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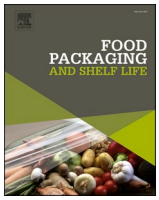

# Kinetics of carvacrol release from active paper packaging for fresh fruits and vegetables under conditions of open and closed package

Antonio López-Gómez [a], Alejandra Navarro-Martínez [a], Alberto Garre [b], Asunción Iguaz [a], Paulina Maldonado-Guzmán [c], Ginés Benito Martínez-Hernández [a,*]

[a] Food Safety and Refrigeration Engineering Group, Department of Agricultural Engineering, Universidad Politécnica de Cartagena, Paseo Alfonso XIII 48, 30203 Cartagena, Murcia, Spain
[b] Department of Agricultural Engineering & Institute of Plant Biotechnology, Universidad Politécnica de Cartagena, Murcia Paseo Alfonso XIII, 48, 30203, Spain
[c] Department of Food Sciences, Universidad Autónoma de Aguascalientes, 20130 Aguascalientes, Mexico

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ABSTRACT

The carvacrol release kinetics from active packaging (including carvacrol-βcyclodextrin inclusion complex) was studied under the following possible scenarios found in fresh produce packaging and marketing facilities: different storage temperatures (2, 8, 15 and 22 °C), relative humidity (60% and 95% RH), as well as different packaging conditions (open or closed). Release kinetics for the closed and open packaging systems were described using first-order and *n*-order power law kinetics, respectively. Increasing temperature and RH enhanced the carvacrol release rate. The release rate (*k*) increased by 1.3–1.7-fold when the RH was augmented from 60% to 95%. An initial release burst effect was observed with the highest *k* rate ($0.14/2.0 \times 10^{-2}$ $1/day^n$) under open active packaging at 8/15 °C (95% RH). In conclusion, the use of active packaging will ensure a proper essential oil release, with even a higher initial release (burst effect) in open packages, leading to a potential extension of the product shelf life.

## 1. Introduction

The reduction of current levels of fruit and vegetable waste (estimated as high as 45–55%), which is mainly due to product quality loss, is a main challenge to be solved by food technologists and engineers (FAO, 2023). In addition, current consumer trends show an increased interest in natural food products (without chemicals and using natural antimicrobials) along with sustainable packaging (e.g. paper and cardboard), avoiding petrochemical-based materials like plastics (Carocho et al., 2015; Otto et al., 2021). One kind of active packaging consists of incorporating active compounds (with antimicrobial, antioxidant, or other preservative properties) into the packaging material, being the active compound gradually released at the appropriate rate to the atmosphere surrounding the food product and applied to the product surface where it is needed (Han, 2005). Hence, the use of active packaging with different properties can reduce substantially the amount of preservatives added to food products while maintaining the product quality.

The properties of essential oils (EOs) make them ideal for sustainable active packaging systems. EOs are aromatic liquids obtained from different parts of plants (i.e. leaves, flowers, buds, etc.), with a complex composition of 20–60 compounds at different concentrations. Among all EOs components, usually two or three are the major components, representing 20–70% of the total EOs composition. Generally, components that appear in greater concentration are those determining their biological/technological properties (Pavela, 2015). Among these majority groups are: i) terpenes/terpenoids (e.g. carvacrol, thymol, etc.), which represent 90% of all major components of EOs; and ii) aromatic components (e.g. eugenol, cinnamaldehyde, etc.). Carvacrol, a phenolic monoterpene, is the major component of several EOs like oregano, thyme, marjoram, and savory EOs. The carvacrol antimicrobial activity against a wide spectrum of foodborne pathogens (such as *Escherichia coli, Salmonella*, and *Bacillus cereus*) is higher compared with other volatile EOs compounds. This is mainly due to the presence of the free hydroxyl group and the phenol moiety (Rathod et al., 2021). In addition, carvacrol has a high antioxidant activity, even higher when it is encapsulated (Kfoury et al., 2015). Carvacrol also shows a high inhibition of the activity of food-degradative enzymes (López-Gómez et al.,

* Corresponding author.
*E-mail address:* GinesBenito.Martinez@upct.es (G.B. Martínez-Hernández).

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2020). Carvacrol used in food products is classified as GRAS (Generally Recognized as Safe) being approved as a food flavoring (together with other EOs components like thymol, eugenol, citral, etc.) by the European Union and the FDA (EFSA, 2012; FDA, 2016). Nevertheless, the high volatility of carvacrol (like many of the EOs components) makes its encapsulation necessary to allow a controlled release from the active packaging avoiding the burst effect.

Cyclodextrins (CDs) are naturally occurring cyclic oligosaccharides (derived from bacterial enzymatic degradation of starch) of six (α-CDs), seven (β-CDs) or eight (γ-CDs) glucose units linked by α (1−4) bond (Pinho et al., 2014). They have a truncated cone-shaped structure with a hydrophobic central cavity allowing them to include hydrophobic guests, with a relatively hydrophilic external surface (Martin Del Valle, 2004). Hence, EOs can be encapsulated into CDs, being this EOs-CDs inclusion complex soluble in water. In particular, the highest encapsulation efficiencies of several EOs components were achieved in β-CD, compared to α-CD and γ-CD, being even higher in the native β-CD compared with β-CD derivatives (hydroxypropylated-β-CD, randomly methylated β-CD and a low methylated-β-CD) (Kfoury et al., 2015). In addition, β-CD is the most used CD in the industry due to its lower prices compared with α-CD and γ-CD. Vapor EOs released from active packaging including EOs-CD inclusion complexes are more efficient than treatments including liquid EOs applied directly on the food product (López-Gómez et al., 2019; Seo et al., 2015).

