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Preservative of Essential Oil Blends: Control of *Clostridium perfringens* Type a in Mortadella

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HIGHLIGHTS

- The blends of essential oils were effective against vegetative cells of *C. perfringens*.
- The blends of essential oils were antioxidants in the meat product.
- Essential oils have a preservative action in mortadella.

Abstract: The aim of this study was to evaluate the antimicrobial effect of the essential oils of cinnamon, cardamom, clove, oregano, and thyme and their synergism on vegetative cells and endospores of *Clostridium perfringens* type A inoculated in meat sausage (mortadella), as well as the influence of blends on the color, and lipid oxidation through the determination of thiobarbituric acid reactive substances (TBARS index). The anticlostridial action of the oil blends was established. The two added oil blends (Treat. 1: oregano, clove, and thyme; Treat. 2: oregano, clove, and cinnamon) in combination with reduced nitrite content (75 ppm) promoted a lower growth of *C. perfringens* in mortadella stored at 15 °C for 21 days in comparison to treatments containing only 75 ppm of nitrite. The essential oil blends showed antioxidant action and did not alter food color, thus possessing potential application as a preservative for the meat products industry.

Keywords: endospores; residual nitrite; natural antimicrobial; antioxidant activity.

INTRODUCTION

Clostridium perfringens is a bacterium commonly found in several environments such as soil, water, plants, the intestinal tracts of animals and humans, as well as in food processing environments [1]. It can produce 16 types of toxins classified as five toxin types (A, B, C, D and E) based on the enterotoxin production

and four types of extracellular toxins (α, β, ε, and ι) [2]. *C. perfringens* Type A produces the toxin called *C. perfringens* enterotoxin (CPE), one of the most common causes of food poisoning in the USA, with about 1 million cases per year [3]. In most strains of *C. perfringens* capable of causing food poisoning, the *cep* gene that encodes to CPE enterotoxin is in the bacterial chromosome. These strains are highly adapted to food transmission due partly to the high resistance of their spores to heat treatment, chemical preservatives, and low temperature [2]. Because it is a fastidious microorganism, it needs a growth medium rich in nutrients, thus it is commonly associated with food-borne outbreaks related to cured cooked meat sausages, since these meat products are a source of amino acids and other nutrients. Furthermore, *C. perfringens* can grow in the presence of high salt concentration (4-6%) and 300 ppm of sodium nitrite, a curing agent and preservative often used in this product type [4].

Nitrite is the key ingredient in the curing process of cooked meat sausages, and is responsible for the development of the pink coloration of the finished product; it also has antioxidant and bacteriostatic effects [5]. However, nitrite in meat (pH between 5.5 and 6.0) is reduced to nitrous acid, resulting from the hydration of the nitrite oxide produced by the reduction of nitrite, which enables it to react with amines in cured meat products for the formation of *N*- nitrous compounds, especially nitrosamines. The latter have toxic, mutagenic, neurotoxic, nephrotoxic and carcinogenic effects [6], and are also correlated to health problems with brain tumors and leukemia [7].

Although nitrite has several undesirable characteristics, few alternatives are suggested to promote its elimination or reduction in cured meat sausages. Thus, alternative preservatives to nitrite have been studied. Among the natural additives, essential oils (EOs) have been highlighted and widely accepted by consumers, besides being generally recognized as safe (GRAS). EOs are complex blends that can contain between 20 and 60 different components occurring in different concentrations. Usually one of them is the major compound, representing 80% or more of the EO composition, in combination with others in lower contents [8]. Several action mechanisms of EOs on bacteria are hypothesized, such as increase in permeability and alteration of the fatty acid profile of the plasma membrane, alteration of the proton-motive force, anti-quorum sensing activity, effect on membrane proteins, action on ATP and ATPase, effect on cytoplasmic proteins and cytoplasmic coagulation [9].

Although several studies have shown that EOs retain their antimicrobial activity when added to food matrices, evaluations typically involve only one EO and their concentrations are generally high [10-12]. An alternative to this problem is the study of the antimicrobial activity of several EOs in combinations with each other and / or with other conservation technologies [13]. Some recent studies have been reported in this regard.

