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Origanum vulgare extract as a natural additive in fresh cheese

Extrato de *Origanum vulgare* como aditivo natural em queijo fresco

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Highlights

Fresh cheese added with extract of *Origanum vulgare* has good acceptance by consumers.
O. vulgare proved to be effective against thermotolerant coliforms in fresh cheese.
Antioxidant activity of *O. vulgare* seems to be better utilized in fatty cheeses.
The application of spice extracts in foods adds value and safety to fresh foods.

Abstract

Oregano (*Origanum vulgare*) is a spice that has in its extracts and essential oils biological activities of interest, such as antimicrobial and antioxidant. These qualities are in line with the increased interest in natural alternatives for preserving foods such as fresh cheese due to its short shelf life. The objective of this research was to evaluate the acceptance, antibacterial and antioxidant activity of the hydroalcoholic extract of *O. vulgare* incorporated in fresh cheese during cold storage. For this, cheeses were produced with different concentrations of extract (0, 0.5, 1 and 3 g of extract/kg). Acceptance was assessed using a nine-point hedonic scale. The presence of *Salmonella* was investigated and the enumeration of coagulase-positive staphylococci, mesophilic, psychrotrophic and thermotolerant coliforms was performed in the samples. The antioxidant activity was evaluated *in vitro* (DPPH and FRAP assays) and directly in the food (measuring the malondialdehyde content). The data revealed a good acceptance of all cheeses regardless

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of the oregano concentration (mean 7.0 to 7.7). The addition of 0.3 g of oregano extract/kg of cheese promoted the inhibition of thermotolerant coliforms during the storage period. Antioxidant activity was demonstrated *in vitro* but not verified in the cheese. Foods have a complex composition, and the concentration and proportion of different constituents may have influenced the biological actions of the extract. Thus, further research becomes necessary, with variation in storage time and concentrations to know the action of this extract directly in the food model.
Key words: Anti-bacterial. Antioxidant. Dairy product. Natural product. Sensory analysis.

Resumo

O orégano (*Origanum vulgare*) é uma especiaria que possui em seus extratos e óleos essenciais atividades biológicas de interesse como a antimicrobiana e antioxidante. Essas qualidades estão alinhadas com o aumento do interesse por alternativas naturais para a conservação de alimentos, como o queijo fresco, devido ao seu curto prazo de validade. O objetivo desta pesquisa foi avaliar a aceitação, atividade antibacteriana e antioxidante do extrato hidroalcoólico de *O. vulgare* incorporado em queijo fresco durante o armazenamento refrigerado. Para isso, foram produzidos queijos com diferentes concentrações de extrato (0, 0,5, 1 e 3 g de extrato/kg). A aceitação foi avaliada por meio de uma escala hedônica de nove pontos. A presença de *Salmonella* foi investigada e a contagem de estafilococos coagulase-positivos, coliformes termotolerantes, mesófilos e psicrotróficos foi realizada nas amostras. A atividade antioxidante foi avaliada *in vitro* (ensaios DPPH e FRAP) e diretamente no alimento (mensuração do teor de malondialdeído). Os dados revelaram uma boa aceitação de todos os queijos independentemente da concentração de orégano (média 7,0 a 7,7). A adição de 0,3 g de extrato de orégano/kg de queijo promoveu a inibição de coliformes termotolerantes durante o período de armazenamento. A atividade antioxidante foi demonstrada *in vitro* mas, não verificada no queijo. Os alimentos possuem composição complexa, e a concentração e proporção de diferentes constituintes podem ter influenciado as ações biológicas do extrato. Assim, mais pesquisas se fazem necessárias, com variação no tempo de armazenamento e concentrações para conhecer a ação desse extrato diretamente no modelo alimentar.
Palavras-chave: Antibacteriano. Antioxidante. Produto lácteo. Produto natural. Análise sensorial.

