

Sensorial, Microbiological, and Physico-Chemical Analysis of Minas Frescal Cheese with Oregano Essential Oil (*Origanum Vulgare*) Addition

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Abstract This study aims to evaluate, from production to shelf-life, the physico-chemical, sensorial, and microbiological characteristics of minas frescal cheese made with oregano essential oil. Three samples F1, F2, and F3 were prepared with different levels of essential oil concentration and evaluated using the hedonic scale for attributes such as color, flavor, texture, odor, and overall evaluation. The physico-chemical analysis included the determination of moisture, total fat, ash, protein, and carbohydrates which were evaluated by ANOVA and Tukey test ($p < 0.05$). The samples were analysed for *Staphylococcus* coagulase positive, mesophilic, psychrotrophic, coliforms, and *Salmonella* at 0, 7, 14, 21, and 28 days. Results for *Staphylococcus* coagulase positive, aerobic mesophilic, psychrotrophic, coliforms, and *salmonella* were found within the limits established by applicable Brazilian legislation. The sensory acceptance rates were satisfactory, being greater than 70% at all concentrations, suggesting that the insertion of oregano essential oil cheese in the consumer market is feasible at the evaluated concentrations.

Keywords Sensory acceptance, Flavoring, Antimicrobial action

1. Introduction

Even though food additives are added with the intent of improving consumers' health, the demand for chemically added ingredients has been decreasing over time. As consumers give more preference to naturally made food, several studies have been developed in order to find natural substitutes to chemical additives that could perform as well as chemical additives in terms of antimicrobial potential [1].

The use of plant's secondary metabolites additives, for example, has been increasing mainly due to its health benefits as well as reduced impact on the environment. Such metabolites proved to be efficient in terms of antimicrobial properties; therefore, they have great potential to be employed in the food industry, mainly where the risk of contamination during product preparation is high. In that case, the use of natural inhibitory substances is highly attractive [2].

Studies conducted using essential oils extracted from

many different plants have demonstrated that such oils perform well in preventing microorganism's deterioration, having great antimicrobial activity properties [3].

According to the International Standard Organization (ISO) 9235:1997, essential oils are defined as products obtained from plants through steam distillation. In general, they are complex mixtures of volatile and lipophilic substances, generally liquid and odoriferous [2].

Oregano (*Origanum vulgare* Linneus, family Lamiaceae) is a plant with a pleasant smell and has an herbaceous and intense flavour. One of its main components is its oil. Oregano's chopped flowers and leaves are widely used for aromatic and seasoning purposes [3].

According to Souza et al. [1] when studying the antimicrobial potential of spices it is noticeable that oregano is widely used for inhibiting bacteria and fungi contamination in food.

In Brazil, there are several types of both handmade and industrially produced fresh cheeses, produced both by small producers and industries [5].

The dairy industry has great social and economic relevance, especially concerning cheese production, occupying the sixth position in world production. The greatest parcel of the Brazilian annual cheese output

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consists in the production of popular consumption cheeses, such as the minas frescal cheese, mozzarella, ricotta, prato cheese, and parmesan [6]. Among these, the minas frescal cheese has the higher moisture, thus having shorter shelf-life.

Most of the studies about essential oils additions, evaluate their microbiological characteristics without assessing the sensorial quality of the obtained product. Seeking a broader approach, this study aims to evaluate the microbiological, sensorial, and physicochemical properties of minas frescal cheese made with oregano essential oil addition, using three different formulations.

2. Materials and Methods

The three studied formulations of Minas frescal cheese tested in this study were produced in the Laboratory of Food Engineering of State University of Maringa. The ingredients were purchased in local markets in the city of Maringa with exception of the oregano oil that was donated by Ferquim®.

2.1. Processing the Minas Frescal Cheese

The basic formulation of the minas frescal cheese was composed of 97.9% of pasteurized non-homogenized milk, 0.03% of calcium chloride, 1.5% of salt and 0.15% of rennet. Different concentrations of oregano essential oil were used in the samples; the control sample, F1, had no essential oil addition while F2 had addition of 0.0075% and F3 0.015%. These concentrations were defined in preliminary tests. The preparation of the samples was made according to recommendations of FURTADO and LOURENÇO-NETO [7]. Right after production, the samples were submitted to percent composition, microbiological, and sensorial analyses. Part of the product was stored in BOD, at 5°C during 28 days for microbiological analysis.

2.2. Percent Composition Analysis

Both moisture and ashes analyses were carried out according to recommendations by Adolfo Lutz Institute [8]. The lipids analysis was carried out using the Bligh & Dyer – BD method [9], the protein analysis was performed according to recommendations of AOAC [10], and the carbohydrate analyses was carried out using the method of difference.

2.3. Sensorial Analysis

The obtained products were submitted to sensorial analysis using a hedonic scale test in order to check whether there had been representative flavour difference between the minas frescal cheese prepared with oregano oil addition.

A nine-point hedonic scale (9= really liked it; 5= indifferent, and 1= really disliked it) was used to evaluate color, smell, taste, texture, overall appearance, and purchase

intention (with 3-point scale).

