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Antibacterial activity of nisin, oregano essential oil, EDTA, and their combination against *Salmonella* Enteritidis for application in mayonnaise

Avaliação de atividade antibacteriana de nisina, óleo essencial de orégano, EDTA e sua combinação contra *Salmonella* Enteritidis para aplicação na maionese

Janine Passos Lima da Silva[I,*]

Erika Fraga de Souza[I]

Regina Celia Della Modesta[I]

Izabela Alves Gomes[II]

Otniel Freitas-Silva[I]

Bernadette Dora Gombossy de Melo Franco[III]

ABSTRACT

Salmonella Enteritidis (SE) is one of the most important serovars associated with Salmonella gastroenteritis outbreaks in Brazil. The use of natural antimicrobials can be an alternative method of SE control. The antimicrobial effect of two oregano essential oils (OEO1 and OEO2) at 0.1%, 0.2%, 0.5%, 1.0%, or 2.0%; nisin (Nisaplin®) at 0, 6.25, 12.5, or 25 ppm; ethylenediaminetetraacetic acid (EDTA) at 0.0037%, 0.0056%, 0.0075%, 0.0110%, or 0.0150%; and their combination against SE in vitro was studied to be applied in mayonnaise and Russian salad made with the same mayonnaise during storage at 8° or 30°C for 24 hours. OEO was very efficient against SE at all tested concentrations, while nisin and EDTA showed no effect against SE. Ten volatile components were identified in the two OEOs using gas chromatography coupled to mass spectrometry with electron impact ionization, with carvacrol being the major component of both samples. OEO2, containing p-cymene (15.95%) and y-terpinene (6.90%), besides carvacrol (61.66%), resulted in larger inhibition zone than the other OEO (OEO1 don't contains p-cymene or y-terpinene). Nisin in combination with OEO1 or OEO2 had an antagonistic effect at all concentrations. The presence of nisin caused a reduction in essential oil antimicrobial activity ($p \leq 0.05$). Sensory evaluation showed that consumers prefer 0.2% OEO in mayonnaise instead of 0.5% and 1.0% concentrations. Thus, OEO only, at a concentration of 0.2%, was applied in mayonnaise against SE. The Russian salad prepared with mayonnaise plus OEO at 0.2% (wt/wt) caused a reduction of SE when compared with the salad prepared with mayonnaise without OEO. These results indicate that the use of OEO as a biopreservative (natural antimicrobial) can enhance food safety, serving as an additional barrier in helping the Good Manufacturing Practices and the Hazard Analysis Critical Control Point program, fundamental to food safety.

KEYWORDS: Pathogens, Food Safety, Antimicrobials

RESUMO

Salmonella Enteritidis (SE) é um dos sorovares mais importantes associados a surtos de gastroenterite provocados por Salmonella no Brasil. A utilização de agentes antimicrobianos naturais pode ser um método alternativo de controle de SE. O efeito antimicrobiano de dois óleos essenciais de orégano (OEO1 e OEO2) a 0,1; 0,2; 0,5; 1,0 ou 2,0%, nisina (Nisaplin®) a 0; 6,25; 12,5 ou 25 ppm, ácido etilenodiaminotetracético (EDTA) a 0,0037; 0,0056; 0,0075; 0,0110% ou 0,0150 e suas combinações contra SE *in vitro* foi estudado para ser aplicado na maionese e na salada russa com a mesma maionese durante o armazenamento a 8° ou 30°C por até 24 horas. Ambos OEO foram muito eficientes contra SE em todas as concentrações testadas, enquanto nisina ou EDTA não apresentaram efeito contra a SE. Dez componentes voláteis foram identificadas em OEO1 e OEO2, utilizando GC-MS-EI, e carvacrol foi o principal componente de ambas as amostras. OEO com maior efeito antimicrobiano tinha, além de carvacrol (61,66%), p-cimeno (15,95%) e γ-terpineno (6,90%), apresentando maior halo de inibição comparado com o outro OEO. A nisina em combinação com OEO1 ou OEO2 tinha um efeito antagonista em todas as concentrações. A presença de nisina causou uma redução na atividade antimicrobiana de óleo essencial (p £ 0,05). A avaliação sensorial mostrou que os consumidores preferem 0,2% de OEO na maionese em vez de concentrações de 0,5 e 1,0%. Assim, OEO sozinho, com uma concentração de 0,2%, foi aplicado na maionese contra SE. A salada russa preparada com maionese adicionada de OEO em 0,2% (p/p) apresentou uma redução de SE quando comparado com salada russa preparado com maionese sem OEO. Esses resultados indicam que o uso de OEO como um bioconservador (antimicrobiano natural) caracteriza-se como uma barreira adicional para as Boas Práticas de Fabricação (BPF) e programa de HACCP (Análise de Perigos e Pontos Críticos de Controle), fundamental para a Segurança de Alimentos.

