

Schinus terebinthifolius (Anacardiaceae) **essential oil for minas frescal cheese preservation**
Óleo essencial de *Schinus terebinthifolius* (Anacardiaceae) na conservação do queijo minas frescal

Aceite esencial de *Schinus terebinthifolius* (Anacardiaceae) para la conservación del queso minas frescal

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Abstract

Microorganism control in food has been a challenge due to the legislation restrictions of each country and the increase in the demand for food without chemical preservatives. Plant essential oils have antimicrobial action and are promising for the use in the food industry. Our study aimed to evaluate the chemical composition and the antimicrobial effect of *Schinus terebinthifolius* Raddi fruit essential oil on *minas frescal* cheese. The essential oil was extracted by hydrodistillation, and the chemical compounds were identified by gas chromatography coupled to mass spectrometry. The major identified compounds were hydrocarbon monoterpenes (57.0%), mainly: α -pinene (22.2%), limonene (17.0%), carvone (10.2%) and β -phellandrene (7.9%). The cheese was produced, the main microorganisms were isolated, and the minimum inhibitory concentration was determined through broth microdilution test. The cheese samples were soaked. The pink pepper fruit essential oil was added to the cheese samples at the concentration of 20000 μ L/mL superficially or to the micelle. *S. terebinthifolius* fruit essential oil was efficient to control *minas frescal* cheese microorganisms mainly when applied by superficial addition. The essential oil is a potential source of studies to develop applications for the control of microorganisms in cheese such as

minas frescal. Further studies may exploit the impact of the essential oil in *minas frescal* cheese acceptability.

Keywords: Antimicrobial; *Minas frescal* cheese; Natural preservatives; *Schinus terebinthifolius*.

Resumo

O controle de microrganismos em alimentos tem sido um desafio face às restrições da legislação de cada país e pelo aumento da demanda de alimentos sem adição de conservantes químicos. Os óleos essenciais de plantas possuem ação antimicrobiana e são promissores para o uso na indústria de alimentos. O objetivo do nosso trabalho foi avaliar a composição química e o efeito antimicrobiano do óleo essencial do fruto de *Schinus terebinthifolius* Raddi em queijos minas frescal. O óleo essencial foi extraído por hidrodestilação, e os compostos químicos identificados por cromatografia gasosa acoplada a espectrometria de massas. Os principais compostos identificados foram os hidrocarbonetos monoterpênicos (57,0%), sendo os principais: α -pineno (22,2%), limoneno (17,0%), carvona (10,2%) e β -felandreno (7,9%). O queijo foi produzido e os principais microrganismos foram isolados e determinada a concentração inibitória mínima através do teste de microdiluição em caldo. As amostras de queijo foram impregnadas com o óleo essencial do fruto da pimenta rosa na concentração de 20000 μ L/mL por adição superficial ou adicionado à micela. O óleo essencial dos frutos de *S. terebinthifolius* foi eficiente para controle de microrganismos do queijo minas frescal principalmente quando aplicado por adição superficial. O óleo essencial é uma potencial fonte de estudos para o desenvolvimento de aplicações para o controle de microrganismos em queijos tipo minas frescal. Futuros estudos podem explorar o impacto do óleo essencial na aceitabilidade do queijo minas frescal.

Palavras-chave: Antimicrobiano; Queijo minas frescal; Conservante natural; *Schinus terebinthifolius*.

Resumen

El control de microorganismos en los alimentos ha sido un desafío dadas las restricciones de la legislación de cada país y la mayor demanda de alimentos sin la adición de conservantes químicos. Los aceites esenciales de plantas tienen acción antimicrobiana y son prometedores para su uso en la industria alimentaria. El objetivo de nuestro trabajo fue evaluar la composición química y efecto antimicrobiano del aceite esencial del fruto de *Schinus terebinthifolius* Raddi en queso de *minas frescal*. El aceite esencial se extrajo mediante

hidrodestilación y los compuestos químicos se identificaron mediante cromatografía de gases acoplada a espectrometría de masas. Los principales compuestos identificados fueron los hidrocarburos monoterpénicos (57,0%), siendo los principales: α -pineno (22,2%), limoneno (17,0%), carvona (10,2%) y β -felandreno (7,9%). Se elaboró el queso y se aislaron los principales microorganismos y se determinó la concentración mínima inhibitoria mediante la prueba de microdilución en caldo. Las muestras de queso se impregnaron con el aceite esencial del fruto de pimienta rosa a una concentración de 20000 $\mu\text{L}/\text{mL}$ por adición superficial o agregado a la micela. El aceite esencial de los frutos de *S. terebinthifolius* resultó eficaz para el control de microorganismos del queso *minas frescal*, principalmente cuando se aplicó por adición superficial. El aceite esencial es una fuente potencial de estudios para el desarrollo de aplicaciones para el control de microorganismos en el queso fresco de minas. Los estudios futuros pueden explorar el impacto del aceite esencial en la aceptabilidad del queso *minas frescal*.

