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Basil, marjoram, nutmeg and oregano essential oils as natural preservatives of Quark-type cheese

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Abstract

This study aims to evaluate the *in vitro* antimicrobial activity of basil, marjoram, nutmeg, and oregano essential oils to use the essential oil with better results for Quark-type cheese preservation. To verify the shelf life of Quark-type cheese without essential oil (CTL) and Quark-type cheese added with 0.05% essential oil (OE), microbiological and physicochemical analyses were performed for 35 days (4 °C). The acceptability of the product was also evaluated. The oregano essential oil showed strong antimicrobial activity in the disk-diffusion test (34.0 to 51.0 mm), and lower values of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against all species of bacteria tested. A lower mold and yeast count ($p < 0.05$) was observed during storage in OE denoting a positive effect in delaying its deterioration. On the other hand, there was no difference ($p > 0.05$) between the CTL and OE count of total mesophiles and *Enterobacteriaceae*. For the physicochemical parameters evaluated, there were no significant differences between the CTL and OE, thus denoting that the typical characteristics of this cheese were maintained. The Quark-type cheese added with oregano essential oil also showed good acceptability (> 7), which is a way to preserve the product with a natural additive.

Keywords: Quark-type cheese; natural additives; antimicrobial activity; *Ocimum basilicum*; *Origanum manjerona*; *Virola surinamensis*; *Ocimum vulgare*.

Practical Application: This work discusses essential oils as strategies to mitigate the use of artificial additives to improve the shelf life of a Quark-type cheese.

1 Introduction

Quark-type is a fresh, spreadable cheese variety with paste-like consistency that is mainly manufactured with combined acid-rennet curds, in which hydrated acid casein gel particles are dispersed in whey (Lepesioti et al., 2021). Quark-type presents a smooth texture and a mild, slightly sour flavor. Quark-type cheese is made to be consumed without aging the milk, so the milk used for quark cheese must be pasteurized, and the product is kept under refrigeration to ensure the safety of the consumer (Chauhan et al., 2022). Dairy products have substantial health benefits and could be considered an essential component of an equilibrated diet (Lima et al., 2022). In addition, the dairy fat present in cheeses has been reported to influence the consumer's health beneficially, when on a moderate diet inclusion. Therefore, some saturated fatty acids present in milk and dairy products could collaborate to the prevention of chronic inflammatory diseases, cardiovascular disease reduction, and the maintenance of gut microbiota (Bruen et al., 2017; Gómez-Cortés et al., 2018; Feeney et al., 2021; Beltrão et al., 2022).

Quark-type cheese, in addition to being rich in nutrients, is a cheese that has high water activity, favoring the growth of undesirable microorganisms. For this reason, food industries often add a preservative agent to this product, such as potassium sorbate. On the other hand, according to Manassi et al. (2022), the current trend of consumers is the search for differentiated foods, with high nutritional value, natural, healthy, and exempt, or with a reduction in synthetic additives. Consumers are willing to purchase new products and products with new processing and conservation technologies. However, information related to the benefits of the technological change of the product needs to be presented on the label, clearly and attractively (Delorme et al., 2021). In addition, some factors must be taken into account when marketing a differentiated product, such as flavor, consistency, color and purchase independence (Cais-Sokolińska et al., 2021). These factors must be of low discrepancy in relation to the already known products by consumers. Thus, the correct choice of the ingredients to be used is fundamental for the final acceptance of the product. In this way, the food industry's search for natural additives origin is increasing, which has been driving research into products such as essential oils.

Essential oils are mixtures of secondary metabolites of plants that have volatile and aromatic products, and can be stored in various plant organs, usually in leaf, fruit, or bark (Angane et al.,

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2022). Most essential oils are composed of terpenic substances, plus smaller molecules such as alcohols, esters, aldehydes, and short-chain ketones (Amor et al., 2021). Several essential oils have antimicrobial properties and can act as biopreservers, reducing or eliminating pathogenic bacteria and increasing the microbiological quality of food products of animal and plant origin (Angane et al., 2022). Mahmoudzadeh et al. (2022) developed a *Cinnamon essencial* oil nanoemulsion and applied it to milk foodborne pathogens as an antimicrobial agent against *Listeria monocytogenes* and *Escherichia coli* O157:H7. Shojaee et al. (2022) got antioxidant and antifungical action against *Penicillium chrysogenum*, *Aspergillus niger,* and *Saccharomyces cerevisiae*, by appling *Trachyspermum ammi* (Ajowan) and *Nigella sativa* (Black Seed) essential oils. In addition, Sani et al. (2020) applied *Mellissa officinalis* essencial oil microencapsules and Santos et al. (2022) applied *Crotonblanchetianus Baill* essential oil to fermented milk and demonstrate a better sensory profile when essential oils were used. The studies demonstrated the feasibility of applying essential oils in dairy products. Thus, the application of essential oil in Traditional Quark cheese is characterized as a possibility to increase the shelf life and microbiological safety of this product, inhibiting or reducing the growth of pathogenic and spoilage microorganisms, in addition to the development of a clean label product.