Introduction

The production of fresh, less processed foods with reduced addition of preservatives and additives is a challenge for the food industry since less processed products have a reduced shelf life (Vivian et al., 2020). The food industry has sought to avoid the potential harmfulness of synthetic food additives and to develop new functional foods containing health-promoting ingredients. Natural matrices and compounds with antioxidant and antimicrobial properties can serve both purposes (Caleja et al., 2015).

Plants have secondary metabolic pathways that give rise to several compounds specific to certain plant families, genera, or species. They are valuable sources of new biologically active molecules that can act as alternatives to products already available as long as they are safe, effective, and acceptable sources (Souza et al., 2003; Negi, 2012).

The activity of plants commonly used as spices in cheese conservation and their influence on the microbiota, lipid oxidation, and acceptance, both by direct application and through packaging, have been reported (Tavares et al., 2014; Caleja et al., 2015; Presente et al., 2016).

Origanum vulgare, popularly known as oregano, is an aromatic herb. Its leaves are used fresh or dried, for the flavor and aroma they give to food. This spice has a high antimicrobial antioxidant activity due to the presence of phenolic compounds and flavonoids (Oniga et al., 2018; Debiagi et al., 2020).

Despite the efficiency of *O. vulgare in vitro*, in the food matrix, it may have limitations because it interacts with the constituents of the food, raising the concentration necessary for the desired effect and causing negative impacts on organoleptic properties due to its intense flavor (Tajkarimi et al., 2010; Hyldgaard et al., 2012).

Among the so-called fresh and high moisture cheeses, the fresh cheese can be highlighted, as it is widely accepted and features white mass, soft consistency, and no acidity, in addition to its high moisture content (Dias et al., 2016). This cheese does not undergo any maturation process, being packaged and sold fresh, having a shelf life of up to twenty days, provided it is properly refrigerated.

Thus, the objective of this work was to evaluate the acceptance, antibacterial and antioxidant activity of *O. vulgare* extract added to fresh cheese throughout the shelf life of the product.

Material and Methods

Origanum vulgare and extract preparation

Origanum vulgare was cultivated in experimental beds in the medicinal garden located on Campus 2, Universidade Paranaense, municipality of Umuarama, Paraná, Brazil. The geographic coordinates are 23º45'44.9" S and 53º16'17.5" W, the species was registered in the National Management System for Genetic Heritage and Associated Traditional Knowledge under registration number A1B382E. The area covers 30,000 m2 and 400 m elevation.

Leaves and stems were placed on trays and dried in an oven with forced air circulation at 40 ºC for 24 hours. After drying, the material was ground, sieved, and kept at -20°C protected from light until use. The extract was prepared using 40g of the plant for each 1000 mL of 70% ethanol and left under constant agitation in an automatic shaker at room temperature for six hours with subsequent rest for 18 hours. Subsequently, the contents were filtered and the solvent was removed under reduced pressure (Pitaro et al., 2012). The extract was lyophilized and stored in a freezer for further analysis.

Formulation and storage

Fresh cheese was prepared in a small dairy factory, using pasteurized milk, salt (15 g/L), calcium chloride (0.25 mL/L), and microbial protease-based rennet (0.08 mL/L). After homogenization and coagulation, the mass formed was cut with a lyre and fork and the excess whey was drained. Soon after, the mass was collected, shaped into cheese

molds, and kept under refrigeration, until the whey removal process was completed, this being the control cheese (0.0%).

From the initial formulation, three types of cheese were also manufactured: T1, cheese added with powdered *O. vulgare* extract mixed directly into the mass at a concentration of 0.5 g/kg (0.05%); T2, with a concentration of 1 g/kg (0.1%) of extract; and T3, with a concentration of 3 g/kg (0.3%) of extract. Eight units of each type of sample were produced. After dewatering, they were placed in closed packages and kept at a temperature of 4 ºC until end the analyses.