A study [14] evaluated the effect of nutmeg essential oil (NEO) on the oxidative and microbial stability of cooked sausages during refrigerated storage and concluded that a maximum amount of 20 ppm of NEO can be applied to extend shelf life. In a combination of preservatives, Smaoui at al [15] evaluated the combination of *Mentha piperita* EO with a commercial bacteriocin, and observed an interesting biopreservative effect in minced raw beef during cold storage. The addition of the EO combined with bacteriocin delayed the proliferation of deteriorating microorganisms, limited the oxidation of lipids and extended the shelf-life in raw beef. Another study by Stojanović-Radić and coauthors [16] investigated the effect of the basil and rosemary EOs as well as their combination on the growth of *Salmonella enterica* serovar Enteritidis (*Salmonella* Enteritidis) in chicken meat, together with their potential protective spoilage at the two storage temperatures, and obtained positive results suggesting the use of EOs to extend shelf life and prevent pathogen contamination in chicken meat. Šojić and coauthors [17] in a very complete study, evaluated the effect of coriander essential oil (CEO) on microbial growth in cooked pork sausages produced with different levels of sodium nitrite. Both data from physical-chemical analyzes (color, pH, TBARS) and microbiological analyzes suggest potential use of CEO in the processing of cooked pork sausages, contributing to increased quality and shelf-life. Furthermore, Radünz and coauthors [18] evaluated the thyme EO in vitro and in situ in hamburger-like meat products. The encapsulated EO showed antioxidant and antimicrobial activity against pathogenic microorganisms (*Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes* and *Salmonella* Typhimurium) tested *in vitro* and against *Escherichia coli in situ*, showing potential for application as a natural preservative in food.

As EOs have antimicrobial action both on deteriorating and pathogenic microorganisms, antioxidant activity and also add flavor to the product, they are of great interest in the development of additives for meat and meat products. Thus, the aim of this study was to evaluate the antimicrobial activity of EO combinations on vegetative cells and endospore of *C. perfringens* Type A and the preservative and antioxidant action of these combinations in mortadella. This study is important because it shows the action of EOs against

endospore-forming bacteria, and works on the hypothesis that the use of EOs can reduce the amount of nitrite in a meat product, keeping this product safe for the consumer and making it the healthiest.

MATERIAL AND METHODS

Essential oils

Essential oils of *Origanum vulgare* (oregano)*; Thymus vulgaris* (thyme)*; Cinnamomum zeylanicum* (cinnamon); *Syzygium aromaticum* (clove) and *Elettaria cardamomum* (true cardamom) were used, purchased from FERQUIMA Indústria e Comércio Ltda, Vargem Grande, São Paulo, Brazil. The constituents of essential oils were characterized by Gas Chromatography – Mass Spectrometry (GC-MS) (Shimadzu, GCMS-QP2010 Plus), except cardamom essential oil (industry information). The main chemical constituents are presented in Supplementary Material 1.

Microorganism, standardization and maintenance of the inoculum

Clostridium perfringens Type A, INCQS 00130 (ATCC 13124) used in this study was provided by the National Institute of Quality Control in Health (INCQS) of the Oswaldo Cruz Foundation (FIOCRUZ), Rio de Janeiro, RJ, Brazil.

Inoculum maintenance was done according to Dias and coauthors [13]. Inoculum standardization (10^7 CFU/mL) was performed with a growth curve (OD 600 nm) and counting on BHI agar plates, with overlay, supplemented with 0.5% glucose and incubated at 37 °C for 24 h.

The endospores were obtained after strain reactivation, standardization, inoculation on sporulation medium (meat extract: 3 g, tryptone: 5 g, starch: 1 g, manganese sulfate: 10 mg, distilled water: 1000 mL) and incubation at 37°C for 6 days. The culture containing the endospores was subjected to thermal shock (75°C for 15 min/ 0°C for 15 min) and the presence of endospores was confirmed by observation under an optical microscope using the Wirtz-Conklin staining technique. The culture was centrifuged (10,000 x *g*/5 min.) and the endospores were stored in freezing medium. Endospore inoculum was standardized at 10^6 CFU/mL by plating on BHI agar plus 0.5% glucose and incubation at 37 °C for 48 hr.