PALAVRAS-CHAVE: Patógenos; Segurança de Alimentos; Antimicrobianos

[I] Embrapa Agroindústria de Alimentos – CTAA, Rio de Janeiro, RJ, Brasil

[II] Universidade Federal do Estado do Rio de Janeiro (UNIRIO), Rio de Janeiro, RJ, Brasil

[III] Universidade de São Paulo (USP), São Paulo, SP, Brasil

* **E-mail:** janine.passos@embrapa.br

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INTRODUCTION

Recognition of new food-borne pathogens has changed understanding of the epidemiology of food-borne diseases in the past 2 decades. Globally, bacterial pathogens that have gained prominence over the past 20 years include *Salmonella* Enteritidis (SE), *Campylobacter* spp., *Escherichia coli* O157:H7, and related verocytoxigenic spp.[1,2]. Since 1991, SE has been one of the most important serovars involved in *Salmonella* gastroenteritis outbreaks in Brazil[3]. While SE is mainly associated with eggs and poultry products, a wide range of other food items have been implicated as vehicles of SE infection in humans, including meat[4,1]. There is a shortage of new methods to reduce or eliminate food-borne pathogens. The use of antimicrobials combined with existing methods, such as hurdle technology and controlling the numbers and growth of SE, thus remains an important objective for the food industry[5,6,7,8].

The use of natural antimicrobial compounds such as bacteriocins and essential oils of edible plants has attracted interest from the food industry because, for centuries, indigenous plants have been used in herbal medicine to cure many diseases, including enteritis. Oregano essential oil (OEO), from the herb *Origanum vulgare*, has been shown to possess antimicrobial activities against pathogens including SE[9,10,11,12,13,14].

Among bacteriocins, nisin, a lantibiotic class of bacteriocin produced by strains of *Lactococcus lactis*, has found practical application as a food preservative in a number of products. However, the practical application of nisin is limited because of its low stability, reduced activity at high pH, and poor efficacy in certain food matrices. Nisin also has limited effect against Gram-negative bacteria[15,16,17].

Bacteriocin in combination with essential oil can achieve good microbiological safety in food while decreasing the doses of each compound in the product, due to the ability of nisin and the essential oil to act on the cytoplasmic membrane, with additive or synergistic effect, allowing the application of both compounds at lower levels without diminishing their inhibitory effects. Therefore, combined use could be a strategy to overcome these restrictions and further extend the range of applications of nisin in food processing[12,15,16].

Ethylenediaminetetraacetic acid (EDTA) is a chelator that can be used as food additive and can potentiate the effect of weak acid preservatives against Gram-negative bacteria (Ntzimani, Giatrakou, and Savvaidis, 2010). Treatment of Gram-negative bacteria with EDTA makes them sensitive to agents to which they are normally resistant. EDTA also increases the sensitivity of Gram-negative bacteria to nisin[18,19].

The aim of this work was to study the antimicrobial activities of OEO, nisin, EDTA, and their combination against SE *in vitro*, to select the best antimicrobial agent for use in mayonnaise, as well as to evaluate the acceptability of mayonnaise and Russian salad with mayonnaise containing selected antimicrobial agents.

MATERIAL AND METHOD

Culture conditions

Salmonella enterica subsp. *enterica* serovar Enteritidis American Type Culture Collection 13076 samples were stored in tryptone soya agar at 4ºC, resuspended in brain heart infusion (BHI) broth and incubated without agitation at 30ºC overnight.

Antimicrobial agents

Solutions of OEO1 (Fine Ingredients Division, Frutarom Daniel (UK) LTD.) and OEO2 (Flavors Division, Duas Rodas, Brazil) were prepared at the moment of testing by dissolution in 95% ethanol until final concentrations of 0.1%, 0.2%, 0.5%, 1.0%, or 2.0% (vol./vol.) were achieved. A stock solution (1 g/L) of nisin (Nisaplin®, Danisco) was prepared in 0.02 N HCl and stored at –18ºC. Further dilutions to 6.25 ppm, 12.5 ppm, or 25 ppm were prepared from the same solution. Neutral solutions of EDTA (Sigma) – 0.0037%, 0.0056%, 0.0075%, 0.0110%, or 0.0150% (wt/vol.) – were prepared and stored under refrigeration at 4ºC.

Chemical analysis of OEO

The composition of both OEOs was determined by gas chromatography coupled to mass spectrometry with electron impact ionization (GC-MS-IE).

To identify the constituents of OEO1 and OEO2, dilutions of samples with hexane and 1 µL of each sample were injected automatically (ALS injector HP-1100; Hewlett Packard, Palo Alto, CA, USA) in split mode (100:1). Separation and analysis were performed with HP 5890 gas chromatograph II coupled to mass spectrometer (HP 5973 MSD) and EI at 70 eV, monitored by the ChemStation software. Helium was used as carrier gas with constant flow of 1 cm^3/min. An HP-5 fused silica capillary column (30 m x 0.32 mm x 0.25 mm) was used, at temperatures of 250ºC for the injector and 250ºC for the interface between the chromatograph and the detector. The temperature cycle was initial column temperature of 40ºC for 2 min, increased at the rate of 5ºC/min up to 90ºC and then at 10ºC/min to 250ºC, which was maintained for 5 minutes, for a total run of 35 minutes.

