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Antimicrobial activity and thermal stability of rosemary essential oil:β−cyclodextrin capsules applied in tomato juice

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ABSTRACT

Rosemary essential oil (RO) was encapsulated within β-cyclodextrin (β-CD) to offer thermal stability and preserve its antimicrobial activity. The identified RO volatiles were 1,8 cineole (52%), 3-carene (9.6%), and camphor (9.3%). The growth of *Salmonella* Typhimurium, *Listeria monocytogenes, Candida tropicalis,* and *Saccharomyces pastorianus* was inhibited by 10, 10, 4.5, and 1.5 mg of free RO per mL of media, respectively. However, RO exhibited its evaporation at 43 °C, losing 50% of its weight at 100 °C and decomposing at 130 °C. RO:β-CD, at a 16:84 wt ratio, showed the highest presence of RO volatiles (7.33 g of RO/100 g of capsules) and initiated its decomposition at 75 °C, accompanied by a 10% weight loss. These particles underwent decomposition at 290 °C. Infrared analysis of RO:β-CD showed molecular interactions between RO and β-CD. The minimal inhibitory concentrations of the encapsulated RO for *S.* Typhimurium, *L. monocytogenes, C. tropicalis,* and *S. pastorianus* were 14.66, 14.14, 2.05, and 3.07 mg/mL, respectively. RO capsules were stable during tomato juice pasteurization and maintained their antimicrobial activity. In conclusion, the encapsulation of RO by β-CD offered protection to the volatile constituents exposed to high temperatures and maintained its antimicrobial properties after the encapsulation process and pasteurization of tomato juice.

1. Introduction

Microbial contamination is known as a threat to both the safety and quality of foods. Bacterial foodborne pathogens are among the most reported causes of food outbreaks, with fresh fruit and vegetables being the most linked items to this issue (Centers for Disease Control and Prevention, 2018). In addition, yeasts are among the most significant contributors to the spoilage of fresh produce, milk, and deli products (Kopper et al., 2009). Both types of microorganisms compromise the safety and quality of food.

The current trends in food preservatives include the use of effective, safe, and environmentally-friendly agents (Vazquez-Armenta et al., 2017). In this context, plant extracts have been tested as potential food antimicrobial agents (Kumar, Ravishankar, & Juneja, 2017; Ortega-Ramirez et al., 2014). Among plant extracts, rosemary essential oil (RO) is an effective antimicrobial agent against several bacteria and fungi species; and its activity has been attributed to its terpene constituents, mainly 1,8-cineole (Alvarez, Ortega-Ramirez, Silva-Espinoza, Gonzalez-Aguilar, & Ayala-Zavala, 2019). This component can affect microbial viability by disrupting membranes and interacting with vital enzymes (Olmedo, Nepote, & Grosso, 2013). Even when RO has been used as a flavoring and antimicrobial agent on strawberry, meat, tilapia, and zucchini, its application on thermally processed food and beverage is limited by its heat-lability (Ayala-Zavala, Gonzalez-Aguilar, & del-Toro-Sanchez, 2009; Han, Patel, Kim, & Min, 2014; Jiang et al., 2011).

The higher the environmental temperature, the larger the evaporation and oxidation of terpenes; e.g., an 80% of weight loss of cinnamaldehyde was caused by evaporation when increasing the temperature from 25 to 100 °C (Weissinger, McWatters, & Beuchat, 2001). The evaporation temperature of other essential oils initiated around

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45 °C and is finished at 164.2 °C for basil, for lemongrass at 155 °C, and orange oil at 166.7 °C (Martins, Sbaite, Benites, & Maciel, 2011). Several considerations have to be taken to assure an optimal result of essential oils as food additives; these considerations include compatibility between the odor and taste of the essential oil and the treated food (Ayala-Zavala et al., 2009). As well the use of thermal processing can induce volatilization and loss of the added oil, seeing that temperatures in the food processing industry could be higher than 63 °C for pasteurization, and between 100 and 275 °C for baking. From these premises emerged the research question: How the thermal stability of RO can be improved? In this context, this study hypothesized that β-cyclodextrin (β-CD) encapsulation could be applied to RO avoiding its evaporation and maintaining its antimicrobial activity after high-temperature process.

β-CD is a cyclic saccharide of 7 glucose units, capable of trapping non-polar compounds similar to RO terpenes controlling their evaporation; this technique was applied to cinnamon leaf and thyme essential oils, improving their thermal stability. (Ayala-Zavala, Del-Toro-Sanchez, Alvarez-Parrilla, & Gonzalez-Aguilar, 2008; Del Toro-Sánchez et al., 2010). Eugenol:β-CD capsules needed 100 °C temperature to volatilize compared to 50 °C needed for the free eugenol. Similarly, oregano essential oil was encapsulated in modified starch and Arabic gum, improving their thermal stability as indicated by DSC and TGA analysis (Hijo et al., 2015). Therefore, the goal of this study was to encapsulate RO within β-CD to protect the antimicrobial terpenes from volatilization at high temperatures using pasteurized tomato juice as a food model.

2. Materials and methods

2.1. Identification of RO volatiles

RO (W29920-0, Sigma Aldrich) volatile species were identified using a gas chromatograph Varian GC-3400 Cx, coupled with a Saturn 2100T Mass selective detector (GC-MS) (Varian, Mexico), using a DB-5 capillary column (30 m × 0.25 mm) (J&W Scientific, Agilent Technologies, Pennsylvania, EUA). The initial temperature (55 °C) was increased to 65 °C at a heating rate of 1 °C/min. The temperature was held at 65 °C for 3 min and further raised to 290 °C at 10 °C/min rate. The temperature was held at 290 °C for 10 min. During the GC-MS analysis, helium was used as carrier gas at a flow rate of 1 mL/min, the ionization energy was set to 70 eV and the injector and detector temperatures at 100 and 290 °C, respectively. A volume of 1 μL of RO diluted in hexane (500 μL/mL) was injected manually in splitless mode. The identification of RO volatile constituents was done by comparing their mass spectra with those of the Saturn library and NIST 98 library data of the GC-MS system (National Institute of Standard and Technology, Maryland, EUA) (Ortega-Ramirez et al., 2017).

