REVISTA CARIBEÑA DE CIÊNCIAS SOCIALES

# Inhibition of *Salmonella* enteritidis in homemade mayonnaise using oregano essential oil (*Origanum vulgare*)

# Efeito do óleo essencial de orégano (*Origanum vulgare*) sobre *Salmonella* enteritidis em maionese caseira

# Efecto del aceite esencial de orégano (*Origanum vulgare*) sobre *Salmonella* enteritidis en mayonesa casera

DOI: 10.55905/rcssv13n3-004

Received on: Feb 05th, 2024
Accepted on: Mar 04th, 2024

**Bianca Silva Pacheco**
Graduate in Food Technology
Institution: Instituto Federal de Educação, Ciência e Tecnologia do Maranhão
Address: R. Palestina, Q.L n. 10, Anjo da Guarda, São Luís – MA
E-mail: biancapacheco290@gmail.com

**Daniela Aguiar Penha Brito**
Doctor in Animal Science
Institution: Instituto Federal de Educação, Ciência e Tecnologia do Maranhão
Address: Av. dos Curiós, s/n, Vila Esperança, São Luís – MA
E-mail: daniela.brito@ifma.edu.br

**Josilene Lima Serra**
Doctor in Biotechnology
Institution: Instituto Federal de Educação, Ciência e Tecnologia do Maranhão
Address: Av. dos Curiós, s/n, Vila Esperança, São Luís – MA
E-mail: josilene.serra@ifma.edu.br

**ABSTRACT**

The potential of oregano essential oil (OEO) as a natural antimicrobial agent to mitigate *Salmonella* spp. contamination in homemade mayonnaise made from raw eggs should be explored. This study aimed to assess the viability of *Salmonella* Enteritidis in homemade mayonnaise supplemented with OEO while also examining key quality parameters, including pH, titratable acidity, and the count of mesophilic aerobic microorganisms. *In vitro* experiments determined the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of OEO against two strains of *Salmonella* Enteritidis and one of *Salmonella* Typhimurium. Subsequently, homemade mayonnaise, intentionally contaminated with a *Salmonella* Enteritidis strain, was prepared, and OEO was added at concentrations of 0.5% and 1.0%. Bacterial plate counts for *Salmonella* spp. and mesophilic microorganisms, as well as acidity levels, were assessed at 0, 4, 8, and 24 hours of incubation at 30°C. The results indicated the *in vitro* efficacy of OEO, with an MIC of 0.5% and an MBC of 0.0625% against all strains. However, in homemade mayonnaise, *Salmonella* counts averaged 6.0 log CFU/g over the 24 hours at 30°C. Mesophilic microorganism counts also averaged 6.0 log CFU/g over 24 hours, similar to those of *Salmonella*. The pH values ranged from 4.5 to 5.1, and acidity levels remained between 0.31% and 0.39% throughout the 24 hours. In conclusion, oregano essential oil exhibited a bacteriostatic effect, effectively limiting the growth of *Salmonella* Enteritidis in homemade mayonnaise at the evaluated concentrations and time intervals.

**Keywords:** eggs, pH, low acidity mayonnaise, mesophilic microorganisms, antimicrobial.

**RESUMO**

O uso do óleo essencial de orégano como antimicrobiano natural pode ser uma alternativa para reduzir a contaminação por *Salmonella* spp. em maionese caseira à base de ovos crus. Objetivou-se avaliar a sobrevivência de *Salmonella* Enteritidis em maionese caseira adicionada de óleo essencial de orégano. Examinou-se ainda as características de qualidade como pH, acidez titulável e contagem de microrganismos aeróbios mesófilos. Foi testado *in vitro* o OEO pelos testes de Concentração Inibitória Mínima (CIM) e Concentração Bactericida Mínima (CBM) sob duas cepas de *Salmonella* Enteritidis e uma de *Salmonella* Typhimurium. Foi preparada uma maionese caseira contaminada com cultura de uma cepa de *Salmonella* Enteritidis sendo adicionadas as concentrações de 0.5% e 1.0 % de OEO. Foram avaliadas as contagens bacterianas em placas para *Salmonella* spp. e microrganismos mesófilos e acidez presente nas maioneses durante 0, 4, 8 e 24 horas de incubação a 30°C. Os resultados apontaram efetividade *in vitro* do OEO, com CIM de 0,5% e CBM de 0,0625% em todas as cepas. Porém, em maioneses caseiras, observou-se contagens médias de 6.0 log UFC/g no período de 0 a 24 horas a 30°C. As contagens médias de microrganismos aeróbios mesófilos foram de 6.0 log UFC/g ao longo de 24 horas, semelhantes às de *Salmonella*. O pH foi de 4.5 a 5.1 e acidez de 0.31% a 0.39% durante 24 horas. Conclui-se que o óleo essencial de orégano limitou o crescimento de *Salmonella* Enteritidis em maioneses caseiras nas concentrações e tempos avaliados, exercendo um efeito bacteriostático.

