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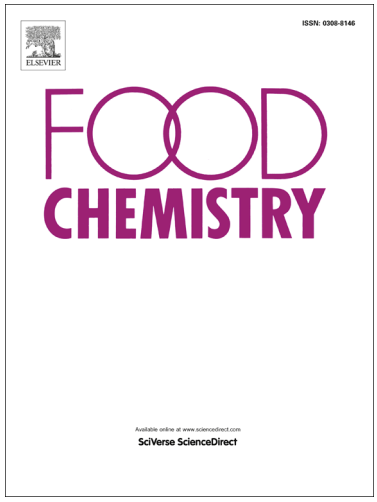

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Antimicrobial and antioxidant activity of unencapsulated and encapsulated clove (*Syzygium aromaticum, L.*) essential oil

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Abstract

Clove (*Syzygium aromaticum*, L.) essential oil is known for its antimicrobial activity against several pathogenic bacteria. Encapsulation of clove oil was proposed as a mean to disguise its strong odor that limits its uses in food industry. Thus, the aim of this study was extraction, encapsulation and assessment of the antimicrobial and antioxidant potential of clove essential oil. The essential oil showed high DPPH scavenging capacity and low hydroxyl radical inhibition. Clove essential oil showed *in vitro* inhibitory and bactericidal effect against *S. aureus*, *E. coli*, *L. monocytogenes* and *S.* Typhimurium. In addition, *in situ* antimicrobial activity of clove oil against *S. aureus* was superior to nitrite. The essential oil particles encapsulated with sodium alginate and emulsifiers, showed high encapsulation efficiency, low antioxidant activity and

strong antimicrobial inhibition. Similar bacterial growth was observed in meat-like products after addition of either particles or nitrite.

Keywords: *Syzygium aromaticum*; encapsulation; bactericidal; emulsifier

1. INTRODUCTION

Over the last few years there has been a significant increase in the population concern about a healthier diet, with a consequent decrease in the consumption of processed foods, mainly due to the presence of potentially carcinogenic preservatives. This trend was lately reinforced by the conclusion of the International Agency for Research on Cancer, a body of the World Health Organization (WHO), that consumption of processed, smoked or cured meat increases the risk of cancer (WHO, 2015).

Faced with this challenge, the industry started search for alternatives to chemical preservatives traditionally used for microbial control, such as the natural compounds present in plants and vegetable oils. Essential oils, also known as volatile or ethereal oils, are hydrophobic, volatile aromatic compounds, which are plants' secondary metabolites.

The essential oil of clove (*Syzygium aromaticum* L.), extracted from the dry floral bud of the clove tree, has antimicrobial and antioxidant activities due to the presence of eugenol and other phenolic compounds. It acts as a bactericide against some of the most important foodborne pathogens including *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes* and *Salmonella* Typhimurium, and has anti-free radical and metal chelating activities (Chaieb et al., 2007). However, due to its intense odor, volatility and instability under ambient conditions (temperature, light and oxygen), its use in the industry is limited. In view of this, encapsulation of this oil appears to be a solution facilitating its application as a food ingredient.

Although there are several studies reporting extraction and encapsulation of clove essential oil, the use of sodium alginate as wall material for food purposes is still an open field of research.

Given the above background, the objective of this study was to extract and chemically characterize clove essential oil (*Syzygium aromaticum* L.) and assess its antioxidant and antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes* and *Salmonella* Typhimurium. Moreover, the efficiency of clove essential oil encapsulation using sodium alginate/emulsifiers system was evaluated as well as the thermal behavior, and antioxidant (*in vitro*) and antimicrobial activity (*in vitro* and *in situ)* of the obtained particles.

2. MATERIAL AND METHODS

Clove dried floral buds were purchased from the local market in the city of Pelotas, RS, and milled with a knife mill (Marconi). The essential oil was extracted according to the Brazilian Pharmacopoeia (Brasil, 2010) by steam distillation using the Clevenger equipment. The process was carried out for 3 h and the obtained clove essential oil was stored in an amber bottle at -18 ºC.

2.2 Extraction yield

The essential oil extraction yield was calculated according to Farmacopéia Brasileira (Brasil, 2010). Results were expressed in percentage.

2.3 Chemical characterization of essential oil

The chemical characterization of clove essential oil was carried out by gas chromatography (GC) coupled to mass spectrometry using a Shimadzu

QP2010 equipment. Clove essential oil (1 µL) was injected in split mode and compound separation was carried out on a Restek Rtx-5MS capillary column (30 m x 0.25 mm x 0.25 µm). GC oven was programed from 40 ºC to 280 ºC at 5 ºC min^{-1}. The volatile constituents of the oil were identified by comparison of both mass spectra and retention index with reference mass spectrum reported in NIST 05 library.

