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# Effect of free and microencapsulated thyme essential oil on quality attributes of minimally processed lettuce

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## ABSTRACT

In the present study, thyme essential oil (TO) was used as a natural antioxidant and antimicrobial agent to enhance the quality and shelf-life of minimally processed lettuce during refrigerated storage. For this purpose, the effect of dipping treatments of free TO (0.5, 1.0, and 1.5 g $L^{-1}$) and microencapsulated TO in β-cyclodextrin (TO:β-CD, 15.8, 31.6, and 47.5 g $L^{-1}$) on quality aspects of minimally processed Romaine lettuce was compared. Quality parameters such as weight loss, color, total phenolics and flavonoids content, antioxidant activity (DPPH), microbial populations, and organoleptic quality were evaluated during 12 d of refrigerated storage. Weight loss of lettuce samples was not affected by treatments with TO. Immediately after application of free TO treatments, the total phenolic and flavonoids content and the antioxidant activity of lettuce increased significantly. However, these improvements were not maintained during storage. Conversely, TO:β-CD treatments did not affect the phytochemical content and antioxidant activity of the samples immediately after application but a significant increase was observed during storage. Moreover, these quality parameters increased as the concentration of the microcapsules in the dipping solution increased. Treatments with free TO were not effective in reducing the microbial load of lettuce compare to control samples during the entire assayed period. Instead, treatments with TO:β-CD (31.6, and 47.5 g $L^{-1}$) exerted a bacteriostatic effect over mesophilic and psychrotrophic bacteria and reduced Enterobacteriaceae and yeast and molds counts throughout the storage period. Additionally, samples treated with microencapsulated TO presented better organoleptic quality scores than control and free TO treated lettuce. From this study, it can be concluded that TO:β-CD microcapsules can be used to control decay and increase the antioxidant health benefits of minimally processed lettuce due to an enhancement of the antimicrobial and antioxidant properties of the essential oil.

## 1. Introduction

Consumption of minimally processed vegetables, including ready-to-eat salads, has increased worldwide in the last decade due to their convenience, fresh-like quality and high nutritional properties. Lettuce is one of the most important ready-to-use products and ranks highly both in production and economic value among vegetables (Allende et al., 2007). However, minimal processing operations, such as selection, washing or cutting, cause an increase in respiration rates and biochemical changes that lead to rapid deterioration of lettuce components, enzymatic browning, and microbial spoilage (Moreira et al., 2008). Therefore, minimally processed lettuce has a shorter shelf life than the whole head. The application of good agricultural and manufacturing practices (GAP and GMP, respectively) and the maintenance of the cold chain are the most important procedures for delaying produce deterioration but further technologies are required to extend the shelf life of minimally processed lettuce.

In this context, a large variety of plant essential oils are used in processed fruit and vegetables in order to reduce or eliminate pathogenic bacteria and to improve overall quality. In particular, thyme essential oil (TO) is obtained from the aromatic plant of thyme (*Thymus vulgaris*) and is recognized for having very interesting antioxidant and antimicrobial properties, although its intense aroma could limit the organoleptic acceptability of the final product (Hudaib and Aburjai, 2007; Cindi et al., 2016). Recent studies have also revealed that TO has the ability to act as a 'signalling compound' that triggers a signal to induce a defense function, increasing the activity of defense-related enzymes and enhancing antioxidant capacity in vegetables (Ben-Jabeur

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et al., 2015; Khumalo et al., 2017). Although the antimicrobial and antioxidant activities of TO have been examined extensively in many *in vitro* studies (Marino et al., 1999; Sacchetti et al., 2005; Viuda-Martos et al., 2007; Tohidi et al., 2017), several authors have observed a reduced efficacy when applied to real food systems due to the volatility and hydrophobicity of TO constituents (Bagamboula et al., 2004; Gutierrez et al., 2009). Moreover, its use as food preservative is often limited due to flavoring considerations since effective antimicrobial doses may exceed organoleptically acceptable levels. One alternative that could solve to a high extent these problems and facilitate its application as food additive is encapsulation (Del Toro-Sánchez et al., 2010). Encapsulation technology has been remarkably developed in the last years for the stabilization, solubilization, and delivery of active components in food industries (Sanguansri and Augustin, 2006).

In particular, microencapsulation in cyclodextrins (CDs) is one of the most effective methods for protecting active compounds against oxidation, heat degradation, as well as masking undesired smell or taste, and increasing solubility (Szente and Szejtli, 2004). Although there are other forms of cyclodextrins (mainly α and γ), β-CD is the most commonly employed due to its size, availability, and low price. β-CD arises from starch degradation *via* enzymes, and is a cyclic oligosaccharide with 7 glucose residues linked by α (1–4) glycosidic bonds. β-CD molecules have a truncated cone shape, with a hydrophobic zone inside and a hydrophilic external surface. The result of this amphipathic property is that β-CD can form soluble and reversible molecular inclusion complexes with poorly water-soluble compounds, resulting in compound solubilization. Moreover, they have the capacity to stabilize the guest molecule against degrading agents (such as oxygen, light, or heat), control volatility properties, mask potentially adverse odors and flavors, and control the release of such encapsulated compounds. In addition, β-CD enjoys ‘generally regarded as safe’ GRAS status (US FDA) for use as additive in food products (Cabral Marques, 2010). Microencapsulation of TO in β-CD has been investigated by several authors, who have characterized the physico-chemical properties of the inclusion complexes (Del Toro-Sánchez et al., 2010; Ponce Cevallos et al., 2010; Tao et al., 2014). Although these microcapsules showed a great potential for practical application in the food industry, further studies with real food systems are required.