**Palavras-chave:** ovos, pH, maionese de baixa acidez, microrganismos mesófilos, antimicrobiano.

**RESUMEN**

Es necesario explorar el potencial del aceite esencial de orégano (OEO) como agente antimicrobiano natural para mitigar la contaminación *por Salmonella* spp. en la mayonesa casera elaborada con huevos crudos. El objetivo de este estudio era evaluar la viabilidad de *Salmonella* Enteritidis en mayonesa casera suplementada con OEO, al tiempo que se examinaban parámetros de calidad clave, como el pH, la acidez titulable y el recuento de microorganismos aerobios mesófilos. Los experimentos *in vitro* determinaron la concentración inhibitoria mínima (MIC) y la concentración bactericida mínima (MBC) de OEO contra dos cepas de *Salmonella* Enteritidis y una de *Salmonella* Typhimurium. Posteriormente, se preparó mayonesa casera, contaminada intencionadamente con una cepa de *Salmonella* Enteritidis, y se añadió OEO a concentraciones del 0,5% y el 1,0%. Se evaluaron los recuentos bacterianos en placa de *Salmonella* spp. y microorganismos mesófilos, así como los niveles de acidez, a las 0, 4, 8 y 24 horas de incubación a 30ºC. Los resultados indicaron la eficacia *in vitro* de la OEO, con una CMI del 0,5% y una CBM del 0,0625% contra todas las cepas. Sin embargo, en la mayonesa casera, los recuentos de *Salmonella* alcanzaron una media de 6,0 log UFC/g durante las 24 horas a 30°C. Los recuentos de microorganismos mesófilos también alcanzaron una media de 6,0 log UFC/g a lo largo de 24 horas, similares a los de *Salmonella*. Los valores de pH oscilaron entre 4,5 y 5,1, y los niveles de acidez se mantuvieron entre 0,31% y 0,39% a lo largo de las 24 horas. En conclusión, el aceite esencial de orégano mostró un efecto bacteriostático, limitando eficazmente el crecimiento de *Salmonella* Enteritidis en mayonesa casera a las concentraciones e intervalos de tiempo evaluados.

**Palabras clave:** huevos, pH, mayonesa de baja acidez, microorganismos mesófilos, antimicrobiano.

## 1 INTRODUCTION

The rise of food industrialization has led to the proliferation of new dietary trends, including increased consumption of processed foods and fast food (BALEM *et al*., 2012). These convenient food options are readily available in cafeterias, restaurants, and from street vendors (BEZERRA *et al*., 2027). Among the various condiments used in these establishments, homemade mayonnaise has gained popularity over the years, surpassing its industrial counterpart (SANT'ANA, 2017).

Homemade mayonnaise is an emulsion prepared from raw eggs and vegetable oil, typically made under cold conditions, featuring low acidity levels. However, these characteristics also pose a risk of transmitting food-borne pathogens, such as *Salmonella* spp. (KEERTHIRATHNE *et al*., 2016). According to data from the Ministry of Health, eggs and egg-based products ranked fifth among the types of food associated with foodborne outbreaks in Brazil from 2009 to 2018 (BRASIL, 2019). Risk factors include contaminated eggs combined with improper storage practices commonly seen in fast food establishments, which create favorable conditions for bacterial growth and infection (BARCELOS *et al*., 2016).

*Salmonella* spp. ranks among the most concerning bacteria for global public health, being one of the leading causes of diarrheal diseases worldwide (WHO, 2018). In the United States alone, *Salmonella* is responsible for an estimated 1.35 million infections, resulting in 26,500 hospitalizations and 420 deaths (CDC, 2023). Typical symptoms include nausea, vomiting, abdominal pain, headache, chills, and diarrhea (BRASIL, 2011).

To enhance the safety of homemade mayonnaise, it is possible to incorporate substances that can reduce the risk of *Salmonella* spp. transmission. In this context, oregano essential oil emerges as a natural and healthier alternative to the preservatives commonly employed in the food industry, backed by antimicrobial efficacy demonstrated in numerous studies (ROBAZZO *et al*., 2016; SILVA *et al*., 2012).

