# Clove Essential Oil – Free and Encapsulated for Antimicrobial Leather

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**Abstract**

Essential oils have antimicrobial properties, with good potential to be used as natural biocides. Microencapsulation is a technological possibility to protect functional natural microbicides and to prevent chemical changes. The performance of the clove essential oil (CEO), free and encapsulated, against bacteria was evaluated. The emulsion extrusion technique was used for CEO encapsulation with alginate and the sol-gel technique was used for the encapsulation with silica. Samples were characterised for antimicrobial activity, size and functional groups present. Strong antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* was observed. FTIR showed the main peaks of the CEO and confirmed its incorporation into the alginate microcapsules obtained and of the silica nanocapsules. The chemical stability of the clove oil after encapsulation gives rise for its bactericidal use in leather manufacturing with advantages of maintaining its properties for more durability and controlled release in leather.

## 1 INTRODUCTION

Essential oils have received interest because of their antimicrobial, antifungal and antioxidant properties and for being of natural origin. Clove essential oil (CEO) contains high levels of eugenol, that has strong biological and antimicrobial activities against a wide range of pathogenic micro-organisms.[1,2] CEO can replace some biocides, conventionally used in the leather industry, which are synthetic and generate environmental risks. The natural biocides are used to prevent the development and growth of bacteria and fungi in pickled hides, tanned leathers (from chromium or vegetable tannin) and finished products during their storage and shipping.[3]

Micro- and nanotechnology in leather manufacturing can lead to sustainable leather and cost-effective improvements to the quality of finished leather with the use of nanoparticles and nanocapsules.

Nanotechnology holds a promise to be one of the major drivers of technology[4] but in the leather industry it has not been explored for commercial nanoparticle/nanocapsule use in the market.[5] Thus many researches are developing advanced solutions to satisfy the growing demand in leather processing.[6] This technology not only improves leather quality but also gives to the user of leather new functionalities. Health protection of the user using a functional antimicrobial leather,[7] diabetic shoes to reduce plantar pressure[8] and scented leather with antimicrobial properties[9] are some examples.

Encapsulation is an effective method which may be used to protect functional natural biocides from reactions with moisture, light, and oxygen.[10] Using an encapsulated dye reduces the wastewater treatment efforts[6] and encapsulated essential oils can be used as natural biocides.[10] These capsules comprise, an active agent surrounded by a natural or synthetic polymeric membrane providing isolation, entrapment, protection or controlled release. This controlled release allows prolonging its useful life, avoiding rapid evaporation and improving its performance.[11] Different techniques are used for encapsulation. Emulsion extrusion is considered as the most common approach of microencapsulation and might be achieved by emulsifying the hydrophobic components in an aqueous solution where gelation occurs (ionotropic or thermal).[12] By using emulsion extrusion for microencapsulation, a broad selection of polymer coatings (shell) and methods of deposition are available, these are easily adaptable to large-scale production.[13] Using sodium alginate for encapsulation produces a new material from renewable sources, replacing synthetic polymers.[14,15]

In the sol-gel process, a network is formed from solution *via* a progressive change of the liquid precursor into a sol, then to a gel and finally to a dry network.[16] Sol-gel encapsulation using tetraethyl orthosilicate (TEOS) as precursor of silica leads to the stabilisation and entrapment of actively released compounds. Besides, $SiO_2$ is non-toxic and safe, thus is environmentally friendly.[17,18] The progress of efficient green synthesis utilising natural agents without the use of toxic, expensive chemicals and high energy consumption have attracted researchers.[19]

Table I presents some work in the field of essential oils and encapsulated products applied in the leather industry.