2.2. Free RO antimicrobial activity

Listeria monocytogenes (ATCC 7644), *Salmonella enterica* subsp. *enterica* serovar Typhimurium (ATCC 14028), *Candida tropicalis* (ATCC 1369), and *Saccharomyces pastorianus* (ATCC 2345) were used to test the hypothesis of this research. Different concentrations of RO (0–20 mg/mL, using twofold dilutions) were used to determine its minimal inhibitory concentrations (MIC) against each microorganism using the micro-well dilution method. In a typical experiment, 5 μL of inocula with an approximately cellular concentration of 1×10^6 CFU/mL and 295 μL of Mueller Hinton (bacteria) or potato dextrose (yeast) broths added with RO were mixed and incubated at 37 °C during 24 h. The cell concentration in the used inocula in each experiment was measured by counting the growth of viable cells on agar, and results from an approximate 5 Log CFU/mL from three different experiments were selected as replicates. The MIC values (mg/mL) were determined as the lowest concentrations of RO to avoid the visual growth of the tested microorganism; this result was further confirmed by optical density (OD) measurements using a microplate reader (Fluostar Omega, BMG Labtech, Chicago, IL, USA), at a wavelength of 600 nm as suggested by the microplate reader company (Pusterla, 2018). Minimal biocide concentrations of RO against bacteria and yeast were detected by examining microbial growth in two concentrations below and above the MIC. The concentrations of RO where no viable microorganisms occurred were selected and expressed as the minimal biocide concentration in mg/mL (Ortega-Ramirez et al., 2017).

The experimental growth data for each bacterial strain was fitted to the Baranyi function using a complementary tool for Microsoft Excel (D-model, J Baranyi, Institute of Food Research, Norwich, UK). The evaluated kinetic parameters included the adaptation or lag time (h), maximum growth rate (μmax, log CFU h^{-1}), and maximum growth in the stationary phase (Ymax, log CFU). These values were calculated using the Baranyi function (Pin, Velasco de Diego, George, Garcia de Fernando, & Baranyi, 2004). The values of the microbial growth parameters were determined and compared before and after exposing the tested microorganisms to RO.

2.3. RO encapsulation with β-CD

RO encapsulation with β-CD was conducted using the co-precipitation method (Del Toro-Sánchez et al., 2010). To test the effect of the RO:β-CD ratio on the load of the essential oil volatiles, this input variable was set to 0:100, 4:96, 8:92, 12:88, and 16:84. A portion of 50 g (± 0.01) of β-CD was dispersed in 500 mL of an ethanol:water (1:2) mixture, and the resulting dispersion was maintained at 55 ± 2 °C on a hot stirrer plate. A predetermined quantity of RO (0, 2.08, 4.35, 6.82, or 9.54 ± 0.01 g) was dissolved in ethanol (10% w/v) and then slowly added to the warm β-CD solution to obtain the ratios mentioned above. During the addition of the oil solution, the β-CD solution was continuously stirred, and the temperature was maintained at 55 °C (± 2 °C). Afterward, the heater was turned off, and the resulting mixture was covered and stirred for 4 h. The final suspension was maintained at 4 °C during 12 h and the precipitated oil:β-CD capsules were recovered by vacuum filtration using Whatman® No. 1 as filter paper and then dried in a convection oven at 50 °C for 24 h. The capsules were removed from the oven and allowed to air-dry at 25 °C in a desiccator for an additional 24 h. The weight of recovered capsules on a dry basis was calculated as the percentage of the initial mass of raw materials (β-CD and RO) which was recovered in the capsules. Finally, the capsules were stored at 25 °C in an airtight bottle. Each starting oil ratio was prepared and analyzed by triplicate.

2.4. Water and RO content in the capsules

The water content of the RO capsules was determined by the gravimetric method, freeze-drying 1 g of capsules (FreeZone ® 77520 series, Labconco Co., Kansas City, MO, USA). The freeze-drying conditions were 24 h at −50 °C at 0.070 mBar and the temperature was raised gradually up to 22 °C. After freeze-drying, the samples were set in a desiccator, and the water content was calculated by subtracting the final capsule weight to the non-dry capsules, the loss of weight was recorded, and the obtained results were expressed as the percentage of water in the capsules. The RO content was measured gravimetrically by mixing 1 g of capsules with 2 mL of distilled water (n = 3), then the slurry was exposed to 120 °C until dryness. This process was repeated three times to assure the complete volatilization of the RO. Weight loss was recorded by subtracting the capsules' weight after the heating cycles to the initial weight of capsules, then the water content was subtracted, and results were expressed as the percentage of RO content in the capsules. These analyses were repeated three times. The RO:β-CD ratio with the highest content of trapped oil was tested as described in Section 2.2 to determine the antimicrobial activity.