In the light of these observations, the objective of this work was to evaluate the antimicrobial activity of basil, marjoram, nutmeg, and oregano essential oils for application in Traditional Quark type cheese, aiming to reach the safety and microbiological quality of the product. In addition, the acceptability of the product from the sensory point of view, regarding the attributes of color, taste, odor, appearance, and global acceptability was also evaluated.

2 Material and methods

2.1 Materials

The essential oils of *Ocimum basilicum* (basil), *Origanum manjerona* (marjoram), *Virola surinamensis* (nutmeg), and *Ocimum vulgare* (oregano) were obtained from Ferquima (São Paulo, Brazil), accompanied by a certificate of purity (> 99%) and origin. Whole milk and commercial rennet, chymosin produced by *Aspergillus niger* var. *awamorii* (with a force of 1 : 3000, Ha La®, CHR HANSEN, Valinhos, Brazil) were used for obtaining the cheese. All culture media and chemical reagents used were of analytical grade.

2.2 Essential oils' antimicrobial activity

The basil, marjoram, nutmeg, and oregano essential oils were tested against *Escherichia coli* ATCC 25922, *Salmonella* Typhimurium ATCC 14028, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11778, and *Listeria monocytogenes* ATCC 19117.

Disk diffusion assay

The detection of antimicrobial activity in the extracts was performed according to the CLSI document M2-A8 (Clinical Laboratory Standards Institute, 2018a), with some adaptations according to Silveira (2012a). The inoculum was prepared from the active culture of each bacterial species, diluted in 0.9% saline solution at a concentration of approximately 10^8 CFU/mL, comparable to the McFarland 0.5 solution standard, verified spectrophotometrically at 625 nm. The suspension was diluted to approximately 10^7 CFU/mL in 0.9% saline solution, and this suspension was used to inoculate Mueller-Hinton agar plates using a sterile swab. Sterile filter paper discs (9 mm in diameter) were impregnated with 25 µL of each essential oil and deposited on inoculated plates and incubated at 36 °C for 18-24 h. Commercial ampicillin disks (10 µL/disk) and chloramphenicol (30 µL/disk) were used as positive controls. The diameter of the zone of inhibition was measured in millimeters. This assay was performed in triplicate and the value was presented as the mean ± standard deviation.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined for bacterial species that are susceptible in the agar diffusion test, using the microdilution method, based on the CLSI document M7-A8 (Clinical Laboratory Standards Institute, 2018b), with some adaptations according to Silveira (2012a). The essential oils were diluted to a concentration of 100 mg/mL in dimethylsulfoxide (DMSO). Subsequently, successive dilution series were prepared, ranging from 10 mg/mL to 0.075 mg/mL, in Mueller-Hinton broth. Then, 100 µL of each diluted solution and 5 µL of the bacterial suspension were added to the wells of the microdilution plates. Sterility controls (Mueller-Hinton broth added with DMSO and no inoculum) and growth controls (Mueller-Hinton broth added with DMSO and inoculum) were also prepared. The plates were incubated at 36 ± 1 °C for 18-24 h and microbial growth was visually detected and confirmed by the addition of 20 µL of 0.5% (w/v) 2,3,5 triphenyl tetrazolium chloride aqueous solution (TTC) with additional 1-hour incubation at the same temperature. The assay was performed in triplicate, and the results were expressed in mg/mL. The MIC was defined as the lowest concentration of the essential oil that inhibited microbial growth.

Determination of Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration (MBC) was determined from the plates used for the determination of MIC, using the methodology described by Celiktas et al. (2007). From each well where microbial growth did not occur, 10 µL were transferred to Tryptone Soy agar (TSA) plates. The plates were incubated at 36 ± 1 °C for 24 h and colony growth was then checked. The assay was performed in triplicate and the results were expressed in mg/mL. The CMB was defined as the lowest concentration of the extract that completely inhibited microbial growth on the plates.

2.3 Quark-type cheese manufacture

Table 1 presents the formulations used for the elaboration of Traditional Quark type cheese, control formulation (CTL), and formulation added with oregano essential oil (OE). For the

Table 1. Formulations used for the elaboration of traditional Quark type cheese, control formulation (CTL), and formulation added with oregano essential oil (OE).

Ingredient	CTL	OE
Whole milk (4% fat)	40 L	40 L
Lactic acid 85% (1 mL/10 L of milk)	4 mL	4 mL
Rennet (9 mL/10 L of milk)	36 mL	36 mL
Calcium chloride 4% (4 mL/10 L of milk)	16 mL	16 mL
Sodium chloride (1.0%)	0.4 g	0.4 g
Oregano essential oil	-	0.05%

manufacture of the cheeses, raw whole milk was subjected to pasteurization at 62-65 °C for 30 min, followed by a temperature adjustment of 37 ±1 °C, then lactic acid, calcium chloride, and commercial rennet were added. Afterward, it was left to rest for 40 to 45 minutes for the curd to be formed. Subsequently, the curd was broken by agitation, whey was removed by muslin-cloth drainage, and sodium chloride was added (Chauhan et al., 2022). For the OE formulation, the essential oil of oregano was added at the final step, in the concentration previously determined.

