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# Hamburger made with mechanically separated poultry meat and essential oil of oregano or peppermint added as an antioxidant

# Hamburguer elaborado com carne mecanicamente separada de aves e adicionado de óleo essencial de orégano ou hortelã-pimenta como antioxidante

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## Highlights

Hamburgers with oregano and peppermint essential oils have high sensory acceptability.
Hamburgers showed the same microbiological results for coliforms at 45 °C for both EOs.
Hamburgers with synthetic antioxidants have a higher antioxidant capacity.
Coagulase-negative *Staphylococcus* growth only occurred on day zero.

## Abstract

The increased production of mechanically separated poultry meat has led to its use in the production of foods of higher commercial value, such as hamburgers. However, hamburgers are more susceptible to oxidation, therefore, the goal of this study was to evaluate the antioxidant and antibacterial properties of hamburgers made with mechanically separated meat (MSM) from broilers and broiler breeder hens supplemented with oregano (*Origanum vulgare*) or peppermint (*Mentha piperita*). Hamburgers with a synthetic antioxidant and three levels of oregano and peppermint essential oils were evaluated using a sensory acceptance test. An

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inclusion level of 0.04% for both essentials oils was chosen for this test to evaluate the physical-chemical compositions as well as the microbiological and antioxidant activities because an inclusion level of 0.08% received a lower score from the evaluators. Higher pH values were found in hamburgers made with broiler MSM (control (6.94) and peppermint essential oil (6.93) and in broiler breeder hens MSM supplemented with peppermint essential oil (6.89). When comparing the origin of MSM, lower pH values were found in the broiler hamburgers supplemented with oregano essential oils (6.83). The total coliform counts at day zero were significantly higher for the hamburgers made with broiler breeder hens MSM with oregano essential oil, however, broilers MSM supplemented with a synthetic antioxidant showed the lowest count when compared to that of the others. For *Staphylococcus* coagulase-negative bacteria, growth was observed only on day zero, with a low count in hamburgers made with broiler breeder hens MSM and broiler MSM supplemented with synthetic antioxidant or peppermint essential oil. Hamburgers made with MSM from broilers and broiler breeder hens supplemented with synthetic antioxidants showed higher antioxidant capacity with an average of 2378.75 and 2265.43 μmols equivalent Trolox/mg, respectively. Our results suggest that the essential oils of oregano or peppermint can be used in the formulation of hamburgers since they presented high sensory acceptability and reduced contamination.
**Key words:** FRAP. *Mentha piperita. Origanum vulgare.* Sensory acceptance. Total coliforms.

## Resumo

O aumento da produção de carne mecanicamente separada (CMS) de aves levou a indústria a buscar seu uso na produção de produtos de maior valor comercial, como os hambúrgueres. No entanto, os hambúrgueres são mais susceptíveis a oxidação, em vista disso, o objetivo desse trabalho foi avaliar hambúrgueres elaborados com CMS de frangos e galinhas pesadas e adicionados de óleos essenciais de orégano (*Origanum vulgare*) ou hortelã-pimenta (*Mentha piperita*) como antioxidantes e antibacterianos. Para o teste de aceitação sensorial, foi avaliado um antioxidante sintético e mais três níveis de óleos essenciais de orégano e hortelã-pimenta. A partir desse teste, foi escolhido o nível de inclusão de 0,04% de ambos os óleos essenciais para realizar as análises físico-químicas, microbiológica e capacidade antioxidante, uma vez que o nível de 0,08% recebeu a menor nota pelos avaliadores. Em relação ao pH, maior valor foi encontrado nos hambúrgueres elaborados com CMS de frango (controle 6,94) e óleo essencial de hortelã-pimenta (6,93) e CMS de galinhas pesadas e adicionados de óleo essencial de hortelã-pimenta (6,89). Ao comparar individualmente a origem da CMS, menor valor de pH foi encontrado para o hambúrguer de frango adicionado de óleo essencial de orégano (6,83). As contagens de coliformes totais, no dia zero, foi significativamente maior para os hambúrgueres elaborados com CMS de galinhas pesadas adicionados de óleo essencial de orégano, porém, a CMS de frangos acrescida de antioxidante sintético apresentou a menor contagem quando comparado aos demais. Já para *Staphylococcus* coagulase negativa, houve crescimento apenas no dia zero, com baixas contagens nos hambúrgueres elaborados com CMS de galinhas pesadas e de frangos adicionados de antioxidante sintético ou óleo essencial de hortelã-pimenta. Hambúrgueres elaborados com CMS de frangos e galinhas pesadas adicionados de antioxidante sintético apresentaram maior capacidade antioxidante, uma vez que apresentaram média de 2378,75 e 2265,43 μmols equivalente Trolox/mg respectivamente para frangos e galinhas pesadas. Conclui-se que os óleos essenciais de orégano ou hortelã-pimenta podem ser utilizados na formulação dos hambúrgueres, uma vez que apresentam boa aceitabilidade e reduzem as contaminações.
**Palavras-chave:** Aceitabilidade sensorial. Coliformes totais. FRAP. *Mentha piperita. Origanum vulgare.*