Determination of antimicrobial activity *in vitro*

The agar well test was used to determine the antimicrobial activities of the investigated compound[20]. Nisin, OEO, EDTA, or their combination were deposited into wells (6 mm) cut into the agar (tryptone soya broth medium with 1% agar; Oxoid, Basingstoke, UK) containing 1 mL of 10^4 colony-forming units (CFU)/mL (pour plate) of an 18-hour SE culture resuspended in BHI broth.

The wells were filled with 35 µL of each solution (prepared as described in Section 2.2): alcohol OEO solution only; acid nisin solution only; neutral EDTA solution only; acid nisin solution plus OEO (35 µL of each); neutral EDTA solution plus nisin (35 µL of each); and EDTA plus OEO (35 µL of each). The diameter of the inhibition zone was measured after incubation at 30°C for 24 hours.

Antimicrobial activity in Russian salad

Ingredients of a standard commercial formulation were used to prepare the mayonnaise: soybean oil, sodium chloride (NaCl), 4% vinegar, EDTA, and emulsifier, as shown in Table 1.

Egg yolk powder was used as emulsifier, with a quality certificate indicating the absence of *Salmonella* sp. The mayonnaise was prepared under laminar flow, with sterilized equipment and solutions. The emulsifier (or egg yolk powder) was incorporated aseptically into the liquid phase consisting of vinegar and aqueous solutions of NaCl and EDTA at 4°C. After mixing these ingredients, soybean oil was slowly poured into the aqueous phase under stirring in sufficient quantity (~ 70%) until a stable emulsion was attained. For this procedure, we used a household blender (Mallory Robot Classic), previously cleaned with 70% alcohol. This mayonnaise base with or without OEO was used to prepare Russian salad.

Antimicrobial activity was measured in Russian salad inoculated with 25 mL of culture in saline solution containing 10^4 CFU/mL SE. The salad was prepared with 225 g of commercial canned mixed vegetables – corn, carrots, and peas (Carrefour SA, Brazil) and 75 g of mayonnaise containing 0.2% OEO2. The procedure was repeated with mayonnaise without OEO, the control sample. Each of the samples was divided into 12 sterile bags of 25 g. Six of them were stored at 8°C and six at 30°C. After 0, 1, 2, 4, 8, and 24 hours, one of the bags was subjected to three decimal serial dilutions, which were plated on xylose lysine deoxycholate agar, in duplicate. After 24 hours' incubation at 30°C, the number of colonies was counted and the results were converted to log CFU per gram of mayonnaise.

Chemical analysis of mayonnaise and Russian salad

The pH and Aw (water activity) were measured in mayonnaise and Russian salad. All experiments were conducted three times, and the results were expressed as the averages of three replications. The pH and Aw were monitored for each batch of mayonnaise prepared.

Sensory evaluation

Table 1. Mayonnaise formula used in the experiment with OEO.

Mayonnaise base (ingredients)	Quantity
Soybean oil	~ 175 mL
Emulsifier	17.5 g
NaCl solution (10%) at 4 ° C	9 mL
EDTA solution (1%) at 4°C	1.25 mL
Vinegar (4% acetic acid)	25 mL
pH	4.22

To conduct sensory evaluation, the project was submitted to and approved by the Ethics in Research Committee of the School of Pharmaceutical Sciences, University of São Paulo.

Mayonnaise and Russian salad, both containing OEO, were used to determine acceptable taste. Acceptability evaluation of mayonnaise was carried out with a commercial brand (Hellmann's, Unilever Brazil LTD.) purchased at a local market in São Paulo city. This mayonnaise was enriched with 0.0%, 0.5%, and 1.0% of OEO1.

The samples were evaluated individually (monadic testing) by 30 consumers, in individual sensory booths under white light at the sensory laboratory of the School of Pharmaceutical Sciences, University of São Paulo.

The mayonnaise samples were spread on toast presented on disposable plates at room temperature. Each sample was coded with three random digits. Saltine crackers and water were supplied to the testers for palate cleansing.

To determine the degree of acceptance of aroma and flavor, a hedonic scale of nine points (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely) was used[21].

Depending on the results obtained with the mayonnaise containing 0.5% or 1% OEO, mayonnaise plus 0.2% OEO2 was prepared for sensory analysis of Russian salad. This salad was presented to 61 consumers in individual sensory booths under white light in the sensory laboratory of the Brazilian Agricultural Research Corporation - Embrapa (Rio de Janeiro, Brazil).

All conditions of the test environment, hedonic test conditions, and acceptance scoring were the same as for the mayonnaise acceptability tests. In this evaluation, the testers were also asked to express their intent to purchase and provided a space for comments about the product. All data were calculated as percentages.

