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Research Article

Chemical Composition and Antimicrobial Potential of *Satureja hortensis* L. in Fresh Cow Cheese

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This study presents data about the chemical composition and antimicrobial effect of *Satureja hortensis* L. used as both dry plant and essential oil, on fresh cow's cheese, in order to extend its shelf-life. The proximate and elemental composition of dry plant of *Satureja hortensis* L. highlights important level of microelements. The content of microelements increases even when small amounts of *Satureja hortensis* in fresh cheese were added. The addition of *Satureja hortensis* dry plant leads to an increase in Fe (13.46–65.54%) and Mn (8.33–88.33%) content of fresh cheese, depending on the amount of plant added. The composition of essential oil isolated from *Satureja hortensis* L. was analyzed by GC-MS and the main compounds found were carvacrol (19.68%), o-cymene (30.86%), and p-cymene (28.07%). In order to use *Satureja hortensis* L. as natural preservative in food industry, in vitro effect of plant extract and essential oil against *Staphylococcus aureus* Gram-positive bacteria was tested. The oil of *Satureja hortensis* L. showed antimicrobial activity at 0.50–1.5%, while the alcoholic extract does not inhibit *Staphylococcus aureus* mycelial growth. The antimicrobial effect of *Satureja hortensis* L. dry plant in various proportions (0.5–1.5%) and essential oil (0.1%; 0.25%; 0.5%), on fresh cow's cheese, was assessed after 3 and 7 days by counting colonies obtained at 30°C. Results have shown that the addition of *Satureja hortensis* L. dry plant and essential oil led to a reduction in the total number of germs, this reduction being more significant when the essential oil was used. Regarding the effect of *Satureja hortensis* L. essential oil against *Staphylococcus aureus* inoculated in fresh cow's cheese, the results highlight that the essential oil of *Satureja hortensis* L. may be a natural solution to prevent the development of this bacteria, while the ethanol extract does not prove to be effective.

1. Introduction

Cheeses are dairy products indispensable to human nutrition due to nutrients with high values necessary for the human body: milk casein (the main component of the coagulum), lipids, lactose and the products that are formed after its fermentation (lactic acid), and a significant amount of mineral salts [1].

The range of cheeses comprises a wide variety of assortments: depending on the production process adopted or the raw material used. Fresh cow's cheese is made from cow's milk, pasteurized, normalized, and cooled to 23–28°C followed by coagulation with the help of coagulum [1].

In the case of cheeses, microbial growth is a major problem that results in product quality deterioration [2]. Among the pathogens, *Listeria monocytogenes, Staphylococcus aureus,* and *Salmonella* species are often associated with outbreaks occurring in food and especially in cheese [3, 4].

In this regard, recently researches studies are focused on the use of natural preservatives to help alleviate this problem.

Many medicinal plants and herbal extracts, in addition to their distinctive flavor, exhibit strong antibacterial properties, which give them the potential to be used as natural preservatives [2, 4].

The volatile oil, extracts, tincture, and oleoresin of *Satureja hortensis* are extensively used as antimicrobial and antioxidant agents in pharmaceutical and food industries. The extracts of *Satureja hortensis* contain caffeic acid derivatives and flavonoids; among them rosmarinic acid is present in high concentration and is mainly responsible for biological

activity of the extracts. *Satureja hortensis* essential oil has a high percentage of carvacrol which is responsible mainly for its biological activity [5].

Satureja hortensis L. is an annual plant belonging to the Lamiaceae family grown in many parts of the world, being one of the most important of the nine species of *Satureja* [6]. In Romania, it is found in spontaneous flora and is used in traditional medicine in the treatment of cardiovascular diseases, thrombosis, muscle aches, stomach, and intestinal disorders. It is also employed as an anti-inflammatory agent in the treatment of rhino-sinusitis but it also has applicability as spice and natural food preservative [7].

