RBSPA
Revista Brasileira de Saúde e Produção Animal

ISSN 1519-9940
Revista Brasileira de Saúde e Produção Animal
http://doi.org/10.1590/S1519-994020240010

Received: 09/03/2024
Accepted: 20/06/2024

Preventive Veterinary Medicine
Editor: José Givanildo da Silva

Effect of applying lime essential oil (*Citrus latifolia*) on the physicochemical and microbiological characteristics of beef meat sausage

Efeito da aplicação do óleo essencial de limão (Citrus latifolia) sobre as características físico-químicas e microbiológicas da linguiça bovina frescal

Leticia de Kássia Reis Frazão[1], Josilene Lima Serra[1*], Geisa Lohuama da Luz Pereira[1], Leidiana de Sousa Lima[1], Rafael Alves Gomes[1], Gleice Karoline dos Santos Alves[1], Anderson Lopes Pereira[2], Adenilde Nascimento Mouchreck[3]

[1] Instituto Federal do Maranhão, São Luís, MA, Brasil
[2] Universidade Federal da Paraíba, Areia, PB, Brasil
[3] Universidade Federal do Maranhão, São Luís, MA, Brasil
* Correspondence to: josilene.serra@ifma.edu.br

ABSTRACT The objective of this study was to investigate the potential of lime essential oil as a substitute for synthetic preservatives in beef sausage, considering consumer demand for healthy meat products produced with natural ingredients. Lime peel essential oil (LEO) was obtained by hydrodistillation and subjected to an evaluation of antibacterial activity by the disc diffusion and microdilution method. Its chemical composition was determined by gas chromatography coupled with mass spectrometry. Three sausage formulations were developed in this study: the first without preservatives (LC), the second with synthetic preservatives (L1), and the third containing 0.5% lime essential oil (L2). Physicochemical and microbiological analyses indicated that all treatments followed current legislation, although the moisture content exceeded the maximum limit. The pH and color varied significantly during refrigerated and frozen storage, reaching stability after 20 days. lime essential oil, with D-limonene as the majority component, proved to be effective in inhibiting microbial growth at a concentration of 0.5%, preserving the physicochemical composition of the sausage. Furthermore, there is a tendency for the color to stabilize during frozen storage. Therefore, 0.5% lime essential oil is a viable and natural alternative for application in meat sausages, such as fresh sausage, and adds a different flavor and aroma to this product.

Keywords hydrodistillation, citrus fruits, microbiological analysis, meat sausages.

RESUMO *O objetivo desse estudo foi investigar o potencial do óleo essencial de limão como substituto para conservantes sintéticos em linguiça de carne bovina, considerando a demanda dos consumidores por produtos cárneos saudáveis e produzidos com ingredientes naturais. O óleo essencial da casca do limão (OEL) foi obtido por hidrodestilação, e submetido a avaliação da atividade antibacteriana pelo método de difusão de discos e microdiluição, assim como, foi determinado a sua composição química por cromatografia gasosa acoplado a espectrometria de massas. Três formulações de linguiça de carne bovina foram desenvolvidas neste estudo: a primeira sem conservantes (LC), a segunda com conservante sintético (L1) e a terceira contendo 0,5% de óleo essencial de limão (L2). As análises físico-químicas e microbiológicas indicaram que todas as amostras estavam em conformidade com a legislação vigente, embora o teor de umidade tenha excedido o limite máximo. Durante o armazenamento refrigerado e congelado, o pH e a cor variaram significativamente, atingindo a estabilidade após 20 dias. O óleo essencial de limão, com d-limoneno como componente majoritário, mostrou-se eficaz na inibição do crescimento microbiano na concentração de 0,5%, preservando a composição físico-química da linguiça. Além disso, evidenciou-se uma tendência de estabilização da cor durante o armazenamento congelado. Portanto, a aplicação de 0,5% do óleo essencial de limão é uma alternativa viável e natural para aplicação em embutidos cárneos, como a linguiça frescal, além de agregar sabor e aroma diferenciados a este produto.*

Palavras-chave *hidrodestilação, frutas cítricas, análise microbiológica, produtos cárneos.*

1. Introduction

Meat products are foods that stimulate great preference among Brazilian consumers, as they are an accessible form of animal-origin protein, low cost, with quick and practical preparation, in

addition to presenting a variety of products available on the market, such as sausages, hamburgers, nuggets, seasoned meats, and others (Vessoni et al., 2019).

Sausage is a meat product obtained using ground meat from different animal species, seasoned, containing other ingredients or not, stuffed in a natural or artificial casing, and subjected to a specific technological process. This product may contain 30 to 35% fat in its composition (Brasil, 2000a; Brasil, 2000b; Brasil, 2017).

According to Vessoni et al. (2019), despite the high consumption of these meat products, consumers still have imminent concern regarding the high content of saturated fats and chemical preservatives. The occurrence of the COVID-19 pandemic affected people's eating habits in two ways, with an increase in binge eating due to anxiety or dietary re-education aimed at healthier habits. In the first case, the consumption of industrialized meat products increased due to the practicality of preparation and greater accessibility. In the second case, the population's concern with healthier habits, including food, led to a reduction in the consumption of industrialized products (Durães et al., 2021).

According to the National Cancer Institute, the consumption of processed meats, such as ham, sausage, salami, and bacon, can cause stomach and intestinal cancer (Instituto Nacional do Câncer, 2022; Ribeiro et al., 2019). The risks associated with the consumption of these products are related to the presence of nitrates and nitrites used as preservatives, which can undergo endogenous nitrosation and become potentially carcinogenic to humans. Recently, nitrate and nitrite have been linked to the risks of breast and prostate cancer, respectively (Chazelas et al., 2022). Therefore, the replacement of these preservatives with natural products has been the subject of many studies.