2.4 Monitoring cheeses storage

Traditional Quark cheese was kept under refrigeration at 4 °C for 35 days. To monitor the shelf life, physical-chemical and microbiological analyses were carried out at 0, 7, 14, 21, 28, and 35 days of storage. Three repetitions of each treatment were performed and the experiment was repeated twice, resulting in an n = 6. The microbiological analyses performed were counting of *Enterobacteriaceae*, molds and yeasts and counting of mesophilic aerobic microorganisms. The physicochemical analysis carried out during the 35 days were pH, water activity, titratable acidity, color, and moisture.

Microbiological analysis

To carry out the microbiological analyses, 25 g of sample were aseptically weighed and 225 mL of 0.1% peptone water were added and homogenized in Bag-mixer® for one minute. From this dilution, successive decimal dilutions were made (Silva et al., 2010). The counting of total aerobic mesophilic microorganisms at 35 °C was performed according to the ABNT NBR ISO 4833-2015 method (Associação Brasileira de Normas Técnicas, 2015). The *Enterobacteriaceae* count was performed according to the ISO method 21528-2:2017 (International Organization for Standardization, 2017). The counting of molds and yeasts was performed according to the method ISO 21527-1: 2008 (International Organization for Standardization, 2008). Results were expressed in colony-forming units per gram of sample (CFU/g).

Physicochemical analysis

The pH was determined using a digital pHmeter with automatic temperature compensation followed the methodology of the Instituto Adolfo Lutz (2008). Titratable acidity (% lactic acid) was evaluated by the titrimetric method according to Pereira et al. (2001). The determination of water activity was performed using the LabTouch equipment (Novasima, LabMasteraw model, Switzerland). The fat content (%) was evaluated using the Van Gulik butyrometer according to Pereira et al. (2001). The determination of moisture (%) was performed by gravimetry at 105 °C following the methodology of the Instituto Adolfo Lutz (2008). Analyses were performed in triplicate and the value was presented as the mean ± standard deviation.

Color analysis

The determination of color parameters was performed through instrumental measurement by colorimeter (Minolta® Color Reader), with a standardized light source, using coordinates L*, a*, and b*, where L* is the chroma associated with luminosity (L* = 0 black, L* = 100 white), a* is the color parameter that ranges from green (a*negative) to red (a*positive), and the b* coordinate, the color parameter that measures blue variations (b*negative) to yellow (b*positive). The analysis was performed in triplicate and the value was presented as the mean ± standard deviation.

2.5 Sensorial analysis

This project was approved by the Ethics Committee of the Federal Institute Catarinence (CAAE 83784018.5.0000.8049). For the sensory evaluation, a new batch of Quark-type cheese added with essential oil was produced, and the microbiological safety of the product was evaluated according to the standards established by the instruction IN n. 60 (Brasil, 2019). The cheeses were stored in a cold chamber at 5 °C until all the results of the microbiological analysis were released. Sensory analyses were performed on the 10th day after manufacture, with 95 untrained panelists, as recommended by the hedonic scale test methodology (Dutcosky, 2013).

2.6 Statistical analysis

Data were submitted to analysis of variance (ANOVA) and differences between means were tested by Tukey's test at a 5% significance level.

3 Results and discussion

3.1 Antimicrobial activity

The results obtained for the disk-diffusion test of essential oils of oregano, basil, marjoram, and nutmeg are shown in Table 2. The oregano essential oil showed the highest zones of inhibition against all the bacteria tested, being characterized as oil with strong antimicrobial activity, that is, with zones of inhibition greater than 18 mm. In addition, it also showed better results when compared to the reference antibiotics used (ampicillin and chloramphenicol) ($p < 0.05$). In the study carried out by Silveira et al. (2012b), on the chemical composition and antimicrobial activity of essential oils from selected herbs grown in southern Brazil against spoilage microorganisms and foodborne pathogens, it was observed oregano essential oil zones inhibition of 26.2 mm against *B. cereus* and 25.4 mm against *L. monocytogenes*, inhibitions lower than those observed in the present work. Pombo et al. (2018) evaluated the antimicrobial effect

of oregano and clove essential oils, at different concentrations, against contaminating bacteria in food. The oregano essential oil showed greater inhibitory potential than the clove oil against the bacteria tested, a result similar to that found in the present work. Gram-positive bacteria (*S. aureus* and *B. cereus*) were more susceptible to essential oils, compared to Gram-negative ones (*E. coli* and *S. enterica*) (Pombo et al., 2018).

The essential oils of marjoram and basil can be considered strong antimicrobials, except for *Listeria monocytogenes* and *Salmonella* Typhimurium, showing moderate inhibition for these two species of bacteria in the present work. Machado et al. (2012) evaluated two species of basil and the difference in antimicrobial activity of essential oil extracted from leaves and flowers. For essential oil extracted from leaves, inhibition zones of 15.5 mm were observed against *S. aureus*, presenting the largest zone of inhibition. Similar results were found by Ferreira et al. (2018) who evaluated the antimicrobial activity of *Ocimum balicum* essential oil against Gram-positive bacteria and showed that the bacteria that had the highest susceptibility were *B. cereus* and *S. aureus*.