Fifty non-trained tasters participated in the test; each one received simultaneously 3 samples of minas frescal cheese with three different concentrations of oregano oil codified with three digits in a randomized order, along with a glass of water at room temperature (25°C) to rinse out their mouth between samples. The test was conducted in the laboratory of sensory analysis of Food Engineering of State University of Maringa in booths with fluorescent lamps (daylight) and with an approving position by the UEM Ethics Committee, CAAE: 04021812.4.0000.0104.

The acceptance rate (IA) of the developed products was calculated through equation 1.

$$IA \% = \frac{X * 100}{N} \quad (1)$$

Where: X = average of sample;

N = maximum score of sample given by tasters

2.4. Microbiological Analyses

Samples were tested on the day of preparation, then at 7, 14, 21, and 28 days of storage for coagulase positive Staphylococci, aerobic mesophilic bacteria, psychrotrophs, thermotolerants, total coliforms (at 35°C and 45°C), and Salmonella according to a methodology suggested by SILVA et al. [11].

2.5. Statistical Analysis of Data

Results were analysed statistically using analysis of variance (ANOVA). The samples which differed significantly had their averages calculated through Tukey at 95% probability by SAS [12].

3. Results and Discussion

3.1. Percent Composition

The percent composition of minas frescal cheese with different oregano oil concentrations is described in table 1.

Table 1. Percent Composition of Minas Frescal Cheese

	F1	F2	F3
Moisture (%)	54.50 ^a ± 0.50	54.12 ^a ± 4.03	56.94 ^a ± 3.69
Ashes (%)	2.77 ^{ab} ± 0.00	2.72 ^a ± 0.00	3.02 ^b ± 0.00
Lipids (%)	15.39 ^a ± 0.00	14.54 ^a ± 0.00	14.50 ^a ± 0.00
Proteins (%)	15.22 ^a ± 0.00	17.42 ^a ± 0.00	17.64 ^a ± 0.00
Carbohydrate (%)	-	-	-

Averages followed by equal letters on the same line do not differ meaningfully concerning acceptance rate ($p \leq 0.05$).

No representative difference was found between the samples regarding moisture, protein, and lipids content. However, the sample with oregano oil concentration of 0.015% (F3) was found to have a higher value of ashes

when compared to the sample with 0.0075% (F2).

In general, we may conclude that despite the occurrence of significative difference regarding ashes, the addition of oregano oil to minas frescal cheese does not have direct influence on the product's chemical composition, which qualifies it to be employed into consumer market.

The average values of moisture found in this study greatly differ from the results presented in the Technical Regulations for Identity and Quality of Dairy Products [13]. According to that legislation, the minas frescal cheese studied herein would be classified of greatly high moisture (55%).

The obtained average lipid content of the samples were: 15.29g/100g for sample F1, 14.54 for sample F2, and 14.50 for sample F3. According to the Technical Regulations for Identity and Quality of Dairy Products [13], the three samples of cheese would be classified as low-fat cheese given that their fat content is within 10 and 24.9 g/100g. Accordingly, the produced cheese samples fit into the minas frescal cheese classification.

Zarbielli *et al* [18] developed a minas frescal cheese enriched with iron, and found 57.9% of moisture content, 2.86 for ashes and 18.3% for protein, values close to our study.

According to Silva [14], the protein content for the samples of minas frescal cheese usually vary from 16 to 22g/100g. Hence, we understand that our results are close enough to the results found in literature.

3.2. Sensorial Analysis

Table 2 presents the results of sensorial analysis of minas frescal cheese.

Table 2. Sensorial analysis for minas frescal cheese

Attributes	F1	F2	F3
Color	7.58 ^a	7.72 ^a	7.80 ^a
Smell	6.84 ^a	7.14 ^a	7.38 ^a
Taste	6.90 ^a	7.56 ^a	7.40 ^a
Texture	6.62 ^a	6.98 ^a	7.20 ^a
Overall Appearance	7.26 ^a	7.26 ^a	7.12 ^a
Purchase Intention	1.94 ^a	2.30 ^a	2.06 ^a
Acceptance Rate	80.66	80.66	79.12

Averages followed by equal letters on the same line do not differ meaningfully concerning acceptance rate ($p \leq 0.05$).

When evaluating the attributes of color, smell, taste, texture, and overall appearance, the samples F1, F2, and F3 did not present representative difference at 5 %. Such fact may be related to the usage of non-trained tasters as well as the relatively small difference in concentration of oregano essential oil used on different samples.

The scores obtained for all attributes varied between "slightly liked it" and "really liked it". Once there are no significative sensorial differences between the standard cheese 0% (F1) and cheeses with concentrations of

0.0075% (F2) and 0.015% (F3), the insertion of minas frescal cheese with oregano oil in the consumer market is feasible.

All the samples had good acceptance receiving more than 70%. Sample F3 had the lowest acceptance due to an unpleasant after taste reported by the tasters probably caused by high oregano oil concentration.

Samples F1 and F2 had the same acceptance rate showing that concentrations up to 0.0075% oregano oil are not noticeable and unpleasant to the taster.