Oregano (*Origanum vulgare* L.), a shrub belonging to the Lamiaceae family, stands out for its antioxidant and antimicrobial properties, primarily attributed to phenolic compounds, particularly thymol and carvacrol. Carvacrol accounts for up to 68% of the total oil composition (PARRA *et al*., 2021). The mechanism of action involves the ability of these active compounds to disrupt the outer membrane of gram-negative bacteria, increasing membrane fluidity and causing the loss of sodium and potassium ions, ultimately inhibiting ATP synthesis, and leading to cell death (TREVISAN *et al*., 2018; OLIVEIRA; SOARES; PICCOLI, 2013).

In light of these considerations, this study aimed to investigate the impact of oregano essential oil on the growth of *Salmonella enterica* serovar Enteritidis in homemade mayonnaise stored at 30°C at different concentrations. Additionally, we will examine pH levels, titratable acidity, and the presence of mesophilic aerobic microorganisms in this product.

## 2 MATERIALS AND METHODS

### 2.1 OREGANO ESSENTIAL OIL

The research was conducted between September 2020 and February 2021 at the Microbiology Laboratory of the Federal Institute of Education, Science, and Technology of Maranhão (IFMA), Campus Maracanã. We utilized oregano essential oil (*Origanum vulgare*) from the commercial brand Phytoterápica®. For the technical specifications of the essential oil used in this study, please refer to Table 1.

Table 1 – Technical specifications of the oregano essential oil used in the study according to the company Phytoterápica LTDA

| Essential oil | Impurities | Density (g/mL, 20ºC) | Origin | Main components |
|---|---|---|---|---|
| Oregano *(Origanum vulgare)* | Free | 0.937 | Türkiye | Carvacrol (49.5 %)<br>Thymol (14.7%)<br>β-phellandrene (13.2%) |

Source: Phytoterápica (2021).

### 2.2 MINIMUM INHIBITORY AND BACTERICIDAL CONCENTRATION OF ESSENTIAL OIL

Initially, we assessed the inhibitory concentration of OEO using both broth microdilution and macro-dilution methods, following a method adapted from Cavalvanti, Almeida, and Padilha (2011). We employed four bacterial strains, including *Escherichia coli* ATCC 25922, and three strains of *Salmonella enterica* belonging to serovars

Enteritidis (2) and Typhimurium (1). These strains were isolated from a broiler chicken production area in Maranhão State (Brazil), and identified through microbiological (BRASIL, 2011) and serological tests conducted by the Oswaldo Cruz Foundation (FIOCRUZ). The strains were stored in nutrient agar at 4°C and reactivated in brain heart infusion (BHI) broth at 37°C for 24 hours.

To determine the Minimum Inhibitory Concentration (MIC), OEO was serially diluted to concentrations of 8%, 4%, 2%, 1%, 0.5%, 0.25%, 0.125%, and 0.0625% using the microdilution technique. The MIC was determined by placing 700µL of BHI broth and 10µL of the bacterial strain adjusted to $1.5 \times 10^6$ microorganisms/mL (equivalent to tube $10^6$ on the MacFarland Scale) into each of the 80 Eppendorf tubes, arranged in eight columns (A to H), with the procedure repeated for each tested microorganism. Subsequently, 700µL of the OEO emulsion from each serial dilution was transferred. The tubes were then incubated in a bacteriological oven at 37°C for 48 hours. The MIC was defined as the highest dilution of essential oil where no bacterial precipitate or turbidity was observed in the culture medium after incubation.

The Minimum Bactericidal Concentration (CBM) was determined by inoculating 10µL aliquots of the dilutions corresponding to the MIC and the two preceding dilutions (2xMIC and 4xMIC) onto Standard Counting Agar (PCA). The Petri dishes were incubated in a bacteriological oven at 37°C for 24 hours. CBM was defined as the lowest concentration that prevented visible growth of the subculture or the formation of more than three Colony Forming Units (CFUs). Concentrations at which more than three CFUs formed were considered inhibitory to bacterial growth (MIC), while concentrations at which no growth or fewer than three CFUs were observed were considered bactericidal (CBM).

## 2.3 HOMEMADE MAYONNAISE FORMULATION

To assess the efficacy of OEO on artificially contaminated homemade mayonnaise, we formulated a base mayonnaise following a method adapted from Robazza *et al*. (2016). The formulation consisted of 61% soybean oil, 19% vinegar (4% acetic acid), 7% NaCl solution (10%), and 13% pasteurized egg yolk (as an emulsifier). Please refer to Table 2 for precise measurements and proportions. The preparation of the mayonnaise was carried out aseptically, using previously sanitized and sterilized utensils and equipment, including a blender.