Trajano et al. (2009) evaluated 10 essential oils against contaminating bacteria in foods and for marjoram essential oil the bacteria that showed the highest zones of inhibition were *B. cereus* (16 mm), *B. subtilis* (25 mm), and *L. monocytogenes* (15 mm). mm). In the study carried out by Valeriano et al. (2012), on the antimicrobial activity of essential oils in pathogenic bacteria of food origin, the results showed higher susceptibility for *E. coli*, and moderate activity against *Salmonella* Enteritidis and *Enterobacter sakazakii* and no antibacterial activity for *L. monocytogenes*.

Nutmeg essential oil was the one that presented lower results for the disk-diffusion test, being characterized as a weak essential oil for all tested bacteria, except for *B. cereus* (Table 2). A similar result was found by Binatti et al. (2016) that evaluated the antimicrobial action of nutmeg essential oil and observed inhibition zones of 9 mm for *S. aureus* and *S.* Typhimurium. For nutmeg essential oil, the MIC and MBC tests were not performed, as this oil showed weak microbial inhibition in the disk-diffusion test.

Table 3 shows the results for the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the essential oil of *Ocimum vulgare* (oregano), *Ocimum basilicum* (basil), and *Origanum manjerona* (marjoram) since these essential oils showed good antimicrobial activity in the disk-diffusion test.

Oregano essential oil showed the highest activities (lower MIC and MBC values) against the tested bacteria, followed by basil and marjoram essential oils. The strong activity of oregano essential oil presented in the disk-diffusion assay was confirmed in the microdilution assay. Monteiro (2015), studying oregano essential oil, observed MIC values of 0.25 µL/mL against *S. aureus*, 0.5 µL/mL against *E. coli,* and 1 µL/mL against *P. aeruginosa*. The differences between the results observed in the present work and the literature could also be related to the different units used to express the results (mg/mL and µL/mL). The work carried out by Araujo & Longo (2016) evaluated the *in vitro* antibacterial action of commercial essential oil of *Origanum vulgare* (oregano) against *E. coli* and *S. aureus*. The MIC was 6.25 µL/mL and the MBC of 12.5 µL/mL for *E. coli* while for *S. aureus*, the MIC and MBC were 12.5 µL/mL. Therefore, this essential oil of *O. vulgare* showed lower antimicrobial activity than that observed in the present work (Araujo & Longo, 2016).

Table 2. Antimicrobial activity of essential oils from *Ocimum vulgare* (oregano) *Ocimum basilicum* (basil), *Origanum manjerona* (marjoram), and *Virola surinamensis* (nutmeg) detected by disk diffusion test (mm)*.

Microrganisms	Oregano	Basil	Marjoram	Nutmeg	Ampicillin	Chloranphenicol
S. aureus	42.6 ± 3.24^{a}	20.7 ± 0.17^{b}	21.4 ± 0.41^{b}	11.0 ± 0.00^{d}	23.7 ± 0.25^{bc}	27.6 ± 0.12^{c}
E. coli	34.0 ± 2.00^{a}	19.0 ± 0.00^{b}	32.1 ± 2.11^{ac}	13.4 ± 0.65^{d}	21.2 ± 0.00^{b}	28.7 ± 0.18^{c}
B. cereus	51.0 ± 7.97^{a}	22.3 ± 0.48^{bc}	22.0 ± 0.31^{bc}	14.1 ± 1.44^{b}	18.2 ± 0.75^{bc}	26.5 ± 0.05^{c}
S. Typhimurium	34.4 ± 1.93^{a}	14.0 ± 0.30^{b}	18.4 ± 2.42^{c}	11.0 ± 0.00^{b}	27.4 ± 0.05^{d}	28.1 ± 0.10^{d}
L. monocytogenes	40.3 ± 0.59^{a}	15.6 ± 1.13^{b}	15.7 ± 1.87^{b}	13.9 ± 1.15^{bc}	35.0 ± 1.22^{d}	11.2 ± 0.05^{c}

*Zone of inhibition including disc diameter, 9 mm. Data refer to the mean of four replicates ± standard deviation. Inhibition degrees: weak: 10-13.9 mm; moderate: 14-18 mm; strong: > 18 mm. [a-c]Means followed by the same letter on the same line are not significantly different ($p < 0.05$).

Table 3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the essential oil of *Ocimum vulgare* (oregano), *Ocimum basilicum* (basil), and *Origanum manjerona* (marjoram) (mg/mL)[a, b].

Microorganisms	Oregano		Basil		Marjoram	
	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus	0.60	0.62	0.075	1.25	1.25	< 10
E. coli	0.075	1.25	0.075	1.25	1.25	< 10
B. cereus	0.075	0.62	0.075	1.25	0.31	< 10
S. Typhimurium	0.31	1.25	1.25	< 10	1.25	< 10
L. monocytogenes	0.62	0.62	1.25	< 10	2.50	2.50

[a]The tests were performed in triplicate, and the results refer to the modal values. [b]In all assays, sterility controls (without the addition of inoculum) and growth controls (Soy Trypticasein TSB broth added with DMSO and inoculum) were maintained.