Statistical analysis

The determination of antimicrobial activity was performed three times in triplicate (nine measurements). The experimental results were subjected to analysis of variance and the Duncan test ($p > 0.05$) using the Statistical Analysis Systems software (SAS Institute, Cary, NC, USA).

RESULTS

Composition of OEO and antimicrobial effect

Using GC-MS-IE, ten volatile components were identified. Figure 1 shows the chromatogram of OEO1.

Figure 2 shows the chromatogram of OEO2.

Carvacrol was the major component of both OEO samples. OEO1 contained carvacrol (93.42%), *β*-caryophyllene, caryophyllene oxide, and terpinen-4-ol, while OEO2 contained

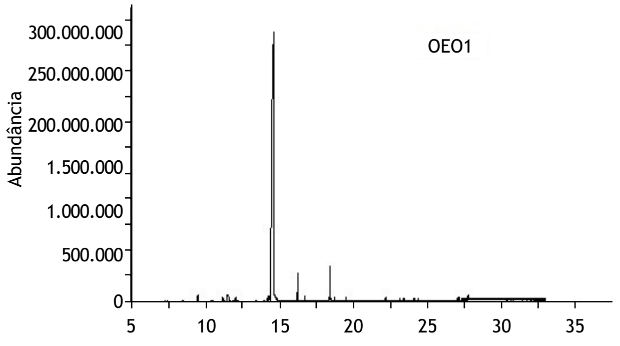

Figure 1. Chromatogram of OEO1.

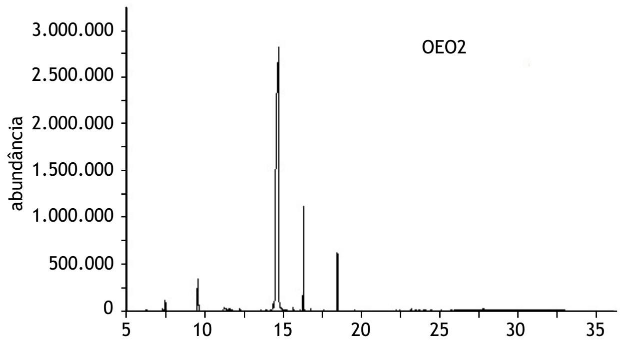

Figure. 2 Chromatogram of OEO2.

carvacrol (61.66%), *α*-pinene, *β*-pinene, *β*-caryophyllene, D-limonene, linalool, caryophyllene oxide, and the monoterpene hydrocarbons *γ*-terpinene (6.90%) and *p*-cymene (15.95%).

Both OEO1 and OEO2 were very efficient against SE at all tested concentrations and the differences in inhibitory activity of OEO1 and OEO2 were not significant ($p > 0.05$), but OEO2, from the Mediterranean, which contained *p*-cymene and *γ*-terpinene besides carvacrol, resulted in larger inhibition zones than the other OEO (Table 2).

The inhibition zone values at 0.2% and 0.5% of OEO2 against SE were not significantly different ($p > 0.05$), and according to the sensory evaluation results, OEO can be added at 0.2% or 0.5% in mayonnaise.

Nisin and EDTA alone, as well as EDTA in combination with nisin, showed no effect against SE. EDTA in combination with OEO1 showed no difference in relation to OEO1 only ($p > 0.05$) (Table 3).

Table 2. Oregano essential oil´s inhibitory activity against *Salmonella* Enteritidis at 30ºC.

OEO	Inhibition zone (mm)*	
(%)	OEO1	OEO2
0.1	10^{e}	11^{d}
0.2	13^{d}	15^{c}
0.5	15^{c}	16^{c}
1.0	18^{b}	19^{b}
2.0	21^{a}	22^{a}

*Inhibition zone includes well (6mm) (for de same column, values followed by the same lowercase letter were not statistically different from each other ($p < 0.05$).

However, EDTA combined with OEO2 showed a difference in relation to OEO2 alone ($p > 0.05$) at concentrations ≤ 0.5% (Table 4).

Nisin combined with EDTA had no effect against SE. In turn, nisin in combination with OEO caused a reduction of the antimicrobial activity of the essential oil ($p \leq 0.05$). An antagonistic effect was observed against SE. The growth inhibition zones obtained by the agar well test are presented in Tables 5 and 6 and Figures 3 and 4.

Considering these results, we decided to apply only OEO2 in mayonnaise and not the antimicrobials combined.

Sensory evaluation

The results indicated that 77% of tasters liked mayonnaise's aroma without the addition of OEO1, while 50% liked it with 0.5% OEO1 and 40% found it pleasant with 1% OEO1 (Figures 5 – 7). The acceptance of the mayonnaise aroma decreased when the concentration of OEO1 was increased from 0.5% to 1%. However, it is important to note that the acceptability of the product was just regular without OEO1 (< 80%).