Sensory analysis

The 9-point Hedonic Scale acceptance test was used. The tasters expressed the degree of like or dislike, in a globalized way. Samples were coded with three-digit numbers and randomized. Preference was obtained by inference (Instituto Adolfo Lutz [IAL], 2008). Analyzes were performed on the 2nd day of shelf life, with 60 untrained tasters. The study was approved by the Ethics Committee for Research Involving Human Beings at UNIPAR (CAEE No. 07800919.7.0000.0109).

Antimicrobial activity of O. vulgare extract applied to fresh cheese

Considering the period of consumption of the product, the analyzes were carried out on days 1 (1st day after manufacture), 5, and 8, with the samples being kept in closed packages, at a temperature of 4 ºC. Three samples were analyzed for each concentration, with analyzes performed in duplicate.

Control (without extract) and treated (with extract) samples were analyzed for *Salmonella* sp., coagulase-positive staphylococci, coliforms at 45 ºC, and bacterial loads of aerobic and psychrotrophic mesophiles were also investigated.

For these microorganisms, excluding *Salmonella,* 25 grams of the sample were homogenized in 225 mL of sterile buffered water and homogenized for two minutes. From this initial 10^{-1} dilution, a series of decimal dilutions were prepared using the same diluent.

Salmonella research

The homogenization procedure was similar to that mentioned above but using buffered peptone water. The homogenized product was transferred to an Erlenmeyer flask and incubated at 35 ºC for 24 hours. Then, 0.1 mL was seeded in a tube containing 10 mL of Rappaport-Vassiliadis (RV) Enrichment Broth, incubated at 42 °C for 24 hours and also 1 mL in 10 mL of tetrathionate broth, added with 0.2 mL of potassium iodide at the time of use and incubated at 35 ºC for 24 hours. After these incubation periods in Rappaport-Vassiliadis medium, a loop was seeded on Xylose Lysine Deoxycholate (XLD) agar and Brilliance *Salmonella* agar.

After 35 ºC for 24 hours, characteristic *Salmonella* colonies were isolated and subcultured in tubes containing slanted trypticase soy agar (TSA) and kept at 35 ºC for 24 hours. From this growth, biochemical tests were performed with triple sugar iron (TSI) agar slant and phenylalanine agar slant and were incubated at 35 ºC for 18-24 hours. The suspected strain was submitted to the

Enterobacteria kit (Newprov) and the strains positive for *Salmonella* were tested against the polyvalent somatic and flagellar sera (Andrews et al., 2001).

Enumeration of coagulase-positive staphylococci

For *Staphylococcus* enumeration, 0.1 mL of the different sample dilutions were seeded with the aid of an L-shaped stick onto plates with Baird-Parker agar added with potassium tellurite and egg yolk solution, incubation was made at 35 ºC for 48 hours, followed by the counting of suspicious colonies on the plate containing between 25 and 250 CFU (colony forming units). Suspicious *Staphylococcus* colonies were isolated and subcultured in tubes with TSA slant and incubated at 35 ºC for 24 hours, followed by catalase test, Gram stain, and tube coagulase test with rabbit plasma (Lancette & Bennett, 2001). To calculate the number of CFU (colony forming units) per gram, the number of confirmed colonies was multiplied by 10 and by the inverse plate-count dilution factor.

Mesophilic count

Counting was performed using the Pour Plate Method on Plate Count Agar (PCA), where 1 mL of each dilution was deposited on sterile plates and then a volume of approximately 20 mL of molten PCA was added. After homogenization and solidification of the agar, the plates were incubated (35ºC for 48 h) and counting of CFU in plates containing between 25 and 250 CFU and obtaining the values of CFU/g (Morton, 2001).

Psychrotrophic count

Counting was performed by seeding 0.1 mL of each dilution on the surface of PCA plates and distributing it with a sterile L-shaped glass rod. After incubation (7 ºC for 10 days), colonies were counted on the plate containing 25 and 250 CFU, and later the calculation of CFU/g was made, also according to Morton (2001).