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**TABLE I**
**Application of free essential oils and encapsulated products in leather reported in the literature**

| Agent | Substrate | Step | Micro-organism | Goal |
|---|---|---|---|---|
| | | Free oil | | |
| Myrtle[20] | Raw sheepskin | Soaking | Mezophyll bacteria | Bactericide |
| Sığla[21] | Raw sheepskin | Soaking | *B. brevis, B. cereus*, *B. subtilis, S. aureus, P. aeruginosa P. fluorescens* | Bactericide |
| Oregano[22] | Raw sheepskin | Pickling and tanning | *A. niger, A. alternate*, *P. rubrum and T. viride* | Fungicide |
| Cinnamon, garlic, clove and star anise[23] | Salted goatskin processed to wet-blue | Post-tanned | *A. niger, P. citrinum*, *A. alternate and R. stolonifer* | Fungicide |
| Cedar and coriander[24] | Chrome-tanned bovine leather | Final dressing composition | *A. niger* | Leathers with antifungal performances |
| Eucalyptus and lavender[25] | Salted bovine hide processed to chromed or vegetable leather | Fatliquoring | *E. coli, P. aeruginosa*, *Cocci* and *B. cereus* | Alternative preservatives |
| Thymes[26] | Salted bovine hide processed to chromed or vegetable leather | Fatliquoring | *S. aureus, B. cereus*, *E. coli* and *P. aeruginosa* | Alternative preservative |
| | | Encapsulated oil | | |
| Clove[7] | Wet-blue goat | Post-tanning | *E. coli, S. aureus*, Viscous red round yeast, *A. niger and P.citrinum* | Antimicrobial leather |
| Orange and lavender[9] | Wet-blue sheep | Post-tanning | *B. cereus, B. subtilis*, *A. fumigatus* and *M. phaseolina* | Scented leather |
| Olive and tea tree[27] | Leather | Finishing | *E. coli and A. niger* | Antibacterial leather for public vehicles |
| Tea tree[10] | Leather | Finishing | *E. coli, B. subtilis*, *K. pneumoniae* and *S. aureus* | Footwear |
| Castor[28] | Wet-blue goat | Lubrication | — | Reduction of pollution |
| | | Encapsulated products | | |
| Acid Black 210 dye[29] | Wet-blue | Dyeing | — | Better penetration |
| Safranin O dye[6] | Wet-blue goat | Dyeing | — | Reduction of wastewater |
| Tannins *Acacia mearnsii*[30] | Cattle hide and wet-blue | Tanning or retanning | *S. aureus, E. coli A. niger and C. sp.* | Improvement performance |
| Chlorhexidine digluconate[8] | Leather | Final stage | *S. aureus, E. coli, P. aeruginosa, C. albicans*, *K. pneumoniae, E. faecalis and S. epidermidis* | Diabetic shoes |
| N-octadecane[31] | Wet-blue goat | Retanning | — | Thermo responsive function |
| DNS-86, hexadecane, dodecyl mercaptan[32] | Leather | Finishing | — | Temperature control |
| DNA[33] | Skin | Tanning | — | Leather marking |

This present study evaluates the antibacterial activity of clove essential oil free and encapsulated. Emulsion extrusion was used for the encapsulation with alginate and the sol-gel technique was used for the encapsulation with silica.

## 2 MATERIALS AND METHODS

### 2.1 Materials

Sodium alginate (Dinâmica, Brazil), tetraethyl orthosilicate ($Si(OCH_2CH_3)_4$, Merck, >98%), clove essential oil (Delaware, Brazil), 2-thiocyano-methylthiobenzothiazole (TCMTB, Buckman, >33%), calcium chloride (Dinâmica, Brazil), were purchased and used as received.

### 2.2 Encapsulation process

Encapsulation of CEO was performed following the techniques of emulsion extrusion[13] for alginate and sol-gel[34] for silica. The composition of the capsules is presented in Table II.

For the microcapsule with a sodium alginate wall, sodium alginate was dissolved in distilled water to produce an alginate suspension. Afterwards, sodium alginate suspension and clove oil (1 or 3w/v%) were mixed and homogenised in a 200mL beaker with stirring at 300rpm for 15 minutes by a magnetic stirrer. The alginate-oil emulsion was then dropped into a collecting water solution containing calcium chloride solution (1w/v%) using a syringe. The resulting microcapsules were allowed to harden in the $CaCl_2$ solution for 5 minutes. The oil-loaded alginate capsules were then rinsed with distilled water and filtered. This procedure took 30 minutes.