The results indicated that 97% liked mayonnaise's flavor without addition of OEO1, while only 24% liked the flavor with 0.5% OEO1 and 3% liked it with 1% OEO1 (Figures 8 – 10). The acceptability of the flavor decreased considerably as the content of OEO1 increased.

These results indicate the impossibility of using substantial concentrations of OEO1 in mayonnaise to improve the antimicrobial effect. Therefore, the microbiological analyses to determine the survival of SE in Russian salad were carried out with 0.2% OEO2, which showed no difference in relation to 0.5% OEO2 ($p > 0.05$).

Table 3. Antimicrobial activity of EDTA combined with OEO1 against *Salmonella* Enteritidis at 30ºC measured by the agar well test.

OEO1 (%)	EDTA (%)					
	0.0037	0.0056	0.0075	0.0110	0.0150	0.0000
0.1	*9^{A,c}	7^{B,d}	9^{A,b}	9^{A,b}	9^{A,c}	10^{A,e}
0.2	10^{A,c}	10^{A,c}	10^{A,b}	10^{A,b}	10^{A,c}	13^{A,d}
0.5	11^{A,c}	12^{A,c}	11^{A,b}	11^{A,b}	10^{A,c}	15^{B,c}
1.0	18^{A,b}	15^{B,b}	19^{A,a}	19^{A,a}	15^{B,b}	18^{A,b}
2.0	21^{A,a}	20^{A,a}	21^{A,a}	21^{A,a}	21^{A,a}	21^{A,a}

*inhibition zone includes well (6 mm).
**each value is the average of nine measurements.
***for the same line, values followed by the same uppercase letter did not differ significantly from each other ($p < 0,05$); for the same column, values followed by the same lower case letter were not statistically different from each other ($p < 0.05$).

Table 4. Antimicrobial activity of EDTA combined with OEO2 against *Salmonella* Enteritidis at 30°C measured by the agar well test.

OEO2 (%)	EDTA (%)					
	0.0037	0.0056	0.0075	0.0110	0.0150	0.0000
0.1	*8[A,c]	7[B,d]	8[A,c]	8[A,c]	8[A,d]	11[C,d]
0.2	10[A,b]	9[A,c]	11[A,b]	10[A,b]	10[A,c]	15[B,c]
0.5	11[A,b]	10[A,c]	12[A,b]	11[A,b]	10[A,c]	16[B,c]
1.0	19[A,a]	15[B,b]	19[A,a]	19[A,a]	16[B,b]	19[A,b]
2.0	21[A,a]	20[A,a]	21[A,a]	21[A,a]	21[A,a]	22[A,a]

*inhibition zone includes well (6 mm).
**each value is the average of nine measurements.
***for the same line, values followed by the same uppercase letter did not differ significantly from each other ($p < 0,05$); for the same column, values followed by the same lowercase letter were not statistically different from each other ($p < 0.05$).

Table 5. Antimicrobial activity of nisin combined with OEO1 against *Salmonella* Enteritidis at 30°C measured by the agar well test.

Nisin concentration (ppm)	Diameter* of inhibition zone (mm) of OEO1 different concentrations				
	0.1%	0.2%	0.5%	1.0%	2.0%
0	10.4[a**]	13.1[a]	15.0[a]	17.7[a]	21.0[a]
6.25	7.6[b]	11.6[b]	12.4[b]	15.0[b]	20.4[b]
12.50	11.3[c]	11.7[b]	13.2[c]	15.4[b]	19.7[c]
25.00	12.0[d]	12.3[c]	12.8[c]	16.3[c]	19.3[c]

*The diameter of inhibition zone includes well (6mm).
**for the same column, values followed by the same lowercase letter were not statistically different from each other ($p < 0.05$).

Table 6. Antimicrobial activity of nisin combined with OEO2 against *Salmonella* Enteritidis at 30°C measured by the agar well test.

Nisin concentration (ppm)	Diameter* of inhibition zone (mm) of OEO2 different concentrations				
	0.1%	0.2%	0.5%	1.0%	2.0%
0	11.2[a**]	14.8[a]	16.4[a]	18.9[a]	22.1[a]
6.25	11.9[b]	13.7[b]	13.8[b]	18.1[b]	20.8[b]
12.50	10.1[b]	13.0[b]	14.2[b]	17.3[b]	20.2[b]
25.00	11.1[b]	12.8[b]	13.7[b]	16.2[b]	19.3[b]

*The diameter of inhibition zone includes well (6mm).
**for the same column, values followed by the same lowercase letter were not statistically different from each other ($p < 0,05$).

The aroma and flavor acceptability of Russian salad with **mayonnaise** containing 0.2% OEO2 showed optimal acceptability: in this test, 95% liked the aroma (Figure 11) and 93% liked the flavor (Figure 12).