The EOs-CDs inclusion complex formation, which was early described by Cramer et al. (1967) in five elementary steps, is a dynamic equilibrium allowing the guest to diffuse reversibly from the CD cavity because of several factors like storage temperature and relative humidity (RH) (Rodríguez López, 2017). Temperature increments enhance the EOs release from the CD-inclusion complex by several hypotheses: i) acceleration of Brownian motion of molecules; ii) the higher the temperature, the greater the extent of damage to wall material (material's aperture greaten, cracks, etc.) leading to speed up the release of the core material; and/or iii) as the temperature rises, the energy of the gas molecules increases, increasing the percentage of activated molecules (compiled by Ren et al. (2018)). Attending to RH, the increase of water molecule concentrations in the surrounding environment leads to imbalances of the CD-EOs bounds, dissociation of the inclusion complex, and finally release of the guest molecule, which is replaced by water molecules (Martin Del Valle, 2004; Ponce-Cevallos et al., 2010).

The effects of RH and temperature on the EOs release kinetics from CD inclusion complexes have been only studied from the powder form of such EOs-CD inclusion complexes (Huang et al., 2018; Ren et al., 2018). Nevertheless, the EOs release kinetics may change when the inclusion complexes are applied on the active packages, as previously observed in our preliminary experiments (unpublished data). It may be explained since the EOs-CD inclusion complex is embedded in the cellulose fibers of the cardboard package material after their application. In addition, the lacquer type used for the application of the EOs-CD inclusion complex on the package surface may also influence the EOs release kinetics (unpublished data). In that sense, it is highly necessary to study the EOs release kinetics once the inclusion complex is applicated on the active package surface. In addition, the available literature (Huang et al., 2018; Ren et al., 2018) only refers to *in vitro* conditions in closed conditions (≈2 L recipients) for EOs-CD inclusion complexes in powder form, not being previously addressed real conditions of refrigerated rooms of horticultural facilities (i.e. open system) directly on active packages including the EOs-CD inclusion complex."

Taking this into consideration, there is a high interest to model release kinetics of carvacrol (one of the most antimicrobial and antioxidant EOs components) from active packaging to be used by the fruit and vegetables industry. Consequently, the fruit and vegetable shelf life could be potentially extended with EOs active packaging leading to lower food waste. Hence, this study aimed at characterizing the effect of different RH (60% and 95%), storage temperatures (2, 5, 15 and 22 ºC) and different packaging conditions (open or closed), common in fresh fruits and vegetables packaging facilities and produce retail, on the carvacrol release kinetics from active packaging with a coating including carvacrol-βCD inclusion complex.

## 2. Materials and methods

### 2.1. Materials

Carvacrol (99.5% purity) was obtained from Merck (Dusseldorf, Germany). *β*-Cyclodextrin Kleptose®10 (hereinafter referred to as *β*CD) was provided by Roquette (Lestrem, France). Water-base lacquer UKAPHOB HR 530 (ammonia-free anionic copolymer; pH (10%) 8–10; viscosity (20 ºC) max. 100 mPa×s; with 30% total solids concentration), which is authorized for food contact surfaces (EC, 2004), was acquired from Schill+Seilacher GmbH (Böblingen, Germany). This is a common lacquer type used for carboard packaging of fruit and vegetables: 1) it is easily dissolved in water to reach the appropriate density to ensure an appropriate spraying through sprayer nozzles; and 2) when dried improves impermeabilization of the cardboard surface against the high humidity levels maintained (to reduce water loss of plant products) in the cold rooms of horticultural facilities. Recycled kraft paper sheets (50 g/cm$^2$) were obtained from Bioencapsulation and iPackaging S.L. (Fuente Álamo, Region of Murcia, Spain).

### 2.2. Active packaging preparation

The carvacrol−*β*CD inclusion complex was prepared using the kneading method (Manolikar & Sawant, 2003). Briefly, 1 g of carvacrol was mixed with 7.6 g of *β*CD (following a 1:1 cavacrol:*β*CD molar ratio) in a mortar with 3 mL of ethanol, kneaded for 45 min and finally maintained in a vacuum desiccator at room temperature for at least 72 h (it will reduce the surface carvacrol that is not trapped in the *β*CD cavity). This carvacrol−*β*CD inclusion complex prepared using the same methodology and equipment has been fully characterized (thermogravimetry, Fourier-transform infrared spectroscopy, scanning electron microscope, etc.) (Buendía-Moreno, Sánchez-Martínez, et al., 2020; Buendía-Moreno, Soto-Jover, et al., 2020), showing a high encapsulation efficiency of 87.3%.

The carvacrol−*β*CD inclusion complex was dissolved (at different concentrations as shown in the next paragraph) in diluted lacquer before spraying on the kraft paper. The lacquer was previously diluted to a final solid concentration of 8.5% to compensate for the addition of the EOs−*β*CD inclusion complex as lacquers with solid content ≥ 30% may be difficult to spray on the paper or cardboard surface.

The active packaging was prepared to obtain load levels of the carvacrol−*β*CD inclusion complex of 100, 500 and 1000 mg/m$^2$, which would be equivalent (based on the previous carvacrol:*β*CD ratio of 1:7.6 g) to entrapped 11.6, 58.1 and 116.3 mg of carvacrol per m$^2$ of paper. The three selected load levels of the carvacrol−*β*CD inclusion complex (hereinafter called C100, C500 and C1000) were selected between the minimum dose to observe the benefits on the product quality during storage (López-Gómez et al., 2020) and the maximum dose without transferring of EOs-related off-flavors to the product (based on preliminary tests). Active packaging material was prepared one day before the experiments.

### 2.3. Carvacrol release tests from the active packaging

The carvacrol release from the active packaging (at the three coating levels of C100, C500 and C1000) was studied accounting for the effect of three technological factors: storage temperature (2, 8, 15 and 22 ºC), relative humidity (RH) (60% and 95%) and the use of an open/closed packaging system.