Palabras clave: Antimicrobiano; Queso de *minas frescal*; Conservante natural; *Schinus terebinthifolius*.

1. Introduction

Minas frescal fresh cheese is obtained by enzymatic coagulation of the nonpolar fraction of milk casein which is separated but not matured (Brasil, 2004). This cheese is very appreciated in Brazil and has an estimated consume of 119,561.7 kg/year in 2019 (Veríssimo et al., 2019). *Minas*-type cheese has fat content ranging from 25.0% to 44.9% and moisture over 55.0% (Brasil, 2004). Due to its physical and chemical characteristics, it is an ideal means for the growth of pathogenic rotting microorganisms (Carvalho et al., 2007) and, therefore, with shelf life of up to 20 days (ABIQ, 2020).

Chemical preservatives are utilized to control microorganism growth in fresh cheese (Gonçalves et al., 2011). The preservatives have been associated with toxic reactions in the metabolism as well as with allergy triggering, behavioral changes and possible risks of cancer development (Polônio & Peres, 2009). Potassium sorbate (Ordoñez et al., 2020) and natamycin (Aparício et al., 2016) have usually been applied in the superficial treatment with fungicide and bactericide functions. Sodium nitrate has also been reported to be used to avoid swelling of *minas frescal* cheese (Mello, 2007), and to inhibit microorganism contamination, specially *Clostridium* bacteria (Perry, 2004).

Koul et al. (2008) alerted that the use of synthetic fungicide could develop resistant fungal strains and recommended essential oil of biological base to be used as fungicides, boosting the search for plant antimicrobials (Anyanwu & Okoye, 2017). Sivakumar & Bautista-Baños (2014) recommended the utilization of essential oils to preserve food; Burt (2004) advise concentrations from 0.1 to 6% of essential oils to preserve food and as aromatizing agents which are also generally considered safe (GRAS) (Burt, 2004; Sivakumar & Bautista-Baños, 2014; Tedesco et al., 2020). Therefore, essential oils are considered promising for food preservation because they present antimicrobial and antioxidant activity (Bouhdid et al., 2010).

Schinus terebinthifolius Raddi, from the family Anacardiaceae, popularly known as pink pepper (Brazilian pepper tree) or aroeira in Brazil (Gomes et al., 2013) is a native plant of the Atlantic Forest (Cesário & Gaglianone, 2013). *S. terebinthifolius* fruit is very utilized in culinary and has approximately 70 g of essential oil/kg (Bortolucci et al., 2019). *S. terebinthifolius* essential oils present antimicrobial activity mainly against Gram-positive bacteria and fungi from the genus *Candida* (Pires et al., 2004). Thus, due to the antimicrobial activity of the oil and the high perishability of minas-type cheese, our study aimed to determine the chemical composition of *S. terebinthifolius* fruit essential oil, and to apply the oil on *minas frescal* cheese. Our results showed that the oil can be used to control microorganisms in cheese and expand the utilization of plant compounds to preserve food.

2. Method

2.1 Vegetal matter acquisition and *S. terebinthifolius* fruit essential oil extraction

Ripe fruits of *S. terebinthifolius* were harvested in December 2015 in Juranda, northern region of Paraná State, Brazil (S24°21'28.2456" and WO52°36'6276" and altitude of 419 m). The botanical identification was carried out and an exsiccate was deposited in the Herbarium of West Paraná State University - UNIOESTE, under the registration number 1717. This species was registered in the System of the Brazilian Genetic Heritage and Associated Traditional Knowledge (SisGen, acronym in Portuguese) under the registration number A67A798. *S. terebinthifolius* fruits were fragmented with water (1:10) in an industrial blender for 5 min and immediately submitted to hydrodistillation in a modified Clevenger device for 2 h (Santos et al. 2013). After the distillation, the essential oil was stored in an amber flask at 4 °C (Brasil, 2010).