## Introduction

The Brazilian poultry sector is well-known around the world for the production and export of broiler chicken meat. In 2021, the country produced 14.329 million tons and remained the world's third largest producer and exporting 4.610 million tons in the same year (Associação Brasileira de Proteína Animal [ABPA], 2022). In addition, the search for protein sources of available animal origin at a low cost has boosted meat science and technology research, including the use of culled chickens (Libório et al., 2018). In this context, it can be mentioned its use in the) in hamburger preparation.

According to Espíndola (2012), the high demand for chicken meat consumption is justified by the combination of its lower price when compared to beef and its capacity to convert cereals into meat in the shortest possible breeding time. Furthermore, because the processing of chicken meat generates several byproducts considered waste by the industry, the parts with the lowest cut value, such as the neck and back, are abundant and can be used to develop new products for consumption or as an alternative ingredient for existing products (Sposito, 2014), such as mechanically separated meat (MSM).

MSM capitalizes on the loss of meat portions, that would otherwise be wasted, thereby assisting the economy and serving as a great source of proteins and allowing for the improvement of several food products, such as sausages and breaded meat (Santos et al., 2020).

According to the Normative Instruction n° 4 of March 31, 2000, MSM is derived from the process of grinding and separating meat from animal bones and is used as an ingredient for the preparation of specific products (Ministério da Agricultura Pecuária e Abastecimento [MAPA], 2000). MSM is obtained primarily from parts of the carcass such as the neck, back and remaining pieces of boning (Real et al., 2018).

Processed products, such as hamburgers, are susceptible to oxidation, mainly due to the lipids contained in MSM, making it necessary to use antioxidants (Gomes et al., 2016) to slow the deterioration, rancidity, and loss of color (Real et al., 2018).

However, the use of some synthetic antioxidants in food has been a challenge due to their instability and potential toxic and carcinogenic effects on human health to the point where several countries have restricted or banned their use, this has led consumers and the industry to seek alternatives to these synthetic additives, including the use of natural antioxidants (Leão et al., 2017).

According to Pires et al. (2017), natural product studies have been explored since the 1980s for the prevention or extension of food shelf life. Jayasena and Jo (2014) investigated the potential use of essential oils, such as oregano essential oil, as natural antioxidants in meat by-products. In addition, due to the antimicrobial characteristics of peppermint (*Mentha piperita* L.) and its use as a condiment in Brazil (Radaelli et al., 2016), it was evaluated in the present study.

This study aimed to evaluate the antioxidant effects of essential oils from oregano (*Origanum vulgare* L.) or peppermint (*Mentha piperita* L.) in hamburgers made of MSM from broilers and broiler breeder hens, as well as the product acceptability of these essential oils.

## Material and Methods

Hamburgers were produced from MSM of broilers and broiler breeder hens from a slaughterhouse under the supervision of the Federal Inspection Service (SIF, Portuguese) located in the northwest region of Paraná.

The hamburger formulations were adapted from C. E. Silva (2013), and their compositions are listed in Table 1. After preparation, the hamburgers were frozen -15°.C for up to two weeks, until sensory acceptance tests were carried out.