Tetraethyl orthosilicate (TEOS) was used as the silica precursor in base-catalysed route. A 1:12:0.04 molar ratio of TEOS: water: catalyst (0.2M NaOH solution) was employed. CEO (2.5% v/v) was dissolved in NaOH solution until complete dissolution followed by the addition of TEOS. The reaction was maintained at room temperature under constant agitation, requiring a gelation time of 24 hours. The resulting material (SG2.5) was dried at room temperature and milled in a porcelain mortar.

**TABLE II**
**Composition of the capsules of CEO**

| Technique | Assay | Clove oil* | Alginate* | Silica* |
|---|---|---|---|---|
| Extrusion emulsion | E330 | 3 | 3 | – |
| | E130 | 1 | 3 | – |
| Sol-gel | SG2.5 | 2.5 | 0 | 10 |

*$g.100g^{-1}$ solution

### 2.3 Antimicrobial activity

In order to assess the antimicrobial capacity of inhibition by the CEO, a natural biocide, it was compared to a commercial biocide, TCMTB. The samples were tested against two micro-organisms: the Gram-positive strain *Staphylococcus aureus* ATCC 25923 and the Gram-negative strain *Escherichia coli* ATCC 25922. The inhibitory effect of the samples on the tested bacteria was performed using the agar disc-diffusion method NCCLS35 with the modifications. *Ca.* $10^6 CFUmL^{-1}$ suspensions of the tested micro-organisms were used to inoculate the agar plates. Sterile paper discs (9mm in diameter) were impregnated with 25µL of the samples and placed on the centre of the inoculated plates. Encapsulated oil testing was performed using the agar well-diffusion method. A hole with a diameter of 6 to 8mm was punched aseptically with a sterile cork borer or a tip, and a volume of the encapsulated oil was introduced into the well. The plates were incubated at 36°C for 24 hours. The diameter of the inhibition zones was measured in millimeters. The tests were performed in triplicate, and the results are presented as the mean ± standard deviation. The degree of inhibition references were scaled: strong, moderate, weak and no inhibition detected.[36]

### 2.4 Size

Capsules of alginate were observed using a stereo microscope (Model SZX16, Olympus) attached to a digital camera. Capsules of silica were observed under dynamic light scattering (DLS) performed on a Zetasizer Nano ZS (He-Ne laser 633nm) with a scattering angle of 173°C, this sample was diluted with deionised water.

### 2.5 FTIR

Fourier Transform Infrared spectroscopy of the materials and microcapsules were recorded in the range 650-$4000cm^{-1}$ with 32 scans and $4cm^{-1}$ of resolution on a Frontier ATR-FTIR spectrophotometer (Perkin Elmer, USA).

## 3 RESULTS AND DISCUSSION

Antimicrobial activity of free CEO, encapsulated CEO and TCMTB, against the micro-organisms is shown in Table III. CEO and TCMTB showed strong antimicrobial activity for the tested organisms, *Staphylococcus aureus* and *Escherichia coli*. Moreover, the effect of CEO was better against *S. aureus* than TCMTB. Therefore, CEO is an alternative natural biocide that can be used against these bacteria. Encapsulated clove oil E330 showed strong activity for both micro-organisms. But, when the concentration of clove oil was lower in the E130 capsules, it only showed strong activity for *S. aureus*. When encapsulated with silica SG2.5 had strong inhibition of *S. aureus* and weak inhibition of *E. coli*.

The capsules of alginate measured 2mm diameter and are considered microcapsules (Table III, Fig. 1a). However, the distance between the leather fibres is 3.0µm.[7] Even though these microcapsules cannot be used to permeate inside the leather fibre structure, they can be used directly in leather shoes/goods (by consumers) to avoid microbial attack and damage.

Using silica it was possible to develop nanocapsules with 73.2% of the nanocapsules of size 39.16nm and 26.8% of 130.3nm size. The bimodal distribution shows a variation in particle size, that can penetrate various levels of pore size presented in the collagen matrix of leather.[37] The agglomerates of silica nanocapsules are shown in the figure (Table III, Fig. 1b).