2.4 Total phenolic content of essential oil

The total phenolic content of clove essential oil was determined by the Folin-Ciocalteau method (Swain & Hills, 1959). Results were expressed as mg of gallic acid equivalents per gram of sample (GAE mg g^{-1}).

2.5 Essential oil encapsulation

An aqueous solution of sodium alginate (2%) (GastronomyLab) was prepared under agitation at 70 °C and allowed to stand for 24 h at 4 °C. After that, the sodium alginate solution was homogenized in Ultra-Turrax (Metabo) at 12000 rpm for 5 min followed by the addition of the emulsifiers glycerol monostearate (0.5%) (Vogler) or polyoxyethylene sorbitan monolaurate (0.5%) (Tween 20) (Synth). Clove essential oil (1%) was added to the mixture and homogenized in Ultra-Turrax at 12000 rpm for 10 min. The mixture of sodium alginate, emulsifier when used and essential oil was added to a calcium chloride (Vetec) solution (5%) under stirring in Ultra-Turrax as described in the previous step. The solution was filtered under vacuum, particles were washed with distilled water until pH 7.0 of the washing was reached and dried in an air circulation oven (Cienlab) at 30 °C for 48 h. Particles were stored in a glass vial at -18 °C until analysis. Three set of particles were prepared: sodium alginate

and clove essential oil (AO); sodium alginate, glycerol monostearate and clove essential oil (AMO) and sodium alginate, polyoxyethylene sorbitan monolaurate and clove essential oil (ATO). Control samples were produced in the same way, however in the absence of essential oil.

2.6 Encapsulation efficiency

The efficiency of encapsulation (EE) of the phenolic compounds present in clove essential oil was estimated according to the method proposed by Rutz et al. (2013). Thus, 0.1 g of particles were homogenized in Ultra-Turrax (Metabo) with 10 mL of methanol and left for 24 h. After this period, the solution was centrifuged at 4200 rpm, at 25 °C for 15 min, and 0.5 ml of the supernatant was collected for the analysis. Total phenolic content of collected fractions were evaluated by the Folin-Ciocalteau method (Swain & Hills, 1959), as described in item 2.4. The EE was given as a percentage of phenolic compounds, according to the equation:

$$EE(\%)\ \frac{Total\ phenolic\ content\ of\ the\ oil - Phenolic\ compounds\ from\ particles}{Total\ phenolic\ content\ of\ the\ oil} x\ 100$$

2.7 Differential Scanning Calorimetry

Thermal analysis of clove essential oil and particles was performed by Differential Scanning Calorimetry using TA Instruments model DSC Q20 equipment. For each sample, 5 mg were heated in aluminum containers at a rate of 10 °C min^{-1} between -25 °C and 300 °C, with a nitrogen flow of 50 mL min^{-1} (Rutz et al., 2013).

2.8 Antimicrobial activity

The antimicrobial effect of essential oil and particles was studied using three methodologies: disk diffusion, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Antimicrobial effects on the standard strains of *Salmonella* Typhimurium (ATCC 13311), *Escherichia coli* O157: H7 (ATCC 43895), *Listeria monocytogenes* (ATCC 7644) and *Staphylococcus aureus* (ATCC 10832) were tested at the 0.5 McFarland turbidity standard (1.5×10^8 CFU mL^{-1}). The disk diffusion results were expressed in cm ± standard deviation of the three equidistant measurements from the formed zone, MIC was expressed as mg mL^{-1} and MBC with microorganism growth or microorganism inhibition of growth.

2.8.1 Disk diffusion

Disk diffusion analysis was performed according to the protocol proposed by the Clinical and Laboratory Standards Institute manual- CLSI (CLSI, 2015).

2.8.2 Minimum Inhibitory Concentration

MIC was determined according to the method described by Cabral et al. (2009) with minor modifications. The essential oil and particles were tested at four different concentrations: 292.66 mg mL^{-1}; 60.93 mg mL^{-1}; 3.047 mg mL^{-1}, and 0.3047 mg mL^{-1}. Microbial growth was evaluated by turbidity readings at a wavelength of 620 nm using spectrophotometer (Biochrom EZ Read 400) at the time of preparation and after 24 h of incubation. MIC was considered the lowest concentration of essential oil, which prevents bacterial growth in the culture medium.

2.8.3 Minimum bactericidal concentration

MBC was performed according to the method described by Cabral et al. (2009) with minor modifications.

2.9 Antioxidant activity

The antioxidant activity was determined using the following spectrophotometric methodologies: scavenging of DPPH (2,2-diphenyl-1-picryl-hydrazyl) radical according to the method proposed by Brand-Willians et al. (1995) with modifications, and inhibition of hydroxyl radical (Vinholes et al., 2011). Antioxidant activity was expressed as percentage of DPPH scavenging and percentage of inhibition of hydroxyl radical.