All culture media used were from the Himedia, Mumbai, India and reagents were from VETEC, Sigma-Aldrich Brazil, Duque de Caxias, Brazil.

Minimum bactericidal (MBC) and sporicidal concentration (MSC) of essential oils

The determination of the minimum bactericidal (MBC) and sporicidal concentrations (MSC) was performed using the BHI broth supplemented with 0.5% glucose added with 0.5% Tween (BHI+G+T). The essential oil concentrations evaluated ranged from 0 to 8% (v/v), varying with fractions of 0.25%. The essential oils were diluted in BHI broth containing Tween 80 (v / v) in the desired concentrations. Aliquots of 20 µL of the standardized culture of vegetative cells or spore suspension were transferred to tubes containing 5 mL of BHI+G+EO and incubated at 37 °C/24 h under anaerobic conditions obtained with the addition of mineral oil. After this period, pour plate technique with an overlay was used by adding 1 mL of the culture plated in BHI+ agar+G and incubated at 37 °C/24 h. MBC and MSC were considered as the lowest concentration of each oil where there was no visible growth on the plates. Experiments were performed in triplicate with three repetitions.

Determination of antimicrobial activity of essential oil combinations

The five essential oils isolated were selected for three-oil combination assessments. The sets of three essential oils were formed by mixing the five oils randomly until a reasonable number of combinations to be evaluated were obtained. Table 1 shows the different ratios relative to the MBC and MSC of each oil used in the combinations.

Table 1. Matrix of the simplex centroid mixture design of essential oils concentration employed for *in vitro* bactericidal and sporicidal activity.

Run	Independent variables *		
	Essential oil 1	Essential oil 2	Essential oil 3
1	100	-	-
2	-	100	-
3	-	-	100
4	50	50	-
5	50	-	50
6	-	50	50
7	67	17	17
8	17	67	17
9	17	17	67
10	33	33	33

*100 (MBC and MSB); 50 (0.5MBC; and 0.5MSC); 67 (0.67MBC; and 0.67MSC); 33 (0.33MBC; and 0.33MSC); 17 (0.17MBC; and 0.17MSC).

Manufacture of meat sausage: mortadella

The mortadella was composed of beef, 56.5%; pork backfat, 14.5%; water/ice, 20%; cassava starch, 5%; salt, 2%; polyphosphate, 0.5%; antioxidant Fixamax®, 1%; and nitrite 75ppm (sodium nitrite), for all treatments., All additives were kindly provided by IBRAC® (Brazil). Two combinations of essential oils were used: Treat 1 containing 0.165; 0.066; and 0.165% of oregano, clove, and thyme, respectively; and Treat 2 containing 0.165; 0.066; and 0.066% of oregano, clove, and cinnamon, respectively.

The mortadella was shredded into a cutter (Sire, Filizola S.A., Brazil) and the batters were stuffed into 65-67 mm diameter synthetic bags (polyamide) (Viskase®, Brazil) in order to obtain mortadella samples of about 450 g. Cooking was carried out by immersion in a water bath until the internal temperature reached 70-72 °C (measured with thermocouple).

The samples were then inoculated with 10^7 CFU/g of *C. perfringens* vegetative cells, homogenized in Stomacher (Metroterm®, Brazil) (490 strokes/2 min) at room temperature, and separated in 10 g portions in plastic (Unipac Darlon, Brazil, 50 µm thickness) vacuum-sealed packages and stored at 15 °C ± 1 °C. Mortadella destined for physical and chemical analyses (without microorganism) were stored at 4.4 °C. The mortadella were analyzed after 0; 7; and 21 days of storage. The experiment was performed in three replicates.

