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Physicochemical and sensory analysis of Coalho Cheese supplemented with different concentrations of essential oil of oregano (*Origanum vulgare*)

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Abstract

The objective of this study was to analyze the physicochemical, microbiological, and sensory characteristics of coalho cheese supplemented with different concentrations of oregano oil. This study used a completely randomized experimental design with four levels of oregano oil and five storage days. However, fat content and fat in dry matter presented a significant difference ($p < 0.0001$) with the inclusion of oregano oil. The sensory attributes had no significant difference. Regarding sensory analysis and purchase intention, all cheeses were well accepted by most consumers, indicating that they can be used as natural additives in coalho cheese.

Keywords: natural additive; quality; acceptance.

Practical application: The essential oils from certain plants have been studied for their antimicrobial properties and their potential use as substitutes to standardize aromatic properties and reduce microbial load present in leaves.

1 INTRODUCTION

The milk is one of the most consumed animal products worldwide. Being a complex food that contains high concentrations of macro- and micronutrients, milk is important for human nutrition and development, as well as a significant source of income for the global population's survival (Siqueira et al., 2019). Due to its high nutritional value, it becomes an excellent medium for the growth of microorganisms, some of which can cause diseases. Therefore, the use of preservation methods such as processing and the use of additives are necessary to ensure increased shelf life and quality of the milk intended for commercialization (Vidal & Saran Neto, 2019).

The increase in milk production and processing that has occurred in recent years is mainly due to new dietary trends (Barlowska et al., 2018). Milk and dairy products are appreciated by consumers for their health benefits (Sobral et al., 2023). Consumers seek products that have quality, food safety, sensory attractiveness, and nutritional value (Linares et al., 2017). There is consumer demand for cheese made by traditional methods. The sensory qualities of cheese produced by traditional methods differ from those produced on an industrial scale (Kuznicka & Łapinska, 2014).

Among the thousand varieties of cheese, the coalho cheese is widely consumed by the population of the Northeast region of Brazil. It is considered to have medium to high moisture content, semi-cooked or cooked, and fat content in the total solids ranging from 35% to 60% (Lima et al., 2021; Muniz et al., 2022; Santos, W. F. et al., 2022; Sobral et al., 2023; Tadjine et al., 2020). It is usually marketed within 10 days of production and stored at temperatures of up to 12°C (Brasil, 2001). Coalho cheese is commonly consumed fresh (Tadjine et al., 2020), without maturation, although it can undergo a maturation process if preferred.

Since it is primarily consumed fresh, the use of additives is necessary to extend its shelf life. Additionally, due to its medium to high moisture content, this type of cheese is more susceptible to microbial proliferation. The current concern of the industry is to develop new products with fewer chemical additives, such as preservatives, in their formulation. This has led the food industry to seek alternative compounds to achieve their objectives related to the stability of their final products against the action of microorganisms that can cause deterioration and/or foodborne diseases (Santos, W. F. et al., 2022).

In this context, essential oils from certain plants have been studied for their antimicrobial properties and their potential use as substitutes to standardize aromatic properties and reduce microbial load present in leaves (Marcial et al., 2016; Santos, D. G. et al., 2022). There are numerous reports associating the chemical composition and antimicrobial properties of essential oils from various species of Origanum, as well as their application in various commercial preparations as antimicrobials and antioxidants

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(Castilho et al., 2012; Farias et al., 2017). Therefore, the substitution of traditional preservatives with essential oils to increase shelf life could be an alternative. However, a balanced amount of essential oil is necessary to prevent significant changes in the characteristic aroma and physicochemical composition of the cheese (Marcial et al., 2016; Santos, D. G. et al., 2022). Thus, the objective of this study was to analyze the physicochemical, microbiological, and sensory characteristics of coalho cheese supplemented with different concentrations of Essential Origanum Oil.

2 MATERIALS AND METHODS

2.1 Study site

The study was carried out at the Dairy School belonging to the Department of Animal Science of the Center for Agricultural Sciences (SCAS) of the Universidade Federal da Paraíba (FUP), located in the municipality of Areia, PB.

2.2 Raw material

The oil used in this study was Now Foods Oregano Essential Oil—100% pure, non-transgenic, and gluten-free vegan.