2.10 *In situ* antimicrobial activity

A burger-like meat product was formulated in order to determine the *in situ* antimicrobial effect of unencapsulated and encapsulated clove essential oil. Based on the results found *in vitro,* an artificial contamination of this meat product was conducted with *S. aureus*. Treatments were prepared as follows: 57% lean beef, 10% swine fat, 15% ice, 15% ice water and 3% soy protein. Further, the essential oil, encapsulated essential oil particles, sodium nitrite (preservative) and 1 mL of *S. aureus* bacterial inoculum ATCC 10832, pre-grown in Tryptone Soy Broth (TSB) medium for 24 h containing approximately $1.5x10^{8}$ CFU mL^{-1}, were added separately. The burger-like meat were molded in Petri dishes with plastic wrappers and kept under refrigeration for 15 days at 4 °C. The following treatments were applied: standard control without inoculum; control with *S. aureus* inoculum; control with 0.25% of sodium nitrite; treatment

with *S. aureus* and 1 µL g^{-1} of clove essential oil; treatment with *S. aureus* and 0.0239g g^{-1} of sodium alginate + clove essential oil particles; and treatment with *S. aureus* and 0.0071 g g^{-1} of sodium alginate + glycerol monostearate + clove essential oil particles. *S. aureus* enumeration was carried out following the recommendations of Downes and Ito (2001) with modifications. Manual counting of bacterial colony forming units was performed after 48 h of incubation and results expressed in CFU g^{-1}.

2.12 Statistical analysis

Statistical analysis of the results of antioxidant and antimicrobial activity determination was performed by analysis of variance with post-hoc Tukey's test (p <0.05). Analysis of *in situ* antimicrobial activity was performed by analysis of variance (ANOVA) with post-hoc Fisher's least significant difference test, LSD (p <0.05).

3. RESULTS AND DISCUSSION

3.1 Extraction yield

The yield of clove essential oil was 1%, which is lower than yields reported in the literature, with values in the range from 1.87% to 8.88% (Hernández-Ochoa et al., 2014). This low essential oil production may be related to the clove variety, soil and climate characteristics of the region where the clove was produced (Alma et al., 2007), in addition to the drying and extraction conditions.

3.2 Terpenes

Chromatographic analysis of clove essential oil revealed presence of three terpenic compounds, of which eugenol was the most abundant accounting for

56.06% of total peak area, followed by caryophyllene (39.63%) and α-caryophyllene (4.31%) (Table 1).

Table 1. Compounds present in clove (*Syzygium aromaticum*, L) essential oil.

Peak	Retention time (min)	Peak area (%)	Compound
1	18.498	56.06	Eugenol
2	20.038	39.63	Caryophyllene
3	20.819	4.31	α-Caryophyllene

Chaieb et al. (2007) found 36 compounds in clove essential oil, where eugenol (88.58%) was the major compound followed by eugenol acetate (5.62%) and β-caryophyllene (1.39%). Eighteen compounds were detected in the clove essential oil studied by Alma et al. (2007), of which eugenol (87%) was the most representative one. Thus, eugenol constitutes the major component of clove essential oil. The low number of compounds identified in the present study may be related to the method and time of extraction of the essential oil (3 h in Clevenger). Alma et al. (2007) for instance, extracted the clove essential oil using an industrial distiller for 3 h.

3.3 Total phenolic compounds content

The total phenolic compounds content in the essential oil was 9.07 GAE mg g^{-1}, which is lower than values reported by Babaoglu et al. (2017) (18.59 mg GAE.g^{-1}), which suggests that the concentration is dependent on the oil extraction method and characteristics of the sample. The major phenolic compound present in clove is eugenol.

3.4 Encapsulation efficiency

In the present study, effect of emulsifiers with different Hydrophilic - Lipophilic Balance (HLB) values on the emulsification efficiency was evaluated. HLB values were 3.8 and 16.7 for glycerol monostearate and polyoxyethylene sorbitan monolaurate, respectively. This means that the former has low ratio of hydrophilic/hydrophobic groups and the latter high ratio. HLB values inform the solubility in oil or water, and may be used to predict the type of emulsion to be formed. Thus, emulsifiers with low HBL values (3 to 6) are predominantly hydrophobic and emulsifiers with high HBL values (8 to 18) are predominantly hydrophilic.