2.2 Gas chromatography-mass spectrometry of *Schinus terebinthifolius* fruit essential oil

The chemical identification of the essential oil was done by a gas chromatographer (Agilent 7890 B) coupled to a mass spectrometer (Agilent 5977A) (GC-MS), equipped with a capillary column fused with silica HP-5MS UI Agilent (30 m x 0.250 mm x 0.25 µm). The analysis conditions were as following: injector temperature at 220 °C, injection volume of µL and injection rate in split mode (1:30). The initial column temperature was 60 °C (2 min), with heating ramp of 2°C/min up to 180 °C (4 min). From 180 °C to 260 °C, a heating ramp of 10 °C/min was determined. From 260 °C to 300 °C, a heating ramp of 40 °C/min was utilized (Cavalcanti et al., 2015). The transfer line was kept at 285 °C, and the ionization source and quadrupole at 230 °C and 150 °C, respectively. The utilized carrier gas was helium with a flow of 1 mL/min. The detection system was EM in “scan” mode, in the mass/charge (m/z) rate of 40-550 m/z with 3-min “solvent delay”. The oil samples were diluted in the ratio of 1:10 with dichloromethane. The chemical compounds were identified comparing their mass spectra to the mass spectra from WILEY 275 as well as comparing their retention indices (RI) which were obtained by utilizing a homologous n-alkane standard series (C7-C26) (Adams, 2017).

2.3 Isolation of *minas frescal* cheese natural microbiota

In order to produce *minas frescal* cheese, *in natura* milk was kept at 70 °C for 30 minutes and cooled in water bath to 34 °C. Next, a commercial chymosin coagulating agent, 75 International Milk Coagulating Unit - IMCU, liquid Estrella® curd (4 mL for each 8 L of milk) was used. The mixture was kept at rest for 50 min until coagulation. The resulting curd was cut in 1-2 cm cubes with a sterile cutter, slowly agitated (5 min) and then 10 g of cooking salt (NaCl) was added. After that, the precipitate was separated in perforated plastic molds (5 cm x 5 cm x 5 cm) to allow liquid draining for 12 hours under refrigeration (7 ± 0.5 °C). Later, they were removed from the molds and stored in closed polypropylene recipients at 8° C (Ribeiro, 2015).

After the cheese preparation, the microorganisms, naturally found in cheese, were isolated to determine the minimum inhibitory concentration (MIC) of the essential oil. Samples of 1g from different parts of the cheese were collected, ground and homogenized in an Erlenmeyer flask containing peptone water at 0.1%. Serial dilutions were carried out in peptone water at 0.1% next, 1 mL of each dilution was sown in 15 mL of standard agar

medium count pattern (PCA) (Himedia®) in Petri dishes by *Pour Plate* technique, and incubated for 24 hours in an oven at 35 °C. The microorganism growth was evaluated and quantified by total count method. The microorganism isolation was done in nutrient agar medium by streak method and the identification was carried out by Gram coloration. Only plates with growth lower than or equal to 300 colonies per plate were considered for the identification and isolation of microorganisms.

2.4 Antimicrobial activity of the essential oil against isolated microorganisms by broth microdilution

Broth microdilution method using 96-well microplate was utilized for the assays according to the Clinical and Laboratory Standard Institute (CLSI) and the protocols M27-A2 and M7-A5, modified for natural products (CLSI, 2015). A replication in the culture medium of the isolated strains was done for 8 hours before determining the minimum inhibitory concentration (MIC). Next, for each isolated microorganism, a standard suspension in saline solution was obtained and adjusted to 0.15 of McFarland scale in order to obtain 1.5×10^4 CFU/mL. The essential oil solution was prepared with distilled water using Tween 80 (2%, volume/volume; Sigma–Aldrich, USA) as emulsifier, from an initial concentration of 40,000 µg/mL. Then, the serial dilution of 1:2 wells in the microplate, containing 100 µL of culture medium, was carried out. After the essential oil dilution, 5 µL of the inoculum was added to all column wells. Each microplate includes positive controls (culture medium without the essential oil) and negative controls (non-inoculated culture medium). The microplates were incubated at 35 °C for 24 h. The antibiotics streptomycin (Sigma P7794) and ketoconazole (Sigma-Aldrich®) were used as positive control (1 mg/mL in sterile saline solution), and tween aqueous solution as negative control. The reading was done with the addition of 10 µL of developer, 2, 3,5-cloreto of triphenyl tetrazolium (Reatec®) at 1.0% in each well, followed by microplate incubation at 37° C for 10 min. The minimum inhibitory concentration (MIC) of the essential oil was defined as the lowest concentration capable of inhibiting bacterial growth.