Plant essential oils are natural products widely used in meat products, such as hamburgers and sausages, due to their antioxidant and antimicrobial properties. These oils have demonstrated a promising role in controlling the growth of pathogenic microorganisms, such as *Escherichia coli* and *Staphylococcus aureus*, in addition to helping to prevent lipid oxidation and preserve the color of foods (Sharafati-Chaleshtori et al., 2015; Gahruie et al., 2017; Sharma et al., 2019; Pateiro et al., 2021).

Lime essential oil (*Citrus latifolia*) is a bioactive compound rich in polyphenols and acts as an antioxidant, with D-limonene as its main terpene. Another biological property of this essential oil is its antibacterial action, which sparks interest in its application as a natural preservative in foods. Additionally, the Food and Drug Administration considers it safe for intentional use in foods (Teixeira et al., 2013; Ben Hsouna et al., 2017; Food and Drug Administration, 2023).

The presence of antioxidant compounds in *Citrus latifolia* extracts reveals great potential for maintaining the quality and shelf-life of marinated chicken meat by reducing the levels of mesophilic aerobic bacteria, inhibiting the oxidation of lipids and proteins, stabilizing color, and improving its acceptability (Lotfy et al., 2023; Budiarto et al., 2024).

Considering the antioxidant and antibacterial potential of *Citrus latifolia* essential oil, this work aimed to apply the essential oil extracted from lime peel (*Citrus latifolia*) in preparing beef sausage as a substitute for synthetic preservatives, thus proposing healthier meat product forms.

2. Material and methods

2.1. Obtaining lime essential oil

Tahiti limes (*Citrus latifolia)* were purchased from a supermarket chain in São Luís, Maranhão, Brazil, to obtain the essential oil. The essential oil was extracted from the lime peel by the hydrodestillation process using the Clevenger system in the Chemistry laboratory at the IFMA −

Campus Maracanã (Santos et al., 2004). The density was determined using a pycnometer, and the yield was expressed on a dry basis, according to the method described by Santos et al. (2004).

2.2. Antimicrobial activity of essential oils

Antibacterial activity was evaluated by the disc diffusion method as referenced by Clinical and Laboratory Standards Institute (2015) and two strains of standard bacteria were used, *E. coli* ATCC 25922 and *S. aureus* ATCC 29213. The bacterial suspension was prepared in saline solution, and the turbidity of this solution was determined using the MacFarland scale (initial concentration of 10^7 CFU/g). This solution was inoculated on plates with Mueller Hinton Agar (Merck, Darmstadt, Germany) and the essential oil (75 µL) embedded in sterile filter paper discs. Later, the discs were transferred to plates containing Muller Hinton Agar. The plates were incubated at 37°C for 24 hours, and transparent inhibition zones were measured using a millimeter ruler. The broth dilution method evaluated the minimum inhibitory concentration (MIC) according to Lertsatitthanakorn et al. (2014). The Brain Heart Infusion broth (Kasvi, S.A, Spain) was supplemented with an initial concentration of the essential oil and subjected to serial decimal dilutions in tubes, with subsequent inoculation of 0.1 mL aliquots of the standardized bacterial suspension and incubation of the tubes at 37°C for 24 hours. The MIC was considered the lowest concentration that inhibited bacteria growth.

2.2. Chemical composition of essential oil by gas chromatography (GC-MS)

The chromatographic analyses were conducted at the Central Analytical Laboratory of the University of São Paulo (USP). The lime essential oil constituents were identified using the Gas Chromatography technique coupled with Mass Spectrometry (GC-MS). The compounds detected were identified using the mass spectra database of the National Institute of Standards and Technology (NIST105, NIST21) and WILEY139.

2.3. Preparation of beef sausage with lime essential oil

The meat product was processed at the Meat Technology Laboratory at IFMA − Campus São Luís Maracanã, in Brazil. All raw materials used in this study were obtained from a local supermarket in São Luís, Maranhão (Table 1). First, a cut of the *Semimembranosus* muscle (topside cut) and animal fat (pork) was used to prepare the meat products. The meat was cleaned, and the skin and other tissues were removed. Next, the meat and fat were ground. The essential oil concentration used was 0.5%. Three formulation treatments were evaluated: control beef sausage, without the addition of preservatives and without the addition of lime essential oil (LC); beef sausage with an addition of 0.005% curing salt and 0.5% ascorbic acid (L1); and beef sausage with an addition of 0.5% lime essential oil (L2).

The mixture of ground meat and fat was divided into polyethylene trays to add condiments and other ingredients (salt, garlic, onion, black pepper, curing salt, nutmeg, antioxidant and chili pepper). The curing salt (Nutri.com, São Paulo, Brazil) used as a synthetic preservative was based on sodium nitrate and nitrite and added at a concentration of 0.005%. In addition, ascorbic acid (ISOFAR, Rio de Janeiro, Brazil) was used as an antioxidant and added to the curing salt at a concentration of 0.5%. The ingredients were manually mixed until a homogeneous mass was obtained. The mixture was embedded in synthetic casings, and then the weight and length of the sausages were standardized. Sausages were refrigerated for a maximum period of 5 days for pH and color analyses, and another part of the sausages were frozen at −18 ºC for 10 days until further physicochemical and microbiological analyses.