Application of TO to minimally processed lettuce could protect the product against spoilage microorganism’s proliferation, improve its antioxidant content, and prevent losses of natural antioxidants. The main goal of this study was to evaluate the effect of TO as a natural antioxidant and antimicrobial agent in order to enhance the quality and shelf-life of minimally processed lettuce during refrigerated storage. For this purpose, free TO and inclusion complexes between TO and β-CD were applied and evaluated as postharvest treatments for commercial applications.

## 2. Materials and methods

### 2.1. Materials

Thyme essential oil (TO) was obtained by steam distillation from leaves of *Thymus vulgaris* plant of Spanish origin (Las Boticarias, Buenos Aires, Argentina). Volatile content of TO was quantified previously (Ansorena et al., 2016) and the main component was thymol (65.8%), followed by ρ-cymene (19.1%) and carvacrol (6.1%). β-CD (Cavamax W7 food grade) was provided by Wacker Biochem, USA.

### 2.2. Thyme oil microencapsulation

TO:β-CD inclusion complex was prepared using the co-precipitation method reported by Ayala-Zavala et al. (2008b). Briefly, 80 g of β-CD were dissolved in 800 mL of an ethanol:water (1:2) mixture by magnetic stirring at 55 °C. Thyme oil (6.96 ± 0.01 g) dissolved in ethanol (10% w/v) was slowly added to the warm β-CD solution to reach a TO:β-CD ratio of 8:92 (% w/w). As reported previously, these microcapsules provide the maximum inclusion of TO volatile compounds in β-CD (Del Toro-Sánchez et al., 2010). The resultant mixture was covered and stirred for 4 h at ambient temperature and then stored overnight at 4 ºC. The precipitated TO:β-CD inclusion complexes were recovered by filtration and then dried in a convection oven at 50 °C for 24 h. Finally, they were stored in a desiccator at 25 °C. As published previously, TO:β-CD microcapsules presented a 2.08% w/w of thymol content (Del Toro-Sánchez et al., 2010).

### 2.3. Plant material and treatments application

Heads of Romaine lettuce (*Lactuca sativa*, type Cos, variety Logifolia) were grown and harvested at optimal maturity in Sierra de los Padres (Mar del Plata, Argentina) and immediately transported to the laboratory. The outer lettuce leaves and the core were removed and discarded. The remaining leaves were washed by hand in running tap water to eliminate undesired soil residues. Then leaves were cut transversely in 2-cm portions and washed with chlorinated water ($150\,\mu L\,L^{-1}$) for 3 min at ambient temperature using a weight to volume ratio of 1/10 w/v, followed by rinsing in tap water for 3 min. Lettuce portions were centrifuged in a salad spinner to remove water excess. The samples were divided into three lots: one for control samples (untreated) and the other two for treatments application: lettuce portions were dipped separately in aqueous solutions (1/10 w/v ratio) of free TO or microencapsulated TO in β-CD during 3 min following the protocol of Scollard et al. (2016) with modifications. Three different concentrations of free and microencapsulated TO were tested, which corresponds to a thymol content of 0.33, 0.66, and 0.99 g of thymol per L of solution. Solutions of free TO (0.5, 1.0, and 1.5 $g\,L^{-1}$, referred in the text as TO-1, TO-2 and TO-3, respectively) were prepared by dispersing a predetermined amount of TO in 1 L of sterile water and vigorously mixed by shaking during 30 min at 30 °C to obtain stable dispersions. Solutions of microencapsulated TO in β-CD (15.8, 31.6, and 47.5 g of microcapsules per L of solution, referred in the text as TO:β-CD-1, TO:β-CD-2, and TO:β-CD-3) were analogously prepared, by adding the corresponding amount of microcapsules in 1 L of water to reach the same thymol content than that of free TO treatments as thymol is the main active compound of the essential oil. Treatments were performed by duplicate.

After the application of each treatment, lettuce portions were drained and 30 g of plant material were aseptically transferred to standard polypropylene food grade containers (15 × 9 x 5 cm, Cotnyl SA, Buenos Aires, Argentina) of 25 μm of thickness (with $O_2$ and $CO_2$ permeabilities of 38 and 230 $cm^3\ mm\ m^{-2}\ d^{-1}\ atm^{-1}$, respectively, measured at 23 °C and 0% RH, and a water vapor permeability of 6 g $m^{-2}\ d^{-1}$; determined at 38 °C and 90% HR). Trays were covered with a lid of the same material, sealed with parafilm without initial atmosphere modification, and stored at 5 °C for 12 d. At days 0, 3, 6, 9 and 12 of storage, samples were taken for phytochemical, microbiological and sensorial determinations.

The whole experiment was repeated independently three times.

### 2.4. Weight loss

Trays were weighed after the application of treatments, and at each sampling day during the storage period. Results were expressed as percentage of weight loss relative to the initial weight.

### 2.5. Superficial color

Superficial color of lettuce leaves was determined by measuring L*, a*, and b* chromaticity coordinates of the CIE-Lab scale with a colorimeter (LoviBond, RT500, Neu-Isenburg, Germany). L* indicates lightness, a* indicates chromaticity on a green (−) to red (+) axis, and b* indicates chromaticity on a blue (−) to yellow (+) axis (CIE, 1978).