2.5 Application of essential oil to *minas frescal* cheese

The cheese samples were added with essential oil at the concentration of 20000 µL/mL by superficial application or by addition to the mass after coagulation at a concentration based

on the results of MIC for isolated microorganisms. For the superficial application, a pipette was used for the dripping of the essential oil diluted in distilled water with tween at the concentration of 20 mg/mL, using 500 μ L (20 μ L/cm²). In the treatment of sample by mixture, the same amount of essential oil was added in the cheese mass when preparing it.

The cheese without essential oil addition was utilized as control. Later, the samples were stored in closed plastic recipients under refrigeration at 8° C for 15 days. Each treatment was carried out in triplicate and analyzed at 0, 5 and 10 days of storing. The antimicrobial activity was determined by quantifying the total aerobic mesophilic microorganisms (APHA, 2001). After the preparation, samples of 1 g were collected from the analyzed cheese. The portions were separately diluted in peptone water at 0.1% and diluted in series. After the dilutions, 1 mL of each dilution in 15 ml of PCA medium in Petri dish was sown and incubated for 24 hours at 35°C. Next, the total microorganism count was carried out.

2.6 Statistical analysis

All assays were done in triplicate. The results were expressed in arithmetic mean \pm standard deviation and analyzed by analysis of unidirectional variance (ANOVA) followed by Tukey HSD test (honestly significant difference) with $\alpha = 0.05$ to determine the statistical significance of the results. The analysis was done using Statistica® 8.0 software.

3. Results

Data related to the chemical composition of pink pepper fruit essential oil are shown in Table 1. Forty-five compounds were found, and the major class was hydrocarbon monoterpenes (57.0%), and the main ones were α -pinene (22.2%), limonene (17.0%), carvone (10.2%) and β -phellandrene (7.9%).

In the evaluation and isolation of cheese microbiota, the presence of Gram-positive bacteria and septate filamentous fungus was observed. The average values of MIC for the isolated bacterium 1 was 6.67 ± 2.88 mg/mL and for the isolated bacterium 2 was 13.34 ± 5.77 mg/mL, and for the fungus 1.9 mg/mL. For the positive control, the average MIC value was $6.25 \pm < 0.01$ mg/mL for streptomycin and 0.35 ± 0.12 mg/mL for ketoconazole, respectively (Table 2). There was no significant difference ($p \leq 0.05$) when comparing the average MIC values of the control streptomycin to isolate 1; however, when compared to isolated 2, the essential oil presented MIC 2-fold higher than the positive control ($p \leq 0.05$).

These results show the potential of pink pepper fruit essential oil as a development inhibitor of isolated contaminating microorganism of cheese.

Table 1. Chemical composition of *Schinus terebinthifolius* fruit essential oil.

Peak	RT	^a Compounds	RI _{Lit.}	^b RI _{calc.}	Relative area (%)	IM
Hydrocarbon Monoterpenes						
1	6.01	α -thujene	924	915	0.60	a,b,c
2	6.33	α -pinene	932	925	22.24	a,b,c
3	7.59	sabinene	969	961	0.39	a,b,c
4	7.70	β -pinene	974	964	0.74	a,b,c
5	8.30	myrcene	988	979	1.89	a,b,c
6	8.87	α -phellandrene	1002	992	6.21	a,b,c
7	9.84	limonene	1024	1015	17.02	a,b,c
8	10.02	β -phellandrene	1025	1019	7.89	a,b,c
Oxygenated monoterpenes						
9	12.84	γ -terpinene	1054	1075	0.64	a,b,c
10	13.30	α -pinene oxide	1099	1092	0.35	a,b,c
11	13.84	<i>trans</i> -pinocarveol	1139	1137	1.85	a,b,c
12	17.91	citronellal	1148	1164	0.31	a,b,c
13	18.21	4-terpineol	1174	1169	0.52	a,b,c
14	18.34	8- <i>p</i> -cyneol	1179	1171	0.60	a,b,c
15	19.34	α -terpineol	1186	1186	1.23	a,b,c
16	20.57	4- <i>cis</i> -caranone	1200	1205	0.58	a,b,c
17	22.80	carvone	1239	1244	10.19	a,b,c
18	23.27	<i>p</i> -menth-1-en-7-al	1273	1252	0.81	a,b,c
19	24.28	ni	-	1267	0.27	a,b,c
20	25.12	hydroxy citronellal	1286	1280	2.47	a,b,c
21	25.96	limonene-10-ol	1289	1292	0.71	a,b,c
22	26.48	γ -terpinen-7-al	1291	1299	0.49	a,b,c
23	26.86	carvacrol	1299	1306	0.22	a,b,c
Hydrocarbon Sesquiterpenes						
24	27.93	δ -elemene	1335	1325	2.80	a,b,c
25	28.72	ni	-	1339	0.28	a,b,c
26	28.92	α -cubebene	1345	1342	0.61	a,b,c
27	29.90	α -longipinene	1350	1357	0.33	a,b,c
28	30.82	Cyclosativene	1369	1372	0.25	a,b,c
29	30.97	α -copaene	1374	1374	0.63	a,b,c
30	31.17	β -patchoulene	1379	1377	0.55	a,b,c
31	31.39	β -cubebene	1387	1380	1.25	a,b,c
32	31.91	β -elemene	1389	1388	0.27	a,b,c
33	32.46	<i>cis</i> -caryophyllene	1407	1397	0.64	a,b,c
34	32.75	α -gurjunene	1409	1401	1.07	a,b,c
35	33.10	<i>trans</i> -caryophyllene	1419	1407	0.85	a,b,c
36	33.31	α - <i>trans</i> -bergamotene	1432	1412	0.60	a,b,c
37	34.91	α -humulene	1452	1450	0.37	a,b,c
38	36.22	germacrene D	1484	1463	2.93	a,b,c
39	37.02	α -selinene	1498	1470	0.36	a,b,c
40	38.77	γ -cadinene	1513	1502	0.32	a,b,c
41	40.47	δ -cadinene	1522	1535	0.67	a,b,c