The storage temperatures were selected based on the recommendations for chilling injury-resistant produce (2 ºC; e.g. broccoli), for chilling injury-resistant produce at ≥ 8 ºC (e.g. tomatoes, citrus fruits,

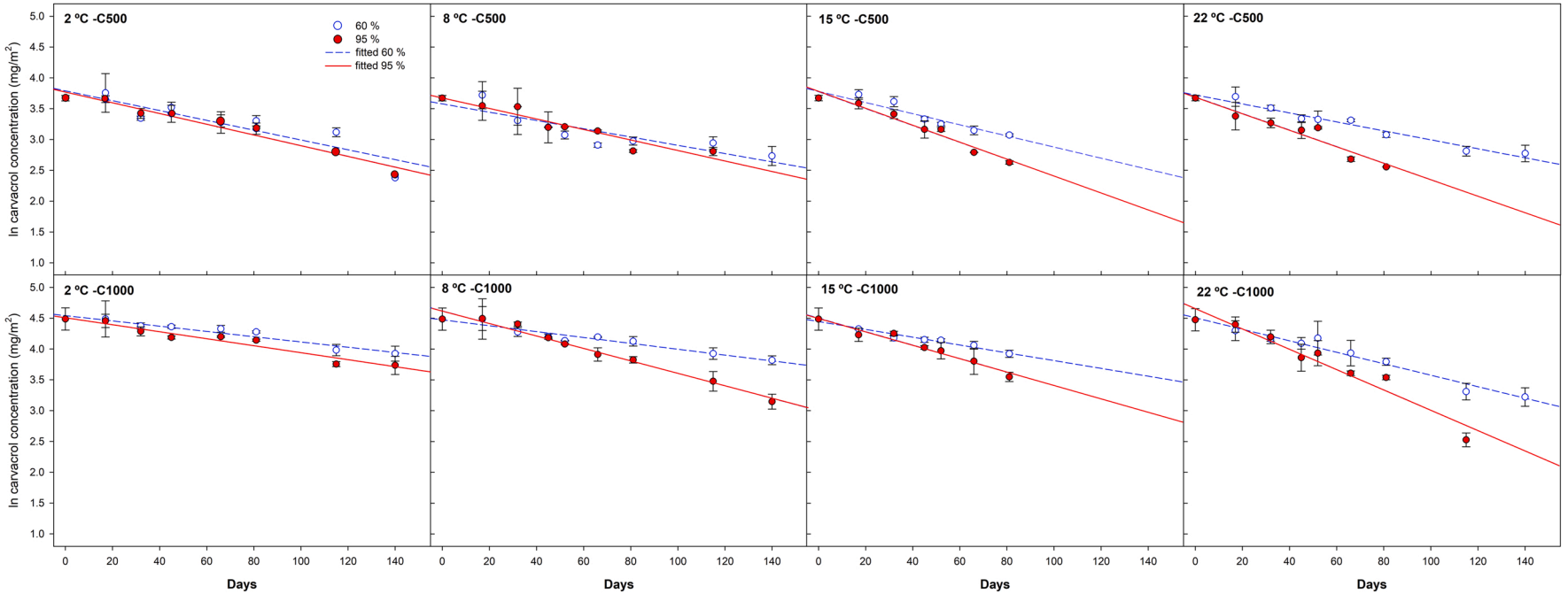


**Fig. 1.** Carvacrol release kinetics (1st order) from closed active packaging at two load levels (500 mg/m$^2$ (C500) and 1000 mg/m$^2$ (C1000) carvacrol:βCD inclusion complex) under different temperatures (2, 8, 15 and 22 ºC) and relative humidities (60% and 95%) during a release test (number of replicates (n)= 3 ± standard deviation (SD)).

**Table 1**
Release rates (estimate±standard error) for carvacrol in a closed active packaging at different initial doses (500 and 1000 mg/m$^2$ carvacrol:βCD inclusion complex), storage temperatures (2, 8, 15 and 22 ºC) and relative humidities (60% and 95%) (number of replicates (n)= 3 ± standard deviation (SD)).

| Temperature (ºC) | Relative humidity (%) | Initial dose (mg/m$^2$) | *k* (1/day) | $R^2$ |
|---|---|---|---|---|
| 22 | 60 | 1000 | 0.0093 ± 0.0008 | 0.83 |
| | | 500 | 0.0072 ± 0.0005 | 0.90 |
| | 95 | 1000 | 0.0155 ± 0.0012 | 0.87 |
| | | 500 | 0.0129 ± 0.0011 | 0.85 |
| 15 | 60 | 1000 | 0.0063 ± 0.0007 | 0.82 |
| | | 500 | 0.0087 ± 0.0008 | 0.86 |
| | 95 | 1000 | 0.0109 ± 0.0010 | 0.85 |
| | | 500 | 0.0134 ± 0.0008 | 0.94 |
| 8 | 60 | 1000 | 0.0047 ± 0.0006 | 0.73 |
| | | 500 | 0.0068 ± 0.0008 | 0.74 |
| | 95 | 1000 | 0.0100 ± 0.0006 | 0.93 |
| | | 500 | 0.0084 ± 0.0009 | 0.78 |
| 2 | 60 | 1000 | 0.0039 ± 0.0009 | 0.46 |
| | | 500 | 0.0064 ± 0.0010 | 0.68 |
| | 95 | 1000 | 0.0056 ± 0.0005 | 0.86 |
| | | 500 | 0.0080 ± 0.0005 | 0.92 |

etc.), usual temperature of refrigerated open-expositors in supermarkets for fruit and vegetables (15 ºC) and room temperature (22 ºC). The RH of 60% was selected as the common RH in uncontrolled ambient places, whereas a RH of 95% was chosen as the recommended value for cold storage rooms for fresh fruits and vegetables to reduce weight losses (mainly water loss due to transpiration) during postharvest handling (Kader, 2002).