The milk used in the research was supplied by the Milk Cattle Breeding Sector of the Animal Science Department of the Agrarian Science Center of the Universidade Federal da Paraíba, located in Areia, PB. The milk was collected during milking in the morning from crossbred cows on pasture, after which it was transported to the dairy school where platform analyses were performed to verify the quality of the milk. Analyses were performed to verify the composition of the milk through ultrasound, using the Master Classic Complete equipment (AKSO, Produtos Eletrônicos Ltda., São Leopoldo, RS) to evaluate the fat, protein, lactose, total dry extract (TDE), defatted dry extract, density, and pH contents.

2.3 Pasteurization of the milk and production of the coalho cheese

The milk was processed by slow pasteurization at 65°C for 30 min, and the time was marked Bonly after the milk reached the treatment temperature.

Cheese manufacturing process:

- For cheese making, after pasteurization at 65°C was performed, the milk was cooled for subsequent addition of the HA-LA liquid coagulant;
- Addition of the liquid coagulant was done according to the manufacturer's recommendations;
- Cutting of the paste after resting for 40 min;
- Homogenization;
- Desorbing;
- Pre-cooking the dough, heating the whey to 75°C;
- Second homogenization;
- In the second desiccation, 15 mL of whey was removed to mix with the essential oil, and 5 ml was used for each treatment where the essential oil was added;
- For the salting of the mass, 45 g was used for each 10 L of milk used;
- Addition and inclusion of the essential oil in the dough, after being mixed with some whey, the amount of essential oil added was 20, 40, and 80 μL/kg dough;
- Vacuum packing and storage at 12°C;
- The storage lasted 60 days for the analyses, and on days 0, 15, 30, 45, and 60 days after manufacture, one cheese of each treatment was removed to perform the physicochemical analyses;
- A total of 40 cheeses weighing about 0.5 kg were produced, of which 20 cheeses were produced in the first repetition and 20 in the second repetition;
- The repetitions were the days of manufacture, two repetitions being 2 days of manufacture.

2.4 Experimental design

This study used a completely randomized design with 4 × 5 factorial, being 4 levels of oregano oil inclusion (0, 20, 40, and 80 μL/kg mass) treatments and 5 stages of physicochemical analysis (0, 15, 30, 45, and 60 days) and two repetitions being the manufacturing days. Each treatment consisted of the production of coalho cheese made with cow's milk, added with oregano essential oil in different concentrations:

- Treatment 1: coalho cheese without adding essential oil (control);
- Treatment 2: cheese with oregano essential oil (20 μL/kg mass);
- Treatment 3: cheese with oregano essential oil (40 μL/kg mass);
- Treatment 4: cheese with oregano essential oil (80 μL/kg mass).

These concentrations were the averages obtained based on studies in the area, seeking to achieve the most favorable level for microbial control and consumer acceptability.

2.5 Physical-chemical analysis of the coalho cheese

The analyses of TDE, fat, and fat in dry extract (FDE) and ash were performed at the Dairy School of CCA/UFPB and the Laboratory of Food Analysis and Animal Nutrition of SCAS/FUP. The coalho cheese samples were analyzed in duplicate according to the methodology of Normative Instruction 30, June 26, 2018, to determine the fat content, using Gerber's Lacto-butyrometer.

FDE was obtained by the following relationship (Equation 1):

$$FDE = \frac{\%\ Fat \times 100}{\%\ TDE} \quad (1)$$

For the determination of protein, we used the methodology according to Normative Instruction 30 of June 26, 2018, by the determination of total nitrogen from the Kjeldahl method. The analysis of total solids and ash also followed the same normative instruction.

2.6 Microbiological analysis of the coalho cheese

Enterobacteriaceae were cultured on Mac-Conkey (MC) Agar and incubated for 24 h at 35°C. After this cycle, the colonies were subjected to biochemical identification using the methods of glucose and lactose fermentation, VM-VP test, citrate utilization, motility evaluation, and production of indole and H2 S (Ferreira & Silva, 2021).

Aliquots of 0.1 mL of the dilutions were transferred to sterile Petri plates containing Hektoen Enteric Agar culture medium for surface plating. Subsequently, the plates were incubated inverted at 37°C/24 h and, after incubation, three characteristic *Escherichia coli* colonies were selected from each plate and submitted to Indol, Methyl Red, Voges-Proskauer, and Simmons Citrate biochemical tests for confirmation.

2.7 Sensory analysis of coalho cheese

The sensory analysis was performed in individual booths under controlled environmental conditions, at a temperature of around 23°C (ISO, 1988). Ten testers were duly selected and trained (ISO, 1993). The tests took place in two sessions and ten tasters, totaling 80 samples.