Oxygenated Sesquiterpenes						
42	41.74	spathulenol	1577	1573	1.50	a,b,c
43	42.08	caryophyllene oxide	1582	1584	0.49	a,b,c
44	45.23	1,10-di-epi-cubenol	1618	1620	2.24	a,b,c
45	45.70	cubenol	1645	1630	0.28	a,b,c
46	46.77	α -cadinol	1652	1648	0.21	a,b,c
47	53.02	aristolene	1756	1761	0.32	a,b,c
48	72.56	ni	-	2292	0.67	a,b,c
Total identified					97.50	
Hydrocarbon Monoterpenes					56.98	
Oxygenated Monoterpenes					20.97	
Hydrocarbon Sesquiterpenes					14.50	
Oxygenated Sesquiterpenes					5.05	

RT = retention time; ^a**RI** = identification based on calculated retention index (RI) utilizing a homologous standard series of n-alkanes c7 to c26 n-alkanes in the column (HP-5MS); ^b**Compounds** = compounds listed in elution order in the column HP-5MS UI; ^c Identification based on the comparison of mass spectra found in Wiley 275 library; Relative area (%): percentage of area occupied by the compound in the chromatogram; **IM** = identification methods; ni = non-identified; Source: Authors.

Table 2. Minimum inhibitory concentration (MIC) of *Schinus terebinthifolius* fruit essential oil and the controls streptomycin and ketoconazole (mg/mL).

	Essential oil (mg/mL)	Streptomycin (mg/mL)	Ketoconazole (mg/mL)
<i>Isolated 1</i>	6.67 \pm 2.88 ^a	6.25 \pm < 0.01 ^a	-
<i>Isolated 2</i>	13.34 \pm 5.77 ^b	6.25 \pm < 0.01 ^a	-
<i>Isolated 3</i>	1.87 \pm 0.88 ^b	-	0.35 \pm 0.12 ^a

*Small letters on the same line differ among themselves according to Tukey test ($p \leq 0.05$). Source: Authors.

The average count results of total aerobic mesophilic microorganisms (CFU/g) of the cheese samples submitted to treatments with essential oil by superficial application or addition to the mass are shown in Table 3. The *in situ* test presented similar tendency for the development of microorganisms in all treatments during 15 days of refrigerated storage, with a gradual increase in the cell count during this period. However, the addition of essential oil resulted in a kinetic difference of the bacterial growth. In the control treatment (without addition of essential oil), the microbial count increase from 6.78 log CFU/g up to 8.49 log

CFU/g in the 15-day storage period. The treatment of essential oil superficial application presented a lower bacterial count between the treatments, from 5.73 log CFU/g up to 8.34 log CFU/g. In the 10-to-15-day period, the cheese samples added with essential oil had log CFU/g from 10 to 12% lower ($p \leq 0.05$) than the control.

Table 3. Total microorganism count (CFU/g) of *minas frescal* cheese submitted to treatments with *Schinus terebinthifolius* fruit essential oil by superficial application or addition to the cheese mass.