Two different packaging conditions were studied: open system and closed system. The former simulates fruits and vegetables packaged in boxes without covers, whereas the closed system simulates boxes covered with plastic liners or wrapped pallets of stacked boxes. For the open system, active paper rectangular pieces (5 × 5 cm) were placed inside a cardboard box (40 × 30 × 9 cm) stacked between 2 other similar boxes, allowing air circulation through the vents of boxes (such vents are common in boxes for fresh fruit and vegetables to allow proper air circulation). Then, this stacked open system was placed in two cold rooms (3.0 × 3.0 × 3.0 m$^3$) at 2 and 8 ºC with controlled RH (using standard ultrasonic humidifiers) at the recommended level for fresh fruit and vegetables (95% RH). Another two cold rooms (same dimensions) were used at 15 and 22 ºC, but with controlled RH at 60% to simulate real conditions of supermarket open-expositors and external ambient at room temperature, respectively.

For the closed system, active paper squares (5 × 5 cm) were placed on the porcelain dish of a glass desiccator (internal volume of 2 L; internal diameter 22 cm). In the bottom of the desiccator were placed 200 mL of saturated $K_2SO_4$ or $KNO_3$ solutions to achieve 60 or 95% RH, respectively. The desiccators were then placed in the four rooms described above at 2, 8, 15 and 22 ºC. The frequency of sampling was done every 15 days up to 140 days (except for C100 samples: 80 days).

### 2.4. Analysis of carvacrol released from the active packaging

The amount of carvacrol released to the air was estimated from the remaining carvacrol content in the active packaging. Briefly, each active paper sheet was cut into smaller squares (0.5 ×0.5 cm) and placed inside a 20 mL-glass gas chromatography (GC) vial. Then, 8 mL of ultrapure water was added, and the vials were crimp-capped. Subsequently, vials were gently shaken for 10 min in an orbital shaker (Stuart SSL1; Osa UK at 220 rpm at room temperature, followed by an ultrasound bath (50 Hz, 1.6 A, 390 W; Ultrasons, J.P. Selecta; Barcelona, Spain) sequence (3 min at 50% + 3 min rest + 3 min at 50%). Then, 2 mL of hexane was injected in the vials, vortexed for 1 min, and allowed to rest for 30 min. Finally, vials were centrifuged at 3320 ×*g* for 10 min. The upper phase was then used as the carvacrol extract to be analyzed by GC.

The carvacrol extracts were analyzed in a gas chromatograph coupled to a mass spectrometer (GC−MS model 6890 (Agilent Technologies; Palo Alto (USA)). Carvacrol separation was achieved on a 30 m × 0.25 mm × 0.25 μm capillary column (CP8982 VF17ms; Agilent Technologies). The carrier gas was helium with a constant flow of 2.8 mL/min$^1$ and pressure of 264.8 kPa. The injection was performed in splitless mode. The oven temperature was held at 50 ºC for 1 min after injection, then programmed to reach 235 ºC after 10 min and held at 235 ºC until 29.5 min. Mass spectrometer (MS) was set in electronic impact mode (70 eV) with a mass range of 40–400 amu. Source and MS quad temperatures were 230 and 150 ºC, respectively. The carvacrol peak was identified by its mass spectra compared to data from the NIST05a.L database (National Institute for Standards and Technology). Carvacrol was quantified with a carvacrol standard (Sigma, USA) and expressed in mg/m$^2$. Three replicates (3 active paper sheets) were analyzed per sampling point.

### 2.5. Kinetic modelling of carvacrol release from the active packaging

The carvacrol release from the active packaging was described using kinetic models of 1st or *n*-order power law depending on the response observed. The 1st order kinetics model assumes a linear relationship between ln of the carvacrol concentration (ln *C*) in the active packaging and the elapsed storage time (*t*), as shown in Eq. (1) where $C_0$ is the initial concentration in the active packaging. In this model, the slope of the relationship is quantified by the release rate constant (*k*), with higher values indicating a faster carvacrol release from the active packaging paper sheet.

$$\ln C = \ln C_0 - k \cdot t \quad (1)$$

The *n*-order power law kinetics model is shown in Eq. (2). The main difference between this model and 1st-order kinetics is the exponentiation of the elapsed storage time by coefficient *n*, which introduces a curvature in the inactivation kinetics ($n < 1$ indicates an upwards curvature and $n > 1$ a downwards one). Note that the 1st-order reaction kinetics can be seen as a particular case of *n*-order power law kinetics where $n = 1$.

$$\ln C = \ln C_0 - k \cdot t^n \quad (2)$$

The most suitable kinetic models to describe the experimental data were identified by model selection based on the Akaike Information Criterion (AIC), as this index accounts for both the overall error of the fit and model parsimony (number of parameters) (Akaike, 1998). As an additional check, we tested whether the estimate of n was significantly different from zero (t-test; $\alpha = 0.05$). Both approaches resulted in the same conclusions.

Some of the conditions studied showed a relationship between the release rate, *k*, and the storage temperature *T*. Those cases were described using the linear model shown in Eq. (3).

$$k = k_{ref} - a \cdot (T - T_{ref}) \quad (3)$$

In this secondary model, $k_{ref}$ is the inactivation rate at the reference temperature $T_{ref}$ (7 ºC). The strength of the temperature effect is quantified by the slope parameter *a*.

The 1st order kinetics model (Eq. 1) and the secondary model (Eq. 3) were fitted to the experimental data by linear regression (Bates & Watts, 1988) using the functions included in R version 4.2.1 (R-Team, 2016). The *n*-order power law kinetics is non-linear, so it was fitted by the Levenberg-Marquardt algorithm (Moré, 1978) using the minpack.lm package from R ("CRAN - Package minpack.lm," n.d.).