In all tests, the samples were randomly placed in trays with random three-digit codes. The samples were divided into equal portions of 5 g on Styrofoam plates, marked with a random three-digit code. To avoid the possible effects of the order of presentation, the samples were presented to the panel members following different orders (Macfie et al., 1989). Consumers evaluated the cheese samples and were asked to mark the option that best suited the product about overall acceptance using a 9-point unstructured hedonic scale (Sobral et al., 2023). The tasters described how much they liked or disliked the attributes: odor, appearance, taste, texture, and overall impression. A scale of 5 points recommended by Meilgaard et al. (2006) was used to evaluate the intention of purchasing: 1 (Certainly I would buy it); 2 (Probably I would buy it); 3 (Maybe I would buy it/Maybe I wouldn't buy it); 4 (Probably I wouldn't buy it); and 5 (Certainly I wouldn't buy it).

2.8 Statistical analysis

Data were submitted for analysis of variance, and Tukey's test compared the means at 5% probability using the PROC GLM of the Statistical Analysis System Institute (SAS, 2010) statistical package. The Ryan-Einot-Gabriel Welsch test compared the means of the sensory attributes at a 5% probability level.

3 RESULTS

The physicochemical variables had no effect ($p > 0.05$) on the interaction of oil × days of storage (Table 1). It is observed that the variables such as total solids ($p = 0.9415$), protein ($p = 0.6063$), and ash ($p = 0.3506$) did not have significant differences according to the inclusion of oregano oil in the cheese formulation. The fat ($p \leq 0.0001$) and FDE ($p < 0.0001$) presented significant differences according to the inclusion of oregano oil (Table 1). The fat and the fat in the dry extract reduced the value as the oil was added to the cheese formulation, and this reduction was around 12%.

The microbiological data are presented in Table 2, and it is observed that the values were below the indicative of food contamination, considering the inclusion of oregano oil and the shelf life.

The attributes such as appearance ($p = 0.1341$), color ($p = 0.8089$), eyeball ($p = 0.3271$), typical aroma of coalho cheese ($p = 0.1165$), attractive aroma ($p = 0.8010$), soft texture ($p = 0.0780$), salty taste ($p = 0.2729$), and general evaluation ($p = 0.3470$) did not differ significantly according to the inclusion of oregano oil in the cheese formulation (Table 3). However, statistical differences were found in typical oregano aroma ($p < 0.0001$), homogeneous texture ($p = 0.0071$), bitter taste ($p = 0.0086$), typical coalho cheese taste ($p = 0.0050$), and typical oregano flavor ($p < 0.0001$) (Table 3).

The attribute typical oregano flavor increased its acceptance according to the inclusion of oregano in the formulation, and there was an increase of approximately 70%. The attribute homogeneous texture increased with the inclusion of oregano

Table 1. Physicochemical characteristics of coalho cheese added from different concentrations of essential oil of oregano (*Origanum vulgare*).

Effects	Total solids (%)	Fat (%)	Fat in dry extract (%)	Protein (%)	Ashes (%)
Level of oregano essential oil (µl)					
0	52.33[a]	24.53a	46.89a	23.03[a]	3.23[a]
20	51.91[a]	22.24b	42.94b	22.86[a]	3.58[a]
40	52.07[a]	21.62c	41.52b	23.86[a]	3.28[a]
80	51.91[a]	21.50c	41.42b	23.04[a]	3.35[a]
Storage (days)					
0	52.41	22.50	43.01	23.35	3.51
15	52.08	22.64	43.48	23.29	3.43
30	52.02	22.33	42.93	23.45	3.25
45	52.30	22.43	42.90	22.12	3.25
60	51.47	22.46	43.65	23.77	3.40
SEM	1.74	0.41	1.70	1.80	0.44
P-value					
Oil – O	0.9415	< 0.0001	< 0.0001	0.6063	0.3506
Storage – S	0.8440	0.6446	0.8556	0.4422	0.7007
O*S	0.9567	0.2801	0.9572	0.9127	0.5279

SEM: standard error of the mean. Different letters in the column differ from each other by Tukey's test at the level of 5% probability.

oil, and the control group differed from the treatment with 40 and 80 µL of oil, being statistically similar to the treatment with 30 µL, where the evaluators felt slightly more lumpiness in the cheese-added essential oil.