Coliforms at 45°C

The counting was performed using the Most Probable Number (MPN) technique, using Sodium Lauryl Sulfate Broth (48 h at 37 ºC), in the presumptive step. The positive tubes (gas in the Durham tube) were inoculated in EC Broth (24 h at 45 ºC) to confirm thermotolerant coliforms (Food and Drug Administration [FDA], 1992).

Study of the antioxidant potential of the extract

Antioxidant activity assessment - DPPH assay

The scavenging activity (DPPH) was performed according to the methodologies of Thaipong et al. (2006) and Choi et al. (2006). DPPH (1,1-Diphenyl-2-picrylhydrazine) - 0.024g of DPPH was diluted in 100 mL of methanol. A volume of 10 mL of this solution was added to 45 mL of methanol and the absorbance was read at 515 nm, correcting it to close to 1.1. Then, 0.15 mL of the sample containing different concentrations of the lyophilized extract was added to 2.85 mL of solution. After 24 hours in the dark and at room temperature, the absorbance was

determined at 515 nm. The scavenging activity was expressed as % efficiency of free radical scavenging (% = (1 - Asample/Acontrol) x 100). The synthetic antioxidant Butylated hydroxytoluene - BHT (0.2 mg/mL) was used as a positive control. To obtain the EC50 of the O. vulgare extract, linear regression was performed in the statistical program Prism 5.0.

Total antioxidant capacity - FRAP assay

The FRAP assay was performed according to the authors (Pulido et al., 2000; Benzie and Strain, 1996). The reagent solution was prepared on the day of use as follows: 2.5 mL of a 10 mmol/L solution of TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mmol/L HCl, plus 2.5 mL of FeCl3.6H2O (ferric chloride hexahydrate) 20 mmol/L in water and 25 mL of 300 mmol/L sodium acetate buffer pH 3.6. For the reaction, 900 µL of FRAP reagent prepared on the day, 90 µL of distilled water, 30 µL of sample (different concentrations of the lyophilized extract), and standard or blank (water) were used. It was left for 30 min at 37°C and centrifuged for 5 min (3500 rpm). Absorbance was determined at 595 nm against a blank (white: 120 µL of distilled water + 900 µL of FRAP reagent) and the standard used was Trolox (Sigma). Results were expressed in µM eq. Trolox.

Evaluation of the antioxidant potential of O. vulgare extract in cheese

The lipid oxidation of cheese was evaluated by measuring the malondialdehyde (MDA) content using the 2-thiobarbituric test (Vyncke, 1970). Results were expressed in milligrams of MDA per gram of the sample. The test of thiobarbituric acid reactive substances was based on the reaction of thiobarbituric acid with the decomposition products of hydroperoxides and one of the main products formed in the oxidative process is malondialdehyde, a short-chain aldehyde (Silva et al., 1999). The technique was performed according to Zeb and Ullah (2016) using the lipid fraction of the cheese. The analyzes were carried out on the 7th, 15th, and 30th days of cheese storage due to low percentage of fat in this food.

Statistical analysis

For the sensory analysis, the data were analyzed by the Friedman test considering non-parametric data and with dependence between groups. Descriptive analysis of the results of the preference and sex test was performed. The analysis of microbiological data was performed through descriptive analysis of the variables under study, through the distribution of absolute and relative frequency for categorical variables and measures of central tendency (mean ± standard error of the mean) for continuous variables. Data normality (continuous variables) was verified by the Kolmogorov-Smirnov test. Data were compared using Analysis of Variance (ANOVA), Kruskall-Wallis and Tukey's test. For all tests, a significance level of 5% was considered, using the statistical program BioEstat 5.3 (Ayres et al., 2007).

Results and Discussion

The sensory analysis was carried out with a total of 60 untrained tasters, chosen at random, among which 23 were men and 37 were women, aged between 18 and 42 years. Considering all the tasters, the cheeses had acceptance ranging from 7.0 to 7.7, that is, they were classified as "I liked it regularly" (Table 1). When the answers of the women and all the tasters were analyzed, a difference was observed between the control and the T2, where the sample without extract was better accepted. There was no difference in the acceptance of the different cheese samples for the men tasters ($p>0.05$) who attributed average scores of 7.2, 6.9, 6.7, and 6.6 for the control cheeses, T1, T2, and T3, respectively. There was a higher coefficient of variation for the men's scores and this may have influenced us to not detect differences in the acceptance of cheese by them (Table 1).