2.3. Microbiological analyses

The analyses were conducted according to the parameters established by Normative Instruction 161 (Brasil, 2022b) and methodology recommended by the APHA (2001), which include identification of *Salmonella* sp., quantification of *Escherichia coli* and mesophilic aerobic bacteria.

Table 1 – Beef sausages formulation (%).

Ingredients	Treatments		
	LC	L1	L2
Beef meat	81.4	80.9	80.9
Pork backfat	11.6	11.6	11.6
Onion	4.59	4.59	4.59
Salt	1.7	1.7	1.7
Garlic	0.3	0.3	0.3
Black pepper	0.2	0.2	0.2
Chilli pepper	0.2	0.2	0.2
Nutmeg	0.009	0.009	0.009
Lime essential oil	-	-	0.5
Ascorbic acid	-	0.5	-
Curing salt	-	0.005	-

LC: Control beef sausage without added preservatives; L1: Beef sausage with the addition of 0.005% curing salt 2 and 0.5% ascorbic acid; and L2: Beef sausage with the addition of 0.5% Lime essential oil.

2.4. Physicochemical analyses

The physicochemical analyses of the beef sausage formulations were conducted at the IFMA Chemistry Laboratory – Campus São Luís Maracanã, in triplicate, according to the methodology described by Instituto Adolfo Lutz (2008). Moisture (012/IV), ash (018/IV), proteins (036/IV), and lipids (032/IV) were determined.

2.5. Determination of pH and color

The pH was determined with a skewer pH meter for solids (ASKO, São Leopoldo, Brazil) and color determination using a spectrophotometer (Delta Color, São Leopoldo, Brazil). These analyses were performed while the sausages were refrigerated at a temperature of 5°C (times of 0 and 5 days) and frozen at −18 °C (times of 10 and 20 days). All analyses were performed in triplicate.

2.6. Statistical analysis

The data obtained from the triplicate analyses were subjected to mean and standard deviation calculations using Excel (Microsoft®) software and analysis of variance (ANOVA), with Tukey's test subsequently applied at a level of 5% significance in the comparison of the means using the Past 4.07b statistical program.

3. Results and Discussion

3.1. Chemical composition of lime essential oil

The essential oil extracted from lime showed an average yield of 1.74% (dry basis) for an average biomass of 416 grams. The essential oil obtained was clear and limpid, with a pleasant aroma characteristic of lime and a density of 0.881 kg/m³.

A total of 30 compounds were found in the lime essential oil belonging to the terpene class (Table 2). D-limonene is the majority compound, representing 45.67% of the compounds found in the oil, corresponding to a concentration of 195.26 µg/mL. The second category of compounds most

found were gamma-terpinene and beta-pinene, corresponding to 14.08% and 13.73%, with concentrations of 60.20 and 58.69 µg/mL, respectively.

Table 2 – Chemical composition of the lime (*Citrus latifolia*) essential oil by gas chromatography coupled to mass spectrometry.

Peak	Compound	Retention time (min)	Area (%)	Concentration (µg/mL)
1	Biciclo[3.1.0]hex-2-eno, 2-metil-5-(1-metiletil)-	6.608	0.67	2.84
2	alpha pinene	6.818	2.61	11.15
3	camphene	7.298	0.1	0.41
4	Sabineno	8.190	1.81	7.75
5	beta-pinene	8.295	13.73	58.69
6	beta-myrcene	8.867	2.04	8.70
7	alpha-phellandrene	9.324	0.1	0.43
8	δ-2-careno	9.821	0.31	1.32
9	p-cymene	10.150	5.17	22.11
10	D-limonene	10.408	45.67	195.26
11	beta-ocimene	11.154	0.13	0.55
12	gamma-terpinene	11.574	14.08	60.20
13	δ-2-carene	12.787	0.91	3.89
14	Linalool	13.316	0.77	3.30
15	Carvone epoxide	14.721	0.18	0.75
16	Carvone epoxide	14.927	0.16	0.66
17	Oxirane, 2-(hexin-1-yl)-3-methoxymethylene	15.275	0.11	0.48
18	4-terpineol	16.666	1.06	4.55
19	terpineol	17.278	1.27	5.41
20	decanal	18.029	0.16	0.69
21	Citrol	19.015	0.73	3.14
22	Neral	19.558	1.58	6.77
23	Geraniol	20.208	0.4	1.73
24	Neral	20.889	2	8.55
25	geranyl acetate	25.021	1.24	5.28
26	geranyl acetate	25.841	0.35	1.51
27	beta-elemene	26.118	0.09	0.39
28	Beta-carophyllene	27.199	0.3	1.28
29	Trans-alfa-bergamoteno	27.954	0.87	3.70
30	beta-bisaboleno	30.930	1.41	6.02

Most of the compounds found in lime essential oil are represented by D-limonene, gamma-terpinene, and beta-pinene, which correspond to 73.48%. In comparison, the minority compounds correspond to 25.5%, totaling 99% of the chemical composition of the essential oil. Compounds with

less than 0.3% area are trace compounds, corresponding to the remaining 1% of compounds. The minor compounds, which ranged from 5.17 to 0.3% include p-cymene, α-pinene, β-myrcene, neral, sabinene, β-bisabolene, terpineol, geranyl acetate, 4-terpineol, δ- 2-carene, trans-α-bergamotene, linalool, citrol, Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-, geraniol, geranyl acetate, δ-2-carene, β-carophyllene.

Teixeira et al. (2013) found D-limonene (67.8 to 46.2%), β-pinene (7.98 to 16.55%), and γ-terpinene (9.33 to 13.54%) as major compounds when evaluating 15 genotypes of *Citrus limon*. According to literature data, the main and characteristic compounds of lime essential oil are limonene, γ-terpinene, and β-pinene, as found in this study. Furthermore, these compounds can be used to identify this essential oil.