REVISTA CARIBEÑA DE CIÊNCIAS SOCIALES

Table 2 – Homemade mayonnaise formulation

| Ingredient | Quantity |
|---|---|
| Soybean oil | 80 mL |
| Pasteurized egg yolk | 17.5g |
| Vinegar (4% acetic acid) | 25 mL |
| NaCl solution (10%) | 9 mL |

Source: Author (2021).

First, eggshells were broken, the yolks were separated, and pasteurization was conducted in a water bath for 3 minutes at 61°C with continuous mixing. The pasteurized yolk was then transferred to a blender, mixed with lemon juice and NaCl solution, and slowly blended with soybean oil until a stable emulsion formed. The pH of the mayonnaise was measured using a digital pH meter from the Kasvi brand.

## 2.4 EVALUATION OF OREGANO ESSENTIAL OIL'S EFFECT ON SALMONELLA ENTERITIDIS DEVELOPMENT

The base mayonnaise was divided into 125 mL portions in four bottles. In two of these portions, we added concentrations of 0.5% (v/v) and 1.0% (v/v) of OEO, while the other two served as control samples.

A strain of *Salmonella* serovar Enteritidis was selected randomly, inoculated into brilliant green agar, and incubated at 37°C for 24 hours. After incubation, colonies were transferred to tubes containing saline solution to achieve a concentration corresponding to 0.5 on the MacFarland scale (approximately 1.5 x $10^8$ CFU/mL).

Next, 1 mL of the *Salmonella* Enteritidis culture (diluted to a concentration of 0.5 on the MacFarland scale) was inoculated into the mayonnaise portions containing OEO and one control fraction (positive control). In the other control fraction, 1 mL of saline solution was added, serving as the negative control. The samples were thoroughly mixed using a sterile glass rod for 6 minutes and then incubated at 30°C for up to 24 hours.

After 0, 4, 8, and 24 hours of incubation, 25 grams of each OEO concentration treatment were weighed, diluted in 225 mL of peptone water, and subjected to three serial decimal dilutions. Surface plating was conducted on brilliant green agar, followed by incubation at 37°C for 24 hours. Subsequently, colonies with *Salmonella* characteristics were counted, and the results were expressed as log CFU/g of mayonnaise.

## 2.5 DETERMINATION OF PH, ACIDITY, AND MESOPHILIC MICROORGANISM COUNT

At each evaluation time point, we determined the pH and titratable acidity according to the method recommended by the Adolfo Lutz Institute (2008). To assess acidity, 1.246g of the mayonnaise sample was weighed, transferred to an Erlenmeyer flask, and homogenized with 50 mL of water. A few drops of phenolphthalein solution were added, and titration was performed with 0.01 M sodium hydroxide solution until a pink color was observed.

We also conducted standard counts of aerobic mesophilic microorganisms in the samples at each time point using the deep seeding technique [27]. Briefly, 1 mL of each dilution was seeded in sterile Petri dishes, and 15 to 20 mL of molten standard counting agar (PCA) was added, maintained at 46°C. The inoculum was homogenized with the agar using circular movements on the plates. After the agar surface solidified, the plates were incubated at 35°C for 48 hours. Following incubation, microorganism colony counts were performed, and the results were expressed as Colony Forming Units per gram (CFU/g).

## 2.6 DATA ANALYSIS

We compared each of the OEO concentrations with the positive control and with each other using the chi-square test (χ2) at a significance level of 5%, employing the Microsoft Excel program (2013).

# 3 RESULTS AND DISCUSSION

The results of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) tests for OEO are presented in Table 3. In all *Salmonella* samples tested, the MIC was determined to be 0.5% (equivalent to 4.68 mg/mL), and the MBC was 0.0625% (equal to 0.58 mg/mL). These findings demonstrate the antimicrobial activity of OEO against *Salmonella* strains in the *in vitro* test.

Table 3 – Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) in mg/mL of oregano essential oil against bacterial strains

| Strain | MIC | MBC |
|---|---|---|
| *Escherichia coli* | 4.68 mg/mL | 0.58 mg/mL |
| S. Enteritidis | 4.68 mg/mL | 0.58 mg/mL |
| S. Enteritids | 4.68 mg/mL | 0.58 mg/mL |
| S. Typhimurium | 4.68 mg/mL | 0.58 mg/mL |

Source: Author, 2021.