Enumeration of Clostridium perfringens in mortadella

The mortadella were homogenized in 90 mL of 0.1% (m/v) peptone water in Stomacher Metroterm® (490 beats/min) for 3 min. Aliquots of 1 mL of the appropriate dilutions were plated on SPS agar (Sulfite Polymyxin Sulfadiazine) using the plating technique in depth with overlay and incubated at 37 ºC/24 h. Analyses were performed in triplicate with three repetitions.

*Evaluation of Lipid oxidation (TBASs index) and overall color difference (ΔE *)*

TBARS index analyses were performed according to the methodology described by Raharjo and coauthors [19] with modifications [20].

Evaluation of the objective color of products was carried out using a Konica Minolta® CM-600D portable spectrophotometer colorimeter, following the recommendations for cured products (21). To calculate the color indexes, the illuminant A and the CIELAB color system were established. Overall color difference (*ΔE **) was calculated using the equation $(\Delta E^*) = [(L^* - L\text{ref})^2 + (a^*-a\text{ref})^2 + (b^*-b\text{ref})^2]^{0,5}$, the reference parameters being those of the control at time 0.

Statistical analyses

The essential oils combinations were geared using the Simplex-Centroid Design [22] and showed in Table 1. The results (growth/non-growth) of the different assays of the combinations among essential oils were submitted to principal component analysis (PCA) using *Chemoface 1.5 software*. Results for antimicrobial action of the oil combinations on *C. perfringens* in mortadella and physical and chemical

analyses were submitted to analysis of variance (ANOVA), and the averages were compared through the Tukey test at 5% significance level, using Sisvar software version 5.6.

RESULTS

Minimal bactericidal (MBC) and sporicidal concentration (MSC) of essential oils and its combinations

MBC was 0.5% for oregano and thyme essential oils and 0.2% for cinnamon, clove, and true cardamom oils. The major components of essential oils used in this study were 80.67% of eugenol in clove oil, 73.11% of carvacrol in oregano; 50.89% of thymol and 24.97% of p-cyneme in thyme oil, and 84.52% of E-cinnamaldehyde in cinnamon. Cardamom essential oil showed 41.45% of α-terpineol (data provided by the industry). All these components are known for their antimicrobial and antioxidant activity [23-24].

Based on the MBC, several blends containing three essential oils were prepared with different concentrations (Table 1), in attempting to increase synergy and reduce oil concentrations.

In vitro antimicrobial activity of the different essential oil blends on vegetative cells of *C.perfringens* is shown in Table 2**.** The runs 1, 2 and 3 correspond to 100% of the MBC of the individual oils. Among the blends containing two oils (50: 50% of their MBC) represented by runs 4, 5 and 6 and containing 0.1% clove and 0.25% oregano; 0.25% oregano and 0.25% thyme; 0.25% thyme and 0.1% cinnamon; and 0.1% of true cardamom and 0.1% of cinnamon were bactericidal.

Table 2. Bactericidal activity *in vitro* of essential oil blends against vegetative cells of *C. perfringens* type A

Run	Essential oils blends*					
	Cl:Or:Th	**Cl:Or:Car**	**Cl:Or:Cin**	**Or:Th:Car**	**Or:Th:Cin**	**Th:Car:Cin**
1	+	+	+	+	+	+
2	+	+	+	+	+	+
3	+	+	+	+	+	+
4	+	+	+	-	+	-
5	-	-	-	-	-	+
6	+	-	-	-	+	+
7	+	+	-	-	+	-
8	+	+	-	-	+	+
9	+	+	-	-	+	+
10	+	+	+	-	+	-

(+) bactericidal activity; (-) without bactericidal activity. *Essential oils - Cl: Clove; Or: Oregano; Car: True Cardamom; Cin: Cinnamon; Th: Thyme.