3.2. Antibacterial activity of lime essential oil

The lime essential oil did not show antibacterial activity against *E. coli* by the disc diffusion or broth dilution method. An inhibition zone of 9 mm was found for *S. aureus*, and none of the oil dilutions inhibited the growth of this bacterium (Table 3). Regarding the antibiotic used as control, it appears that the evaluated strains were sensitive to gentamicin, presenting inhibition halos of 17 and 18 mm for *E. coli* and *S. aureus*, respectively.

Table 3 – Antibacterial activity and minimum lime essential oil inhibitory concentration.

	Inhibation halo (mm)[1]		
Microorganism	Lime essential oil	Gentamicin (20 µg/g)	MIC (mg/mL)
E. coli ATCC 25922	0	17 (S)	0
S. aureus ATCC 29213	9	18 (S)	0

[1] R - Resistant (≤12 mm); I - intermediary (13-14 mm) and S - Sensitive (≥15 mm) (Clinical and Laboratory Standards Institute, 2012).

These results demonstrate that only the pure essential oil applied had a bacteriostatic action on the growth of *S. aureus*. According to Altun and Yapici (2022), the chemical composition of essential oils is a determining factor for their antibacterial activity. These authors report that essential oils containing aldehydes or phenols, such as citral, carvacrol, eugenol, or thymol, have strong antibacterial activity, such as oregano essential oil. On the other hand, monoterpenes are less efficient. Limonene, γ-terpinene, and β-pinene belong to the class of monoterpenes. This fact may justify the low efficiency in inhibiting bacteria in this study and the need for higher essential oil concentrations (Ben Hsouna et al., 2017).

Another factor that influences the antibacterial activity of essential oils is the cell wall composition of the target bacteria. Most essential oils applied to bacteria are more effective against Gram-positive bacteria (Delaquis et al., 2002). Gram-negative bacteria have an outer membrane that reduces the permeability of antimicrobials, which may have restricted the diffusion of terpenes present in lime essential oil (Nascimento et al., 2010; Araújo et al., 2015). Therefore, only the action of essential oil was observed against *S. aureus*.

Nieto-Velázquez et al. (2021) also reported that lime essential oil did not affect the growth of *E. coli*, even at concentrations of 0.872 g/mL, six times higher than that applied in this study. Other studies report that other Citrus species and other plant parts have antibacterial activity. For example, Ben Hsouna et al. (2017) verified the antibacterial activity of essential oil from the *Citrus limon* flower, both pure and diluted, against Gram-positive and Gram-negative bacteria and fungi. Moreover, Lemes et al. (2018) verified the antibacterial action of the essential oil from peels of *Citrus aurantifolia*

in concentrations of 200 µg/mL against Gram-positive bacteria isolated from the oral cavity of humans.

3.3. Physicochemical composition

The results of the physicochemical analyses of the beef sausages are presented in Table 4. There were no significant differences between the sausage formulations evaluated in this study for the parameters of moisture, proteins, lipids, and ash, demonstrating that adding 0.5% of lime essential oil did not affect its physicochemical composition.

Table 4 – Proximate composition of beef sausage formulations.

	Treatments		
Parameters	LC	L1	L2
Moisture	73.90 ± 0.79[a]	73.66 ± 1.94[a]	71.51 ± 0.99[a]
Protein	21.55 ± 0.75[a]	21.68 ± 0.83[a]	21.30 ± 1.26[a]
Lipid	22.47 ± 0.91[a]	22.89 ± 0.46[a]	22.39 ± 0.59[a]
Ash	2.63 ± 0.13[a]	1.25 ± 0.39[a]	2.40 ± 0.10[a]

Means ± Standard Deviation. Values with identical lowercase letters in the same line do not differ in the analysis of variance – ANOVA and Tukey's test ($p > 0.05$). Treatments: LC: Control beef sausage without added preservatives; L1: Beef sausage with the addition of 0.005% curing salt 2 and 0.5% ascorbic acid; and L2: Beef sausage with the addition of 0.5% lime essential oil.

Moisture levels ranged from 71.51% to 73.90%, above that for fresh sausages (70%). Meanwhile, the protein and lipid contents found in the treatments follow current legislation, establishing a minimum protein content of 12% for the product and a maximum of 30% for lipid content, respectively (Brasil, 2000b). The high moisture content in the sausage may be related to other condiments present in its formulation, such as onion and fresh garlic. According to the Brazilian Food Composition Table, the moisture content of the *Semimembranosus* muscle (topside cut) used in sausages is 68.6%. The meat content added to the other condiments used in the formulations may have influenced the final moisture value. This table also provides the carbohydrate, protein, lipid, and ash values of the meat cut used in this study, corresponding to 0%, 21.2%, 8.7%, and 1.0%, respectively (TACO, 2011).

3.4. pH determination and color analysis

The pH results evaluated during 0, 5, 10, and 20 days are expressed in Table 5 for the three beef sausage formulations stored under refrigeration and freezing. There were no significant differences in pH values for the LC treatment during storage. On the other hand, there were significant differences in the L1 and L2 treatments. The L1 and L2 treatments showed decreased pH on the 5th day during the refrigerated storage period. The pH of the L1 and L2 treatments increased on the 10th day and decreased during freezing storage.