Our study achieved lower and more effective antimicrobial concentrations compared to those reported by Reis *et al*. (2020), who found that OEO (containing 72% carvacrol and 2% thymol) was effective against *Salmonella* spp., with MIC concentrations ranging from 0.9% to 1% and an MBC of 0.9% for different essential oils. In the case of the standard *E. coli* strain, OEO exhibited inhibitory and bactericidal concentrations between 1.0% and 1.1%. The MIC values in our study were lower than those reported by Parra *et al*. (2021), who found an MIC of 0.078 mg/mL for *Salmonella enterica* and 0.156 mg/mL for *Escherichia coli* when evaluating oregano essential oil from the Atacama Desert. However, our MBC values (1.25 mg/mL for *Salmonella* and 2.50 mg/mL for *E. coli*) were lower compared to their results. The effectiveness of OEO is attributed to the high concentrations of thymol and carvacrol, which disrupt the bacterial outer membrane, leading to cell death (TREVISAN *et al*., 2018).

When assessing the impact of OEO in artificially contaminated mayonnaise (Table 4), we observed no significant difference (P>0.05) in the *Salmonella* Enteritidis count between mayonnaise without OEO and those with OEO at concentrations of 0.5% and 1.0%. Bacterial growth remained consistent, with similar average counts between the samples with OEO added (0.5%: 6.10 to 6.35 log CFU/g and 1.0%: 5.35 to 6.25 log CFU/g) and those without OEO (6.10 to 6.35 log CFU/g) over the evaluated periods. These results indicate that the addition of 0.5% and 1.0% OEO had no significant impact on bacterial growth at 30°C for 24 hours, a period during which the bacteria remained active in the medium.

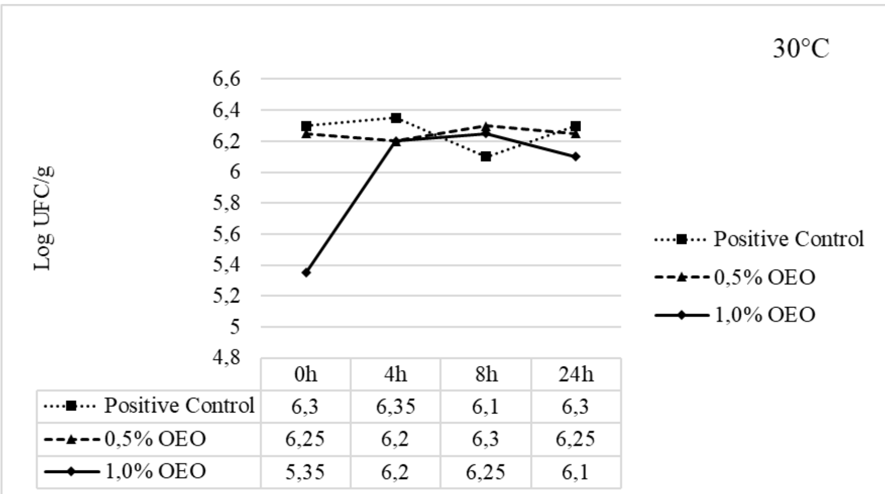
Table 4 – Counts of *Salmonella* and mesophilic microorganisms in mayonnaise with and without addition of oregano essential oil at 30 °C

| Mayonnaise | *Salmonella* spp. | | Mesophilic microorganisms | |
|---|---|---|---|---|
| | CFU/g | Log CFU/g | CFU/g | Log CFU/g |
| | | 0 h incubation time | | |
| Control - | 0 | 0 | 7.6 x $10^3$ | 3.90 |
| Control + | 2.0 x $10^6$ | 6.30[a] | 2.18 x $10^6$ | 6.30[a] |
| 0.5% OEO | 1.86 x $10^6$ | 6.25[a] | 2.14 x $10^6$ | 6.30[a] |
| 1.0% OEO | 2.2 x $10^5$ | 5.35[a] | 2.32 x $10^6$ | 6.35[a] |
| | | 4 h incubation time | | |
| Control - | 0 | 0 | 6.5 x $10^3$ | 3.80 |
| Control + | 2.2 x $10^6$ | 6.35[a] | 2.82 x $10^6$ | 6.45[a] |
| 0.5% OEO | 1.62 x $10^6$ | 6.20[a] | 2.38 x $10^6$ | 6.35[a] |
| 1.0% OEO | 1.6 x $10^6$ | 6.20[a] | 1.54 x $10^6$ | 6.20[a] |
| | | 8 h incubation time | | |
| Control - | 0 | 0 | 4.0 x $10^2$ | 2.60 |
| Control + | 1.22 x $10^6$ | 6.10[a] | 1.5 x $10^6$ | 6.20[a] |
| 0.5% OEO | 2.18 x $10^6$ | 6.30[a] | 1.62 x $10^6$ | 6.20[a] |
| 1.0% OEO | 1.7 x $10^6$ | 6.25[a] | 1.54 x $10^6$ | 6.20[a] |
| | | 24 h incubation time | | |
| Control - | 0 | 0 | 2.2 x $10^3$ | 2.35 |
| Control + | 1.98 x $10^6$ | 6.30[a] | 2.06 x $10^6$ | 6.30[a] |
| 0.5% OEO | 1.84 x $10^6$ | 6.25[a] | 1.38 x $10^6$ | 6.10[a] |
| 1.0% OEO | 1.22 x $10^6$ | 6.10[a] | 1.44 x $10^6$ | 6.15[a] |