### *Hamburger development for sensory analysis*

**Table 1**
**Percentage of ingredients used in the preparation of hamburgers - control, oregano EO (0.04%, 0.06% and 0.08%) and peppermint EO (0.04%, 0.06% and 0.08%)**

| Ingredients | Control | EO 0.04% | EO 0.06% | EO 0.08% |
|---|---|---|---|---|
| Mechanically separated meat | 30% | 30% | 30% | 30% |
| Chicken thigh and over thigh | 44.75% | 44.81% | 44.79% | 44.77% |
| Hydrogenated vegetable fat | 10% | 10% | 10% | 10% |
| Textured soy protein | 4% | 4% | 4% | 4% |
| Salt | 1.25% | 1.25% | 1.25% | 1.25% |
| Water | 8.5% | 8.5% | 8.5% | 8.5% |
| Garlic powder | 1% | 1% | 1% | 1% |
| Chilli powder | 0.4% | 0.4% | 0.4% | 0.4% |
| Sodium erythorbate | 0.1% | --- | --- | --- |
| Oregano or Peppermint EO | | 0.04% | 0.06% | 0.08% |

EO: Essential Oil.

### *Sensory acceptance test*

To evaluate sensory acceptance, a total of 480 hamburgers of approximately 30 g each were prepared: 240 with oregano essential oil (EO) (*O. vulgare* L.) and 240 with peppermint EO (*M. piperita* L.). The selection criteria for tasters (30 volunteers, students and employees) at the Universidade Paranaense, Umuarama Campus, Paraná, included consuming meat products and enjoying chicken meat. The number of tasters was defined based on the work conducted by Verruma-Bernardi et al. (2010). The tasters were provided with a Free and Informed Consent Term that was read and signed before the analyses were carried out after approval by the Ethics and Research Committee Involving Human Experimentation (CEPEH) of the Universidade Paranaense (number CAAE 58204316.7.0000.0109).

For the test, the seven-point structured hedonic scale was used, ranging from “I disliked it a lot” (grade 1) to “I liked it a lot” (grade 7) adapted by Tavares et al. (2007). For each test, there was a standard hamburger formulated with synthetic antioxidants (0.1% sodium erythorbate) and three levels of oregano EO (*O. vulgare* L.) or peppermint (*M. piperita* L.) (0.04%, 0.06% and 0.08%) prepared with MSM from broilers or broiler breeder hens. The essential oils of oregano and peppermint were purchased from Laszlo, São Paulo, SP, Brazil.

### *Definition of the inclusion level of essential oils*

After defining the inclusion level of natural antioxidants (oregano and peppermint EOs) based on the sensory acceptance test, hamburgers were prepared considering two origins of MSM (broiler chickens and broiler breeder hens) and three antioxidants (synthetic - sodium erythorbate, natural - oregano EO and natural peppermint EO).

These hamburgers were evaluated for antioxidant capacity after seven days of storage at -20 ºC, physical-chemical analyses after the preparation of the hamburgers, and microbiological analysis at times zero, seven, 15, 30, and 60 days of storage at -20 ºC.

### *Determination of the total antioxidant capacity of hamburgers – by ferric reducing antioxidant power (FRAP) assay*

To determine the total antioxidant capacity of the hamburgers using the FRAP assays, four hamburgers samples (24 samples total) from each treatment were analyzed (two sources of MSM (broilers and broiler breeder hens) and three antioxidants (synthetic - sodium erythorbate, natural oregano EO and natural peppermint EO) after storage for seven days at -20 ºC.

The samples were thawed at a refrigerated temperature 4o.C for approximately 12 h, and the antioxidant activity was analyzed.

Using a FRAP assay carried out according to the methods described by Pulido et al. (2000) and Benzie & Strain (1996) and adapted for hamburgers following the methodology described by Fachinello et al. (2018).

### *Physical-chemical analysis of hamburgers*

After defining the inclusion level of essential oils, the percentage of dry matter, crude protein, ash and pH in the samples were analyzed according to the methodology described by the Instituto Adolfo Lutz [IAL] (2008).