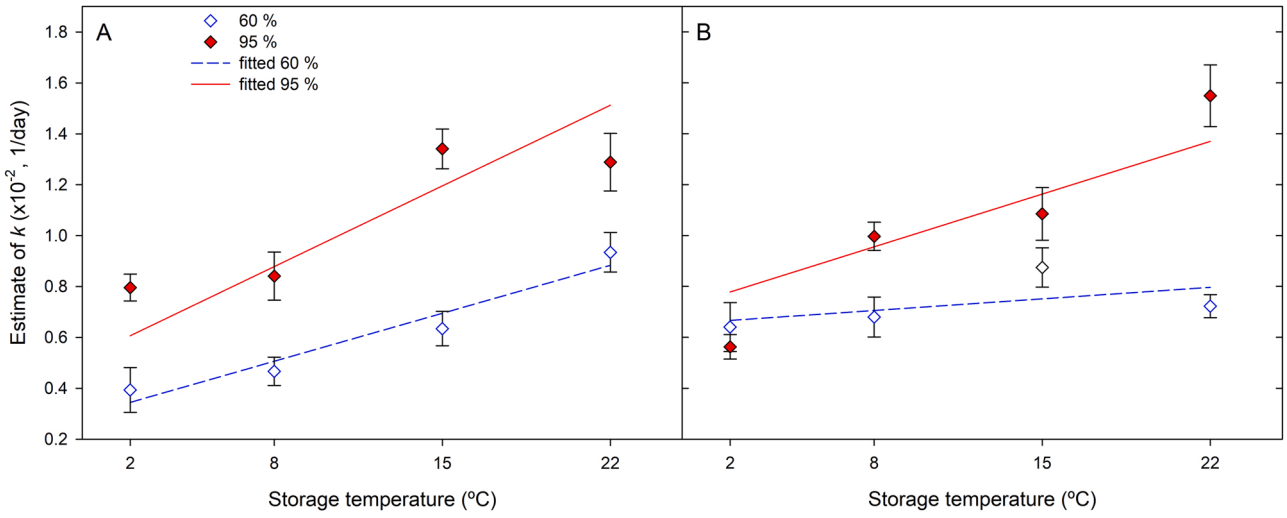


**Fig. 2.** Relationship between the release rate (*k*, in 1/day) and the storage temperature for the carvacrol release from closed active packaging at two load levels (A, 1000 ; and B, 500 mg/m$^2$ carvacrol:βCD inclusion complex) under different relative humidities (60% and 95%) (number of replicates (n)= 3 ± standard deviation (SD)).

## 3. Results and discussion

### 3.1. Carvacrol release kinetics from the active packaging under closed system conditions

The initial carvacrol content of C100, C500 and C1000 samples was 8.6, 39.3 (Figs. 1,3) and 88.8 mg/m$^2$ (Figs. 1,3), respectively. These measured carvacrol contents were close to the theoretical packaging loads (11.6, 58.1 and 116.3 mg/m$^2$, respectively, based on the carvacrol: CD encapsulation ratio of 1:7.6), which implies loading efficiencies on the active packaging of 70–76%. Such load deviation may be owed to: (i) the CD:carvacrol inclusion complex encapsulation efficiency, and (ii) possible carvacrol release during the inclusion complex dissolution into the aqueous lacquer before and during spraying on the active packaging paper sheet.

Among the 3 active packaging levels (C100, C500 and C1000), no remarkable carvacrol content changes during the storage study were observed for C100 (omitted in Fig. 1). This finding was also corroborated by fit of the kinetic model, with *k* not significantly different from zero ($\alpha < 0.05$). It may be explained because the low loading level of C100 is close to the detection limit of the analysis method (≈1 mg/m$^2$), so the release kinetics are masked by the experimental error. Consequently, C100 data are not included in Table 1. Conversely, higher carvacrol release was observed for C500 and C1000 active packaging (Fig. 1).

On the other hand, the carvacrol release from C500 and C1000 samples had a clear variation throughout the experiment (Fig. 1). Both the Akaike Information Criterion (AIC) and the significance of the order parameter (*n*) confirmed that the release kinetics for these conditions followed 1st order kinetics. The fitted models are illustrated in Fig. 1, whereas the model parameters of the primary model are provided in Table 1. In most cases, the 1st order kinetic model has a good fit of the data, with $R^2$ values higher than 0.7. Only the experiments at 2 ºC with a RH of 60% show a relatively poorer fit. This is most likely due to experimental noise in these experiments being larger than for the rest (larger error bars in Fig. 1). Therefore, for consistency with the rest of the analysis, the linear model is used.

**Table 2**
Kinetic carvacrol release parameters estimated in a closed active packaging under two load doses (500 and 1000 mg/m$^2$ carvacrol:βCD inclusion complex) (reference temperature of 7 ºC) (number of replicates (n)= 3 ± standard deviation (SD)).

| Relative humidity (%) | Initial dose (mg/m$^2$) | $k_{ref}$ (×10$^{-2}$, 1/day) | $a$ (×10$^{-3}$, 1/day °C) | $R^2$ |
|---|---|---|---|---|
| 60 | 1000 | 0.480 ± 0.040 | 0.269 ± 0.048 | 0.94 |
| 60 | 500 | 0.700 ± 0.060 | 0.068 ± 0.070 | 0.30 |
| 95 | 1000 | 0.833 ± 0.072 | 0.453 ± 0.080 | 0.94 |
| 95 | 500 | 0.926 ± 0.095 | 0.296 ± 0.107 | 0.79 |