Among the blends containing three oils, in the several ratios, a majority showed bactericidal activity, suggesting synergistic/additive antimicrobial effect among the essential oils. Among the assays containing clove, oregano and cinnamon, only one containing 0.07% clove, 0.17% oregano and 0.07% cinnamon (run 10) was bactericidal. However, the tests containing oregano, thyme and true cardamom oils did not show antimicrobial activity. Thyme, true cardamom and cinnamon were bactericidal in the assays where the ratio of thyme oil was 17% of the MBC (run 8 and 9). At higher concentrations, there was no bactericidal activity, showing a probable antagonism between thyme oil and the others. Observing the PCA (Figure 1A) of the essential oils combinations, oregano and clove oils are highlighted with the greatest contribution to the bactericidal effect of the different tested combinations. These results were similar to those obtained by Dias and coauthors [13], who found that blends containing the highest ratios of oregano essential oil were more effective against *C. perfringens* type A. The phenolic constituents present in essential oils alter the permeability and break the cell membrane leading to the inhibition of functional properties and leakage of the internal content, leading to cell death [25]. True cardamom and thyme oils showed lower intensity effects on vegetative cells of *C. perfringens.*

The antimicrobial action of essential oils on *C. perfringens* endospores was not as effective. The sporicidal activity for blends of two oils was restricted to combinations containing true cardamom (4% thyme

and 4% true cardamom, 4% clove and 4% true cardamom, and 3.65% oregano and 4% true cardamom) (run 4, 5 and 6). For blends containing three oils, only two combinations were sporicidal: oregano 4.86%, thyme 1.36%, and true cardamom 1.36%; and clove 1.36%, oregano 4.86%, and true cardamom 1.36%. The important presence of true cardamom oil in blends with sporicidal actions is evidenced in the PCA (Figure 1B). Besides the essential oil of true cardamom, clove and oregano contributed to sporicidal activity of blends. The activity of thyme and cinnamon essential oils was less expressive. Although some blends showed sporicidal activity, synergism cannot be affirmed, since the combination of essential oils did not act on the endospores in most of the tests. The difference between MBC and MSC can be explained through the endospore structure of *C. perfringens*, since these have high resistance to several environmental stressors [26].

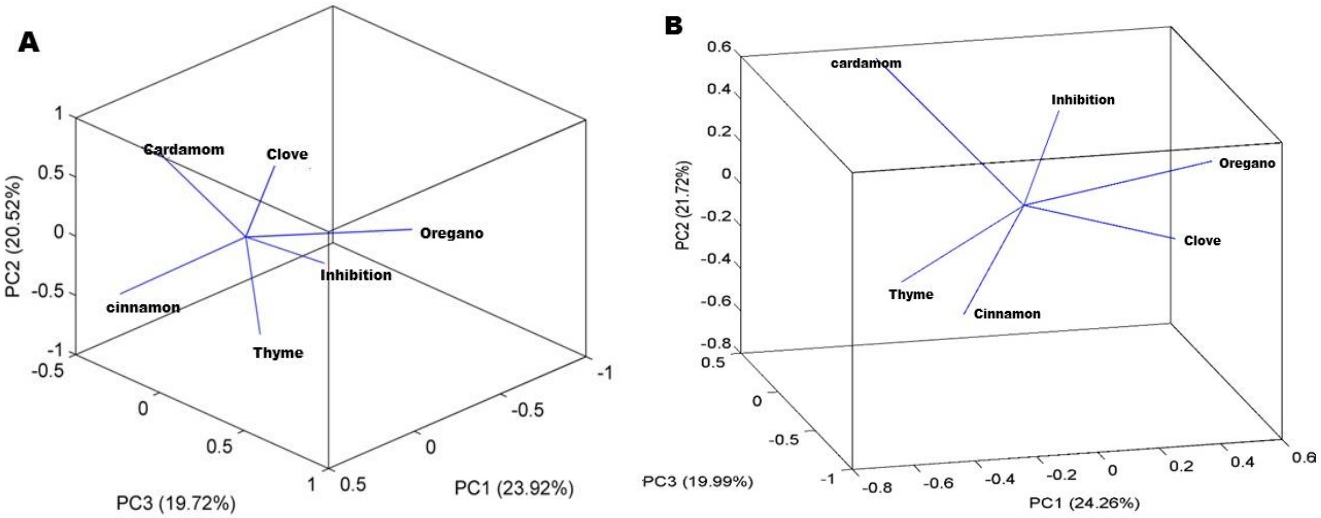

Figure 1. Weights of Principal Component Analysis (PCA) inhibition of *C.perfringens* type A (A) vegetative cells and (B) endospore from different combinations of oregano, thyme, cloves, cardamom, and cinnamon essential oils.