Counts in Log CFU/g followed by the same letters on the same row do not exhibit statistically significant differences from each other at a 5% probability level, as determined by the chi-square test ($\chi 2$). Similarly, counts followed by the same letters in the same column do not significantly differ from the control sample or those with the addition of oregano essential oil based on the statistical analysis.
Source: Author, 2021.

The survival of *Salmonella* spp. within 24 hours at 30°C was evident, maintaining a stable count close to 6.0 log CFU/g (Figure 1). The addition of OEO to mayonnaise samples did not reduce the bacterial population to safe levels, as the infectious dose for *Salmonella* is > 5.05 Log CFU/g (BRASIL, 2011).

Figure 1- Growth of *Salmonella* Enteritidis in mayonnaise with and without addition of oregano essential oil at 30°C within 24 hours.




Source: Author, 2021.

These results contrast with those of Robazza *et al*. (2016), who observed different outcomes when studying the growth of a standard strain of *Salmonella* Enteritidis in mayonnaise with the addition of 0.7% and 1.4% OEO at various temperatures (8°C, 25°C, and 36°C). Their study suggested that OEO might cause increased cell death at temperatures higher than refrigeration and a concentration of 1.4%. However, they also noted that concentrations above 1% of OEO negatively affected the acceptability of mayonnaise formulated with OEO.

Aerobic mesophilic microorganism counts showed no significant differences (P>0.05) between samples with OEO and the positive control, with average counts remaining at 6.0 log CFU/g over 24 hours. This indicates the limited effectiveness of OEO against these microorganisms, consistent with the fact that *Salmonella* grows at mesophilic temperatures (SILVA *et al*., 2017). However, when compared to the negative control, which showed contamination with counts close to 103 CFU/g at most time intervals, the conditions under which this study was conducted (30°C) allowed for the growth of these microorganisms (SILVA *et al*., 2017).

It is worth noting that despite adhering to good aseptic practices during homemade mayonnaise production, mesophile counts exceeded the recommended limits for industrial mayonnaise, which typically should not exceed $10^2$ CFU/g (BRASIL, 2019). The reduced efficacy of OEO against *Salmonella* in mayonnaise may be linked to the diminished *in vitro* antimicrobial potency of agents when applied to foods, due to the influence of macromolecules and minerals present (LEUSCHNER; ZAMPARINI, 2002; RATTANACHAIKUNSOPON; PHUMKHACHORN, 2010).

Furthermore, the lipid content in mayonnaise may directly impact OEO's performance against *Salmonella*, as the high fat content can create a protective barrier around the bacteria (SMITH-PALMER; STEWART; FYFE, 2001). It is also possible that OEO is absorbed by the lipid component, rendering it ineffective, as the microorganism could easily multiply in the aqueous phase (SILVA; FRANCO, 2012).

Table 5 presents the pH and titratable acidity values measured at different time intervals in the mayonnaise. The pH values ranged from 4.5 to 5.1, conditions that may have allowed bacterial survival, as a pH of < 4.2 in egg-based foods can inhibit bacterial growth (KEERTHIRATHNE *et al*., 2016). *Salmonella* tends to thrive in foods with a pH range between 4.5 and 9.3, with optimal growth occurring between 6.5 and 7.5. It requires a minimum pH of approximately 5, with viability diminishing in pH ranges below 4 (ASAE, 2012).