**TABLE III**
**Inhibition zone of CEO free and encapsulated and TCMTB and size of the capsules**

| Inhibition zone (mm)* | | | |
|---|---|---|---|
| Sample | *S. aureus* | *E. coli* | Diameter |
| CEO free | 36.33 ± 2.22 | 19.00 ± 0.67 | - |
| TCMTB | 30.00 ± 0.00 | 31.33 ± 0.44 | - |
| E330 | 18.33 ± 0.44 | 17.33 ± 0.89 | 2mm |
| E130 | 23.00 ± 0.67 | — | 130.3 ± 45.23nm |
| SG2.5 | 18.67 ± 0.89 | 12.67 ± 0.04 | 39.16 ± 9.27nm |

*Inhibition including 9mm disc diameter expressed as the mean of three replicates ±SD. Micro-organism resistant test, represented with (—) sign. Reference inhibition degrees: >18mm (strong) 14–18mm (moderate) and 10-13mm (weak).

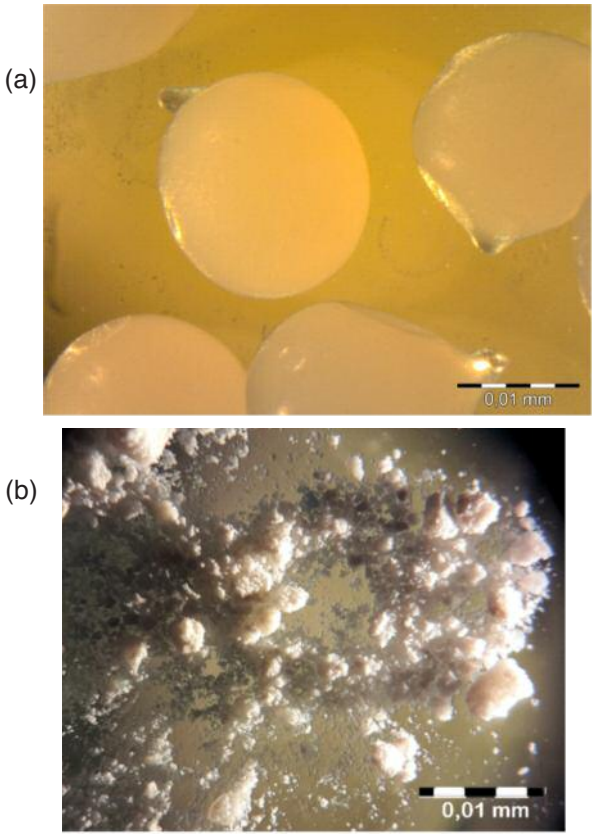


**Figure 1**. Microscope images (x40 magnification) of capsules: (**a**) of alginate E130 and (**b**) of silica SG2.5.

FTIR spectra (Fig. 2) of CEO showed peaks at 3518cm$^{-1}$ of stretching vibrations O-H, at 2937, 1612, 1366cm$^{-1}$ attributed to C-H, C=C, O-H, respectively and some special peaks at 1265cm$^{-1}$ (C-OH axial)[38] and at 1033cm$^{-1}$ (C-O-C axial symmetric).[7]

Microcapsules of alginate – E130 and E330 (Fig. 3a) presented peaks at 3252cm$^{-1}$ (O-H vibration) due to the presence of the great amount of water. The peak at 1415cm$^{-1}$ is attributed to the asymmetrical stretching of COO-. The bands at 1081 and 1029cm$^{-1}$ correspond to guluronic units of C-H stretching present in sodium alginate. The peak at 947cm$^{-1}$ corresponds to the C-H group of the ring pyranose. The CEO is presented at 1594cm$^{-1}$ (C=C) and 1265cm$^{-1}$ (C-OH axial).