Table 1
Taster acceptance profile (mean ± standard error of the mean) performed with a 9-point hedonic scale for fresh cheese without oregano extract (Control), fresh cheese with 0.5 g of oregano extract/kg (T1), fresh cheese with 1g of oregano/kg (T2) and fresh cheese with 3g of oregano extract/kg (T3), on the second day of storage

Treatments	Tasters (n=60)	Men (n=23)	Women (n=37)
Control	7.73±0.18^{a}	7.22±0.39	8.05±0.15^{a}
T_1	7.43±0.16^{ab}	6.87±0.30	7.78±0.15^{ab}
T_2	7.03±0.18^{b}	6.65±0.34	7.27±0.2^{b}
T_3	7.12±0.20^{ab}	6.61±0.34	7.43±0.23^{ab}
CV%	19.08	23.98	14.75

CV: Coefficient of variation. Means followed by different letters differ by Friedman test ($P<0.05$).

In a study by Presente et al. (2016), essential oil of *O. vulgare* added to fresh cheese was used. According to the tasters, regarding acceptance, the cheeses with essential oil of *O. vulgare* and the control were the ones that obtained the best indexes, not differing statistically. The essential oil of *O. vulgare* presented acceptance values of 7.5 ± 1.86 and the control of 7.6 ± 1.12. Therefore, they fall between "I liked it moderately" and "I liked it a lot", which shows that the product was well accepted by the evaluators.

Costa et al. (2009) used fresh Minas cheese from spiced goat's milk, with dry *O. vulgare* extract, at concentrations of 0.0%, 0.1%, 0.5%, and 1%. Considering the flavor attributes and global evaluation, a higher score was obtained for the control cheese and cheeses added with 0.1% and 0.5% of the extract. The flavor notes were 7.42 (0.0%), 7.08 (0.1%), 6.26 (0.5%), and 4.17 (1.0%), in the order of preference of the cheeses, the results showed that 47.2% of the tasters considered the unseasoned cheese as the best and the

one seasoned with 1.0% of *O. vulgare* as the worst, that is, the control cheese and low concentrations are well accepted by tasters, while the one with the highest concentrations was not well accepted.

It can be observed that the studies evaluating fresh cheese and other types of cheese have a good acceptance with or without the use of seasoning, varying the grades depending on their concentration. In our work, regardless of the concentration, all samples were accepted by the panelists, signaling that the oregano extract should be better explored as a natural additive in cheese.

Microbiological analyzes did not detect the presence of *Salmonella* in cheese samples, regardless of the addition or not of *O. vulgare* extract.

The antimicrobial action of *O. vulgare* extract was also evaluated for bacterial growth of coagulase-positive staphylococci. On the first day of storage, there was a count of 2.80 log CFU/g for coagulase-positive staphylococci in the control, indicating that the cheeses produced are within the standards determined by the Brazilian Legislation. In treated cheeses, there was a growth of coagulase-positive staphylococci within sanitary standards. The extract of *O. vulgare* promoted a decrease in the bacterial count, especially at a concentration of 3 g/kg. However, there was no significant difference between the groups, even when compared to the control. On the fifth and eighth days of storage, the value of 2.50 log CFU/g was verified for coagulase-positive staphylococci in the control, indicating that there was no increase in the number of *Staphylococcus* during the storage period. The addition of O. vulgare extract did not significantly influence the quantification of coagulase-positive staphylococci (Table 2).