### *Microbiological analysis of hamburgers*

Total coliforms, coliforms at 45 ºC, *Salmonella* spp., and coagulase-negative *Staphylococcus* strains were evaluated, according to the methods described by N. Silva et al. (1997). Three samples from each treatment were analyzed at time zero, with 18 samples for each analysis, totaling 72 samples. In addition, at times seven, 15, 30, and 60 days of storage, a sample of hamburger from each treatment group was analyzed.

*Statistical analysis of data*

For the analysis of sensory acceptance, statistical analysis was performed using the *Kruskal-Walli's* test. When the results were significant, the means were compared using the *Student-Newman-Keuls* test at a 5% significance level. After defining the level of inclusion of oregano and peppermint EOs, statistical analysis was performed considering two different types of MSM (broiler and broiler breeder hens), two types of aromatic herbs (oregano and mint EOs) and a control group (with a synthetic antioxidant). All results were evaluated using factorial variance analysis, using the statistical program BioEstat 5.0 (Ayres et al., 2007), and means were compared using the Tukey test with a significance level of 5%.

## Results and Discussion

The sensory acceptance test was performed with three levels of inclusion (0.04%, 0.06% and 0.08%) of oregano or peppermint EO in hamburgers made with mechanically separated meat from broilers or broiler breeder hens. The lowest grade for the oregano EO was given to the hamburgers made with MSM from broiler hens and the highest level (0.08%) of oregano EO ($P<0.05$) when compared to that of the other levels with the exception of hamburgers made with MSM from broiler breeder hens and the highest level of oregano. There were no significant differences in sensory acceptance for peppermint EOs at different levels (Table 2). Thus, the lowest inclusion level was chosen for both EOs in other tests (physical-chemical, microbiological, and antioxidant tests).

When comparing the results of the sensory acceptability test for each source of MSM (broiler or broiler breeder hen) separately, hamburgers made with 0.08% oregano EO showed less acceptability compared to the others, however, this difference was not observed in hamburgers made with broiler breeder hen MSM. These results demonstrate that the level of oregano EO inclusion can interfere with product acceptability and the origin of MSM can influence the acceptability of broiler breeder hens.

EOs have several functions that provide numerous benefits, such as antibacterial, antifungal and antioxidant effects. *M. piperita* EO is composed mainly of two phytochemical constituents, menthol and mentone (Souza et al., 2021), which are responsible for its refreshing taste. The inclusion of peppermint EO, even at low doses, may have accentuated the flavor, making it difficult for tasters to give a different grade. However, it demonstrated that the acceptability was high, since the grade was close to five or above, which is close to the "I liked it slightly" grade.

**Table 2**
**Preference test for the inclusion of three levels (0.04%; 0.06% and 0.08%) of essential oil of oregano or peppermint with students and employees of the Veterinary Hospital of University Paranaense**

| Treatments | Oregano | Peppermint* |
|---|---|---|
| Control Broiler | 5.53[a] | 5.52 |
| Broiler 0.04 EO | 5.03[a] | 5.38 |
| Broiler 0.06 EO | 5.57[a] | 5.67 |
| Broiler 0.08 EO | 3.97[b] | 5.00 |
| Control Hen | 5.67[a] | 4.90 |
| Hen 0.04 EO | 5.77[a] | 5.85 |
| Hen 0.06 EO | 5.47[a] | 5.55 |
| Hen 0.08 EO | 4.86[ab] | 5.05 |

Means followed by different letters in the column differ by the SNK test (P<0.05)
* Not significant according to the *Kruskal-Wallis* test (P>0.05).
EO: Essential oil.

Pires et al. (2017) compared the sensory acceptability of broilers hamburgers with high doses of natural antioxidants made with 0.10%, 0.15% and 0.048% green tea extracts from Camellia sinensis and did not find differences between them, however, the sensory acceptability differed from the sample with the synthetic antioxidant butylhydroxyanisole (BHA). Hamburgers made with 0.093% rosemary extract (*Rosmarinus officinalis*) showed similar acceptability to those made with the synthetic antioxidant BHA. These results demonstrate that acceptability differs not only in the levels of inclusion, but also in the origin of the plant used, whether in the form of extracts or essential oils.