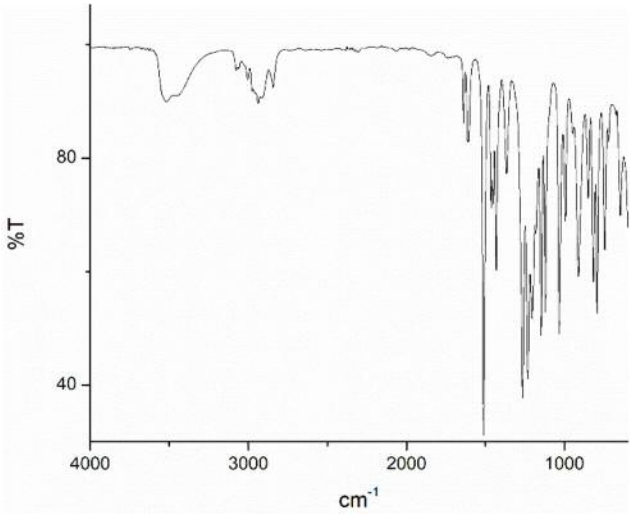


**Figure 2**. FTIR spectra of CEO.

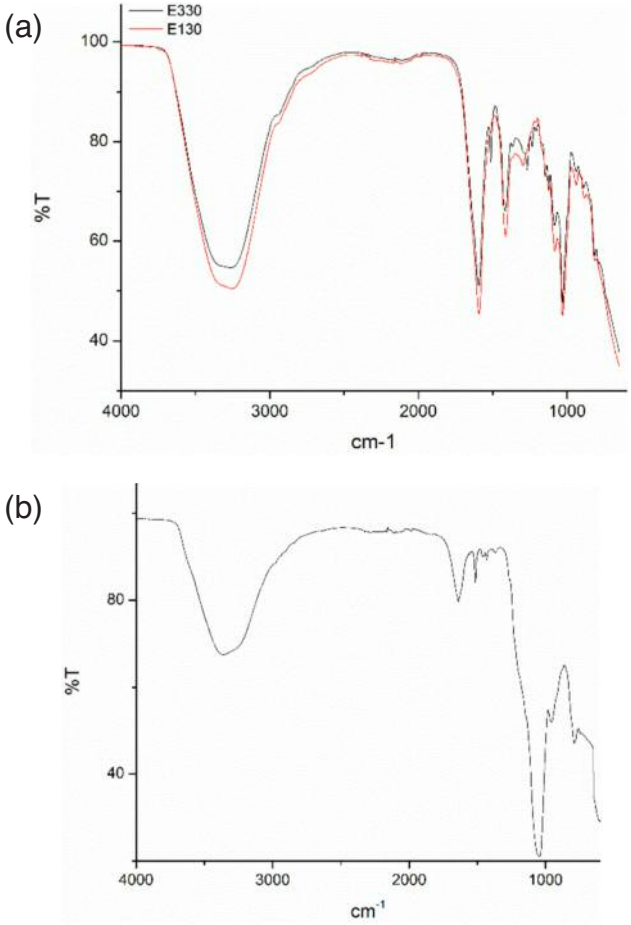


**Figure 3**. FTIR spectra of CEO encapsulated (**a**) with alginate E130 and E330, (**b**) with silica SG2.5.

FTIR of silica nanocapsules SG2.5 (Fig. 3b) shows the silica present at 1043 (Si-O-Si (chain) stretching), 956 (Si-O angular deformation) and 790cm$^{-1}$ (OH, geminal stretching and Si-O, symmetric stretching).

The bands present in the free CEO (1638.12; 1511.48 and 1431.2 referent to C-C stretching vibrations in the phenyl ring)[39] were at 1637, 1514.15 and 1431.74cm$^{-1}$ when encapsulated. CEO exhibited infrared band shifts toward higher wavenumbers after encapsulation, which in turn may provide insight into potential sites for interaction with the silica framework. These band shifts, suffered by the organic chemical groups, can result from a strong rearrangement of such chemical functions.[40]

## 4 CONCLUSION

From this study, it was concluded that clove essential oil can be used as a natural antibacterial agent in the leather industry. The encapsulation can maintain the chemical stability of the oil and its antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* can acquire a time controlled release property. The alginate CEO microcapsules can be used over the leather surface in finishing or can be sprayed on leather goods. The developed silica CEO nanocapsules are smaller than the pore sizes of the leather and can be applied during wet leather processing in drums.

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