According to Burt (2004), the MIC of oregano essential oil is usually 0.05% to 0.12% for *E. coli* and *S. aureus*.

Basil essential oil showed good activity against *S. aureus*, *E. coli,* and *B. cereus* in the MIC and MBC assay. Machado et al. (2012) observed results of 0.32 µg/mL for basil essential oil extracted from leaves against *S. aureus* and *E. coli*. For the essential oil obtained from basil flowers, they found 0.38 µg/mL against *S. aureus* and 3.90 µg/mL against *E. coli*. Silveira et al. (2012b), when evaluating basil essential oil, observed MIC, 2.5 mg/mL, 1.25 mg/mL, and 1.25 mg/mL against *S. aureus*, *E. coli,* and *L. monocytogenes*, respectively. For MBC, 2.5 mg/mL, 1.25 mg/mL, and 2.5 mg/mL were observed against *S. aureus*, *E. coli,* and *L. monocytogenes*, respectively, i.e. lower results than those observed in the present work. According to Burt (2004), most studies that investigate the action of essential oils concerning pathogenic microorganisms in foods verify that, in most cases, the oils are slightly more active for Gram-positive bacteria than for Gram-negative bacteria. This is because Gram-negative bacteria are less susceptible to the action of essential oils due to the existence of an outer membrane that surrounds the cell wall (peptidoglycan layer), which restricts the diffusion of hydrophobic compounds through the outer wall (Burt, 2004).

Marjoram essential oil showed moderate activity against *B. cereus* and weak antimicrobial activity for the other bacteria tested in the MIC and MBC assays. In the research carried out by Walker et al. (2014), among the four essential oils evaluated, the one with the best antimicrobial activity was wild marjoram oil (*Thymus mastichina*) in which the MIC was 50 µL/mL. On the other hand, Arroyo et al. (2017) found weak antimicrobial activity for marjoram essential oil for all bacteria tested, which were *E. coli*, *S. aureus*, *B. subtilis,* and *P. aeruginosa*. Changes in chemical composition, geographic location, seasonality, part of the plant extracted for essential oil, and extraction methods directly affect the biological activities of essential oils (Celiktas et al., 2007; Van Vuuren et al., 2007). Taking into account the results obtained in the present study, the oregano essential oil showed the best antimicrobial properties. Therefore, it was the oil selected for addition to Traditional Quark-type cheese.

3.2 Microbiological analysis during storage

The microbiological analyses over the 35 days of storage of the control (CTL) traditional Quark-type cheese with 0.05% oregano essential oil (OE) added are shown in Figure 1. There was a difference ($p < 0.05$) in the count of molds and yeasts from the fourteenth day of storage, that is, from that day onwards it was possible to verify the efficiency of the oregano essential oil added as a preservative in the OE sample (Figure 1a). Ribeiro (2015) evaluated Minas Frescal cheese added with microencapsulated pink pepper essential oil and the counts resulted in 6.2 x 10^1 CFU/g for molds and yeasts for the control sample and 5.4 x 10^1 CFU/g for the cheese added with essential oil, i.e. higher results that found in the present work considering the same period. According to Britto & Rossi (2005), molds and yeasts are indicators of the efficiency of the process of sanitizing equipment and utensils, heat treatment during food processing, and the storage conditions of raw material. In cheeses, they are responsible for deterioration when stored under inadequate conditions (Siqueira, 1995; Franco & Landgraf, 2005). According to Pinto (2017), the count of molds and yeasts can be performed as an indicator of shelf life. Therefore, analyzing this microbiological parameter, it is possible to say that the addition of essential oil contributed to the conservation of Quark-type cheese, reducing the deterioration of the product at the end of the 35 days of storage.

For the counting of mesophilic aerobic microorganisms, there was no significant difference ($p > 0.05$) between the control cheese (CTL) and the cheese added with oregano essential oil (OE) (Figure 1b). For mesophilic aerobic counting, a growth from 6.6 x 10^2 CFU/g (day 0) to 2.0 x 10^9 CFU/g (day 35) was observed for the control cheese, as well as for the cheese added with essential oil, which increased from 6.3 x 10^2 CFU/g (day 0)