In one sensory study of minas frescal cheese enriched with iron, with nine point scale, the authors obtained for the sample without added iron and containing different amounts of fat, an average of 6.3 for taste and texture, and 5.8 for smell, this values were lower than those found in this research [18].

3.3. Microbiological Analysis

According to Technical Regulations for Identity and Quality of Dairy Products [13], the microbiological limits for cheeses with high humidity content, tempered, spiced or with herbs addition or other ingredients are of 10^2 for Coliforms at 45°C/g, 10^3 Staphylococcus coagulase positive (UFC/g) and absence for *Salmonella*/25 g.

Results of mesophilic and psychrothrophic micro-organisms are presented in Table 3.

Table 3. Microbiological properties of cheese samples at different periods of time

	Sample/Time (days)				
	0	7	14	21	28
Mesophilic aerobic microorganisms (UFC/g)					
F1	4.19×10^2	4.22×10^2	4.86×10^2	5.4×10^2	5.4×10^2
F2	4.25×10^2	3.81×10^2	5.40×10^2	5.4×10^2	5.4×10^2
F3	4.55×10^2	3.18×10^2	4.99×10^2	5.4×10^2	5.4×10^2
Psychrotrophs (UFC/g)					
F1	$<10^2$	4.5×10^2	$>10^4$	$>10^4$	$>10^4$
F2	$<10^2$	0.5×10^2	$>10^4$	$>10^4$	$>10^4$
F3	$<10^2$	$>10^2$	$>10^4$	$>10^4$	$>10^4$

For all periods assessed, the count of total coliforms and thermotolerants were under 10 UFC/g, well below the limit value of 10^2 UFC/g set by Brazilian legislation.

The present study observed the absence of *Salmonella* and *Staphylococcus Coagulase positive* for all samples analyzed at all periods, showing that the produced samples performed well regarding microorganism of great relevance to public health having its control importantly related to the product quality.

Cardozo *et al.* [25] produced an Ultra-Filtered (UF) fresh Minas cheese and observed that the product had low counts of mesophilic and coliform as well as a total absence of *S. aureus*, *L. monocytogenes*, and *Salmonella* spp. The authors also mention that the Minas cheese UF presented a pH value conducive to microbial contamination.

There were two observations done through statistical analysis: the significative time for mesophilic aerobic microorganisms was achieved at 1% while the treatment, however, was not significative. The behavior of mesophilic aerobic microorganisms is presented on Figure 1.

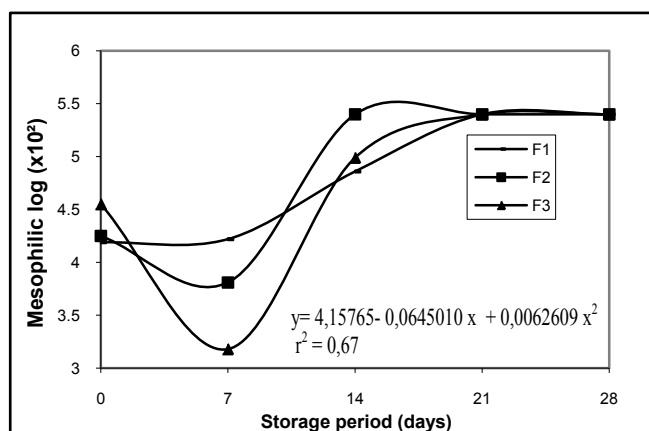


Figure 1. Regression for mesophilic aerobic microorganisms at periods assessed (0, 7, 14, 21 and 28 days)

In a different study, Salimena et al. [3] added clove essential oil in concentrations of 0.78%, 1.56%, and 3.12% to minas frescal cheese. The microorganism analysis of mesophilic, psychrotrophic, coliforms, and *S. aureus* showed results similar to the ones presented in this study. The authors concluded that essential oils have promising antimicrobial characteristics to combat pathogenic and detrimental microorganisms.

According to Franco e Landgraf [16], most food begin to demonstrate alterations when concentrations of aerobic mesophilic microorganisms get higher than 10^6 UFC/g. In those cases, the food should be considered suspicious since it has a greater chance of being contaminated by pathogenic and detrimental microorganisms.

Regarding psychrotrophic microorganisms, Santos et al. [17] concluded that alterations of proteolytic and/or lipid nature appear when the count of such microorganisms reaches 10^7 UFC/g. The authors also reached the conclusion that the essential oils have promising antimicrobial characteristics to combat pathogenic and detrimental microorganisms.

4. Conclusions

Concerning the physical and chemical parameters, the addition of oregano oil did not have direct influence on the cheese composition. The sensorial analysis of all samples did not show significative differences for the attributes assessed (color, taste, smell, texture, overall appearance, and purchase intention). The resulting acceptance rate was above 70%, which is considered good. In addition, a low concentration was observed for every microorganism researched, which is compliant with the microbiological standards required by law.

Thus, the conclusion is that combining minas frescal cheese with oregano essential oil would be welcomed by the consumers market, especially because the oil acts as a natural food preservative for cheese, in contrast with chemical additives, causing shelf-life to be increased.

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