Regarding the bitter taste, it was observed that it increased with the inclusion of oregano oil, with the control group being statistically different from the treatment with 80 μL, while the control group was statistically similar to the treatments of 20 and 40 μL. The treatments of 20, 40, and 80 μL were statistically similar, and the essential oil provided a more bitter taste sensation in the cheeses.

Regarding the typical taste of coalho cheese, there was a decrease in the value of the attribute with the inclusion of oil, which occurred in contrast to the typical taste of oregano, which increased due to the inclusion of oil in the formulation. The taste attribute and global evaluation, where the results ranged from 6.38 to 7.13 and 6.22 to 7.06, equivalent to "I liked it a little" to "I liked it moderately" for the two attributes, respectively. Therefore, there was no rejection in any of the evaluated cheeses, including the one with the highest addition of essential oils.

Regarding purchase intention, the control group had the highest purchase intention (100%), while the 20, 40, and 80 μL presented about 33.33% of intentions to probably not buy, and only the 80 µL treatment presented 8.33% of people who would not buy (Figure 1).

However, 20, 40, and 80 μL showed a good purchase intention, even presenting this percentage of disapproval. Of the cheeses added to oregano essential oil, 20 μL showed an acceptance of about 91.67%, 40 μL showed an acceptance of about 83.34%, and 80 μL showed an acceptance of about 83.34%. Of all the treatments, only 80 μL showed a "Would Not Buy" intention of about 8.33%.

4 DISCUSSION

Fat is one of the most variable components in milk, being affected by the type of animal feeding, breed, species, and lactation stage, among other factors. Normative Instruction Nº 30, of June 26, 2001, classifies coalho cheese as medium to high humidity, semi-cooked or cooked paste, presenting the fat contents in total solids varying between 35 and 60% (Brasil, 2001). It can be observed that the cheeses presented results that varied from 41.52 to 46.84% for the treatments and from 42.90 to 43.65% concerning the days, within the parameters established by NI N°. 30, in which the days of evaluation of the cheeses did not interfere in their composition.

Table 2. Microbiological analysis of coalho cheese with the addition of oregano essential oil.

Microorganism	Level of oregano essential oil (µl)				
	Days	0	20	40	80
Enterobacteriaceae (UFC/g)	0	1,8 x 10^2	2 x 10^4	0	0
	15	10^3	2,7 x 10^2	3 x 10^3	3 x 10^3
	30	2 x 10	0	0	0
	45	3 x 10^5	0	5 x 10	2,01 x 10^5
	60	3 x 10^5	5,9 x 10^4	3 x 10^5	3 x 10^5
E. Coli (UFC/g)	0	0	0	0	0
	15	0	0	0	0
	30	0	0	0	0
	45	0	0	0	0

Table 3. Sensory attributes of coalho cheese with the addition of oregano (Origanum vulgare) to its formulation.

Attributes	Level of oregano essential oil (μL)				SEM	*P*-value
	0	20	40	80		
Appearance	2.63	3.38	3.63	3.53	1.66	0.1341
Coloring	4.95	4.51	4.88	4.64	1.78	0.8089
Hole	2.25	3.08	3.03	3.14	1.86	0.3271
Aroma						
Typical coalho cheese	5.34	4.16	3.97	3.79	2.42	0.1165
Typical oregano	1.40c	3.24b	3.90ab	4.72a	1.86	< 0.0001
Attractive	6.32	6.50	6.61	6.09	1.93	0.8010
Texture						
Soft	5.97	4.85	4.97	5.48	1.66	0.0780
Homogeneous	2.98b	4.50a	4.05ab	4.28a	1.59	0.0071
Flavor						
Salty	3.08	3.60	3.85	3.86	1.55	0.2729
Bitter	1.11b	1.81ab	1.98ab	2.55a	1.42	0.0086
Typical coalho cheese	6.75a	5.60ab	5.10ab	4.50b	2.18	0.0050
Typical oregano	1.15b	4.30a	4.55a	5.19a	1.92	< 0.0001

SEM: standard error of the mean. Different letters on the line differ from each other by the Ryan-Einot-Gabriel Welsch test at the level of 5% probability

The fat contents varied from 21.50 to 24.53% for the treatments and from 22.33 to 22.64% for the days. Similarly, Gomes and Medeiros (2012) found fat percentage in coalho cheese that ranged from 23.30 to 27.30%, while Freitas Filho et al. (2012) found values for fat content that ranged from 12.36 g/100 g to 42.40 g/100 g.