The tasters were asked to comment about the product. **Regarding** the aroma, only six consumers made comments: they found no change between this or any other mayonnaise and identified a "slight oregano aroma." Twenty-nine consumers commented about the flavor. Some of them felt that the vegetables affected the mayonnaise flavor. In contrast, others said that the mayonnaise was "spicy, but did not overpower the flavor of the food." Some also stated that the mayonnaise flavor was very pronounced, suggesting decreasing the amount of OEO2. Other consumers identified a disagreeable aftertaste in the sample containing OEO2. A single consumer defined the initial taste as acidic but said the final flavor was pleasant. Some consumers found the flavor to be different from what they were accustomed to, but pleasant, while others defined a "slight taste" that improved the vegetables' flavor. Some other consumers suggested culinary preparations such as green salads, sandwiches,

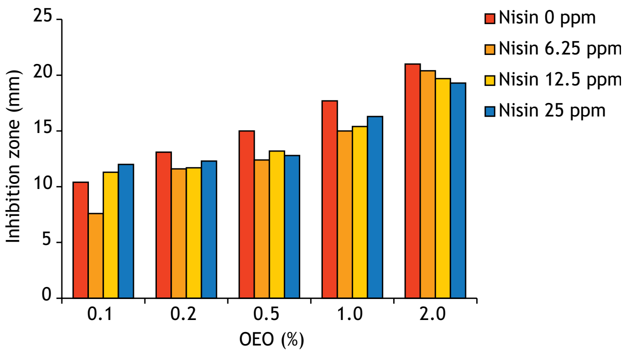

Figure 3. Effect of OEO1 only or combined with nisin at 6.25 ppm, 12.5 ppm, or 25 ppm against *Salmonella* Enteritidis at 30°C.

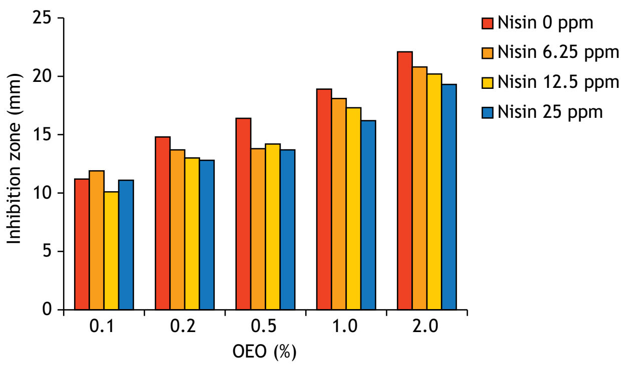

Figure 4. Effect of OEO2 only or combined with nisin at 6.25 ppm, 12.5 ppm, or 25 ppm against *Salmonella* Enteritidis at 30°C.

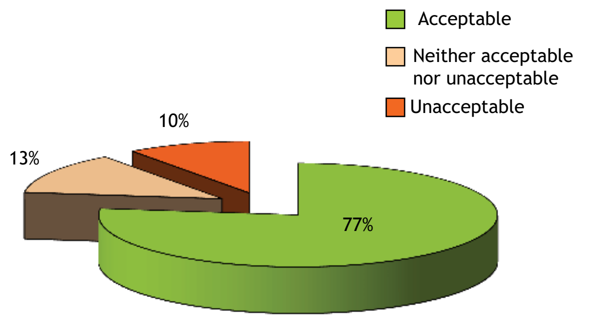

Figure 5. Acceptability of mayonnaise aroma without oregano essential oil.

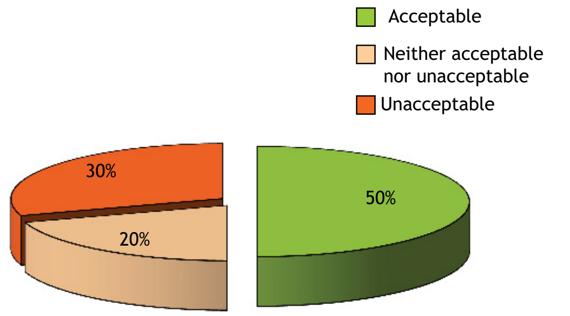

Figure 6. Acceptability of mayonnaise aroma with 0.5% oregano essential oil.

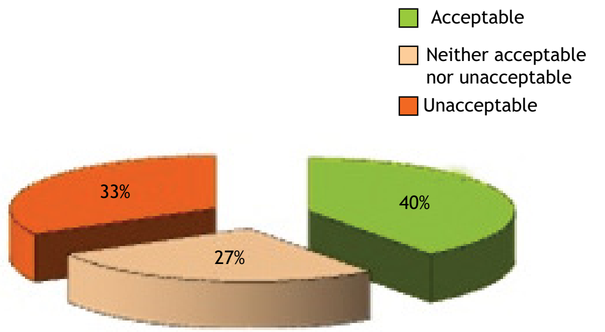

Figure 7. Acceptability of mayonnaise aroma with 1% oregano essential oil.