The pH value of fresh meat is an important parameter that directly influences the growth of microorganisms and can vary from 5.3 to 6.5. The lower the pH, the slower the growth rate of pathogenic bacteria. Meat with a high pH (equal to or greater than 6.0) is more prone to microbial deterioration than meat with a pH lower than 6.0, as it favors the growth of these microorganisms (Brasil, 2022a). The sausages analyzed in this study had a pH lower than 5.8, indicating they are suitable for consumption. Interestingly, the pH of the L1 sample produced with curing salt and ascorbic acid was lower than the other samples. Furthermore, it is also observed that the pH of the

L2 formulation with the addition of lime essential oil was the highest at the end of the 20th day of the storage period.

Table 5 – pH values of beef sausage formulations stored under refrigeration and freezing for 0 to 20 days.

	0 days	5 days	10 days	20 days
Treatments	T = 25 ºC	T = 5 ºC	T = −18 ºC	T = −18 ºC
LC	5.39 ± 0.04^{aA}	5.33 ± 0.04^{aA}	5.68 ± 0.02^{aA}	5.66 ± 0.01^{aA}
L1	5.44 ± 0.20^{aA}	5.25 ± 0.05^{bA}	5.64 ± 0.08^{cA}	5.45 ± 0.01^{aB}
L2	5.57 ± 0.10^{aA}	5.39 ± 0.02^{bA}	5.72 ± 0.02^{cA}	5.70 ± 0.01^{aC}

Means ± Standard Deviation. Values with identical lowercase letters in the same row and values with capital letters in the same column do not differ in the analysis of variance – ANOVA and Tukey's test ($p > 0.05$). Treatments: LC: Control beef sausage without added preservatives; L1: Beef sausage with the addition of 0.005% curing salt 2 and 0.5% ascorbic acid; and L2: Beef sausage with the addition of 0.5% lime essential oil.

Animal fat lipolysis and the formation of free fatty acids can cause a reduction in pH in meat products (Tomovic et al., 2020). Monoterpenes, such as D-limonene and β-pinene present in lime peel essential oil, are reported in the literature to have antioxidant activity, acting to block free radicals, which can delay lipolysis and prevent pH reduction (Budiarto et al., 2024).

The use of lemon essential oil in ground beef showed an increase in the pH of the meat with the addition of 0.06 and 0.312% of the essential oil during 10 days of storage at 4°C, from 5.59 and 5.57 to 6.24 and 6.19, respectively (Ben Hsouna et al., 2017). This differs from the study by Xin et al. (2022), who found a decline in pH value when applying 0.150 µL/g of lemon seed essential oil to meat aged at 4°C for 15 days, which reduced it from 6.52 to 5.69.

To date, there is no data in the literature on the use of lime essential oil in beef sausage. When analyzing our results, it appears that adding lime essential oil in the L2 formulation promoted a slight increase in pH compared to the control formulation (LC) at time 0. Furthermore, the increase in pH during the 20 days of storage under freezing may be related to the antioxidant action exerted by this oil, which is rich in monoterpenes and delayed the lipid oxidation of this product.

The color of the sausages was measured by the L*, a*, and b* parameters, which represent the luminosity and the intensity of the red and yellow colors, respectively (American Meat Science Association, 2012). All treatments showed a significant increase in L* and a* values over time, indicating that the sausage became lighter and increased the red color intensity with storage. The L1 and L2 treatments also showed a significant increase in b* values between 0 and 10 days, indicating an increase in the intensity of the yellow color in the sausage over time. According to Xin et al. (2022), lime essential oil is rich in antioxidant bioactive compounds, such as terpenes, that can inhibit the oxidation of myoglobin, as well as the rancidity of fatty acids, resulting in better luminosity and color stability. The L1 and L2 treatments presented L*, a*, and b* values that were generally higher than the LC treatment, demonstrating that the synthetic preservatives and essential oil added changed the color of the sausage more than the absence of preservatives.

When evaluating different smoked salami formulations, Tomovic et al. (2020) observed that the pH remained stable in the presence of 75 and 150 mg/kg of nitrites and that Juniperus communis L. essential oil generated a slight increase in pH. However, no significant differences existed between 0 to 0.10 µg/g concentrations. For the color, the authors found that L* reduced, and the a* and b* parameters remained stable.

Table 6 – Color parameters (L*, a*, and b*) of beef sausage formulations stored under refrigeration and freezing for 0 to 20 days.

		Storage period			
		0 days	5 days	10 days	20 days
Parameter	Treatments	T = 25 ºC	T = 5 ºC	T = −18 ºC	T = −18 ºC
L*	LC	50.76 ± 2.11^{aA}	64.96 ± 3.44^{bB}	67.97 ± 2.30^{bB}	68.24 ± 1.22^{bB}
	L1	52.29 ± 0.15^{aA}	67.00 ± 3.31 ^{bB}	68.12 ± 1.53 ^{bB}	69.03 ± 0.52 ^{bB}
	L2	50.28 ± 0.02^{aA}	70.49 ± 1.83^{bB}	75.89 ± 0.08^{cC}	71.14 ± 0.31^{bC}
a*	LC	10.36 ± 3.12^{aA}	14.22 ± 4.05^{bA}	15.04 ± 1.49^{bA}	16.51 ± 1.12^{bA}
	L1	11.73 ± 1.54^{aA}	14.59 ± 3.49^{bA}	18.27 ± 1.38^{bB}	18.85 ± 0.51^{bA}
	L2	7.92 ± 1.13^{aB}	13.10 ± 1.04^{aA}	14.01 ± 0.33 ^{bA}	14.34 ± 0.59 ^{bA}
b*	LC	11.14 ± 4.26^{aA}	21.75 ± 6.07^{bA}	23.85 ± 4.61^{bA}	22.09 ± 1.38^{bA}
	L1	12.02 ± 2.39^{aA}	28.08 ± 4.13^{bA}	31.09 ± 1.61^{bA}	26.74 ± 3.72^{bA}
	L2	11.57 ± 0.40^{aA}	25.28 ± 0.83^{bA}	24.56 ± 1.65^{bA}	30.61 ± 1.66^{bA}