Antimicrobial activity of essential oils on C. perfringens in mortadella

Two blends with bactericidal action were added to the mortadella. The sets were defined by pre-tests (data not shown) with the objective of promoting a pleasant aroma and reducing the sensory impact when applied to the meat product; Treat. 1 containing 0.17, 0.07, and 0.17% of oregano, clove, and thyme, respectively; and Treat. 2 containing 0.17, 0.07, and 0.07% of oregano, clove, and cinnamon, respectively.

Growth of *C. perfringens* on mortadella plus nitrite (75 ppm) and different combinations of essential oils, maintained at 15 °C, was followed up for 21 days. The vegetative cell count was not significantly affected ($P>0.05$) during storage time. However, there was a significant difference ($P<0.05$) among treatments, with a decrease in the CFU/g count in treatments containing essential oils (Table 3) in relation to the control.

Table 3. Vegetative cells of *Clostridium perfringens* (Log UFC/g) in mortadella added with 75 ppm of nitrite and essential oil blends, stored at 15 °C for 21 days.

Treatments	Log CFU/g
CONTROL	6.07 [a]
TREAT. 1	5.59 [b]
TREAT. 2	4.66 [b]

Control: 75 ppm of nitrite; TREAT 1: 75 ppm of nitrite + essential oils of oregano (0.17%), clove (0.07%) and thyme (0.17%); TREAT 2: 75 ppm of nitrite + essential oils of oregano (0.17%), clove (0.07%) and cinammon (0.07%). Averages followed by different letters in the same column differ among themselves by Tukey test at 5% probability.

Nitrite at the concentration of 75 ppm (control), without the addition of essential oils, did not inhibit the growth of *C. perfringens*, showing the highest number of CFU/g (6.07 Log CFU/g) in this treatment (control) during storage. Oliveira and coauthors [10] worked with the same strain used in this study and observed that an addition of 200 ppm of nitrite in the formulation of mortadella, a higher concentration than that used as control in this study, reduced 3 cycles log the population of *C. perfringens* on mortadella.

The blend of oregano, clove and cinnamon oils showed the highest antimicrobial activity, with a reduction of 1.41 Log CFU/g in relation to the control. Although the elimination of *C. perfringens* did not occur, the results suggest the possibility of using essential oils for the control of this microorganism.

Both essential oil blends reduced the growth of *C. perfringens* in the mortadella, however, they were not able to completely inhibit the bacterial growth. Studies with vacuum-packed ground pork showed that oregano essential oil acted in synergism with sodium nitrite (50-100 ppm) inhibited the germination of endospores and growth of *C. botulinum* (type A, B and E) [27]. The synergism between the essential oil of *Satureja montana* and sodium nitrite, leading to growth inhibition of *C. perfringens* inoculated in mortadella, was also observed by Oliveira and coauthors [10].

In general, the efficiency of several natural antimicrobial agents can probably be reduced by certain food components (water, lipids, carbohydrates, and proteins) [28], since the essential oils appear to be stable and maintain their antimicrobial activity even after being subjected to temperatures of 100 °C [29], and that high levels of proteins and/or lipids in food matrices seem to protect microorganisms from the action of essential oils [30]. On the other hand, this study used an inoculum of 10^7 CFU/g, aiming at better detection in cell counts. However, it is known that initial contamination by *C. perfringens* is generally low and the infectious dose is 10^6 CFU/g [2].