Zero-order (extended release), 1st (immediate mode) and Avrami's equation kinetics have been applied in the literature to describe EOs release from CD inclusion complexes (Huang et al., 2018; Ren et al., 2018; Shiga et al., 2001, 2014). Ren et al. (2018) found a similar goodness of the fit ($R^2$) using either 1st order or Avrami's equation kinetics during an EO release study (eucalyptus EO-βCD inclusion complex) under refrigeration (5 ºC) and ambient temperature (26 ºC). For the samples in a closed system, we observed a clear relationship between the release rate (*k*) and the storage temperature, as well as an effect of the RH (Figs. 1 and 2). Attending to RH, carvacrol release kinetics at 95% RH were faster than at 60% RH. In particular, *k* values at 60% RH were $0.70 \times 10^{-2}$ and $0.48 \times 10^{-2}$ 1/day, respectively for C500 and C1000, while *k* values increased to $0.926 \times 10^{-2}$ and $0.833 \times 10^{-2}$ 1/day, respectively for C500 and C1000 at 95% RH. The observed higher release at higher RH has been previously reported in other EOs release studies using EOs-CD inclusion complexes in powder form. Such enhanced EOs release may be explained by the inclusion complex dissociation due to the higher content of surrounding water molecules at higher RH levels (Martin Del Valle, 2004; Ponce-Cevallos et al., 2010). The RH-enhanced carvacrol release was higher at C500 compared with C1000 in the closed packaging system (Figs. 1 and 2). The resulting binding between the CD and the guest molecule (i.e. carvacrol) is not rigid or permanent but is an equilibrium governed by a constant, the strength of which depends on two factors: the relative size of the host molecule (i.e. CD) and the interactions established between CD and the guest molecule (Kfoury et al., 2018; Martin Del Valle, 2004). The effect of the first factor may be neglected in our study as the CD cavity size was always constant (only one CD type was used, i.e. β-CD). Attending to the second factor, a dynamic CD-guest equilibrium may be the result of the driving force (net energy) that allows the guest molecule encapsulation into the CD (Martin Del Valle, 2004). Hence, the higher RH-enhanced carvacrol release in C500 compared with C1000 may be due to the higher carvacrol content in the surrounding atmosphere of C1000 under the closed packaging system, which hampered the carvacrol release from the CD-carvacrol system to the air-carvacrol system, and contrariwise.