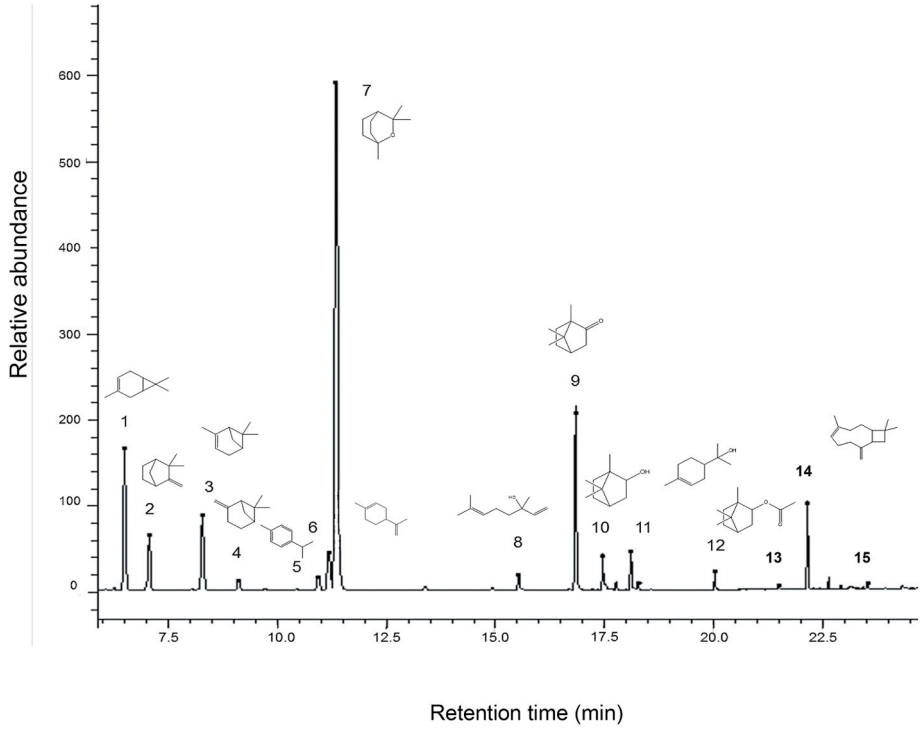

Fig. 1. Volatile profile of RO (GC-MS). 1) 3-carene, 2) camphene, 3) α-pinene, 4) β-pinene, 5) β − cymene, 6) D-limonene, 7) eucalyptol, 8) β-linalool, 9) camphor, 10) borneol, 11) α-terpinol, 12) bornil acetato, 13) copaene, 14) cariofilene, 15) γ − cadinene.

2.5. Infrared analysis (IR)

The IR spectra of the β-CD and both free and encapsulated RO were recorded using an infrared spectrophotometer FTIR Nicolet, Protege 460 (Nicolet, Madison, WI, USA). Scanning conditions were as follows: wavenumber range, from 4000–400 cm^{-1}; resolution, 4 cm^{-1}; number of scans, 64; scan speed, 0.63; detector, DTGS. RO was recorded on KBr plates. β-CD, a physical mixture of RO and β-CD, and capsules RO:β-CD, were recorded using KBr pellets. An Aldrich Library of Infrared Spectra was used for identification (Del Toro-Sánchez et al., 2010).

2.6. Thermogravimetric analysis and differential scanning calorimetry (TGA-DSC)

TGA-DSC curves of free and encapsulated RO were obtained in a Thermal Analysis SDT2960 unit using alumina crucibles. In a typical measurement, 2 mg of sample was placed in the crucible and exposed to a constant flow rate of N_2 (23 mL/min). A heating rate of 10 °C/min was set, and the temperature was increased in the range of 25–400 °C. The results were expressed as the first derivative of the signals of weight and heat flow against temperature.

2.7. Antimicrobial stability of RO during tomato juice pasteurization

Three treatments were tested on tomato juice: 1) control juice, 2) juice + free RO (1.5 mg/mL), and 3) juice + RO encapsulated in β-CD (25 mg of capsules from the 16:84 RO:β-CD ratio were applied per mL of juice; this concentration was equivalent to 1.3 mg of free RO/mL); the added oil concentrations were selected in base to the obtained MIC against *S. pastorianus*. Fresh tomato juice was obtained from fully ripe saladette tomatoes purchased in a local market in Hermosillo, Sonora, Mexico. The fruit was disinfected and cut, and the halves were processed by using a nutribullet® extractor. After, a triplicate of 100 mL of juice was treated with the previously described concentrations of free and encapsulated RO; continuous stirring was applied to assure complete dispersion of the treatments.

The juice samples were maintained at 25 or 65 °C for 30 min, upon which they were conditioned to 25 °C and inoculated with *S. pastorianus*. *S. pastorianus* was selected among the microorganism tested in Section 2.2, considering its resistance to the pH of the juice and for using tomato pectin as the main carbon source (ATCC 2345), and the desired cell concentration in the inocula (1×10^6 CFU/mL) was achieved as described in the same section. Subsequently, the inoculated samples were stored for 15 days, and the survival cells were counted at days 0, 5, 10, and 15 during storage at 5 °C. Each sample was analyzed by triplicate according to the serial dilution method. Thus, 1 mL of sample was taken in 9.9 mL of peptone water for dilutions (10^{-2}, 10^{-4}, 10^{-6}). Further, 1 mL of each dilution was plated in triplicate in Petri dishes with potato dextrose agar and incubated at 27 °C for 5 days; finally, each plate was counted and the results reported as Log CFU/mL.

2.8. Statistical analysis

The experiments were based on a completely randomized design with equal replications. Analysis of variance was done using the NCSS statistical software (NCSS, 2016). Mean comparisons of the studied parameters among treatments were done using the least significant difference (LSD) test at the 5% level ($P < 0.05$).

3. Results

3.1. Characterization of free RO: identification of RO volatiles and antimicrobial activity

Volatiles of RO were identified mainly as monoterpenes, being the most predominant 1,8 cineole or eucalyptol (52%), 3-carene (9.6%), camphor (9.3%), and α-pinene (6.1%). Minor concentrations of *cis*-sabinol, 6-caffenol, γ-cadinene, and copaene were also detected (Figs. 1 and 2). Ten mg/mL of free RO were found as MICs for *S.* Typhimurium and *L. monocytogenes* (Table 1). Yeasts were more sensitive to RO with MIC values of 4.5 and 1.5 mg/mL, for *C. tropicalis* and *S. pastorianus*, respectively. The MBCs for *S.* Typhimurium, *L. monocytogenes*, *C.*