High EE values were observed for all particles: 83.60% for sodium alginate + polyoxyethylene sorbitan monolaurate + clove essential oil (ATO) particles, 90.02% for sodium alginate + clove essential oil (AO); and 92.12% for sodium alginate + glycerol monostearate + clove essential oil particles (AMO). According to the obtained results, characteristics of the emulsifier influenced the EE, since the use of the glycerol monostearate increased the EE of the oil compared to the polyoxyethylene sorbitan monolaurate. However, due to the high EE value obtained for the sample without emulsifier (AO), it is assumed that the high stirring speed used in the preparation of the mixture of the alginate and oil solution and properties of the alginate were sufficient to produce an emulsion. A possible explanation can be the interaction of carboxyl and hydroxyl groups of alginate with the polar groups present in the clove essential oil, such as the hydroxyl present in eugenol, via dipole bonds, dipole-dipole and hydrogen bonds. However, it can be suggested that the presence of the glycerol

monostearate emulsifier affords encapsulation of apolar compounds such as caryophyllene.

EE results reported in the literature are dependent of the wall material, emulsifier and encapsulated oil. Sebaaly et al. (2015) obtained EE of 57.9% to 84.6% for clove essential oil encapsulated with soybean phospholipids. Both oil concentration and the speed applied during the encapsulation step have a direct effect on the formation of the emulsion and consequently on the EE.

3.5 Thermal analysis

DSC thermograms of clove essential oil, wall material and particles are depicted in Figure 1. According to these results clove essential oil, an endothermic event was observed at 240.50 °C. This event was attributed to the boiling of the compounds present in the clove essential oil, mainly eugenol, whose endothermic event in clove essential oil was observed at 270.2 ºC (Santos et al., 2009).

Endothermic peaks of the wall material were observed in thermograms as well: for AO at 175.31 ºC, for AMO at 69.46 ºC and 173.15 ºC, and for ATO at 154.48 ºC. Endothermic peak of sodium alginate is presumably due to the cleavage of the carboxylate-calcium bond, formed in the reaction between sodium alginate and calcium chloride during encapsulation process. This event was reported to occur at 197 °C by Abulateefeh and Taha (2015). According to the authors, the thermogram obtained for sodium alginate in the absence of calcium chloride is different from the one observed in the present study, displaying an endothermic event at 85 ºC due to the loss of water. Different

endothermic events observed for the cell wall materials, were influenced by the presence of emulsifiers.

Endothermic events were also observed in the thermograms of AO at 181.19 °C, AMO at 69.06 °C and at 171.93 °C, and ATO at 165.31 °C. The characteristic endothermic event of clove essential oil was not identified in the thermograms of AO and AMO particles while this event was displaced for ATO particles. In all cases the behavior is related to the interaction of the matrix with the essential oil, being indicative of the encapsulation process (Rutz et al., 2013).