Means ± Standard Deviation. Values with identical lowercase letters in the same row and values with capital letters in the same column do not differ in the same column do not differ in the analysis of variance – ANOVA and Tukey's test ($p > 0.05$). Treatments: LC: Control beef sausage without added preservatives; L1: Beef sausage with the addition of 0.005% curing salt 2 and 0.5% ascorbic acid; and L2: Beef sausage with the addition of 0.5% Lime essential oil.

3.5. Microbiological quality

Table 7 presents the microbiological analysis results for the beef sausage formulations. These results were satisfactory for all microorganisms evaluated, being within the standards required by current legislation and indicating that the addition of lime essential oil did not affect the microbiological quality of this product (Brasil, 2022b). The results obtained for the quantification of mesophilic aerobic bacteria were satisfactory. They ranged from 1.7 x 10^4 to 4.5 x 10^4 CFU/g, following the microbiological standards (Brasil, 2022b). A higher count of mesophilic aerobic bacteria was found in the L1 treatment (with the addition of 0.005% curing salt and 0.5% ascorbic acid) compared to the L2 treatment (with the addition of 0.5% of lime essential oil), which showed a reduction in contamination by these bacteria.

Table 7 – Microbiological analyses of beef sausages after 10 days of freezing at − 18°C.

	Treatments			Legislation	
Microorganisms	LC	L1	L2	m	M
Total coliforms	Abs	Abs	Abs	Abs	
Salmonella sp.	Abs	Abs	Abs	Abs	
E. coli	Abs	Abs	Abs	Abs	
Mesophilic aerobic bacteria	2.1 x 10^4	4.5 x 10^4	1.7 x 10^4	10^5	10^6

m: minimum; M: maximum; Abs: Absent (Brasil, 2022b). Treatments: LC: Control beef sausage without added preservatives; L1: Beef sausage with the addition of 0.005% curing salt 2 and 0.5% ascorbic acid; and L2: Beef sausage with the addition of 0.5% lime essential oil.

Lime essential oil applied in sausages has little been explored in the literature, but it is widely used in meat cuts, especially chicken. A recent meta-analysis study on the quality and safety of

additives based on citrus fruits indicates the efficiency of natural extracts in reducing the population of mesophilic aerobic bacteria in chicken meat, corroborating with the data obtained in this study (Budiarto et al., 2024).

Replacing synthetic preservatives with essential oils in fresh sausage is a natural alternative that has demonstrated great potential for controlling spoilage bacteria in this product. In comparing the antibacterial efficiency of four nanoemulsions produced with *Mentha piperita*, *Punica granatum*, *Thymus vulgaris*, *Citrus limon*, and chitosan in cuts of beef, Lotfy et al. (2023) found that the lemon nanoemulsion had greater efficiency against *E. coli*.

4. Conclusion

Based on the results obtained in this study, it is concluded that the addition of 0.5% essential oil extracted from lime peel in beef sausage formulations presented beneficial effects for bacteriostatic activity on mesophilic aerobic bacteria compared to the sample with synthetic preservative without affecting the physicochemical composition of the product and maintaining pH and color stability during frozen storage. These data show that lime essential oil is a viable alternative for application in meat sausages, adding a different flavor and aroma to this product.

Acknowledgments

To the Federal Institute of Education, Science and Technology of Maranhão - IFMA - Campus São Luís - Maracanã and the Institutional Scientific Initiation Scholarship Program – PIBIC – FAPEMA.

Declaration of Conflict of Interest

The authors declared no conflicts of interest.

CRediT authorship contribution statement

Leticia de Kássia Reis Frazão: Data curation, Formal analysis, Writing – original draft; **Josilene Lima Serra:** Data curation, Formal analysis, Project administration, Writing – original draft, Writing – review & editing; **Geisa Lohuama da Luz Pereira:** Formal analysis, Writing – original draft; **Leidiana de Sousa Lima:** Formal analysis, Writing: review; **Rafael Alves Gomes:** Formal analysis; **Gleice Karoline Alves:** Formal analysis; **Anderson Lopes Pereira:** Writing – review & editing; **Adenilde Nascimento Mouchreck:** Formal analysis, Writing – review & editing.

References

Altun, M., & Yapici, B. M. (2022). Determination of chemical compositions and antibacterial effects of selected essential oils against human pathogenic strains. *Anais da Academia Brasileira de Ciências, 94*(1), Article e20210074. https://doi.org/10.1590/0001-3765202220210074.

American Meat Science Association. (2012). *Meat Color Measurement Guidelines* (2nd ed). Champaign. 136 p.

APHA. (2001). *Compendium of methods for the microbiological examination of foods* (4th ed). American Public Health Association. 4199 p.

Araújo, L. S., Araújo, R. S., Serra, J. L., & Nascimento, A. R. (2015). Composição química e susceptibilidade do óleo essencial de óregano (*Origanum vulgare L.*, família Lamiaceae) frente à cepas de *Escherichia coli*, *Staphylococcus aureus* e *Salmonella choleraesuis*. *Boletim Centro de Pesquisa de Processamento de Alimentos, 33*(1), 73−78. http://dx.doi.org/10.5380/cep.v33i1.43808.