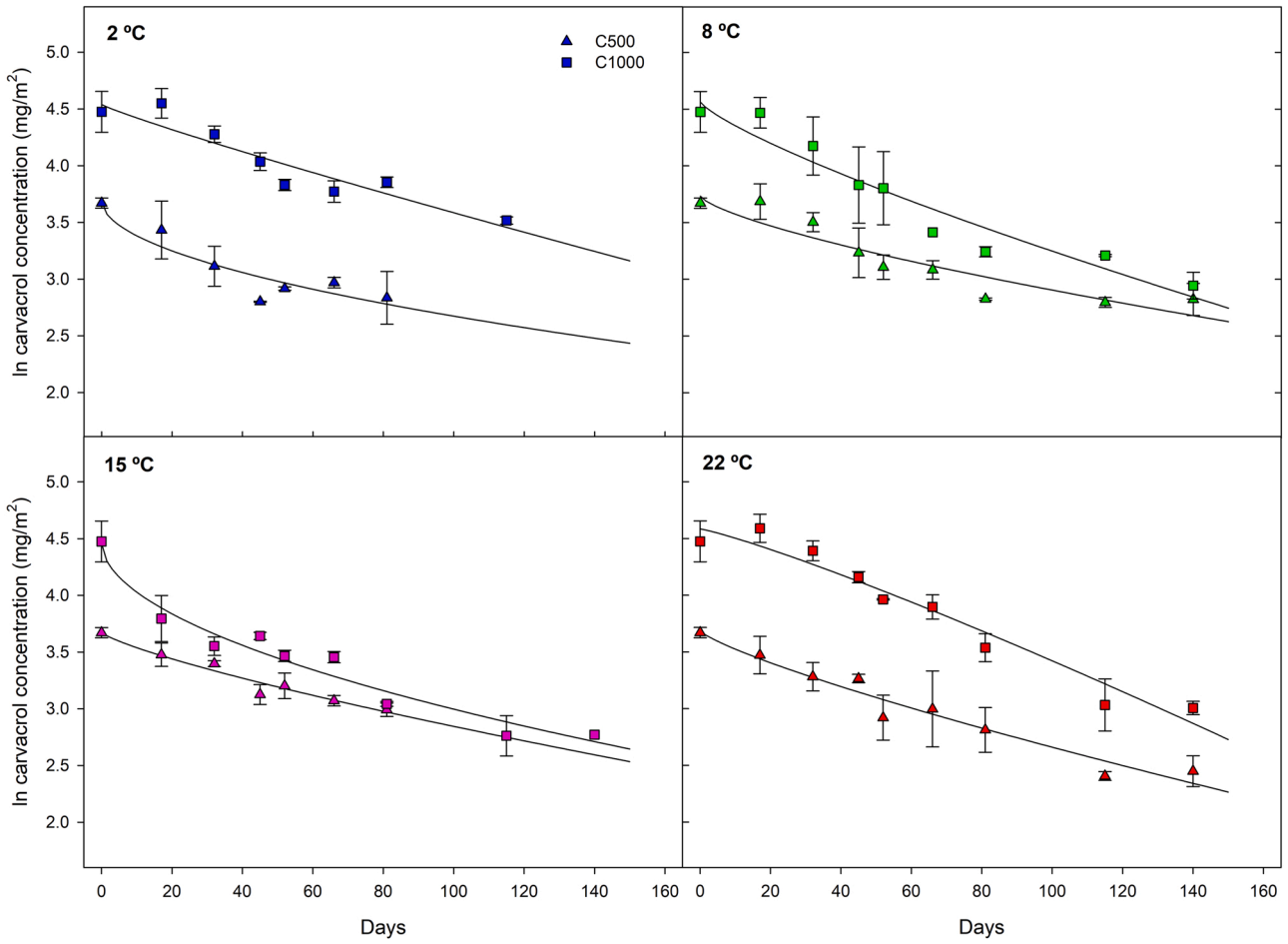


**Fig. 3.** Carvacrol release *n*-order power law kinetics from open active packaging at two load levels (500 mg/m$^2$ (C500) and 1000 mg/m$^2$ (C1000) carvacrol:βCD inclusion complex) under different temperatures (2, 8, 15 and 22 ºC) during a release test (number of replicates (n)= 3 ± standard deviation (SD)). Relative humidities for 2 and 8 ºC were 95% (as recommended in controlled refrigerated facilities for fruit and vegetables), while for 15 and 22 ºC were 60% (real conditions in usual uncontrolled conditions of supermarket open-expositors and external ambient at room temperature).

**Table 3**
Kinetic carvacrol release parameters estimated in an open active packaging under two load doses (500 and 1000 mg/m$^2$ carvacrol:βCD inclusion complex) (number of replicates (n)= 3 ± standard deviation (SD)).

| Temperature[a] (ºC) | Initial dose (mg/m$^2$) | *n* | *k* (×10$^{-2}$, 1/day$^n$) | ln $C_0$ (mg/m$^2$) |
|---|---|---|---|---|
| 2 | 500 | 0.52 ± 0.16 | 1.02 ± 0.25 | 3.68 ± 0.09 |
| 8 | 500 | 0.71 ± 0.14 | 0.77 ± 0.11 | 3.73 ± 0.08 |
| 15 | 500 | 0.78 ± 0.13 | 0.79 ± 0.10 | 3.68 ± 0.04 |
| 22 | 500 | 0.80 ± 0.14 | 1.03 ± 0.13 | 3.69 ± 0.08 |
| 2 | 1000 | 0.91 ± 0.19 | 0.95 ± 0.11 | 4.54 ± 0.08 |
| 8 | 1000 | 0.80 ± 0.14 | 1.41 ± 0.24 | 4.56 ± 0.11 |
| 15 | 1000 | 0.53 ± 0.07 | 2.05 ± 0.34 | 4.46 ± 0.08 |
| 22 | 1000 | 1.15 ± 0.14 | 1.14 ± 0.09 | 4.58 ± 0.07 |

[a] Relative humidities for 2 and 8 ºC were 95% (as recommended in controlled refrigerated facilities for fruit and vegetables), while for 15 and 22 ºC were 60% (real conditions in usual uncontrolled conditions of supermarket open-expositors and external ambient at room temperature).

The relationship observed between the release rate (*k*) and the storage temperature was described by a linear secondary model. The models support the results discussed in the previous paragraphs. Fig. 2 depicts the curves fitted to the parameter estimates, whereas Table 2 includes the parameters of the secondary model and the goodness of fit index. The experiments with an initial dose of C1000 show an excellent agreement between the fitted model and the observed data ($R^2$ >0.9). On the other hand, the fit for C500 is only good for the RH of 95% and poor for the data obtained for a RH of 60%. It may be explained by the initial lower carvacrol release (better observed at 15 and 22 ºC) at 60% compared with 95% RH, while such effect was masked by the initial higher releases due to a higher carvacrol dose (C1000) than the RH effect. On the other side, the secondary model (Eq. 3) is fitted to the estimates of the primary model (Eq. 1). Therefore, any error in these estimates will propagate to the secondary model. Because the accuracy of the slope estimates depends on the range covered by the data in the y-axis, experiments with an initial concentration of C500 will, in most cases, result in more uncertainty in the estimates for *k*. This uncertainty is then propagated to the secondary model.

Regardless of the model limitation, the fitted models emphasize the effect of the RH on the kinetics of carvacrol release. Focusing on the experiments with a C1000 dose due to their lower uncertainty, the RH affects both the release rate at the reference temperature and the sensitivity of the system to temperature changes. According to the fitted model, for a product stored at 7 ºC, a change in the RH from the 60% to the 95% will result in almost a 2-fold increase in the release rate (from 0.00480 1/day to 0.00833 1/day). This effect is even larger to higher temperatures, as evidenced by the slope parameter (*a*) being almost twice as large at 95% ($0.453 \times 10^{-3}$) than at 60% ($0.269 \times 10^{-3}$). Higher storage temperatures accelerate the molecular Brownian motion increasing the speed of EOs escaping from the inclusion complex (Ren et al., 2018). Subsequently, released EOs molecules from the inclusion complex are substituted by water molecules, being this process more intense at higher RH environments. Nevertheless, EOs-CD interactions

(Van der Waals and hydrogen bonds) remain less affected at refrigeration temperatures with the lower release of guest molecules (Cramer et al., 1967). An intermediate situation was observed with similar $k$ values for both C500 and C1000 (the same trend at either 95 or 60% RH) at 15 ºC.

Based on these results, storage of fresh fruits and vegetables with closed (using plastic liners or wrapping) active packaging at ambient temperatures would lead to a higher carvacrol release from the active packaging aiming to control microbial growth and inhibit quality-degrading enzymes of plant products, while reducing metabolic rates leading to lower production of volatile compounds (e.g. ethylene), and increasing then the product shelf life at such unrecommended storage temperatures. Nevertheless, the lower EOs release at refrigeration temperatures may be counterbalanced with higher RH, such as those recommended (90–95% RH) inside the cold rooms in the horticultural facilities to avoid product water loss (product dehydration).