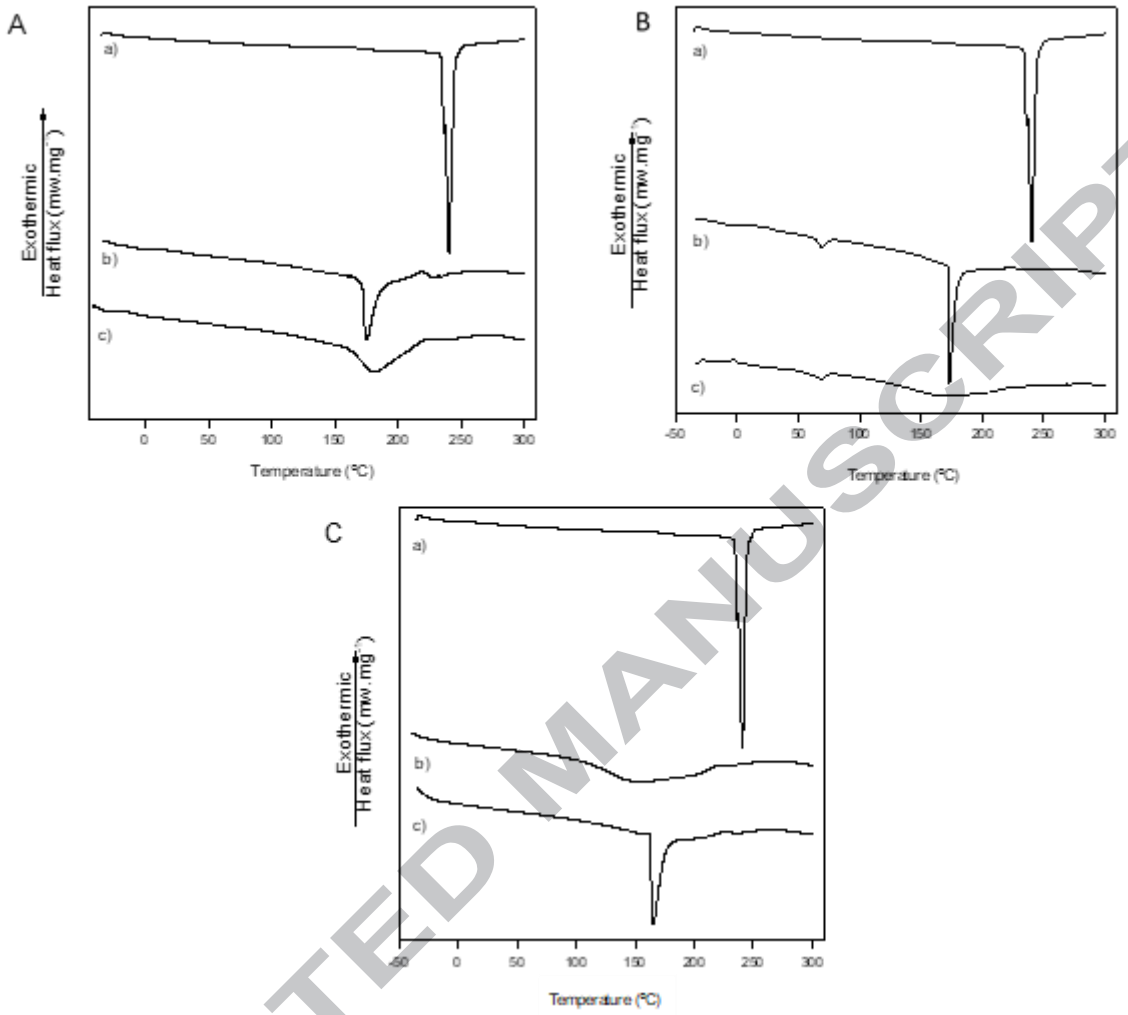

Figure 1. (A) Thermal behavior of sodium alginate and clove (*Syzygium aromaticum*, L.) essential oil particles. a) clove essential oil b) sodium alginate; c) sodium alginate and clove essential oil particle; (B) Thermal behavior of sodium alginate, glycerol monostearate and clove (*Syzygium aromaticum*, L.) essential oil; a) clove essential oil; b) sodium alginate and glycerol monostearate; c) sodium alginate, glycerol monostearate and clove essential oil particle; (C) Thermal behavior of sodium alginate, polyoxyethylene sorbitan monolaurate and clove (*Syzygium aromaticum*, L.) essential oil; a) clove essential oil; b) sodium alginate and polyoxyethylene sorbitan monolaurate; c) sodium alginate, polyoxyethylene sorbitan monolaurate and clove essential oil particle.

3.6 Antimicrobial activity

3.6.1 Disk diffusion

The inhibition zones of clove essential oil were 2.83 cm, 2.81 cm, 2.47 cm and 2.22 cm for *S. aureus*, *E. coli*, *L. monocytogenes* and *S.* Typhimurium, respectively. The presence and size of the inhibition zone indicate the susceptibility of the bacteria to the essential oil. When inhibition zones are smaller than 0.7 cm, a sample is considered to be non-active against bacteria. Inhibition zone diameter greater than 1.2 cm indicates satisfactory inhibitory effect (Arora & Kaur, 1999). Thus, the clove essential oil evaluated in this study displayed satisfactory inhibitory effect against all bacteria evaluated. However, wide variation of inhibitory effect of clove essential oil was reported in the literature.

In the present study an inhibition zone of 2.83 cm was observed against *S. aureus*. This value was lower than the inhibition zone of 3.2 cm reported by Silvestri et al. (2010). The inhibition zone for *E. coli* was found to be 2.81 cm, which is larger than the inhibition zone of 1.97 cm reported in the literature (Silvestri et al., 2010). Clove essential oil inhibition zone for *L. monocytogenes* was 2.47 cm. The inhibition zone formed by clove essential oil against *S.* Typhimurium was 2.22 cm. This value was higher than inhibitions zones reported by Silvestri et al. (2010), which were 1.1-1.5 cm and 1.57 cm, respectively. The presence of the inhibition zone formed by the clove essential oil against studied bacteria is possibly due to its lipophilic characteristic, which allows an interaction between the oil and the lipids of the cell membrane of the bacterium, altering its permeability.

3.6.2 Minimum inhibitory concentration

Results of determination of minimum inhibitory concentration of unencapsulated and encapsulated clove essential oil against *S.* Typhimurium, *L. monocytogenes*, *S. aureus* and *E. coli* bacteria are presented in the Table 3.

Table 3. Minimum inhibitory concentration of unencapsulated and encapsulated clove (*Syzygium aromaticum*, L.) essential oil against *Salmonella* Typhimurium, *Listeria monocytogenes*, *Staphylococcus aureus* and *Escherichia coli* bacteria.

Bacteria	Concentration (mg mL^{-1})			
	Oil	AO	AMO	ATO
S. Typhimurium	0.304	7.96	23.66	12.46
L. *monocytogenes*	0.304	7.96	23.66	12.46
S. *aureus*	0.304	7.96	23.66	12.46
E. *coli*	0.304	7.96	23.66	12.46

Particles: AO=Sodium alginate and clove essential oil; AMO=Sodium alginate, glycerol monostearate and clove essential oil; ATO=Sodium alginate, polyoxyethylene sorbitan monolaurate and clove essential oil.