Influence of essential oils on lipid oxidation and overall color difference of mortadella

Essential oils showed antioxidant activity, a result observed by the TBARS Index (Table 4).There was no significant interaction between storage time and treatments; however, treatments and time showed a significant influence on the TBARS index. Rancidity can be detected in values above 0.5 mg MDA/kg, emphasizing that the highest value obtained after 21 days of storage was close to a safe level regarding rancidity detection.

Table 4. Effect of nitrite and different combinations of essential oils on TBAR levels of mortadella during storage at 4.4 °C for 21 days

Treatments	(mg MA/Kg)	Storage (days)	(mg MA/Kg)
CONTROL	0.72 ^{a}	1	0.48 ^{a}
TREAT. 1	0.39 ^{b}	7	0.50 ^{ab}
TREAT. 2	0.44 ^{b}	21	0.57 ^{b}

Control: 75 ppm of nitrite; TREAT 1: 75 ppm of nitrite + essential oils of oregano (0.17%), clove (0.07%) and thyme (0.17%); TREAT 2: 75 ppm of nitrite + essential oils of oregano (0.17%), clove (0.07%) and cinammon (0.07%). Averages followed by the same letter in the column did not differ among themselves by Tukey test at 5% probability.

Food color is very important to both consumers and the food industry, being a fundamental parameter used in quality control. This sensory characteristic, although subjective, is also responsible for the induction of the global sensation resulting from other characteristics, such as aroma, flavor and texture of foods. There was significant interaction ($P<0.05$) among treatments and storage time of mortadella for overall color difference (ΔE^*) (Table 5). However, the values between the differences did not reach 3. According to Ramos and Gomide [21] global differences smaller than 3.0 cannot be detected by the human eye.

Table 5. Overall color difference (ΔE^*) in mortadella added with nitrite and different combinations of essential oils, stored at 4.4 °C for 21 days.

	Storage (days)		
Treatments	**1**	**7**	**21**
CONTROL	0^{aA}	1.38^{bA}	1.48^{bA}
TREAT. 1	1.03^{aB}	1.30^{bA}	1.47^{bA}
TREAT. 2	0.56^{aB}	1.36^{bA}	1.68^{cA}

Control: 75 ppm of nitrite; TREAT 1: 75 ppm of nitrite + essential oils of oregano (0.17%), clove (0.07%) and thyme (0.17%); TREAT 2: 75 ppm of nitrite + essential oils of oregano (0.17%), clove (0.07%) and cinammon (0.07%). Averages followed by the same capital letter in the column, and by the same lower case letter in the line there is no difference between them by the Tukey test at 5% probability.

The results found in this study show that there is a bactericidal action of essential oils against *C. perfringens* type A, which is dependent on the chemical composition of each essential oil. Moreover, the difference between MBC and MSC is observed, the latter being a higher concentration showing that the resistance structure of the C. perfringens endospore prevents the penetration of essential oils into the

structure and does not cause enough damage to lead to inactivation of the microorganism. Thus, the association with other conservation technologies needs to be assessed.

Overall, it was observed that the combination of essential oils is synergistic. The effect of the combined oils is greater than the isolated EOs. In fact this is very important as it allows the decrease in concentration required for antimicrobial action. In practice, this helps to reduce sensory changes when essential oils are added to foods and ensures that their use is economically viable for the industry.

Finally, the results showed that the essential oils when applied in the nitrite-reduced mortadella decreased the cell count of *C. perfringens* and also showed antioxidant action, which may help to increase the shelf life of meat products and ensure a safe product for the consumer. Furthermore, the use of essential oils did not impact the color of the meat product, which is very important since coloration is the first aspect observed by the consumer.

Although the results are promising, essential oils can cause a change in the flavor of foods. Thus, studies should be developed in an attempt to reduce the sensory impact and allow consumer acceptance.

CONCLUSION

The blend of oregano, clove and cinnamon oils showed greater activity against vegetative cells of *C. perfringens* Type A;

The essential oil blends reduced the growth of *C. perfringens* in mortadella, showed antioxidant action and did not alter the characteristic food color, thus possessing potential application as a preservative for the meat products industry, proving to be an alternative for partial or total replacement of nitrite.

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