Time	Superficial application	Addition to the mass	Control
0	5.73 ± 0.49 ^{aA}	5.84 ± 0.22 ^{aA}	6.78 ± 0.01 ^{aB}
5	6.52 ± 0.02 ^{bA}	7.83 ± 0.02 ^{bB}	6.78 ± 0.01 ^{aC}
10	7.89 ± 0.01 ^{cB}	7.20 ± 0.20 ^{cA}	8.00 ± 0.01 ^{cB}
15	8.34 ± 0.01 ^{dA}	8.40 ± 0.01 ^{dB}	8.49 ± 0.01 ^{dC}

*Averages followed by the same lowercase letters in the same columns and the same uppercase letter in the lines do not differ among themselves by Tukey test ($p \leq 0.05$). Source: Authors.

4. Discussion

Our results showed that *Schinus terebinthifolius* Raddi essential oil has inhibitory activity in *minas frescal* cheese microbiota. The oil contains mainly monoterpenes, principally α -pinene, limonene, carvone and β -phellandrene. For α -pinene, antimicrobial activity has been reported in the literature against *Streptococcus pyogenes* (MIC 0.132 mg/mL), methicillin-resistant *Staphylococcus aureus* (MIC 0.210 mg/mL), methicillin-gentamicin *Staphylococcus aureus* (MIC 0.256 mg/mL), *Streptococcus pneumoniae* (MIC 0.172 mg/mL), *Haemophilus influenzae* (MIC 0.126 mg/mL) and *Escherichia coli* (MIC 0.98 mg/mL) (Yang et al. 2015). In studies by Sieniawska et al. (2013), α -pinene exhibited antimicrobial activity against *Staphylococcus epidermidis* (MIC 0.65 mg/mL) and inhibited biofilm formation (MBIC – minimal biofilm inhibitory concentration 1.25 mg/mL). The

isomer (+)- α -pinene has antimicrobial activity and synergic effect with ciprofloxacin bactericide against *Staphylococcus aureus* MRSA (MIC 4.15 mg/mL), as well as presents antifungal activity and inhibits the biofilm formation of *Candida albicans* yeast (MIC 3.12 mg/mL), and activity against *Cryptococcus neoformans* (MIC 0.117 mg/mL), *Rhizopus oryzae* (MIC 0.390 mg/mL) (Silva et al. 2012). Silva et al. (2012) reported that (+)- α -pinene inhibited the secretion of phospholipase and esterase enzymes by microorganisms.

For limonene, antimicrobial activities have been reported against Gram-positive bacteria, *Staphylococcus aureus* (MIC 0.06 mg/mL), *Bacillus subtilis* (MIC 0.06 mg/mL), and Gram-negative *Salmonella* sp. (MIC 0.60 mg/mL), *Pseudomonas aeruginosa* (MIC 0.60 mg/mL) (Teneva et al., 2019). In studies by Li et al. (2019), limonene showed antibacterial activity against *S. aureus* (MIC 0.62 mg/mL), *B. subtilis* (MIC 1.25 mg/mL), *Micrococcus luteus* (MIC 2.5 mg/mL) and *Escherichia coli* (MIC 1.25 mg/mL).

The antimicrobial activity of *S. terebinthifolius* Raddi essential oil can be explained by the minimum inhibitory concentrations obtained by monoterpene compounds. Therefore, the compounds of pink pepper fruit essential oil may have acted on the control of microorganisms of *minas frescal* cheese. Dannenberg et al. (2016) showed, for ripe fruit essential oil, high antimicrobial activity with MIC values of 1.7 mg/mL, 6.8 mg/mL and 0.85 mg/mL against *S. aureus*, *L. monocytogenes* and *B. cereus*. Also, the essential oil exhibited biopreservative activity of *frescal* queijo. For these authors, *S. terebinthifolius* essential oil is a potential biopreservative, but not yet much used by the food industry.

5. Conclusion

In our study, 45 compounds were identified in *Schinus terebinthifolius* fruit essential oil and the major class was hydrocarbon monoterpenes (57.0%), and the main compounds were: α -pinene (22.2%), limonene (17.0%), carvone (10.2%) and β -phellandrene (7.9%). *S. terebinthifolius* fruit essential oil was efficient to control microorganisms of *minas frescal* cheese, mainly by superficial application. The essential oil is a source of studies for the development of applications to control microorganisms in *minas frescal* cheese. Further studies may explore the impact of the essential oil on the acceptability of *minas frescal* cheese.