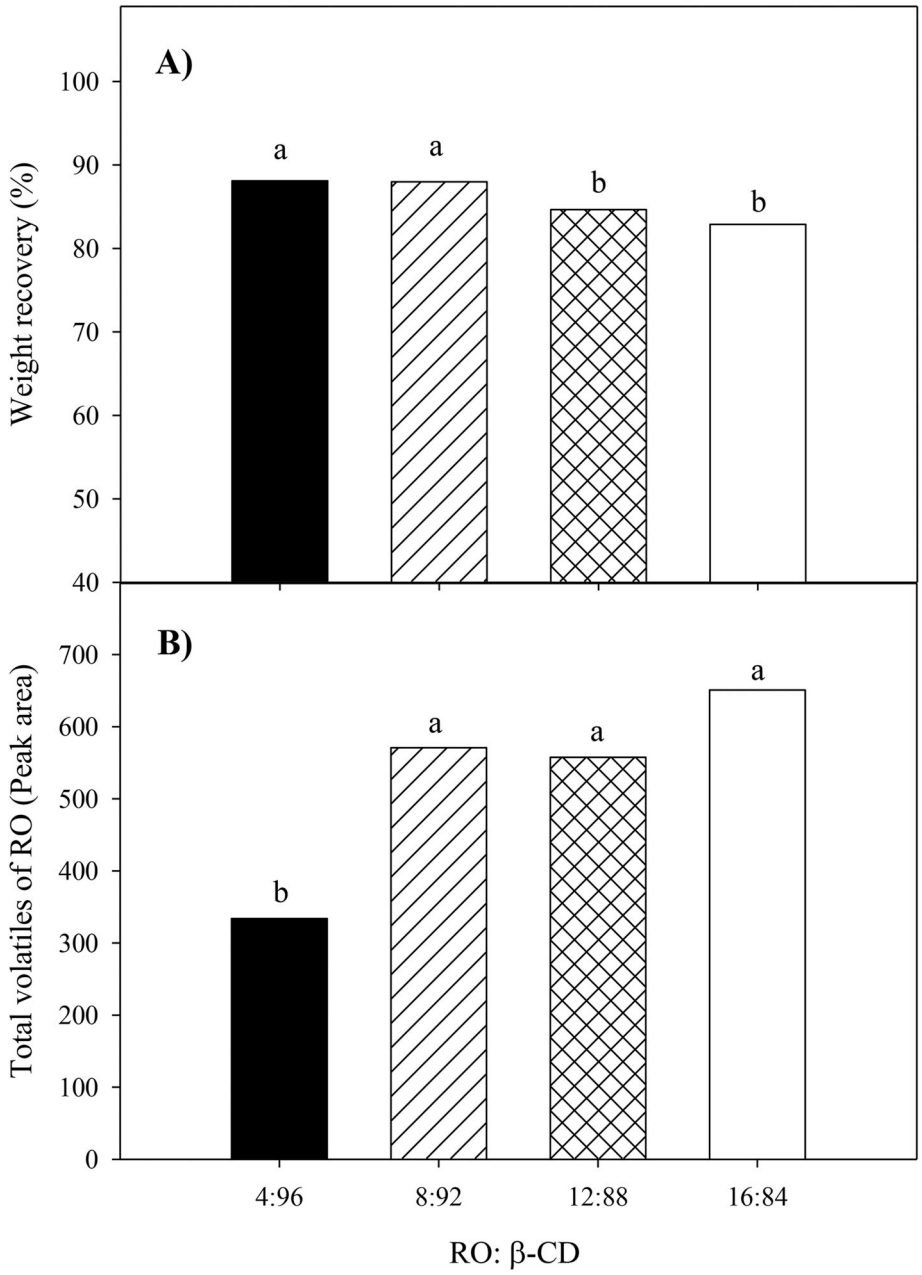

Fig. 2. A) Final weight recovered from the encapsulation process of RO in β-CD. B) Relative quantification of total volatiles of RO encapsulated within β-CD. Different literals among bars indicate significant differences among treatments ($p < 0.05$).

Table 1
Minimal inhibitory (MIC) and biocide concentration (MBC) of free RO against food pathogenic bacteria and yeast. Results expressed as mg of RO per mL of media.

Bacteria	MIC/MBC (mg/mL)	Yeast	MIC/MBC (mg/mL)
S. Typhimurium	10/12	*C. tropicalis*	4.5/6.5
L. monocytogenes	10/14	*S. pastorianus*	1.5/4.5

tropicalis, and *S. pastorianus* were 12, 14, 6.5, and 4.5 mg/mL, respectively. Free RO showed antibacterial and antifungal capacity, being the yeasts more sensitive than bacteria.

When *S.* Typhimurium was treated with 8 mg/mL of free RO, it showed an extension of 6.3 h of the adaptation period concerning the control bacteria (Table 1S). For *L. monocytogenes* exposed to 8 mg/mL of free RO, the extension of the adaptation phase was 8.6 h concerning the control. *C. tropicalis* and *S. pastorianus* exposed to 2.5 and 0.8 mg/mL showed an extension in the lag phase of 47.8 and 15.8 h concerning the controls.

3.2. RO encapsulation

The final weight of the capsules was lower than the initial mass of β-CD and RO used as raw materials. Overall, the more the initial amount of RO used in the experiments, the lower the recovery in the capsules (Fig. 2A). The recoveries in the ratios with less oil were 88.09% and 87.98% (4:96 and 8:92), compared with the 84.6 and 16.84% recorded for the 12:88 and 16:84 ratios. No significant differences were observed ($p \geq 0.05$) between the 4:96 and 8:92 ratios, nor between the 12:88 and 16:84 ratios. These results indicated the saturation of the CD system. Thus, an increase in the amount of oil added to the system did not increase the mass of the product obtained. This result was verified by

the relative volatile content found in the capsules. The ratio RO:βCD that presented the highest total volatile area was 16:84, and therefore, a higher number of terpenes (Fig. 2B), followed by 12:88, 8:92, and 4:96. These results showed that, although there was a higher recovery in the weight of capsules in the lower RO ratio, the content of trapped volatiles was higher in the highest RO ratio.

The maximum capture of RO by β-CD possibly occurred at 8:92, since the recovery of trapped volatiles did not increase (Fig. 2). Although the 16:84 ratio had the highest relative volatile value, an 7.77 ± 0.014% of RO was recovered from the capsules (w/w), and a 0.44 ± 0.008% of water content; no significant difference was found with the intermediate proportions. Considering these results, the antimicrobial experiments were done with the 16:84 (Table 2). As observed for free RO, bacteria required higher concentrations of RO capsules ($p \leq 0.05$) 200 and 150 mg/mL (*S.* Typhimurium and *L. monocytogenes*, respectively) to reach the MIC, compared with yeasts 28 and 20 mg/mL (*C. tropicalis* and *S. pastorianus*, respectively), without differences between both bacteria, nor between both yeasts. Considering the RO content of 7.77% in the capsules, the MIC/biocide concentrations needed were 14.66/19.4, 14.14/15.5, 2.05/3.5, 3.07/1.94, for *S.* Typhimurium, *L. monocytogenes, C. tropicalis,* and *S . pastorianus*, respectively. Comparing these concentrations with those of the free RO, it can be perceived that the encapsulation did not affect the antimicrobial activity of RO. It is also noted that both free and encapsulated RO showed a greater antifungal than antibacterial activity. *C. tropicalis* and *S. pastorianus* 50 and 68 mg/mL, respectively, without significant difference ($p \geq 0.05$) between both bacteria, neither between both yeasts.