The clove essential oil showed inhibitory effect up to the concentration of 0.304 mg mL^{-1} against *S. aureus*, *E. coli*, *L. monocytogenes* and *S.* Typhimurium bacteria. Thus, this effect was independent of the characteristics of the microorganism membranes. The action of clove essential oil is ascribed to the presence of eugenol. This compound promotes the rupture of the bacterial cytoplasmic membrane, increasing its permeability allowing the extravasation of the ions and the loss of intracellular proteins, which leads to the cell death (Devi et al., 2010).

Strong antimicrobial activity is attributed to oils with MIC up to 0.5 mg mL^{-1}, moderate activity to the oils with MIC between 0.6 to 1.5 mg mL^{-1}, and poor antimicrobial activity to the oils with MIC above 1.6 mg mL^{-1} (Duarte et al., 2007). Therefore, the clove essential oil used in the present study showed a

strong antimicrobial activity. Our result (MIC=0.3047 mg mL^{-1}) was similar to the MIC found by Silvestri et al. (2010), which was 0.3 mg mL^{-1}. The MIC for clove essential against *E. coli* was 0.3047 mg mL^{-1}, this result was lower than values reported by Silvestri et al. (2010) (0.400-0.600 mg mL^{-1}). MIC for *S.* Typhimurium reported by Beraldo et al. (2013) (0.0400 mg mL^{-1}) was higher than values of the oil in the present study. MICs for *L. monocytogenes* were also lower than those reported in the literature. Beraldo et al. (2013) found MIC of 0.800 mg mL-1. The differences observed between MIC values may be due to the type of the culture of the sample, concentrations of substances and differences in extraction methods of essential oils (Rio & Recio, 2005).

Diluted essential oil particles showed inhibition of bacterial growth of *S.* Typhimurium, *L. monocytogenes*, *S. aureus* and *E.coli.* In the present study, no difference of inhibition between gram-positive and gram-negative bacteria was observed. Hill et al. (2012) found MIC of 0.693 mg mL^{-1} for clove essential oil encapsulated with β-cyclodextrin against *S.* Typhimurium, which is lower than values obtained in the present study. Clove essential oil encapsulated with lipids were reported to have MICs in the range from 0.224 to 0.856 mg mL^{-1} against *S. aureus* (Meneses et al., 2017), which is also lower than values observed in the present study. Similarly, MIC for *E. coli* in the range between 0.228 to 0.9999 mg mL^{-1}, reported by Meneses et al. (2017), was also lower than values observed in our study. MIC for *L. monocytogenes* ranging from 7.96 to 23.66 mg mL^{-1} among treatments, were higher than values reported by Hill et al. (2012) for *L. inoccua* for clove essential oil encapsulated with β-cyclodextrin (MIC of 1.155 mg mL^{-1}). The differences between MIC values obtained in this work and reported in the literature can be due to the differences in wall

materials, encapsulation processes, particle size, encapsulation efficiency and interactions between essential oil and encapsulating agents.

3.6.3. Minimum bactericidal concentration

Results of determination of minimum bactericidal concentration (MBC) are presented in the Table 4.

Table 4. Minimum bactericidal concentration of unencapsulated and encapsulated clove (*Syzygium aromaticum*, L.) essential oil against *Salmonella* Typhimurium, *Listeria monocytogenes*, *Staphylococcus aureus* and *Escherichia coli.*

Bacteria	Concentration (mg mL^{-1})			
	Oil	AO	AMO	ATO
S. Typhimurium	0.304	nd	23.66	nd
L. *monocytogenes*	0.304	nd	nd	nd
S. *aureus*	0.304	79.6	23.66	nd
E. *coli*	0.304	nd	nd	nd

*Minimum bactericidal concentration; nd – not detected. Particles: AO=Sodium alginate and clove essential oil; AMO=Sodium alginate, glycerol monostearate and clove essential oil; ATO=Sodium alginate, polyoxyethylene sorbitan monolaurate and clove essential oil.

Clove essential oil showed bactericidal effect against all tested microorganisms up to a concentration of 0.304 mg mL^{-1} (Table 4). In the present work, MBC for clove essential oil against *L. monocytogenes* was higher than those reported by Beraldo et al. (2013) (0.180 mg mL-1) and by Devi et al. (2010) (0.250 mg mL^{-1}). Devi et al. (2010) obtained MBC of 0.250 mg mL^{-1} against *Salmonella typhi*, which is lower than value obtained in the present study.