The results of the physical-chemical analysis demonstrated that there were no differences in the percentages of moisture, ash and crude protein between the hamburgers (Table 3).In accordance with Ordinance SDA No. 724, of December 23, 2022, regarding the Technical Regulation of Hamburger Identity and Quality, some physicochemical parameters have minimum and maximum values established for fat and total carbohydrates of 25% and 3%, respectively, at maximum allowed, and a minimum content of 15% protein (MAPA, 2022). In this study, the fat content showed variable results, therefore, the results are not presented in Table 3. However, despite the variability, the results were between the limits required by the Brazilian legislation for hamburgers.

Moisture, ash and pH do not have standard values established for MSM in the current legislation. The data obtained in this study can serve as a resource for the standardization of physical-chemical parameters. Ash levels are indicative of the functional properties of some food products; therefore, they are a useful parameter for determining the nutritional value of some foods. Ash is composed of potassium, sodium, calcium, magnesium, aluminum, iron, copper, manganese and zinc at higher concentrations (Gonçalves et al., 2009).

**Table 3**
**Mean ± standard error of the percentage of dry matter (%), ash (%), crude protein (%) and the pH of hamburgers made with mechanically separated poultry meat and added essential oil of oregano or peppermint (control broiler, broiler + essential oil of oregano, broiler + essential oil of peppermint, hen control, hen + essential oil of oregano and hen + essential oil of peppermint)**

| Treatments | Moisture | Ashes * | Crude protein* | pH |
|---|---|---|---|---|
| Control Broiler | 35.81±0.36 | 1.91±0.09 | 13.04±0.19 | 6.94±0.01[a] |
| Broiler + oregano EO | 33.51±0.41 | 2.09±0.07 | 12.88±0.20 | 6.83±0.01[c] |
| Broiler + peppermint EO | 35.48±0.88 | 1.88±0.11 | 13.03±0.18 | 6.93±0.01[a] |
| Control Hen | 35.95±0.25 | 2.17±0.25 | 14.64±1.10 | 6.86±0.02[bc] |
| Hen + oregano EO | 35.02±1.25 | 2.06±0.27 | 14.84±0.39 | 6.82±0.01[c] |
| Hen + peppermint EO | 35.81±1.01 | 2.13±0.18 | 14.49±0.26 | 6.89±0.01[ab] |

EO: Essential oil included in the concentration of 0.04%.
* Not significant according to ANOVA.
Means followed by different letters differ by the Tukey's test ($P<0.05$).

Pires et al. (2017) found an average amount of 2.21% ash in chicken hamburgers, which is consistent with the results of this study.

Higher pH values were observed in hamburgers made with broiler MSM (control and peppermint EO) and broiler breeder hen MSM supplemented with peppermint EO, when compared to those made with broiler MSM and oregano EO, broiler breeder hen control and broiler breeder hen MSM with oregano EO (Table 3).

Similar pH values in broiler MSM were found by Sposito (2014); the pH varied between 6.20 and 5.97 in MSM samples containing 0.5% and 3.0% acerola in powder, respectively, at 45d of storage at -18 °C. Bigolin et al. (2014) compared the pH values in relation to the shelf life of chicken MSM and found a variation between 6.04 and 6.54 on the 1st and 5th day of shelf life, respectively.

In the present study, when comparing the pH separately for each type of MSM (broiler or broiler breeder hens) a lower pH was obtained for hamburgers made with broiler MSM supplemented with oregano EO (pH 6.83) compared to that of the others (Table 3). A lower pH was also found for the hamburgers made with broiler breeder hen MSM supplemented with oregano EO (pH 6.82), but the result did not differ for the hamburgers made with synthetic antioxidants (broiler breeder hen control - pH 6.86).

The pH of food not only has a direct impact on the rate of microorganisms multiplication, but also interferes with the intrinsic quality of food during storage, heat treatment or any other type of treatment, in other words, pH is directly responsible for the deterioration of food products (Sposito, 2014). However, there is no specific legislation for pH.