Table 5 – Averages of pH and titratable acidity in mayonnaise samples at 30°C for 24 hours

| Parameter | 0h | 4h | 8h | 24h |
|---|---|---|---|---|
| pH | 5.1±0.0 | 4.5±0.0 | 4.7±0.0 | 4.7±0.0 |
| Acidity (% acid) | 0.39±0.0 | 0.31±0.0 | 0.31±0.0 | 0.31±0.0 |

Source: Author, 2021.

Regarding titratable acidity, initial acidity (0 hours) in mayonnaise was 0.39%, which decreased to 0.31% and remained stable over 24 hours. While the acidity levels in this study were lower than those reported by Tavakoli *et al*. (2021), who observed acidity variations of 0.56% to 0.73%, they were similar to values reported by Zhu, Li, and Chen (2012) when evaluating mayonnaise formulated with vinegar, with acidity variations of 0.18% to 0.30%.

Despite optimal temperature conditions for bacterial growth, *Salmonella* Enteritidis populations remained stable, with counts close to 6.0 log CFU/g after 4 hours, both in mayonnaise with OEO and without (Figure 1). This may be attributed to the acidity in the mayonnaise, which created unfavorable conditions for bacterial growth during the logarithmic phase. While the acidity did not lead to cell death, acids like acetic acid present in the vinegar used in mayonnaise may have inhibited bacterial growth within 24 hours. Zhu, Li, and Chen (2012) demonstrated the inhibitory effect of vinegar on *Salmonella* Enteritidis inoculated in mayonnaise made from unpasteurized eggs.

## 4 CONCLUSION

The addition of commercial oregano essential oil (OEO) at concentrations of 0.5% and 1.0% to homemade egg-based mayonnaise, combined with suitable acidity conditions, effectively restrained the growth of *Salmonella* Enteritidis when stored at a temperature of 30°C for 24 hours, demonstrating a bacteriostatic effect.

To enhance the efficacy of OEO, future studies can explore the potential synergistic effects of combining OEO with other essential oils derived from spices or natural products in homemade mayonnaise. Additionally, investigating the antimicrobial properties of OEO at various concentrations and temperature ranges would provide valuable insights for food safety applications.

REVISTA CARIBEÑA DE CIÊNCIAS SOCIALES

## REFERENCES


ASAE – Autoridade de Segurança Alimentar e Econômica. ***Salmonella.*** Lisboa. 2012. Disponível em: <https://www.asae.gov.pt/seguranca-alimentar/riscos-biologicos/salmonella.aspx#:~:text=A%20salmonelose%20%C3%A9%20considerada%20uma,ra%C3%A7%C3%B5es%20ou%20de%20%C3%A1gua%20contaminados> Acessado em: 31 jul 2021.

BALEM, T. A. *et al*. As transformações alimentares na sociedade moderna: a colonização do alimento natural pelo alimento industrial. **Revista Espacios**. 38: 5-17. 2017.

BARCELOS, I.B. *et al*. Pesquisa de *Salmonella* spp. e *Listeria Monocytogenes* em Saladas Contendo Maionese Comercializadas em Restaurantes Localizados no Município de JI – Paraná, Rondônia, Brasil. **Journal Health Science**.18:159-62, 2016.

BEZERRA I. N. *et al*. Consumo de alimentos fora do lar no Brasil segundo locais de aquisição. **Revista de Saúde Pública**. 51: 1-8. 2017.

BRASIL – Ministério da Saúde. **Surtos de Doenças Transmitidas por Alimentos no Brasil.** Dados epidemiológicos. Brasília; 2019**.** Disponível em: <https://portalarquivos2.saude.gov.br/images/pdf/2019/fevereiro/15/Apresenta----o-Surtos-DTA---Fevereiro-2019.pdf.> Acessado em 01 set. 2023.

BRASIL – Agência Nacional de Vigilância Sanitária. Instrução Normativa n. 60, de 23 de dezembro de 2019. **Listas de padrões microbiológicos para alimentos.** Available at: <http://portal.anvisa.gov.br/documents/10181/4660474/IN_60_2019_.pdf/8b764b8f5172-4bfc-a855-bc73972ee96f/>. Accessed on: Jul. 01, 2021.

BRASIL – Ministério da Saúde. Secretaria de Vigilância em Saúde. Manual técnico de diagnóstico laboratorial de Salmonella spp.: diagnóstico laboratorial do gênero Salmonella / Ministério da Saúde. Secretaria de Vigilância em Saúde, Fundação Oswaldo Cruz. Laboratório de Referência Nacional de Enteroinfecções Bacterianas, Instituto Adolfo Lutz. – Brasília: Ministério da Saúde, 2011. 60p. Disponível em: <https://bvsms.saude.gov.br/bvs/publicacoes/manual_tecnico_diagnostico_laboratorial_salmonella_spp.pdf> Acessado em: 01 set. 2023.