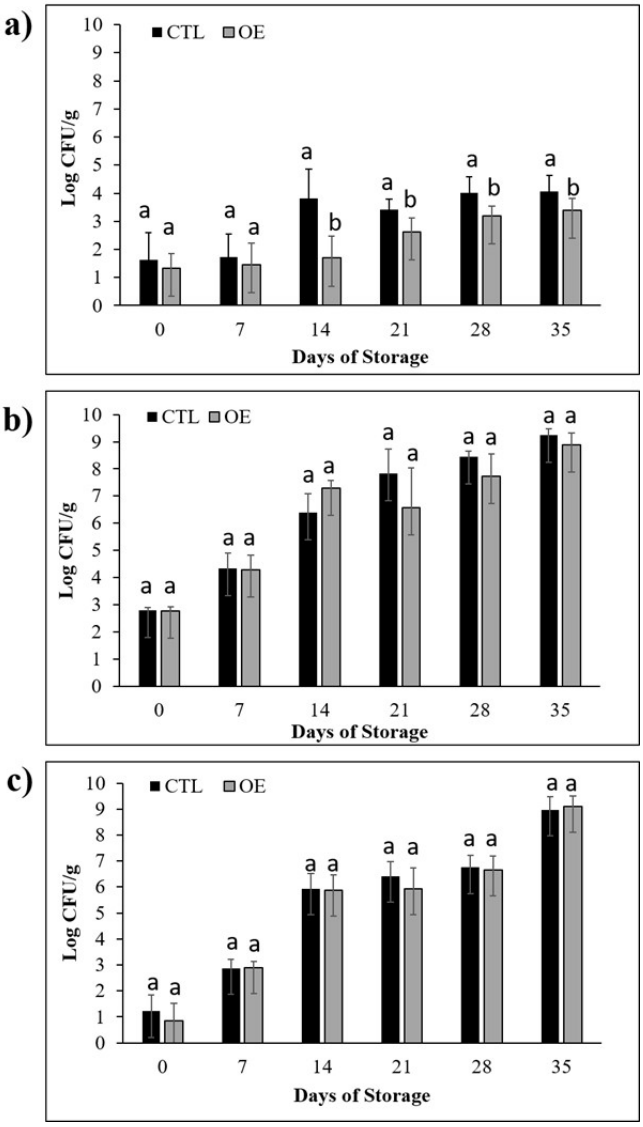

Figure 1. Counts of molds and yeasts (a), mesophilic aerobic microorganisms (b), and *Enterobacteriaceae* (c) in control Quark-type cheese (CTL) and Quark-type cheese added with oregano essential oil (EO) at a concentration of 0.05% over 35 days of cold storage. [a-b] Means followed by different letters on the columns are statistically different by Tukey's test at a 5% significance level.

to 1.7 x 10^9 CFU/g (day 35). A different result was found in the research carried out by Hafemann et al. (2015) that evaluated the addition of oregano essential oil in ricotta and observed a growth of 10^1 CFU/g on day 0 for mesophilic aerobic microorganisms to 4.6 x 10^3 CFU/g at 15 days of storage. According to Ribeiro (2015), when it comes to the total count of aerobic mesophilic microorganisms, it is practically impossible to obtain counts equal to zero and for products considered fresh, such as Quark-type cheese, there is no legislation with an established standard for this group of microorganisms, however, this analysis is considered as microbiota control.

For *Enterobacteriaceae* counts, there was no significant difference ($p > 0.05$) between the control cheese (CTL) and the cheese added with oregano essential oil (OE) (Figure 1c). There was observed growth from 1.0 x 10^1 CFU/g to 3.8 x 10^9 CFU/g for cheese added with essential oil. Mallet (2011) observed *Enterobacteriaceae* counts values below 10^2 CFU/g during 35 days of storage. According to Brant et al. (2007), the *Enterobacteriaceae* count is not required by the current sanitary legislation, however, as these microorganisms are usually environmental contaminants, the high counts of these microorganisms indicate a deficiency in the hygienic-sanitary quality of the product.

It is worth noting that the Quark-type cheese produced in the present work did not have any type of preservative and/or additive added, only oregano essential oil was used for the preservative purpose, thus, it is evident that with the days of storage, there is an expressive microbial multiplication, since the concentration of essential oil was low (0.05%). According to Mallet (2011), several studies have sought to find a concentration that simultaneously promotes the antimicrobial effect, in minimal inhibitory concentrations, and enhances the flavor, to promote positive sensory changes, contributing to the development of new products exempt or with as few synthetic additives as possible. In addition, there are many *in vitro* studies carried out on the antibacterial activity of essential oils, but few involve the application of these substances in food matrices and the evaluation of their behavior on the microbiological quality of the products. Also, there are several extrinsic factors (such as temperature, packaging, and characteristics of the microorganism) and intrinsic (such as fat, protein, water activity, antioxidants, pH, salt, and additives) of foods that interfere with the effect of essential oils and the action of microorganisms in foods that must be taken into account when choosing a preservative (Mallet, 2011).

3.3 Physicochemical analysis during storage

Table 4 presents the physicochemical results obtained for the shelf life of Quark-type cheese with (OE) and without (CTL) oregano essential oil. There was no significant difference ($p < 0.05$) in fat and moisture contents between the samples (CTL and OE) during the storage time. Regarding fat, according to the Technical Regulation on Identity and Quality of Cheese (Brasil, 1996), the fat contents of cheeses are classified as: skimmed (< 10.0%), low-fat (10.0 to 24, 9%), semi-fat (25.0 to 44.9%), fatty (45.0 to 59.9%) and extra-fat or double cream (≥ 60.0%). In the present work, the fat content of Quark-type cheeses ranged from 13 to 14.58%, classifying them as low-fat cheese. Similar results were observed by Azevedo et al. (2012) and Mallet (2011) where the fat content of Quark-type cheese was 8.92% and 13.03%, respectively.