Ben Hsouna, A., Ben Halima, N., Smaoui, S., Hamdi, N. (2017). Citrus lemon essential oil: chemical composition, antioxidant and antimicrobial activities with its preservative effect against *Listeria monocytogenes* inoculated in minced beef meat. *Lipids in Health and Disease, 16*(1), Article 146. https://doi.org/10.1186/s12944-017-0487-5.

Brasil. (2000a). Ministério da Agricultura e do Abastecimento. Instrução Normativa SDA n° 20, de 31 de julho de 2000. Regulamentos Técnicos de Identidade e Qualidade de Almôndega, de Apresuntado, de Fiambre, de Hambúrguer, de Kibe, de Presunto Cozido e de Presunto. Diário Oficial da União. Retrieved from: https://pesquisa.in.gov.br/imprensa/jsp/visualiza/index.jsp?data=03/08/2000&jornal=1&pagina=55&totalArquivos=88. Accessed July 08, 2023.

Brasil. (2000b). Ministério da Agricultura e do Abastecimento. Instrução Normativa SDA n° 4, de 31 de março de 2000. Regulamentos Técnicos de Identidade e Qualidade de Carne Mecanicamente Separada, de Mortadela, de Linguiça e de Salsicha.Diário Oficial da União. Retrieved from: https://pesquisa.in.gov.br/imprensa/jsp/visualiza/index.jsp?data=05/04/2000&jornal=1&pagina=54&totalArquivos=73. . Accessed October 14, 2023.

Brasil. (2017). Ministério da Agricultura e Pecuária. Decreto n. 9.013, de 29 de março de 2017.Diário Oficial da União. Retrieved from: https://www.gov.br/agricultura/pt-br/assuntos/mpa/legislacao/legislacao-geral-da-pesca/decreto-no-9-013-de-29-03-2017.pdf/view. Accessed October 14, 2023.

Brasil. (2022a). Agência Nacional de Vigilância Sanitária. Instrução Normativa n. 161 de 01 de julho de 2022. Diário Oficial da União. Retrieved from: https://antigo.anvisa.gov.br/documents/10181/2718376/IN_161_2022_.pdf/b08d70cb-add6-47e3-a5d3-fa317c2d54b2. Accessed August 20, 2023.

Brasil. (2022b). Ministério da Agricultura, Pecuária e Abastecimento. Métodos Oficiais para Análise de Produtos de Origem Animal. . Retrieved from: https://www.gov.br/agricultura/pt-br/assuntos/lfda/legislacao-metodos-da-rede-lfda/poa/metodos_oficiais_para_analise_de_produtos_de_origem_animal-_1a_ed-_2022_assinado.pdf. Brasília : MAPA. Accessed August 13, 2024.

Budiarto, R., Ujilestari, T., Rumhayati, B., Adli, D. N., Hudaya, M. F., Sitaresmi, P. I., Widodo, S., Wulandari, W., Wahyono, T., & Sholikin, M. M. (2024). Meta-analysis of citrus-derived additives on chicken meat quality and safety: a comprehensive evaluation of acceptability, physicochemical properties, and microbial contamination. *Poultry Science, 103*(5), Article 103556. https://doi.org/10.1016/j.psj.2024.103556.

Chazelas, E., Pierre, F., Druesne-Pecollo, N., Esseddik, Y., Szabo de Edelenyi, F., Agaesse, C., De Sa, A., Lutchia, R., Gigandet, S., Srour, B., Debras, C., Huybrechts, I., Julia, C., Kesse-Guyot, E., Allès, B., Galan, P., Hercberg, S., Seschasaux-Tanguy, M., & Touvier, M. (2022). Nitrites and nitrates from food additives and natural sources and cancer risk: results from the NutriNet-Santé cohort. *International Journal of Epidemiology, 51*(4), 1106–1119. https://doi.org/10.1093/ije/dyac046.

Clinical and Laboratory Standards Institute. (2012). M07-A10 Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. 9 ed. [s.l: s.n.]. Retrieved from: https://clsi.org/media/1928/m07ed11_sample.pdf. Accessed October 13, 2023.

Delaquis, P. J., Stanich, K., Girard, B., & Mazza, G. (2002). Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. *International Journal of Food Microbiology, 74*(1–2), 101–109. https://doi.org/10.1016/S0168-1605(01)00734-6.

Durães, S. A., Souza, T. S., Gomes, Y. A. R., & De Pinho, L. (2021). Implicações da pandemia da Covid-19 nos hábitos alimentares. *Revista Unimontes Científica, 22*(2), 1–20. https://doi.org/10.46551/ruc.v22n2a09.

Food and Drug Administration. (2024). CFR - Code of Federal Regulations Title 21: Part 182 – substances generally recognized as safe. Retrieved from: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?fr=170.38. Accessed March 03, 2024.

Gahruie, H. H., Hosseini, S. M. H., Taghavifard, M. H., Eskandari, M. H., Golmakani, M. T., & Shad, E. (2017). Lipid oxidation, color changes, and microbiological quality of frozen beef burgers incorporated with shirazi thyme, cinnamon, and rosemary extracts. *Journal of Food Quality, 2017*, Article 6350156. https://doi.org/10.1155/2017/6350156.

Instituto Adolfo Lutz. (2008). *Métodos físico-químicos para análise de alimentos* (5th ed). 1020 p.

Instituto Nacional do Câncer. O câncer embutido. Retrieved from: https://www.inca.gov.br/sites/ufu.sti.inca.local/files/media/document/rrc-32-prevencao-o-cancer-embutido.pdf. Accessed March 29, 2024.