CAVALCANTI, Y. W.; ALMEIDA, L. F. D.; PADILHA, W. W. N. Atividade Antifúngica de Três Óleos Essenciais Sobre Cepas de Candida. **Revista Odontológica do Brasil Central**. 20: 68 -73. 2011.

CDC – Centers for Disease Control and Prevention. National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Foodborne, Waterborne, and Environmental Diseases (DFWED). ***Salmonella***. 23 de agosto de 2023. Disponível em: <https://www.cdc.gov/salmonella/index.html> Acessado em: 01 set. 2023.

INSTITUTO ADOLFO LUTZ. Normas Analíticas do Instituto Adolfo Lutz. **Métodos químicos e físicos para análise de alimentos**. 4. Ed. São Paulo: Instituto Adolfo Lutz, 2008. 1020p.

KEERTHIRATHNE, T. P. *et al*. Review of Temperature, pH, and Other Factors that Influence the Survival of *Salmonella* in Mayonnaise and Other Raw Egg Products. **Pathogens**. 5: 1-11, 2016.

LEUSCHNER, R. G. K.; ZAMPARINI, J. Effects of spices on growth and survival of Escherichia coli O157 and Salmonella enterica serovar Enteritidis in broth model systems and mayonnaise. **Food Control**. 13: 399-404, 2002.

OLIVEIRA, T. L. C.; SOARES, R. A.; PICCOLI, R. H. A Weibull model to describe antimicrobial kinetics of oregano and lemongrass essential oils against *Salmonella* Enteritidis in ground beef during refrigerated storage. Meat Science. 93: 645-651, 2013.

PARRA, C. *et al*. Characterization of *Origanum vulgare L*. from the Atacama Desert Andean Region and Antioxidant, Antibacterial and Enzyme Inhibition Activities. **Molecules**. 26:1-13, 2021.

RATTANACHAIKUNSOPON, P.; PHUMKHACHORN, P. Antimicrobial Activity of Basil (Ocimum basilicum) Oil against *Salmonella* Enteritidis in Vitro and in Food. **Bioscience, Biotechnology, and Biochemistry**. 74: 1200-1204. 2010 Disponível em: https://doi.org/10.1271/bbb.90939.

REIS, J.B. *et al*. Avaliação da atividade antimicrobiana dos óleos essenciais contra patógenos alimentares. **Brazilian Journal Health Review**. 3: 342-363, 2020.

ROBAZZA, W. S. *et al*. Modelagem do crescimento de *Salmonella Enteritidis* em maionese adicionada de óleo essencial de orégano. **Vetor.** 26: 51-60, 2016.

SANT'ANA, A. S. **Quantitative Microbiology in Food Processing: Modeling the Microbial Ecology.** New York: Wiley & Sons, 2017. p. 519-532.

SMITH-PALMER, A.; STEWART, J.; FYFE, L. The potential application of plant essential oils as natural food preservatives in soft cheese. **Food Microbiology.** 18: 463–470, 2001.

SILVA, J. P. L.; FRANCO, B. D. G. M. Application of oregano essential oil against *Salmonella* enteritidis in mayonnaise salad. **Journal of Food Science and Nutri Engineer.** 2: 70-75, 2012.

SILVA, N. *et al*. **Manual de Métodos de Análise Microbiológica de Alimentos e água.** 5 ed. São Paulo (SP): Blucher**;** 2017. 560p.

TAVAKOLI, R. *et al*. Production of Low-fat mayonnaise without preservatives: Using the ultrasonic process and investigating of microbial and physicochemical properties of the resultant product. **Food Science & Nutricion**. 9: 2676–2685, 2021.

TREVISAN, D. A. C. *et al*. Antibacterial and antibiofilm activity of carvacrol against *Salmonella enterica* serotype Typhimurium. **Brazilian Journal of Pharmaceutical. Sciences.** 54: e17229, 2018.

ZHU, J.; LI, J.; CHEN, J. Survival of Salmonella in Home-Style Mayonnaise and Acid Solutions as Affected by Acidulant Type and Preservatives. **Journal Food Protect**. 75: 465-71. 2012.

WHO – World Health Organization. Newsroom. ***Salmonella* (non-typhoidal)**. 2018. Available at: <https://www.who.int/news-room/fact-sheets/detail/salmonella-(non-typhoidal)> Accessed on: Sep. 01, 2023.