In the aerobic mesophilic count on the first day, the control cheese and the treated cheeses (T1, T2, and T3) presented the same average contamination (around 5 log CFU/g), showing that the manipulation necessary for the addition of *O. vulgare* did not affect the microbiological quality of the food. On days 5 and 8 of storage, there was a gradual and equal increase in the number of bacteria between the control cheese and the treated cheeses. Thus, we observed that even the highest concentration of *O. vulgare* used was not able to inhibit the multiplication of microorganisms (Table 2).

Similar to what happened with mesophiles, the concentrations used for the antibacterial analysis of the *O. vulgare* extract in the food did not show any activity on the psychrotrophic bacteria and, over the days, it was also observed that the bacterial load showed a gradual increase (Table 2).

Table 2
Coagulase-positive staphylococci, mesophilic and psychrotrophic counts (Log CFU/g) in fresh cheese without oregano extract (Control), fresh cheese with 0.5 g of oregano extract/kg (T1), fresh cheese with 1g of oregano/kg (T2) and fresh cheese with 3g of oregano extract/kg (T3), during storage days

Time	Control	T1	T2	T3
Coagulase-positive staphylococci				
Day 1	2,80	1,82	2,00	1,70
Day 5	2,50	2,34	2,40	2,07
Day 8	2,50	2,48	2,26	1,92
Mesophilic				
Day 1	5,86	5,60	5,84	5,80
Day 5	6,64	6,72	6,67	6,62
Day 8	7,39	7,45	7,32	7,50
Psychrotrophic				
Day 1	5,81	5,73	5,72	5,83
Day 5	6,49	6,52	6,59	6,60
Day 8	7,67	7,61	7,52	7,65

CFU: Colony Forming Units. Kruskal-Wallis test (P>0.05).

Cheese, as a food that has a high moisture content, can favor the multiplication of microorganisms, both at the time of its manufacture and during its storage, thus reducing its shelf life (Ribeiro et al., 2005). While, the essential oil of *O. vulgare* is composed of about 40 to 70% of carvacrol, in addition to other compounds such as borneol, cineol, terpineol, terpinene, and thymol. Carvacrol is a phenolic compound and may be the main responsible for the antimicrobial activity of the spice, preventing the multiplication of microorganisms (Dolinsky, 2009; Marinelli et al., 2018; Santurio et al., 2007).

In the present study, the *O. vulgare* extract was not effective in inhibiting coagulase-positive staphylococci strains in fresh cheese. Unlike the essential oil, the extract is water-soluble, and the lack of effectiveness may be related to the fact that the cheese is highly moist, and the concentrations tested may have interacted with the water content of the food.

A determining factor to consider for the growth of microorganisms is the temperature range that favors their multiplication, where the greatest growth occurs at its ideal temperature, varying between species. Rosa (2005) analyzed the psychrotrophic bacterial load of Minas frescal cheese and found a bacterial load of 5.67 log CFU/g. As for bacteria from the mesophiles group, a bacterial load of 6.48 log CFU/g was found.

In the works by Sangaletti et al. (2009), who also analyzed Minas fresh cheese, the mean contamination of psychrotrophics and mesophiles on the first day of storage was lower than those found in this work, with

mean values of 3.13 log CFU/g and 3.75 log CFU/ g, respectively. However, on the tenth day, the values reached the averages of 7.74 log CFU/g for psychrotrophics and 6.86 log CFU/g for mesophiles, corroborating the data found in this work. According to Normative Instruction No. 60, of the National Health Surveillance Agency - ANVISA (Instrução Normativa n°60/2019), there is no established value for mesophilic and psychrotrophic bacteria in cheese. However, counting these microorganisms serves to indicate the hygienic-sanitary quality of foods, providing a parameter on their shelf life.

Differently from what was observed for mesophiles and psychrotrophic, the number of thermotolerant coliforms tended not to increase during storage. In the results, the difference in MPN/g of thermotolerant coliforms between the control cheese (without extract) and T3 (3g of *O. vulgare* extract/kg) was observed, showing the antibacterial activity of *O. vulgare* at the highest concentration tested ($p<0.05$), regardless of the number of days of storage. The antibacterial activity of the extract depended only on the amount (concentration), the more extract used, the more effective the result (Table 3).