Referências

ABIQ (2020). Sobre queijos: minas frescal. Website da ABIQ - Associação Brasileira das Indústrias de Queijo. Retrieved from https://abiq.com.br/queijos_ler.asp?codigo=1925&codigo_categoria=16&codigo_subcategoria=37.

Adams, R. P. (2017). *Identification of essential oil components by gas chromatography/mass spectrometry*. (5th ed.), Texensis Publishing, Gruver, TX, USA, 804.

Anyanwu, M. U., & Okoye, R. C. (2017). Antimicrobial activity of Nigerian medicinal plants. *J Intercult Ethnopharmacol*, 6, 240–259. doi: 10.5455/jice.20170106073231

Aparício, J. F., Barreales, E. G., Payero, T. D., Vicente, C. M., de Pedro, A., & Santos-Aberturas, J. (2016). Biotechnological production and application of the antibiotic pimaricin: biosynthesis and its regulation. *Appl Microbiol Biotechnol*, 100(1), 61–78.

APHA (2001). Committee on Microbiological for Foods. Compendium of methods for the microbiological examination of foods. APHA - American Public Health Association. (4th ed.) *Washington: American Public Health Association*, 676, 2001.

Bortolucci, W. de C., de Oliveira, H. L. M., Silva, E. S., Campo, C. F. de A. A., Gonçalves, J. E., Piau Junior, R., Colauto, N. B., Linde, G. A., & Gazim, Z. C. (2019). Schinus terebinthifolius essential oil and fractions in the control of *Aedes aegypti*. *Biosci J*, 35(5), 1575-1587.

Brasil. Ministério da Agricultura, Pecuária e Abastecimento. Aditivos no Regulamento Técnico para Fixação de Identidade e Qualidade do Queijo *minas frescal*. Instrução Normativa nº 4, de 01 de março de 2004. *Diário Oficial da República Federativa do Brasil, Brasília*, 5 mar. 2004.

Brasil (2010). Farmacopeia Brasileira. Agência Nacional de Vigilância Sanitária. Brasília: *Anvisa*, (5th ed.), 546.

Bouhdid, S., Abrini, J., Amensour, M., Zhiri, A., Espuny, M. J., & Manresa, A. (2010). Functional and ultrastructural changes in *Pseudomonas aeruginosa* and *Staphylococcus aureus* cells induced by *Cinnamomum verum* essential oil. *J Appl Microbiol*, 109(4), 1139-1149.

Burt, S. 2004. Essential oils: their antibacterial properties and potential applications in foods-a review. *Int J Food Microbiol*, 94 (3), 223–253.

Cesário, L. F., & Gaglianone, M. C. (2013). Polinizadores de *Schinus terebinthifolius* Raddi (Anacardiaceae) em formações vegetacionais de restinga no norte do estado do Rio de Janeiro. *Biosci J*, 29(2), 458-467.

Carvalho, J. D. G., Viotto, W. H., & Kuaye, A. Y. (2007). The quality of *minas frescal* cheese produced by different technological processes. *Food Control*, 18(3), 262- 267.

Cavalcanti, A. S., Alves, M. S., Silva, L. C. P., Patrocínio, D. S., Sanches, M. N., Chaves, D. S. A. & Souza, M. A. A. (2015). Volatiles composition and extraction kinetics from *Schinus terebinthifolius* and *Schinus molle* leaves and fruit. *Revista Brasileira de Farmacognosia*, 25(4), 356-362.

CLSI - Clinical and Laboratory Standards Institute. 2015. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved Standard, (10th ed.), CLSI document M07-A10. Wayne, PA.

Dannenberg, G. D., Funck, G. D., Mattei, F. J., Silva, W. P., & Fiorentini, Â M. (2016). Antimicrobial and antioxidant activity of essential oil from pink pepper tree (*Schinus terebinthifolius* Raddi) in vitro and in cheese experimentally contaminated with *Listeria monocytogenes*. *Innov Food Sci Emerg Technol*, 36, 120-127.

Gomes, L. J., Silva-mann, R., Mattos, P. P. De, Rabbani, A. R. C. (2013). *Pensando a biodiversidade: aroeira (Schinus terebinthifolius Raddi.)*, São Cristóvão: Editora UFS.

Gonçalves, J. F., Oliveira, W. C., Silva, C. A. O., Cunha, M. R. R., & Pereira, F. R. (2011). Ocorrência de nitratos e nitritos em queijos *minas frescal*, Mussarela, Parmesão e Prato. *Rev Inst Adolfo Lutz*, 70(2), 193-198.

Koul, O., Walia, S., & Dhaliwal, G. S. (2008). Essential oil as green pesticides potential and constraints. *Biopestic Int*, 4, 63-84.