Table 2
Minimal inhibitory (MIC) and biocide concentration (MBC) of 16:84 ratio of RO:β-CD against food pathogenic bacteria and yeast. Results expressed as mg of capsule per mL of media.

Microorganism	MIC (mg/mL)	MBC (mg/mL)
S. Typhimurium	200	250
L. monocytogenes	150	200
C. tropicalis	28	45
S. pastorianus	20	25

3.3. FT-IR

Fig. 3 shows the FTIR spectra of RO, βCD, and RO:β-CD capsules. RO spectra showed signals at 2925-2966 cm^{-1} corresponding to aliphatic groups CH, CH_2, CH_3, aromatics CH 3070 cm^{-1}, and hydroxyl groups at 3461 cm^{-1} of their constituents. On the other hand, bands for cyclodextrin were observed at 3394 cm^{-1} for the stretching of hydroxyl groups, at 2923 cm^{-1} for –C-H and 1643 cm^{-1}. Bands between 1421 and 1343 cm^{-1} represented –C-H at the inner and outer plane. Bands between 1160 and 1029 cm^{-1} correspond to the –C-O-C- tension among glucose rings; besides, the signal recorded at 1081 cm^{-1} corresponded to the –C-O-H- tension of hydroxyl groups. It is important to point out that the most common changes representing the formation of a molecular complex are the shifts of the O–H signals, indicating the occurrence of hydrogen bonds between the oil constituents and the CD hydroxyl groups.

Comparing the IR spectra of free oil and encapsulated, it can be observed that the band at 3461 cm^{-1} disappeared as did the signals at 3,070, and 2966 cm^{-1}, and the signals at 2723 and 2356 cm^{-1} were overshadowed by CD signals. However, the C═O signal of the oil constituents appeared in the capsule spectrum. Also, hydrogen bonding between RO terpenes and CD forming a capsule caused a bathochromic shift of 49 cm^{-1} from 3394 cm^{-1} to 3345 cm^{-1}.

3.4. Thermal stability of RO capsules

Thermogravimetric analysis of free RO showed a single weight loss step that initiated at 40 °C and ended at 140 °C (Fig. 4). The maximum rate of weight loss occurred in the range of 102 with a maximum mass loss at 110 °C. This behavior indicated that RO terpenes exposed to temperatures higher than 40 °C became unstable and evaporated. Differential scanning calorimetry experiments (Fig. 5) confirmed the evaporation of RO, showing an endothermic peak at 117 °C, with a Tmax between 85 °C and 145 °C and vaporization of the oil's terpenes. To verify the previous results on thermal stability in a food system, free and encapsulated RO were added to tomato juice before pasteurization and subsequently inoculated with *S. pastorianus*. A significant yeast reduction was observed during the storage time of tomato juice, with a more effective reduction on the juice added with encapsulated RO compared to the observed results for the juice treated with free RO and control (Fig. 6).

4. Discussion

The identified volatile compounds are similar to those previously reported in the literature (Coy Barrera & Eunice Acosta, 2013). Other authors (Mangena & Muyima, 1999; Romeu, Ferret, & Finalé, 2007) detected 1,8 cineole (21.5–31%), camphor (18–30%), α-pinene (15.3%), and camphene (5.7–6%). The differences in RO volatile concentrations may be attributed to variations in the culture, harvest, and postharvest conditions. The identified compounds have been reported as the active antimicrobial agents of RO; for instance, 1,8 cineole was found to be effective as fruit and vegetables sanitizer, showing values of MIC in the range of 5–20 mg/mL against *L. monocytogenes, Aeromonas hydrophila,* and *Pseudomonas fluorescens* (de Sousa et al., 2012). These values differed from those in previous works (Castaño, Ciro, Zapata, & Jiménez, 2010) that reported values of 0.51 and 28.48 mg/mL for *S.* Typhimurium and *L. monocytogenes*, respectively. Also, α-pinene also showed an inhibitory effect against *Staphylococcus aureus* (Maguna, Romero, Garro, & Okulik, 2006). Camphor and 1,8-cineole at 0.5% inhibited by 70% and 50% potato infection by *Rhizoctonia solani*, respectively (Vaillant Flores et al., 2009).

The inhibitory mechanism of microorganisms exposed to terpenes is not yet well understood. Some authors stated that bioactive compounds in the oil inactivate cellular enzymes when they penetrate the membrane (Zacchino et al., 2017). Furthermore, the inactivation of the cellular enzymes depends on the penetration rate (Ortega-Ramirez et al., 2017). Another possible explanation is that lipophilic components of RO increase the membrane permeability, which eventually leads to its rupture, the release of ions and genetic material contained in the cell and causing the death.

It is noted that the lag phase represents the adaptation period to a new environment before cellular division takes place, and when it is disturbed occurs the inactivation or inhibition of the maximum growth. This behavior was observed in bacteria treated with RO, which extended the lag phase compared with the control runs. This extension may be due to the impairment of cell membrane functionality either by inhibiting the absorption of nutrients or by affecting some essential enzymes or membrane proteins essential for cellular multiplication. Therefore, further studies are needed to explain RO antimicrobial properties. In this work, RO showed a more significant effect against Gram-positive than against Gram-negative bacteria. Explanation of this result is as follows: Gram-negative bacteria have a double cell membrane, which means a double barrier to capture RO compounds and avoid internal damage. In contrast, Gram-positive bacteria have a single membrane, which makes them more sensitive to antibacterial agents. This work also showed that yeasts were more sensitive to the attack of terpenes than bacteria; therefore, the elucidation of the mechanism by which RO constituents interact with yeast requires further.

