation behaviours. Although the combined evaluation of antioxidant migration, oxidation markers, volatile compounds and colour changes provides strong experimental support for the proposed mechanism, additional studies integrating kinetic modelling and microstructural characterization of antioxidant distribution within the packaging/food system would further strengthen its mechanistic validation.

4. Conclusions

In the present study, polyethylene films containing α -tocopherol, manufactured under industrial-scale conditions, exhibited a slight reduction in mechanical performance due to a moderate plasticizing effect and an increase in water vapor permeability, while fully preserving oxygen barrier properties. This preservation is particularly relevant for active packaging applications, as it ensures that oxygen availability remains uncompromised by the packaging material.

When applied to grated PDO Grana Padano cheese, extensive migration of α -tocopherol into the matrix failed to inhibit primary lipid oxidation. Nevertheless, the active packaging successfully reduced colour changes, limited free fatty acid accumulation under accelerated storage conditions, and selectively modulated volatile compounds associated with secondary oxidation pathways. These findings demonstrate that high antioxidant migration alone does not guarantee primary oxidative stability, emphasizing that packaging effectiveness is governed by the temporal synchronization between antioxidant release kinetics and oxidation pathways within the food matrix.

Overall, these results provide clear evidence that antioxidant active packaging performance cannot be predicted solely from active compound loading or release extent. Instead, the successful design of active packaging systems requires a paradigm shift towards engineering release profiles—through optimized polymer formulations, film architectures, and controlled additive distribution—to achieve a dynamic match with the oxidation kinetics of specific food products. This kinetics-driven strategy offers a useful mechanistic framework for developing more efficient active packaging systems at the industrial scale.

CRediT authorship contribution statement

Lucia De Luca: Writing – original draft, Methodology, Formal analysis. **Marika Valentino:** Writing – original draft, Methodology, Formal analysis. **Mariamelia Stanzione:** Writing – original draft, Methodology, Formal analysis. **Giovanna Giuliana Buonocore:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Fabiana Pizzolongo:** Writing – original draft, Supervision. **Elena Torrieri:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization. **Raffaele Romano:** Writing – review & editing, Funding acquisition.

Ethical approval

Ethics approval was not required for this research.

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.lwt.2026.119951>.

Data availability

Data will be made available on request.

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