Bactericidal effect was probably due the microorganisms' sensitivity to eugenol, which increases the bacterial membrane permeability. Differences between the MBC values found in the present study and those reported in the

literature can be due to the variation of the concentrations of oil used to test the bacteria, type of sample and employed method (Rio & Recio, 2005).

The AMO particles were found to promote bacterial killing of *S. Typhimurium* and *S. aureus* at concentration of 23.66 mg mL^{-1}. AO particles promoted bacterial killing of *S. aureus* at the concentration of 79.6 mg mL^{-1}, while no MBC was observed for the ATO particles. Generally, essential oils have lower MBCs for gram-negative bacteria; however we observed a higher bactericidal effect against S. aureus for the particles. This fact can be explained by the higher sensitivity of the gram-positive bacteria to eugenol present in the clove essential oil.

A correlation between MBC and EE was observed as particles with higher EE and essential oil content showed higher bactericidal effect. Different studies have shown that the bactericidal effect of essential oils is dependent of the wall material. For instance, Hill et al. (2012) obtained MBC of 1.115 mg mL^{-1} for clove essential oil using β-cyclodextrin as wall material. Cui et al. (2015) reported similar MBC for clove essential oil encapsulated with liposomes against *S. aureus.* Both authors reported values of MBC lower than the ones in the present study.

In the present study the particles did not display bactericidal effect for *L. monocytogenes*, however MBC of 1.155 mg mL^{-1} for *L. innocua* was reported for clove essential oil encapsulated with lipids. MBC for particles against *E coli* obtained in this work is in agreement with the results reported by Cui et al. (2015), where clove essential oil encapsulated in liposomes did not kill bacteria. The absence of bactericidal activity against *E. coli* and *L. monocytogenes* may

be due to the type of wall material, which may not interact with the bacteria, or to the tested concentrations.

3.7. Antioxidant activity

The DPPH scavenging capacity of the clove essential oil at the concentration of 484.7 μg mL^{-1} was 94.86 %. Lower inhibitions of 28.83 % and 22.13 % were observed for hydroxyl and nitric oxide radicals, respectively, for the essential oil concentration of 12.25 μg mL^{-1}.

The high DPPH scavenging activity observed for the clove essential oil can be explained by a synergistic effect between phenolic compounds, even at low concentrations. In contrast, the lower inhibition values obtained for hydroxyl and nitric oxide radicals may be due to the low interaction of the phenolic compounds with these radicals. A stronger DPPH scavenging activity was found for the clove essential oil under study compared to the results reported in the literature. For instance, Silvestri et al. (2010) reported 45.27 % of scavenging, at the concentration level of 500 μg mL^{-1}, while 92.82 % was reported by Sebaaly et al. (2016) at the concentration level of 10000 μg mL^{-1}.

As far as we are concerned there are no studies reporting the inhibition of hydroxyl radical by clove essential oil. However, their evaluation is extremely important since hydroxyl radical is the main reactive oxygen species that leads to lipid peroxidation and other biological damage to the organism (Hazra et al., 2008).

Results of antioxidant activity determination of the encapsulated clove essential oil are presented in the Table 5. Particles with higher EE (AO and

AMO) also showed higher antioxidant activity for all tested radicals. ATO particles presented significantly lower antioxidant activity than the others.

Table 5. Antioxidant activity of the clove essential oil encapsulated with sodium alginate and different emulsifiers.

Sample	DPPH	Hidroxyl
AO	9.73[a]	8.31[a]
AMO	7.69[b]	7.00[b]
ATO	2.16[c]	6.90[b]

[1]Expressed as percentage of radical scavenging. [2]Expressed as percentage of radical inhibition. Means followed by the same letter in the same colunm are significantly different by the Tukey test ($p<0.05$). Particles: AO=Sodium alginate and clove essential oil; AMO=Sodium alginate, glycerol monoestearate and clove essential oil; ATO=Sodium alginate, polyoxyethylene sorbitan monolaurate and clove essential oil.

All particles showed low antioxidant activity for evaluated radicals, possibly due to the strong interaction between phenolic compounds and the wall material. According to Pramod et al. (2015) sodium alginate has good compatibility with eugenol. As AO particles showed significantly higher antioxidant activity, we can hypothesize that the absence of the emulsifier facilitates release of the oil compounds allowing their interaction with the radicals. On the other hand, ATO particles showed a significantly lower antioxidant activity among all tested particle compositions. This fact can be partly explained by the lower EE value obtained for these particles and also by the chemical interaction between the oil and the emulsifier. Polyoxyethylene sorbitan monolaurate has higher number of carbons than glycerol monostearate, thus the essential oil would be more easily retained in the former.