The molecular size of RO volatiles is an essential factor in the encapsulation process. Kayaci, Ertas, and Uyar (2013) proved that the molecular size and shape of both guest and β-CD are decisive to form the capsules. These results agree with those found by Del Toro-Sánchez

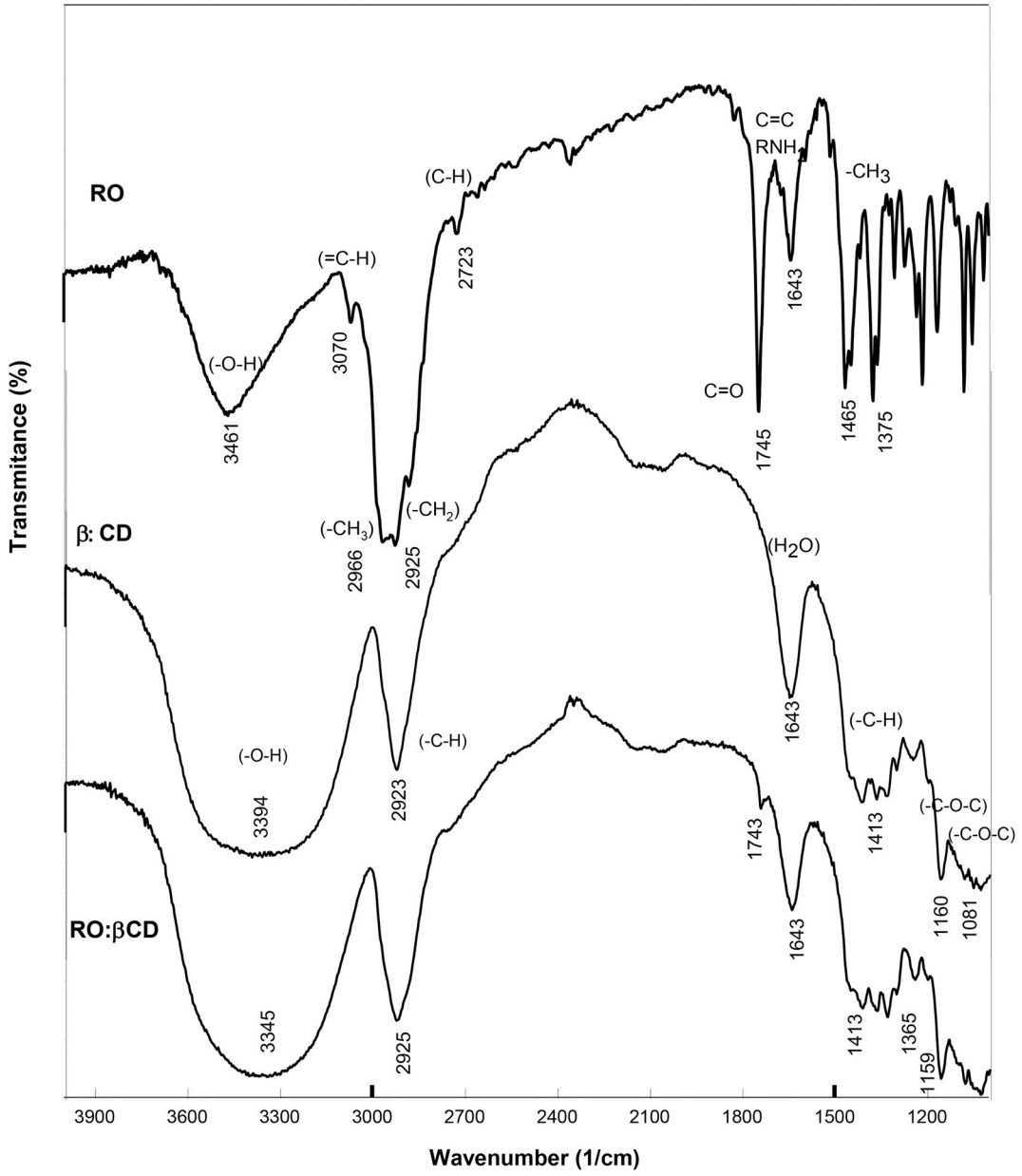

Fig. 3. IR spectra of free RO, β-CD, and RO:β-CD (16:84% w/w) complexes.

et al. (2010), who achieved the highest volatile content in the 16:84 ratio, but the maximum capture of thyme oil in β-CD occurred from the 8:92 ratio onwards. A similar behavior was observed in the encapsulation of cinnamon and garlic oil reported by Ayala-Zavala, Del-Toro-Sanchez, et al. (2008) and Ayala-Zavala, Soto-Valdez, et al. (2008) in which the 16:84 ratio of cinnamon oil in β-CD showed the highest amount of eugenol, and the highest amount of allyl disulfide in garlic oil was found in the proportions 12:88 and 16:84, without significant difference. They also reported a maximum inclusion of allyl disulfide of garlic and eugenol of cinnamon in the ratio 12:88, for both essential oils.

The formation of hydrogen bonding theory for the RO capsules lies on the Planck's law. Considering that changes on the O–H signal can be considered as a response of molecular guest-host interactions, this signal in the free β-CD has a given frequency (v) and when the oil is interacting with the β-CD, O–H- signal change its frequency. Planck's law relates the vibrational energy of molecular bonds throughout the following formula $E = hv$, where h is the Planck's constant ($6.62606896 \times 10^{-34}$ J s). Therefore, when a frequency in O–H signals shift from free β-CD to RO:β-CD (v_{OH} free β-CD = 3394 cm^{-1}, v_{OH} RO:β-CD = 3345 cm^{-1}), this indicated that such molecules were interacting throughout hydrogen bonds. Similar results were observed for garlic and thyme essential oil capsules with β-CD (Ayala-Zavala, Soto-Valdez et al., 2008; Del Toro-Sánchez et al., 2010). Reporting a shift on the frequency of hydroxyl groups from v_{OH} 21 cm^{-1} y 18 cm^{-1} for encapsulating garlic and thyme, respectively; compared with the present study a shift on hydroxyl frequency was of 49 cm^{-1} for RO, this stronger shift indicates a stronger bonding among RO and β-CD, compared to the observed values of bonding with garlic and thyme oils. This interaction is a key part of the capsule stability.