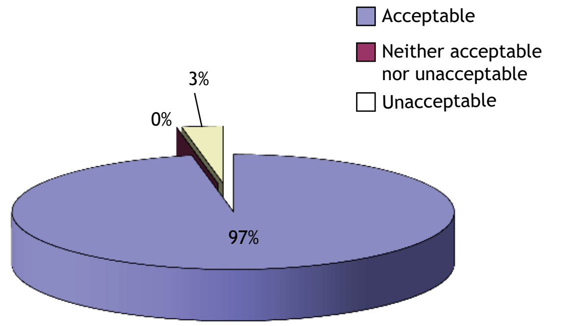

Figure 8. Acceptability of mayonnaise flavor without oregano essential oil.

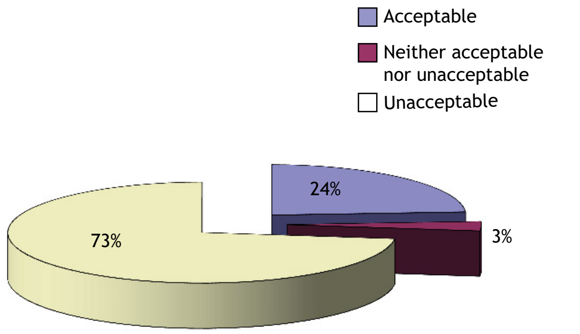

Figure 9. Acceptability of mayonnaise flavor with 0.5% oregano essential oil.

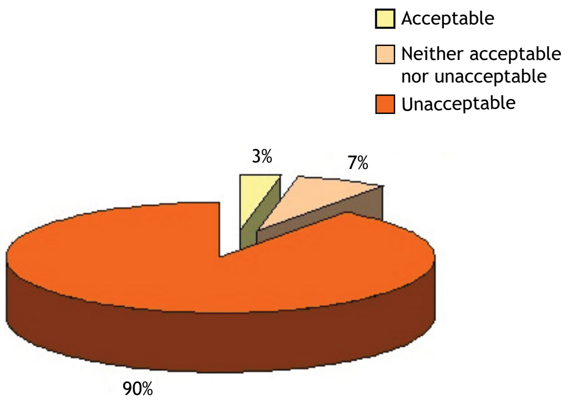

Figure 10. Acceptability of mayonnaise flavor with 1.0% oregano essential oil.

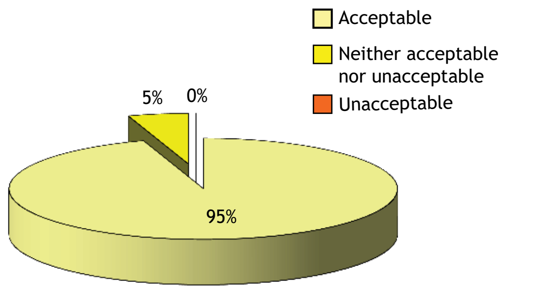

Figure 11. Acceptability of the aroma of Russian salad with mayonnaise containing 0.2% oregano essential oil.

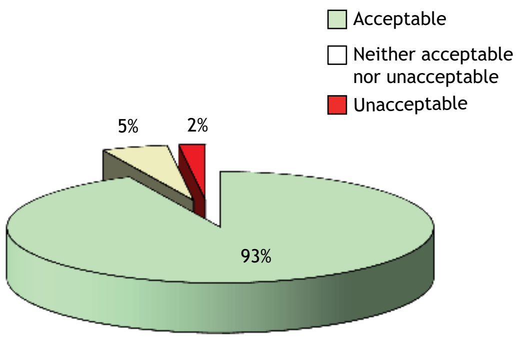

Figure 12. Acceptability of the flavor of Russian salad with mayonnaise containing 0.2% oregano essential oil.

and snacks, which made us believe that using mayonnaise with OEO2 is possible.

Regarding buying intentions, only 74% of consumers said they would purchase the mayonnaise with 0.2% OEO, while 10% said they might buy it and 16% stated they would not (Figure 13).

Chemical analysis of mayonnaise and Russian salad

Chemical analysis of mayonnaise and Russian salad Chemical analyses of mayonnaise without OEO and Russian salad with or without 0.2% OEO showed an increase in pH and Aw (Table 7) when vegetables were mixed in the mayonnaise to make Russian salad, demonstrated that the safety of mayonnaise decreases in culinary preparations, as indicated by the presence of microbial multiplication.

Antimicrobial activity in Russian salad

Antimicrobial activity was measured in Russian salad inoculated with 25 mL of culture in saline solution containing 10^4 CFU/mL SE. The addition of OEO2 at 0.2% in mayonnaise used in Russian salad showed antimicrobial activity against SE. The reducti-on in the count of SE was less than 0.5 log CFU/g for a period of 24 hours at 8°C and for a period of 4 hours at 30°C compared to the control (without OEO2) (Figures 14 and 15).

DISCUSSION

The composition of both OEOs tested is in agreement with the results of previous studies[1,22,23,24,25]. The monoterpene hydrocarbons γ-terpinene (6.90%) and *p*-cymene (15.95%) acted synergistically with carvacrol.