The moisture content of all samples was above 55%, classifying this type of cheese as with very high moisture content (Brasil, 1996). In the research carried out by Presente et al. (2016), the conservation of fresh cheeses added with oregano essential oil was evaluated and moisture contents of 57.6% (essential oil) and 56% (control) were observed, respectively. Higher results were observed for this matrix by Azevedo et al. (2012), Gonçalves (2009) and Mallet (2011), 78.37%, 79.84% and 73.14%, respectively. The high moisture content was also reflected in the a_w of the samples, which varied between 0.93 and 0.94. High a_w is a factor that directly interferes with microbial development, especially the growth of pathogenic microorganisms (Tavares et al., 2019).

Regarding pH, the results were close to neutrality at the beginning of the shelf life, however, at the end of the 35 days of storage, an increase in acidity and a consequent decrease in pH can be observed. This behavior may be directly associated with the microbial development observed in microbiological analysis since several microorganisms produce organic acids through their metabolism (Siqueira, 1995; Franco & Landgraf, 2005). Gonçalves (2009) analyzing the chemical characterization of Quark-type cheese added with different probiotics, found acidity values from 0.52-0.58 and pH values of 4.38 and 4.5, results similar to the present work. In another study with Quark-type cheese, Cardarelli (2006) observed pH values from 4.29 to 4.64. On the other hand, Mallet (2011), Azevedo et al. (2012), and Presente (2015) that also evaluated Quark-type cheese found higher acidity values of 0.77%, 1.11%, and 0.87%, respectively. This variation may be directly related to the amount of lactic acid or starter

Table 4. Physicochemical analyses evaluated during storage of Quark-type cheese added with 0.05% oregano essential oil (OE) and without essential oil (CTL).

Time (days)	Acidity (acid lactic, %)		pH		a_w		Moisture (%)		Fat (%)	
	CTL	OE	CTL	OE	CTL	OE	CTL	OE	CTL	OE
0	0.09 ± 0.02^a	0.09 ± 0.02^a	6.75 ± 0.06^a	7.15 ± 0.82^a	0.938 ± 0.003^a	0.937 ± 0.001^a	65.12 ± 0.90^a	65.33 ± 0.39^a	13.42 ± 0.80^a	13.00 ± 0.89^a
7	0.14 ± 0.03^a	0.14 ± 0.02^a	5.80 ± 0.19^a	5.55 ± 0.24^a	0.933 ± 0.001^a	0.935 ± 0.002^b	66.16 ± 0.40^a	65.17 ± 1.15^a	13.17 ± 1.04^a	13.00 ± 1.00^a
14	0.15 ± 0.01^a	0.15 ± 0.01^a	5.80 ± 0.04^a	5.70 ± 0.15^a	0.937 ± 0.001^a	0.936 ± 0.001^a	65.88 ± 1.06^a	66.64 ± 2.44^a	12.83 ± 0.76^a	13.67 ± 0.58^a
21	0.16 ± 0.01^a	0.200 ± 0.03^b	5.53 ± 0.07^a	5.54 ± 0.07^a	0.939 ± 0.002^a	0.938 ± 0.001^a	65.34 ± 1.79^a	66.60 ± 1.29^a	13.83 ± 2.36^a	14.00 ± 2.18^a
28	0.29 ± 0.09^a	0.26 ± 0.15^a	5.77 ± 0.17^a	5.85 ± 0.04^a	0.939 ± 0.001^a	0.938 ± 0.001^a	67.76 ± 1.69^a	67.01 ± 1.08^a	14.83 ± 2.47^a	15.67 ± 2.36^a
35	0.35 ± 0.11^a	0.38 ± 0.04^a	5.68 ± 0.07^a	5.85 ± 0.03^a	0.937 ± 0.001^a	0.937 ± 0.002^a	66.33 ± 0.45^a	67.39 ± 1.27^a	14.58 ± 2.35^a	14.25 ± 2.36^a

Means followed by different letters on the lines are statistically different by Tukey's test at a 5% significance level.

Table 5. Color parameters evaluated during storage of Quark-type cheese added with 0.05% oregano essential oil (OE) and without essential oil (CTL).

Time (days)	L*		a*		b*	
	CTL	OE	CTL	OE	CTL	OE
0	84.15 ± 3.16[a]	86.36 ± 4.28[a]	-6.65 ± 0.26[a]	-7.79 ± 0.27[a]	16.28 ± 0.86[a]	16.32 ± 0.85[a]
7	88.96 ± 4.16[a]	86.69 ± 6.19[a]	-7.01 ± 0.57[a]	-6.97 ± 0.63[a]	16.23 ± 0.58[a]	16.66 ± 0.47[a]
14	90.34 ± 2.00[a]	88.81 ± 2.19[a]	-6.59 ± 0.57[a]	-6.55 ± 0.56[a]	16.73 ± 0.80[a]	16.92 ± 0.50[a]
21	88.78 ± 2.42[a]	87.98 ± 1.91[a]	-6.90 ± 0.21[a]	-7.04 ± 0.42[a]	16.59 ± 0.24[a]	17.65 ± 1.21[a]
28	90.16 ± 1.62[a]	88.10 ± 3.75[a]	-7.19 ± 0.75[a]	-6.90 ± 0.25[a]	18.46 ± 1.00[a]	19.15 ± 0.81[a]
35	90.83 ± 1.90[a]	86.68 ± 4.40[a]	-7.14 ± 0.52[a]	-6.74 ± 0.96[a]	18.96 ± 1.73[a]	19.89 ± 1.53[a]

Means followed by different letters on the lines are statistically different by Tukey's test at a 5% significance level.