### 3.2. Carvacrol release kinetics from the active packaging under open-system conditions

The release of carvacrol in the open packaging system differed substantially, both quantitatively and qualitatively, from the closed system. From a quantitative point of view, carvacrol release under the open packaging system was faster than for the closed system. For instance, the carvacrol concentration on the packaging was reduced in 1 log unit after 120 days stored at 2 ºC on an open system (Fig. 3), whereas that reduction was not attained in the closed system even after 150 days at the same temperature (Fig. 1). Furthermore, the release kinetics also differ from a qualitative point of view. Conversely to the closed system, where we observed a log-linear relationship between the concentration and the storage time, the observations for the open system had a clear non-linear shape.

These observations are corroborated by the kinetics model, whose parameters are reported in Table 3. As illustrated in Fig. 3, the kinetic models correctly describe the trend of the release kinetics in the open system. The AIC and the significance test for parameter $n$ confirmed the non-linearity of the release kinetics. In most cases, we estimate $n < 1$, indicating an upwards curvature in the release kinetics. This would imply an initial burst release followed by subsequent slower release (Fig. 3). This nonlinear EOs release process from CD inclusion complexes (also observed in other EOs encapsulation methods) has been previously reported (Hosseini et al., 2013; Huang et al., 2018; Martínez-Hernández et al., 2017). In particular, the initial burst release may be explained as lower energy is needed for the release of residual carvacrol molecules adsorbed on the CD surface and the release of carvacrol molecules entrapped near the CD cavity opening, as reported in other EOs-encapsulation systems (Anitha et al., 2011). Hence, the subsequent slower release of the two-step biphasic release behavior may be explained by the higher activation energy needed to allow the disruption of the EOs-CD bounds. The initial burst effect (as well as the subsequent release phase) is desired to be low during the conservation of empty active packages (prior to the plant product loading). Nevertheless, a moderate burst effect is desired when active packages contain perishable food products, such as fresh fruits and vegetables, to quickly achieve high EOs concentrations at the beginning of the product storage and, hence, extend their shelf life.

Carvacrol release kinetics from the open packaging system depended on the initial carvacrol load. Indeed, the faster carvacrol release was observed for C1000 at the higher temperatures with $k$ values of $1.4–2.0 \times 10^{-2}$ $1/day^n$ for samples at 8–22 ºC (Table 3). Hence, the open packaging system avoided high carvacrol accumulation in the surrounding atmosphere which may reduce the release rates, as observed for the closed packaging system. As previously discussed, temperature increments enhance the EOs release from the inclusion complex due to the acceleration of Brownian motion of molecules and higher energy transmitted to carvacrol molecules that are faster released from the CD inclusion complex (Ren et al., 2018). Interestingly, C1000 samples at 22 ºC showed a low $k$ value, and higher $n$ value (1.15), compared to samples at 8 or 15 ºC (Table 3). This finding may be explained as RH at room temperature is lower, with the consequent lower carvacrol release as observed from the absence of the burst effect in C1000 at 22 ºC (Fig. 3).

Studies conducted by our laboratory showed that carvacrol vaporized to air concentrations of 2.3 mg/L induced ≈ 60–65% inhibition of ethylene production (unpublished data). In addition, it is known that EOs in the vapor phase have higher technological efficiency (higher antimicrobial, antioxidant, enzyme-inhibitory effects, etc.) than liquid forms (López-Gómez et al., 2019; Seo et al., 2015). Overall, storage of fruits and vegetables in active packaging under open conditions (packages without plastic liners or wrapping) at refrigeration (8 ºC; RH 90–95%) or common temperatures of expositors in retail and supermarkets (15 ºC) would lead to a higher carvacrol release (with initial burst effect) in the vapor phase, and consequently longer product shelf life.

## 4. Conclusions

There is a high industrial interest about knowing the release kinetics of active compounds from active packaging under real conditions of different temperatures and relative humidities in fruit and vegetable facilities, and during product retail. To the best of our knowledge, previous release studies from cyclodextrin inclusion complexes are related to their powder form, not to the active packaging. Hence, the present study offers the release kinetics of EOs components (carvacrol used as a model) from active packaging under different real conditions of the horticultural facilities studying different storage temperatures and RH, as well as different packaging conditions (open or closed packages). Carvacrol release kinetics from the closed packaging system followed 1st order kinetics, while non-linear kinetics were observed when packages remained uncovered (open). In particular, the highest carvacrol release was observed under the open packaging system at common temperatures during refrigerated storage (8 ºC) and retail (15 ºC) of fruit and vegetables. Nevertheless, when products are stored under closed conditions is not observed the initial burst effect on the carvacrol release kinetics observed in the open system. Hence, the use of active packaging for fruits and vegetables in the horticultural facilities under refrigeration and during retail, will ensure a controlled essential oils release to reach the expected technological properties of essential oils (antimicrobial, antioxidant, etc.) to protect the product. Even, a higher initial release (burst effect) of essential oils is reached under uncovered packages, which is desired since an appropriate essential oil concentration level must be ensured around the product to reach the expected technological properties leading to extension of the shelf life of the product. On the other side, fresh and low relative humidity environments are recommended for conservation of active packages prior to their use for fruit and vegetables.

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## CRediT authorship contribution statement

**Antonio López-Gómez:** Conceptualization, Methodology, Validation, Resources, Writing-Review & Editing, Visualization, Supervision, Project administration, Funding acquisition. **Alejandra Navarro-Martínez:** Investigation, Data Curation, Formal analysis. **Alberto Garre:** Data Curation, Formal analysis. **Asunción Iguaz:** Supervision, Writing-Review & Editing. **Paulina Maldonado-Guzmán:** Investigation. **Ginés**

**Benito Martínez-Hernández:** Conceptualization, Methodology, Resources, Writing-Review & Editing, Visualization, Supervision.

## 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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