Li, Z. H., Cai, M., Liu, Y. S., Sun, P. L., & Luo, S. L. (2019). Antibacterial Activity and Mechanisms of Essential Oil from *Citrus medica* L. var. *sarcodactylis*. *Molecules*, 24(8), 1577.

Mello F. F. Produção de queijo *minas frescal*. *Portal Hexis Científica*. 2007.

Ordoñez, R., Contreras, C., González-Martínez, C., & Chiralt, A. Edible coatings controlling mass loss and *Penicillium roqueforti* growth during cheese ripening. (2020). *J Food Eng*, doi: <https://doi.org/10.1016/j.jfoodeng.2020.110174>.

Perry, K. S. P. (2004). Queijos: aspectos químicos, bioquímicos e microbiológicos. *Quim Nova*, 27(2), 293–300.

Pires, O. C., Taquemasa, A. V. C., Akisue, G., Oliveira, F., Araújo, C. E. P. (2004). Análise preliminar da toxicidade aguda e dose letal mediana (DL50) comparativa entre os frutos de Pimenta-do-Reino do Brasil (*Schinus terebinthifolius* Raddi) e Pimenta-do-Reino (*Piper nigrum* L.). *Acta Farm Bonaer*, 23(2), 176-182.

Polônio, M. L. T., & Peres, F. (2009). Consumo de aditivos alimentares e efeitos à saúde: desafios para a saúde pública brasileira. *Cad Saúde Pública*, 25(8), 1653-1666.

Ribeiro, A. C. Efeito da adição de óleo essencial de pimenta rosa (*Schinus terebinthifolius* Raddi) microencapsulado em queijo *minas frescal*. Dissertação de Mestrado. Alegre. ES. Universidade Federal do Espírito Santo. 10, 2015.

Santos, M. R. A., Lima, R. A., Silva, A. G., Lima, D. K. S., Sallet, L. A. P., Teixeira, C. A. D., & Facundo, V. A. (2013). Chemical composition and insecticidal activity of the essential

oil of *Schinus terebinthifolius* Raddi (Anacardiaceae) on coffee berry borer (*Hypothenemus hampei*) Ferrari. *Rev Bras Pl Med*, 15(4), 757-762. <https://doi.org/10.1590/S1516-0572201300050001>

Silva, A. C. R., Lopes, P. M., Barros De Azevedo, M. M., Costa, D. C., Alviano, C. S., & Alviano, D. S. (2012). Biological activities of α -pinene and β -pinene enantiomers. *Molecules*, 17(6), 6305-16.

Sieniawska, E., Los, R., Baj, T., Malm, A., & Glowniak, K. (2013). Antimicrobial efficacy of *Mutellina purpurea* essential oil and α -pinene against *Staphylococcus epidermidis* grown in planktonic and biofilm cultures. *Ind Crops Prod*, 51, 152-157.

Sivakumar, D., & Bautista-Baños, S. 2014. A review on the use of essential oils for postharvest decay control and maintenance of fruit quality during storage. *Crop Prot*, 64, 27-37.

Tedesco, L., de Campos Bortolucci, W., Silva, E. S., Gonçalves, J. E., Colauto, N. B., Gazim, Z. C., & Linde, G. A. (2020). Fases fenológicas da jabuticaba (*Plinia cauliflora*) e composição química do óleo essencial das folhas e atividade antioxidante. *Research, Society and Development*, 9(9), e396997305-e396997305.

Teneva, D., Denkova-kostova, R., Goranov, B., Hristova-ivanova, Y., Slavchev, A., Denkova, Z., & Kostov, G. (2019). Chemical composition, antioxidant activity and antimicrobial activity of essential oil from *Citrus aurantium* L zest against some pathogenic microorganisms. *Z Naturforsch C J Biosci*, 74(5-6), 105-111.

Veríssimo, J. Cardoso, F. V., Silvério, D.F., Souza, T. C. B., Machado, R. H. C. Análise de Viabilidade Econômica De uma Fábrica de Queijo *minas frescal*. ConBRepro - IX Congresso Brasileiro de Engenharia de Produção. Retrieved from aprepro.org.br/conbrepro/2019/anais/arquivos/09302019_220935_5d92a7c74b73e.pdf

Yang, C., Hu, D., & Feng, Y. (2015). Antibacterial activity and mode of action of the *Artemisia capillaris* essential oil and its constituents against respiratory tract infection-causing pathogens. *Mol Med Rep*, 11(4), 2852-2860.

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