DPPH scavenging activity was lower compared to the results reported in the literature for encapsulated clove essential oil. Sebaaly et al. (2016) obtained DPPH scavenging ranging from 89.3 to 92.31% for clove essential oil encapsulated in cyclodextrin. This result was probably due to the different wall material allowing greater release of the compounds present in the essential oil. Meneses et al. (2017) found DPPH scavenging activity greater than 80% for clove essential oil encapsulated in lipids. These differences in antioxidant activity may be related not only to wall materials, but also to particle characteristics such as size and load, and the method of analysis employed.

3.8 *In situ* antimicrobial analysis

The results of the *in situ* antimicrobial activity determination of unencapsulated and encapsulated clove essential oil carried out in burger-like meat products tested at two time points (0 and 7 days) against *S. aureus* are presented in the Table 6.

Table 6. *In situ* antimicrobial activity for nitrite and unencapsulated and encapsulated clove (*Syzygium aromaticum,* L.) essential oil performed in burger-like meat products against *Staphylococcus aureus* at two different times.

Treatments	***S. aureus* concentration (Log UFC g^{-1})**	
	Tempo 0*	**Tempo 7****
Control without inoculum	3.69^{Aa}	4.47^{bA}
Control with inoculum	4.08^{Ab}	4.90^{bB}
Nitrite	3.47^{Aa}	4.11^{bC}
Essential oil 3,04***	4.70^{Ac}	3.84^{bD}
Essential oil 0,304****	5.0^{Ad}	4.47^{bE}
AO particle 79.6*****	4.0^{Aa}	4.79^{bF}
AMO particle 23.66******	3.47^{Aa}	4.74^{bG}

* Time 0 – sample withdrawal for analysis after the preparation of meat products similar to hamburgers; ** time 7 – sample withdrawal for analysis after 7 days of preparation of meat products similar to hamburgers; *** clove essential oil at 3.04 mg mL^{-1}; **** clove essential oil at 0.304 mg mL^{-1}; *****sodium alginate + clove essential oil at 79.6 mg mL^{-1}; ******sodium alginate

+ glycerol monostearate + clove essential oil at 23.66 mg mL^{-1}. Means followed by the same lower case letters in lines and capital letters on the columns do not differ significant ($p < 0.05$).

It can be observed that essential oils at concentrations of 3.047 mg mL^{-1} and 0.3047 mg mL^{-1} inhibited growth of *S. aureus* more efficiently than commercial preservative nitrite. Nitrite can form nitrosamines that potentially carcinogenic, thus total or partial replacement of this compound by clove essential oil may be a viable natural alternative preservative. However, as nitrites are also responsible for sensory characteristics typical of meat products such as color and taste, their total substitution by oil would also require use of alternatives additives to supply the sensorial characteristics expected of these products.

Clove essential oil particles allowed *S. aureus* growth, but it was also possible to verify growth in samples containing nitrite preservative. The study by Cui et al. (2015) reported that clove essential oil applied to tofu promoted growth control of *S. aureus*; however the concentrations used were different from our study.

The protection of bioactive compounds by such systems as capsules, microstructures and micelles using different wall materials (i. e. cyclodextrin, chitosan, alginate, maltodextrin, gelatin, among others), has been extensively studied. The use of encapsulated bioactive compounds as food preservatives for red meats, chicken, cheese, milk, fruit and vegetables has demonstrated a great potential for industrial applications as a mean to improve food safety (Rakmai et al., 2018; Cui et al., 2018a; Cui et al., 2018b; Rakmai et al., 2017a;

Rakmai et al., 2017b; Cui et al., 2016a; Cui et al, 2016b; Cid et al., 2014; Cid et al., 2013; Astray et al., 2010; Astray et al., 2009).

4 Conclusion

Clove essential oil has strong scavenging activity determined by the DPPH method, and can be used for the control of free radicals. It also presented strong *in vitro* inhibitory and bactericidal action against *S. aureus*, *E. coli*, *L. monocytogenes* and *S.* Typhimurium. Furthermore, it showed *in situ* inhibitory effect against *S. aureus* tested in meat products similar to burgers, being therefore an alternative to chemical preservatives that are potentially carcinogenic.

Sodium alginate showed high efficiency of clove essential oil encapsulation. Resulting particles displayed lower antioxidant activity and greater inhibitory action compared to the unencapsulated oil. These properties were independent of the evaluated microorganism, but the *in vitro* bactericidal effect depended on both the wall material and the microorganism. Particles also showed bactericidal effect against *S. aureus* and *S.* Typhimurium, being therefore a viable alternative preservative. The *in situ* application of the particles in ground meat products allowed some bacterial growth, which was similar to the one observed in the presence of chemical preservative nitrite.

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The essential oil of clove has strong antimicrobial and antioxidant activity

The encapsulation process inhibits the characteristic odor of clove essential oil

Application of the particles in ground meat products has similar action to nitrite

Clove essential oil can be new alternative to synthetic food preservatives