Table 3
Most Probable Number of thermotolerant coliforms (MPN/g) in fresh cheese without oregano extract (Control), fresh cheese with 0.5 g of oregano extract/kg (T1), fresh cheese with 1g of oregano/kg (T2) and fresh cheese with 3g of oregano extract/kg (T3), during storage days

Time	Control	T1	T2	T3
Day 1	$4.3x10^{3\,b}$	$2.3x10^{3\,ab}$	$4.3x10^{3\,ab}$	$9.3x10^{2\,a}$
Day 5	$4.3x10^{3\,b}$	$4.3x10^{3\,ab}$	$4.3x10^{3\,ab}$	$9.3x10^{2\,a}$
Day 8	$4.3x10^{3\,b}$	$4.3x10^{3\,ab}$	$2.1x10^{2\,ab}$	$2.3x10^{2\,a}$

*Tukey's test: Different letters indicate a statistical difference between groups ($P<0.05$).

O. vulgare extract had a positive result in inhibition when compared to thermotolerant coliform bacteria, which are indicators of fecal contamination, showing that T3 samples, which were added 3 g of extract/kg, had a decrease in contamination from 4300 (control) to 930 MPN/g on days 1 and 5, and a reduction from 4300 to 230 MPN/g on the eighth day.

Picoli et al. (2006) analyzed the quantification of thermotolerant coliforms in fresh goat cheese in the stages of cheese production and showed positive results, due to good handling practices during all stages of preparation, similar to the present work, where the control cheese was within the acceptable sanitary values for consumption.

The results of antioxidant capacity and antioxidant potential of *O. vulgare* extract were 0.035 mg/mL by EC_{50} - DPPH and 75 nmol eq. Trolox/mg extract by FRAP assay.

The TBARS test revealed that the cheese, regardless of treatment, had little oxidation and, therefore, the action of *O. vulgare* was not statistically significant.

However, in Figure 1 we see an increasing trend in malonaldehyde levels in the control, T1 and T2 samples reaching 0.081, 0.094 and 0.072 µmols MDA/g on the 30th day of storage, while at T3 we observed stabilization in the oxidation levels between the 15th and 30th days with 0.039 µmols MDA/g.

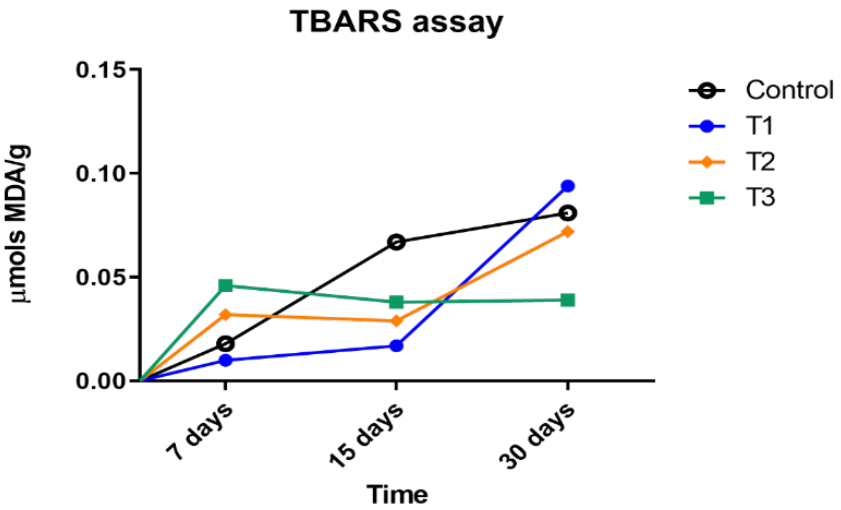

Figure 1. Mean values of malondialdehyde (µmol MDA/g) in control fresh cheese (without extract), T1 (0.5 g of *O. vulgare* extract/kg), T2 (1 g of *O. vulgare* extract/kg), and T3 (3 g of *O. vulgare* extract/kg) on the 7th, 15th and 30th days of storage.