The total coliform count in hamburgers evaluated on day zero was significantly higher for those made with broiler breeder hen MSM and supplemented with oregano EO than for those made with broiler MSM and supplemented with peppermint EO (Table 4). The hamburgers made with broiler MSM supplemented with synthetic antioxidant showed the lowest total coliforms count compared to those of the other hamburgers. However, after seven and 15d of storage at -20°C all hamburgers made with broiler MSM had higher total coliform counts than those made with broiler breeder hen MSM (Table 4).

The sanitary quality of products intended for consumption is critical to ensure that they do not endanger consumer health (Vieira, 2012). When product quality is compromised, some changes become noticeable, such as changes in the color, flavor and texture of the product, and the production of compounds with toxic potential (Bigolin et al., 2014).

**Table 4**
**Mean ± standard error of $\log_{10}$ of total coliforms, coliforms at 45 ºC and coagulase-negative *Staphylococcus* on days zero, seven and 15 of stocking broiler and hen MSM hamburgers with or without 0.04% oregano EO or 0.04% peppermint EO as an antioxidant**

| Treatments | Total coliforms CFU $g^{-1}$ | Coliforms at 45 ºC CFU $g^{-1}$ | Coagulase-negative *Staphylococcus* CFU $g^{-1}$ |
|---|---|---|---|
| | | Day zero | |
| Control Broiler | 2.34±0.09$^{c}$ | 2.53±0.19 | 3.91±0.07$^{bc}$ |
| Broiler + oregano EO | 2.77±0.06$^{ab}$ | 2.33±0.17 | 4.37±0.10$^{a}$ |
| Broiler + peppermint EO | 2.71±0.07$^{b}$ | 2.46±0.18 | 3.91±0.03$^{bc}$ |
| Control Hen | 2.81±0.07$^{ab}$ | 1.98±0.07 | 3.72±0.09$^{bc}$ |
| Hen + oregano EO | 3.07±0.07$^{a}$ | 2.49±0.12 | 4.14±0.06$^{ab}$ |
| Hen + peppermint EO | 2.82±0.07$^{ab}$ | 2.46±0.04 | 3.66±0.17$^{c}$ |
| | | Seven days | |
| Control Broiler | 2.06±0.10$^{ab}$ | 2.10±0.15$^{a}$ | 0.00 |
| Broiler + oregano EO | 2.32±0.09$^{a}$ | 1.42±0.42$^{ab}$ | 0.00 |
| Broiler + peppermint EO | 2.40±0.15$^{a}$ | 2.11±0.25$^{a}$ | 0.00 |
| Control Hen | 1.46±0.06$^{cd}$ | 0.00±0.00$^{b}$ | 0.00 |
| Hen + oregano EO | 1.30±0.12$^{d}$ | 1.30±0.30$^{ab}$ | 0.00 |
| Hen + peppermint EO | 1.81±0.05$^{bc}$ | 1.80±0.80$^{ab}$ | 0.00 |
| | | 15 days | |
| Control Broiler | 2.33±0.07$^{ab}$ | 0.00 | 0.00 |
| Broiler + oregano EO | 2.10±0.09$^{abc}$ | 0.76±0.48 | 0.00 |
| Broiler + peppermint EO | 2.38±0.06$^{a}$ | 0.52±0.30 | 0.00 |
| Control Hen | 1.89±0.04$^{cd}$ | 0.00 | 0.00 |
| Hen + oregano EO | 2.04±0.07$^{bc}$ | 0.96±0.33 | 0.00 |
| Hen + peppermint EO | 1.94±0.07$^{c}$ | 1.08±0.39 | 0.00 |

EO: Essential oil included in the concentration of 0.04%.
* Not significant according to ANOVA.
Means followed by different letters differ by the Tukey's test (P<0.05).

There is no specific legislation for total coliform counts in meat products or by-products. However, high counts can provide an indication of the hygienic-sanitary quality of the product (Muniz et al., 2017) and could lead to a reduction in hamburger shelf life.

At 45°C, there were no differences in coliforms between treatments at day zero and at 15 days of storage. However, with seven days of storage, only hamburgers made with broiler breeder hen and supplemented with synthetic antioxidant did not show growth. In hamburgers that showed growth, no differences were detected between the treatments (Table 4).