The protein content found in the cheeses ranged from 22.86 to 23.86% when considering the treatments and from 22.12 to 23.77% when considering the days. In studies conducted with coalho cheese, Freitas Filho et al. (2012) obtained a percentage of protein ranging from 19.14 to 26.41 g/100 g.

The percentage of ash ranged from 3.23 to 3.58% related to the treatments and from 3.25 to 3.51% related to the days. The legislation does not establish limits for the amount of ash in coalho cheese. Nevertheless, Gomes and Medeiros (2012), in a physicochemical analysis of artisanal and industrial coalho cheese, found values ranging from 4.47 to 4.88%. Therefore, the percentage of ash in the cheeses found in this research is closely similar to the results in the literature.

The microbiological results follow the legislation for cheese (Brasil, 2001). All cheese samples indicate acceptable hygienic-sanitary quality throughout the production and demonstrate the microbiological quality required by current legislation so that all samples were fit for consumption and within the reference values for this type of product. Castilho et al. (2012) in a study observed that the antimicrobial activity with the use of oregano essential oil indicates a positive performance in combating the growth of pathogenic and food-deteriorating microorganisms, especially the bacteria *E. coli*, *Listeria monocytogenes*, and *Staphylococcus aureus*. Farias et al. (2017) observed in a study with fresh minas cheese enriched with oregano oil that the microbiological parameters were within the standards required by current Brazilian legislation. Thus, it is concluded that it is feasible to insert the cheese market with the addition of oregano essential oil, mainly because this oil works as a natural preservative in cheeses discarding the use of chemical additives.

Concerning the typical oregano aroma, the results are possibly due to the higher levels of oregano essential oil. The evaluators felt a greater aroma, which is perfectly usual as indeed they had a higher oregano essential oil content. Although there was no statistical difference regarding the attribute "typical aroma of coalho cheese," the average ratings ranged from 3.79 to 5.34, which, on the hedonic scale, would be between "moderately weak" and "indifferent." For the aroma alone attribute, the average rating was 6.38, which, on the hedonic scale, is equivalent to "I liked it slightly." These results corroborate the effect of the essential oil on the perception of the attribute "aroma" by the evaluators, even in the control cheese, which probably may have been because all the samples were delivered at the same time and the aroma of the oil in the other cheeses may have also influenced the evaluation of the control cheese.

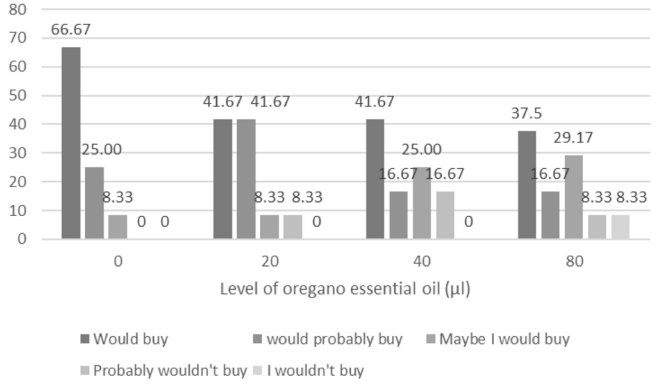

Figure 1. Purchase intention (%) of coalho cheese added of different concentrations of oregano essential oil.

The greater manipulation of the cheeses to add the essential oil may have affected the texture attribute or even the effect of the oil itself on the cheeses during pressing. On the contrary, for the texture attribute, the evaluations ranged between 4.85 and 5.97, showing a certain indifference to the attribute in general. Concerning salt, the evaluations were between 3.08 and 3.86, which shows a low salt perception. The salt was added to the cheese mass equally before separating them for the addition of the essential oil, which can probably explain the result. It is important to highlight the negative effect of salty foods on health of the consumers.

Ideally, when adding the essential oil to the cheese consumers should have minimal sensation of alteration in the aroma and taste as the intention with this additive is the antimicrobial effects. Therefore, finding the dose of essential oil that provides the balance between microbial control, effect on increased shelf life and minimal effect on sensory attributes are of utmost importance.

5 CONCLUSION

Oregano essential oil reduced the fat content and fat content in the dry extract of the cheeses. Regarding the sensory analysis and purchase intention, there was good acceptance of all cheeses evaluated by most consumers, indicating that the Oregano Essential Oil can be used as a food additive in coalho cheese.

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