Nunes and Dias (2010) obtained in their analysis the EC_{50} at 25 µg/mL, while in our work it was 35 µg/mL. It is worth mentioning that the lower the EC_{50} value, the greater the antioxidant activity of the analyzed extract. Through the antioxidant activity of *O. vulgare,* also by the DPPH method, it was observed that at concentrations of 1000 µg/mL, *O. vulgare* had 81.32% of antioxidant activity and that, through the evaluation by the iron reduction method (FRAP), *O. vulgare* showed great antioxidant capacity as iron ion scavenger at the same concentration of 1000 µg/mL, with a reading of 315.11 µmol/g of extract (Matiolli, 2014).

Using the FRAP method, Santos (2009) found that the *O. vulgare* extract also demonstrated antioxidant power (154 µmol of TROLOX/mg sample) compared to other herbs such as *Rosmarinus officinalis* (rosemary), *Origanum majorana* (marjoram), *Thymus citriodorus* (lemon thyme) and *Salvia officinalis* (sage). The extract of *O. vulgare* obtained a value of 75 nmol eq. TROLOX/mg of extract. About the method mentioned above, Jardim et al. (2019) stated that few studies are demonstrating the antioxidant action by the FRAP method. However, it can be applied as an alternative method to assess this ability.

The content of secondary plant metabolites can vary considerably depending on several factors such as climate, weather, environment, conditions of collection, stabilization and even storage can have a

great influence on quality (Gobbo & Lopes, 2007). This may explain the differences found in antioxidant activity even when evaluating plants of the same species.

In ricotta cheese, Santos et al. (2012), through the thiobarbituric acid reactive substances test (TBARS), found a 49.8% reduction in the production of MDA, and Santos (2009) found a percentage in the production of malondialdehyde of 44.98% using the same method in tests with extracts of *O. vulgare,* being used in the oxidative stability of butter. In our study, only one of the treatments, T3, which was the highest concentration, had a tendency to stabilize lipid oxidation, which suggests a need to increase the concentrations of *O. vulgare* extract.

Almeida-Doria et al. (2000) evaluated the antioxidant action of ethanol extracts from *R. officinalis* and *O. vulgare.* It was found that the compounds used retarded the oxidation of soybean oil. However, the natural extracts did not reach the efficiency of TBHQ but were as effective as the BHA + BHT mixture. Gonçalves et al. (2015) reported positive results of the antioxidant activity of *O. vulgare* and its total phenolic compounds and diversity of secondary metabolites, detected by phytochemical screening, which highlights thymol, carvacrol, and eugenol, which are compounds subject to reactions oxidation that occur during food processing and storage because some are unstable.

For a spice to act as a modulator in health promotion, its phytochemical content alone is not enough, the form of preparation and the amount consumed are also important factors (Del Ré & Jorge, 2012). Given the facts mentioned above and the result achieved by T3 in our work, it was observed that there is potential for further research with the extract of *O. vulgare.* Studies show that research involving natural agents should continue as they prove to be of great importance to human health.

Conclusion

The incorporation of different amounts of *O. vulgare* extract to the fresh cheese was well accepted by the group of tasters, with scores similar to the control cheese. Regarding antibacterial activity, the extract was effective in reducing thermotolerant coliforms, an activity highlighted at the concentration of 0.3 g/kg. The antioxidant activity in the food indicates application potential for cheeses with higher fat content. Thus, the research shows that the *O. vulgare* extract has the potential to add flavor to fresh cheese and, in addition, it has an important role in the control of microorganisms that indicate hygienic-sanitary conditions common in this type of food. The extract of *O. vulgare,* still little studied in a food model, can contribute to the safety and flavor of fresh cheese over the shelf life of the product.

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Conflict of interest statement

The authors declare no conflicts of interest.

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