The loss of mass and vaporization of the RO agreed with the boiling temperatures reported for its major terpenes: 1,8 cineole (176 °C), 3-carene (170 °C), camphor (204 °C), and α-pinene (155 °C). The evaporation temperature of the RO reported here (130 °C) was higher than that of orange oil (118.1 °C), but lower than the oil of lemongrass (166.7 °C) and basil (164.2 °C) reported by Martins et al. (2011). The loss of terpenes in RO occurred in a range of temperatures commonly used in food processing; this highlights the importance of seeking

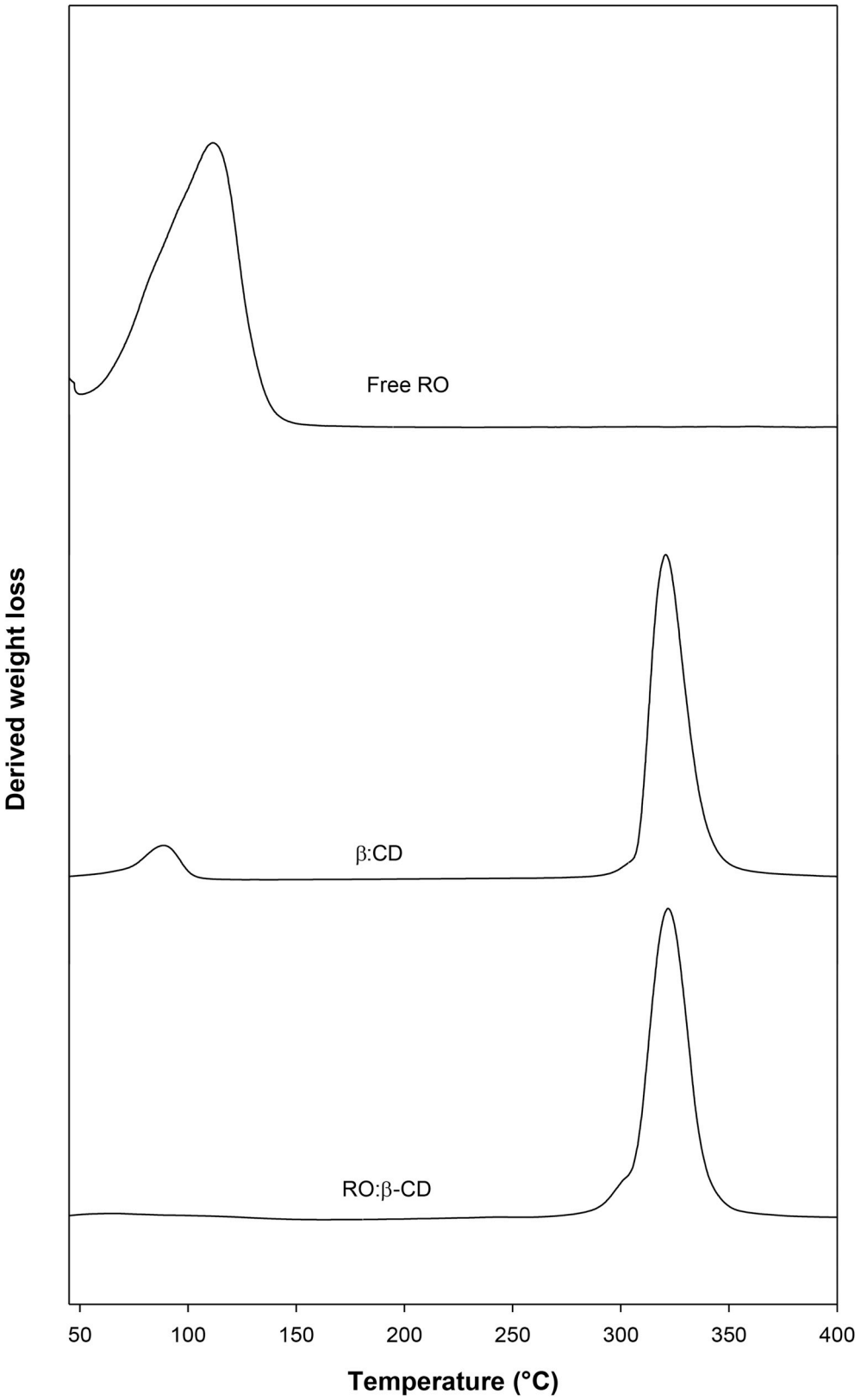

Fig. 4. Derivative weight loss of free RO, βCD, and RO:βCD (16:84).

alternatives for protection, such as encapsulation. The offered protection of β-CD to the trapped RO volatiles could be due to the observed polar interactions involving hydrogen bonding between the oligosaccharide and the oil constituents.

As described before, FTIR spectra indicated the occurrence of these interactions between the studied compounds; thus the bonding restrained the release of RO volatiles. These results proved the positive results of considering β-CD encapsulation as an alternative to stabilize RO and optimize its efficacy as antimicrobial in thermally treated food. Traditionally, the shelf-life of juices has been achieved through thermal