Lemes, R. S., Alves, C. C. F., Estevam, E. B. B., Santiago, M. B., Martins, C. H. G., Santos, T. C. L., Crotti, A. E. M., & Miranda, M. L. D. (2018). Chemical composition and antibacterial activity of essential oils from *Citrus aurantifolia* leaves

and fruit peel against oral pathogenic bacteria. *Anais da Academia Brasileira de Ciências, 90*(2), 1285–1292. https://doi.org/10.1590/0001-3765201820170847.

Lertsatitthanakorn, P., Manwiwattanakun, K., Paengnakorn, N., & Khunkitti, W. (2014). Antibacterial activity of an effective essential oil formulated in liquid soap against skin bacteria. *Chiang Mai Journal of Science, 41*(1), 71–83.

Lotfy, T. M. R., Shawir, S. M. S., & Badawy, M. E. I. (2023). The impacts of chitosan-essential oil nanoemulsions on the microbial diversity and chemical composition of refrigerated minced meat. *International Journal of Biological Macromolecules, 239*, Article 124237. https://doi.org/10.1016/j.ijbiomac.2023.124237.

Nascimento, A. R., Serra, J. L., Martins, A. G. L. A., Filho, J. E. M., Natanael, E. A., & Andrade, L. S. (2010). Inhibitory effect of *Eucalyptus* sp. essential oil, pure and in associated to antibiotics, against strains of *Escherichia coli* and *Staphylococus aureus* isolated from handlers, food, sand and seawater. *Boletim Centro de Pesquisa de Processamento de Alimentos, 28*(1), 141−148. https://doi.org/10.5380/cep.v28i1.17905.

Nieto-Velázquez, N. G., Gomez-Valdez, A. A., González-Ávila, M., Sánchez-Navarrete, J., Toscano-Garibay, J. D., & Ruiz-Pérez, N. J. Preliminary study on citrus oils antibacterial activity measured by flow cytometry: a step-by-step development. *Antibiotics, 10*(10), Article 1218. https://doi.org/10.3390/antibiotics10101218.

Pateiro, M., Munekata, P. E. S., Sant'ana, A. S., Domínguez, R., Rodríguez-Lázaro, D., & Lorenzo, J. M. (2021). Application of essential oils as antimicrobial agents against spoilage and pathogenic microorganisms in meat products. *International Journal of Food Microbiology, 337*, Article 108966. https://doi.org/10.1016/j.ijfoodmicro.2020.108966.

Ribeiro, J. S., Santos, M. J. M. C., Silva, L. K. R., Pereira, L. C. L., Santos, I. A., Da Silva Lannes, S. C., & Da Silva, M. V. (2019). Natural antioxidants used in meat products: A brief review. *Meat Science, 94*(2), 220−227. https://doi.org/10.1016/j.meatsci.2013.01.007.

Santos, A. S., Alves, S., Figueirêdo, F. J. C., & Rocha Neto, O. G. (2004). Descrição de sistema e de métodos de extração de óleos essenciais e determinação de umidade de biomassa em laboratório. *EMBRAPA: Belém*, 1–6.

Sharafati-Chaleshtori, R., Rokni, N., Rafieian-Kopaei, M., Drees, F., & Salehi, E. (2015). Antioxidant and antibacterial activity of basil (*Ocimum basilicum* L.) essential oil in beef burger. *Journal of Agricultural Science and Technology, 17*, 817–826.

Sharma, H., Mendiratta, S. K., Agarwal, R., & Goswami, M. (2019). Optimization of various essential oils and their effect on the microbial and sensory attributes of chicken sausages. *Agricultural Research, 8*(3), 374–382. https://doi.org/10.1007/s40003-018-0367-x.

TACO. (2011). Tabela brasileira de composição de alimentos. 4. ed.Campinas: NEPA-UNICAMP. 161 p. Retrieved from: https://www.gov.br/agricultura/pt-br/assuntos/inspecao/produtos-vegetal/legislacao-de-produtos-origem-vegetal/biblioteca-de-normas-vinhos-e-bebidas/tabela-brasileira-de-composicao-de-alimentos_taco_2011.pdf. Accessed March 29, 2024.

Teixeira, J. P. F., Marques, M. O. M., & Figueiredo, J. O. (2013). Composição química de óleos essenciais de quinze genótipos de limão em duas épocas de colheita. *Citrus Research & Technology, 34*(2), 65−74. https://doi.org/10.5935/2236-3122.20130008.

Tomović, V., Šojić, B., Savanović, J., Kocić-Tanackov, S., Pavlić, B., Jokanović, M., Dordević, V., Parunović, N., Martinović, A., & Vujadinović, D. (2020). New formulation towards healthier meat products: *Juniperus communis* L. essential oil as alternative for sodium nitrite in dry fermented sausages. *Foods, 9*(8), Article 1066. https://doi.org/10.3390/foods9081066.

Vessoni, N. G., Piaia, A. F., & Bernardi, D. M. (2019). Pesquisa de consumo de carne bovina, produtos cárneos, hambúrguer e alimentos funcionais. *FAG Journal of Health (FJH), 1*(4), 25–37. https://doi.org/10.35984/fjh.v1i4.88.

Xin, K., Ji, X., Guo, Z., Han, L., Yu, Q., & Hu, B. (2022). Pitaya peel extract and lemon seed essential oil as effective sodium nitrite replacement in cured mutton. *LWT, 160*, Article 113283. https://doi.org/10.1016/j.lwt.2022.113283.