Table 6. Mean values of the sensory attributes taste, odor, color, appearance, and global acceptability for Quark type cheese without the addition of oregano essential oil (CTL) and with the addition of 0.05% of oregano essential oil (EO).

Sample	Flavor	Odor	Color	Appearance	Global acceptability
CTL	7.78 ± 1.31[a]	7.24 ± 1.32[a]	7.95 ± 1.01[a]	7.59 ± 1.18[a]	7.79 ± 1.05[a]
OE	7.29 ± 1.65[b]	7.77 ± 1.23[b]	8.00 ± 1.00[a]	7.68 ± 1.21[a]	7.49 ± 1.37[a]

Means followed by different letters on the columns are statistically different by Tukey's test at a 5% significance level.

culture used to manufacture this type of cheese, considering that there is no standardization for it (Chauhan et al., 2022).

3.4 Color analysis during storage

Table 5 presents the color analysis performed for the control Quark-type cheese (CTL) and the Quark-type cheese added with 0.05% essential oil (EO). The L* parameter is associated with luminosity (L* = 0 black, L* = 100 white), a* is the parameter that varies from green (-a*) to red (+a*), and the b* coordinate, the color parameter that measures variations from blue (-b*) to yellow (+b*). Regarding color analysis, there was no significant difference ($p < 0.05$) between the control samples and those added with essential oil in relation to luminosity (L*), which presented values around 88, representing a tendency towards white color. For the a* parameter, negative values were observed for all samples, indicating a trend toward green color. Finally, for the parameter b* the values found were all positive, indicating a trend to the yellow color. It was possible to observe that the addition of oregano essential oil did not change the color aspect of Quark-type cheese over 45 days of cold storage.

3.5 Sensory analysis

Table 6 presents the results obtained for the sensory analysis of acceptability of the control Quark-type cheese (CTL) and the Quark-type cheese added with 0.05% oregano essential oil (EO) samples. Quark-type cheese added with oregano essential oil showed a better odor ($p < 0.05$) and was acceptable in terms of flavor. For the attributes of appearance, color, and global acceptability there was no significant difference between the samples added or not with essential oil. In the research carried out by Mallet (2011), on the use of essential oils from condiments in the conservation of Quark-type cheese, the author observed an average flavor attribute of 6.3 for the cheese without the addition of essential oil and for the cheese added with oregano essential oil at a concentration of 0.06%, an average of 4.6. Hafemann et al. (2015), researching the addition of essential oil in ricotta, observed similar results, an average of 6.22 ricotta without the addition of essential oil and 6.12 for ricotta added with oregano essential oil at a concentration of 0.02%. For the global acceptability attribute Mallet (2011) observed averages of 6.8 in cheese without the addition of essential oil and 5.4 in cheese added with oregano essential oil at a concentration of 0.06%. As for the global appearance attribute, the research carried out by Hafemann et al. (2015) found an average of 6.7 for ricotta without the addition of essential oil and 6.62 for ricotta added with essential oil at a concentration of 0.002%. This behavior was the opposite of that observed in our study. Better results were observed in the research by Silva et al. (2014), 7.4 for traditional ricotta and 7.7 for ricotta with the addition of 0.01% oregano. These results are similar to those found in the present work where the oregano essential oil showed better results for some sensorial parameters. In addition, Hafemann et al. (2015) observed in their research an average of 7.64 for traditional ricotta and 7.4 for ricotta with the addition of essential oil at a concentration of 0.002% for the color attribute which represents the results observed in the present work were superior in sensorial quality.

4 Conclusion

The essential oils of marjoram, basil, and oregano showed strong antimicrobial activity against most Gram-positive and Gram-negative bacteria evaluated in the present study, and the essential oil of oregano showed better antimicrobial activity than the other essential oils tested. For this reason, this essential oil was selected for application in Quark-type cheese. The addition of oregano essential oil to Quark-type cheese did not significantly affect the physicochemical characteristics of this product, that is, the typical characteristics of traditional Quark-type cheese were maintained. Regarding the microbiological quality of the product, there was a delay in the growth of molds and yeasts, indicating

an increase in the shelf life with the addition of oregano essential oil at a concentration of 0.05%. The sensory analysis showed that there was no significant difference between the samples added or not of essential oil in relation to the attributes of appearance, color, and global acceptability. The Quark-type cheese added with essential oil had a better odor and was acceptable in terms of flavor. Finally, the addition of oregano essential oil in Quark-type cheese can be characterized as an innovation, resulting in the development of a differentiated product, as it does not have any synthetic additives.

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