It is noteworthy that the thermotolerant group of coliforms at 45°C differs from the total coliforms as a function of the ideal growth temperature, as the bacteria belonging to the thermotolerant group grow at temperatures between 44 and 45°C. The main representative of thermotolerant bacteria is *Escherichia coli* (N. Silva et al., 2017). According to Normative Instruction n°60, of December 23, 2019, regarding microbiological standards for food, and specifically for hamburgers made with poultry meat, the limit allowed for *E. coli* is up to $5x10^2$ CFU $g^{-1}$ (Agência Nacional de Vigilância Sanitária [ANVISA], 2019a). The results obtained in the present study show that despite the presence of coliforms at 45°C, all hamburger samples were within the required standards, since the highest count was 2.53 CFU $g^{-1}$ for the control group of broilers MSM on day zero of storage (Table 4).

For coagulase negative *Staphylococcus,* growth was only observed on day zero (Table 4) with a lower count (P<0.05) for hamburgers made with broiler or broiler breeder hen MSM supplemented with synthetic antioxidants or peppermint EO compared to those made with broiler or broiler breeder hen MSM supplemented with oregano EO. These results indicate that natural antioxidants are efficient in reducing MSM contamination by coagulase-negative *Staphylococcus,* with greater efficacy of peppermint EO.

According to the new legislation IN n°60 on December 23, 2019, there is only specific legislation for coagulase-positive *Staphylococcus,* where, for meat products prepared with poultry meat, semi-prepared, seasoned or not, breaded, chilled or frozen, the allowed limit for coagulase-positive *Staphylococcus* is $5x10^2$ CFU $g^{-1}$ (ANVISA, 2019a). None of the hamburger samples analyzed contained isolated coagulase-positive *Staphylococcus.* Although the current legislation does not specify limits for the presence of coagulase-negative *Staphylococcus* in poultry meat, several authors have reported its presence in broiler carcasses.

According to Wang et al. (2018), some species of coagulase-negative *Staphylococcus* present in meat are classified as potential pathogenic agents and are considered important transmitting agents of diseases because they can transmit toxins and antibiotic resistance factors from other bacteria.

Menezes et al. (2018) analyzed chicken carcasses produced in ten different industries in the state of Minas Gerais and found high counts of *Staphylococcus* spp. Fifty seven carcasses (23.8%) were characterized as positive for coagulase, and the others 183 carcasses (76.2%) were characterized as negative for coagulase. Gabaron et al. (2020) analyzed the carcasses of colonial broilers for the presence of microorganism indicators of contamination and verified the presence of coagulase-negative *Staphylococcus* in 12 (20%) of the 60 carcasses. One of the samples was from cloacae swabs, two were from breast swabs and nine swabs were from the backs of the carcasses.

Valeriano et al. (2012) tested the antimicrobial capacity of several essential oils against enteropathogenic *E.coli*, *Salmonella* Enteritidis, *Listeria monocytogenes*, and *Enterobacter sakazakii* and found that the essential oil of *M. piperita* has higher antimicrobial capacity for *E. coli*, moderate capacity for *Salmonella* Enteritidis and *E. sakazakii*, and low activity for *L. monocytogenes.*

Desam et al. (2019), in Saudi Arabi, found an inhibitory effect on the growth of *Mentha piperita* L. EO against gram positive (*Staphylococcus aureus*, *Micrococcus flavus*, *Bacillus subtilis*, and *Staphylococcus epidermidis*) and gram negative (*Salmonella* Enteritidis) and Marwa et al. (2017) in Morocco, found an inhibitory effect with a concentration of 0.25% of *M. piperita* EO in relation to gram positive bacteria (*S. aureus* and *B. cereus*), when applied to food.

K. B. Silva and Mello (2021) evaluated the antibacterial activity of three different essential oils (*M. piperita*, *Mentha arvensis* and *Ocimum basilicum*), both pure and diluted, against strains of *S. aureus* and *S. enterica.* They found that S. aureus presented sensitivity only for pure basil essential oil (*Ocimum basilicum*) while *S. enterica* was sensitivity to essential oils of peppermint (*M. piperita*) and Japanese mint (*M. arvensis*) both in pure and diluted form (1:2), while for basil essential oil, the bacteria presented sensitivity only in pure form.