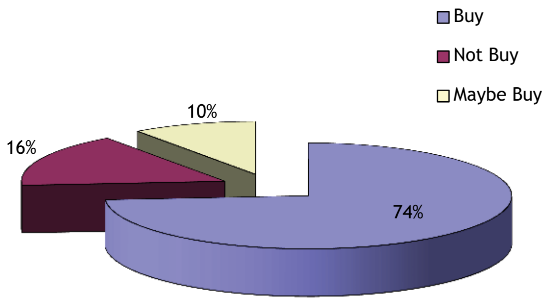

Figure 13. Intent to purchase the mayonnaise with 0.2% oregano essential oil.

The antimicrobial efficacy of essential oils can be attributed to the chemical composition and the proportions in which the components are present and their interactions[4,6,25,26,27]. Synergism between carvacrol and its biological precursor *p*-cymene was observed in *Bacillus cereus* vegetative cells because *p*-cymene probably enables carvacrol to be more easily transported into the cell[28].

Nisin or EDTA only had no effect against SE. It is already known that nisin has no effect against Gram-negative bacteria, including SE[1,29].

The agar well test is a qualitative assay and can introduce errors in testing EDTA only, whereas the inhibition of bacteria by EDTA is presumed to be due to chelation of divalent cations found in the cell wall[18]. However, *Salmonella* Infantis is sensitized either in the presence of EDTA only or when combined with plant extracts[9].

Nisin combined with EDTA at all tested concentrations presented no effect against SE. SE inhibition zones were not observed, showing that both antimicrobial agents studied, alone or in

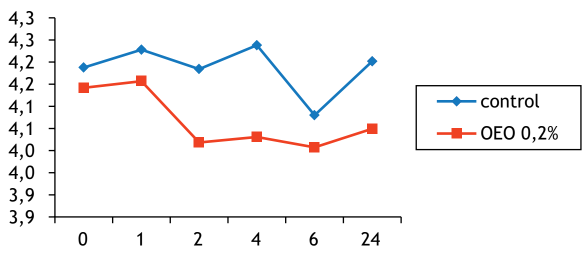

Figure 14. Survival curve of SE in Russian salad prepared with mayonnaise containing 0.2% OEO maintained at 8°C.

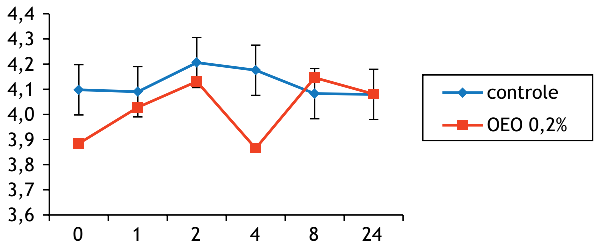

Figure 15. Survival curve of SE in Russian salad prepared with mayonnaise containing 0.2% OEO maintained at 30°C.

Table 7. Values of pH and Aw of mayonnaise, Russian salad without OEO2, and Russian salad with 0.2% OEO2.

	pH*	Aw*
Mayonnaise	4.22	0.955
Russian salad without OEO2	4.79	0.970
Russian salad added 0.2% OEO2	4.81	0.970

*Each value is the average of three experiments.

combination, showed no bacteriostatic or bactericidal effects against SE. These results differ from those reported by Ukuku[17] and Fett (2004), who found a bacteriostatic effect of the combination of nisin and EDTA against a pool of *Salmonella* tested on tryptone soya broth medium containing the antimicrobial solution. A possible explanation for the discrepant results is the difference in the analytical methods used.

An antagonistic effect was observed against SE when nisin was combined with OEO. The addition of OEO caused a reduction of the antimicrobial activity of the essential oil. Several mechanisms could be responsible for the antagonistic effect between nisin and OEO. Nisin might prevent OEO from being taken up by the cells or vice versa. Alternatively, the interaction between nisin and OEO might prevent the OEO from reaching its target of activity or could interfere with its structure or configuration, leading to a reduction in its specific activity[15].

On the other hand, the combination of nisin and thymol (OEO constituent) was very effective against vegetative cells of *B. cereus*, showing a synergistic effect[30]. In another study, when OEO was used in combination with nisin, a synergistic effect was observed against *Listeria monocytogenes,* i.e., the oil enhanced the activity of the bacteriocin[31].

In Russian salad, the presence of 0.2% OEO resulted in a reduction in the SE count, thus forming an effective barrier that inhibits the growth of the pathogen. Increased safety by increasing the concentration of OEO in these products is not always possible because they can become sensorially unacceptable. It follows, therefore, that to reduce the risk of infection by SE in recipes with mayonnaise, other ways must be used besides high temperature and addition of OEO as a natural antimicrobial.

In conclusion, these results indicate that OEO antimicrobial activity against SE is enhanced when combined with nisin or EDTA. Nisin and OEO both have potential applications in improving food safety, but their activity together depends on the target pathogen.

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