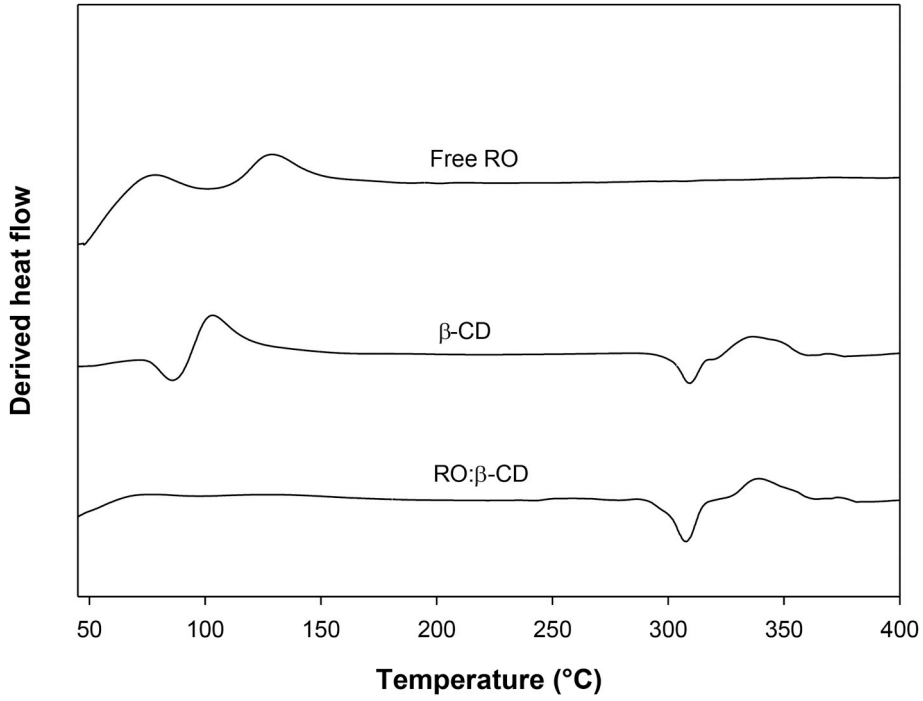

Fig. 5. Derivative heat flow of free RO, β-CD, and RO:β-CD (16:84).

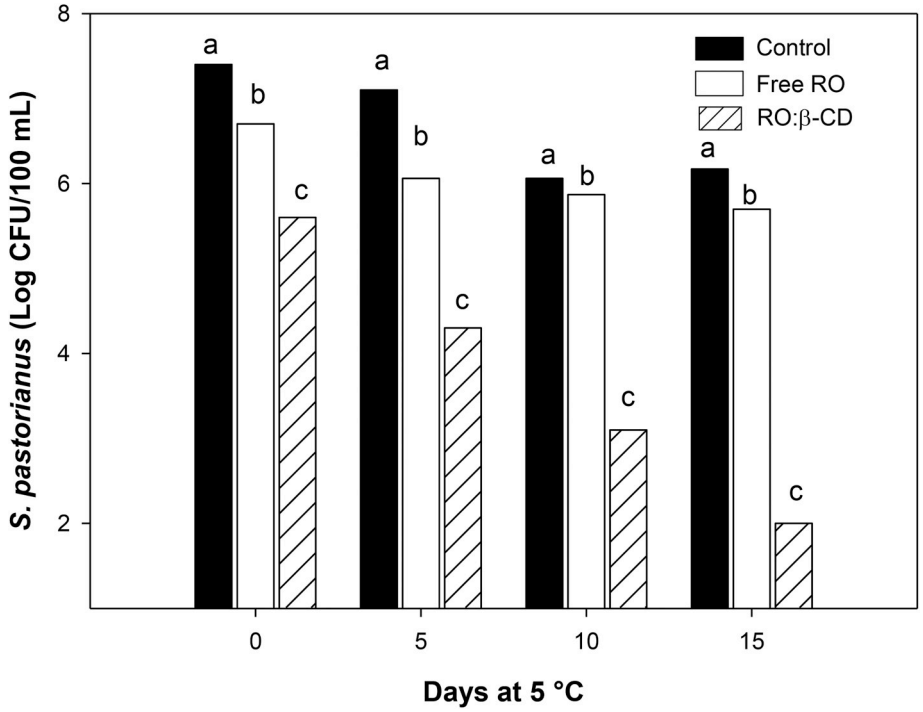

Fig. 6. Antimicrobial activity of free and encapsulated RO against *S. pastorianus* inoculated after pasteurization (63 °C, 30 min) of tomato juice stored at 5 °C. Different literals among bars indicate significant differences among RO treatments during storage time ($p < 0.05$).

processing, and the recommended temperatures for pasteurization by high-temperature short-time are 72 °C–82 °C. In addition to thermal treatment, chemical preservatives such as potassium sorbate and sodium benzoate are widely used to extend the shelf-life of juices.

The use of hurdle technologies to assure food safety is recognized as the best option; however, in addition to the odor compatibility among the used essential oil and the treated food, the interaction of the volatile antimicrobial agents with thermal processes is considered to accomplish this goal. Many studies have considered this issue; however, most of them obviated testing this efficacy on real food systems. Eugenol encapsulated in β-CD needed 100 °C temperature to volatilize being a temperature twice higher to that required by the free eugenol, preserving its *in vitro* antibacterial action. Oregano essential oil was encapsulated in modified starch and Arabic gum, improving their thermal stability as indicated by DSC and TGA analysis (Hijo et al., 2015); however, this previous study did not prove this stability in real food systems as was done in the present study. A previous study, evaluated the addition of free RO into tomato juice, vegetable soup, and poultry

burgers, founding that the treated tomato juice and vegetable soup showed lower acceptability than poultry burgers treated with higher concentrations of the free oil (Espina, Garcia-Gonzalo, & Pagan, 2014). The stated limits of tolerance for free RO were lower than 20 μL/L for tomato juice, 20 μL/L for tomato soup, and 200 μL/L (Espina et al., 2014). It has to be mentioned that this study did not measure the antimicrobial efficacy of the used concentration, and only the poultry burgers were cooked at 60 °C after the addition of the oil, then it is evident that the cooking process affected its activity. Therefore, as observed in the present study, the encapsulation of RO by β-CD was an excellent method for protecting the antibacterial action from thermal degradation. The obtained results support the application of RO:β-CD complexes as antimicrobial additives in food systems that require thermal processing and storage in conditions with high temperatures.

5. Conclusions

The β-CD, as encapsulating material, controlled the volatile nature of RO terpenes, avoided their evaporation at high temperatures, maintaining its antimicrobial capacity. These benefits could be related to the observed physicochemical interactions among the oil constituents and the β-CD. In this context, the RO:β-CD capsules could be an option to reduce microbial growth of food exposed to thermal processing.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2019.05.061.

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