All hamburgers had no *Salmonella* spp. at any of the evaluated times (0, seven and 15d of storage).

After seven days of storage, higher antioxidant capacity was verified for hamburgers made with broiler and broiler breeder hens MSM supplemented with synthetic antioxidant, compared to that of the other hamburgers (Table 5).

**Table 5**
**Average antioxidant capacity (µmols equivalent Trolox/mg) of hamburgers made with broiler and hen MSM with or without 0.04% oregano EO or 0.04% peppermint EO as an antioxidant after seven days of storage**

| Treatment | µmols equivalent Trolox/mg |
| --- | --- |
| Control Broiler | 2378.75$^{a}$ |
| Broiler peppermint EO | 319.14$^{c}$ |
| Broiler oregano EO | 319.14$^{c}$ |
| Control Hen | 2265.43$^{b}$ |
| Hen peppermint EO | 293.44$^{c}$ |
| Hen oregano EO | 299.90$^{c}$ |

Means followed by different letters differ by the Tukey test ($P < 0.05$)
MSM: Mechanically separated meat.
EO: Essential oil.

The synthetic antioxidant (sodium erythorbate) is approved for use in meat and meat products (ANVISA, 2019b) with a maximum limit in 100 grams (*quantum satis*). In this study, 0.01% sodium erythorbate had a high antioxidant capacity and was chosen because it is the amount used by some meat processing industries.

Antioxidants needs to be added in meat products and by-products, as they are susceptible to oxidation and, according to Leão et al. (2017), have been associated as the main non-microbiological cause of quality deterioration during meat product processing.

The use of essential oils from aromatic plants has not been recommended, however, their antioxidant potential, which is mainly associated with the presence of phenolic compounds that act as scavengers of free radicals and hydrogen donors to prevent lipid oxidation, justifies their application in meat products and byproducts (Jayasena & Jo, 2014). Considering the adverse effects of synthetic antioxidants, food manufacturers are increasingly looking for alternatives with high antioxidant capacity, such as essential oils, which contain a number of important phenolic compounds and natural antioxidants that can be found in herbs, spices and fruits (Leão et al., 2017).

Oliveira (2017) compared the antioxidant capacities of essential oils from allspices (*Pimenta dioica* Lindl.), baleera (*Varronia curassavica* Jacq.) and clove (*Eugenia caryophyllata* Thunb.) with the synthetic antioxidants added in fresh chicken sausage and verified that the natural antioxidants, especially the oils obtained from allspice and clove were effective in delaying lipid oxidation because they had a very high index of antioxidant activity.

Prete et al. (2020) found that the content of phenolic compounds in oregano EO was 340.12 μg EAG (Equivalent gallic acid)/mL and Raeisi et al. (2019) found 179.58 mg of EAG $g^{-1}$ in *M. piperita* essential oil. Although no differences in antioxidant capacity were found between the hamburgers made with oregano EO and those made with peppermint EO, the lower total coliform counts for the hamburgers made with oregano EO and broiler breeder hen MSM after seven and 15 days of storage (Table 4) may be associated with differences in the number of phenolic compounds.

The lower antioxidant capacity observed for hamburgers made with oregano or peppermint EO (Table 5) in relation to the synthetic antioxidant may be associated, according to Jayasena and Jo (2014) to the interaction of some essential oils with food ingredients or structures, which could reduce their activity. The essential oils may interact with the lipid content of the food, causing its activity to be reduced.

However, the adverse effects of synthetic antioxidants on human health have been reported in the literature, such as carcinogenic effects (Leão et al., 2017) which justifies the search for natural antioxidants by the food industry, such as those evaluated in the present study. However, a higher dosage of the natural antioxidant may be needed in order to show similar results to the synthetic antioxidant, which may make its use unfeasible because of the change in the flavor of hamburgers made with broiler or broiler breeder hen MSM.

## Conclusion

The EO of oregano (*O. vulgare* L.) and peppermint (*M. piperita* L.) can be used in the formulation of hamburgers made with broiler and broiler breeder hens MSM, as they have good acceptability and reduced contamination. However, further studies are needed to assess the oxidative stability during longer storage times.

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