Due to antibacterial properties, extracts and essential oil have been used for many thousands of years in food products conservation [2]. *Satureja hortensis* L. had shown antibacterial properties against different food borne pathogens and spoilage bacteria [6, 8]. So, it turned out that the oil showed activity against *Klebsiella oxytoca, Streptococcus pyogenes, Proteus mirabilis, Citrobacter* species and *Acinetobacter* species [6], *Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, Staphylococcus* species, *Pseudomonas aeruginosa, Enterococcus* species, *Enterobacter* species [6, 9], *Streptococcus salivarius, Salmonella typhimurium, Proteus vulgaris, Shigella dysenteriae* RI 366, *Shigella flexneri* NCTC 8516, *Serratia marcescens, Candida albicans* ATCC 10231, *Candida glabrata, Aspergillus flavus*, *Aspergillus niger* ATCC 16404, *Aspergillus parasiticus* [9], *Pseudomonas* species and coliforms [10], *Bacillus cereus* [11], *Salmonella enterica* [12], and *Listeria monocytogenes* [13].

Considering the current trends in the food industry regarding the use of natural compounds as bacterial protection agents, this study aimed to use the dried *Satureja hortensis* L. plant and its essential oil as a natural preservative for fresh cow's cheese. The total plate count analysis in sanitary veterinary control is used as an indicator of hygiene. In our experiment, we aimed to improve the conservability of the cheese by adding *Satureja hortensis* L. extract and oil, using the total plate count method for detection and quantitation of bacteria in food as an indicator. Also, the paper aims are to study the effect of *Satureja hortensis* L. essential oil added in cheese in decreasing the amount of microorganisms with potentially pathogenic, enterotoxin-producing, in our case *Staphylococcus aureus*.

The paper presents originality by the fact that until now limited studies have approached the topic regarding the antimicrobial effect of the *Satureja hortensis* L. plant and oil on dairy products in general and on fresh cow cheese in particular. Also, the fortification of fresh cheese in microelements by using the *Satureja hortensis* L. has not been sufficiently addressed.

The purpose of this study is (i) to obtain fresh cheese enriched with dry plant of *Satureja hortensis* and essential oil in different concentration; (ii) to analyze the proximate composition of raw material (fresh cheese and dry plant of *Satureja hortensis*) and chemical composition of essential oil; (iii) to study the influence of fortification with *Satureja hortensis* dry plant and essential oil on macro- and microelements composition of fresh cheese; (iv) to perform the sensory analysis of mixed fresh cheeses; (v) to study *in vivo* total antimicrobial potential and in particular the effect of mixed cheeses against *Staphylococcus aureus*.

2. Materials and Methods

2.1. Sampling of Plant Material and Obtaining Satureja Hortensis Extract and Essential Oil. Satureja hortensis L. plants from spontaneous flora grown in the west side of Romania were purchased from specialized distribution units (S.C.Plafar). Species identification has been confirmed by the Department of Herbs at BUASVM Timişoara and a voucher sample has been retained. The fresh herb was dried at ambient temperature and ground with the Grindomix Retsch GM 2000 laboratory mill. The essential oil was extracted from 300 g dry plant with 1000 ml water by hydrodistillation using the Clevenger equipment.

In order to study *in vitro* antimicrobial effect, ethanol extract (E) in concentration of 100 μg/mL was obtained using 10 g dry plants with 100 mL extraction solvent. A second work concentration (10 μg/mL) has been obtained by dilution of the initial concentration. The extracts were shaken using a HEIDOLPH PROMAX 1020 Shaker, then filtered, and stored at 4°C until analysis.

2.2. Samples Preparation. Fresh cheese (FC) has been purchased from a local producer and stored at 4°C, until analysis. Two distinguished experiments have been realized: a set of samples consisted of fresh cheese with added plant (FC + P) and a second set of samples consisted of cheese with added essential oil (FC + EO).

In order to analyze the chemical composition and microbiological activity of FC + P samples, 9 samples each of 100 g cheese were weighed and mixed with *Satureja hortensis* L. dry plant (P) in the following concentrations: 0.5%; 1%; 1.5% (FC + P). From these samples, 3 g of each was taken for macro- and microelement analysis, 10 g for detection of total plate count number (TPC), and the rest for sensory analysis. The analysis was done for each experiment in triplicate.

In the second experiment has been used the same set of samples, but the fortification was done with essential oil of *Satureja hortensis* L. in concentration of 0.1%; 0.25%; and 0.5% (FC + EO).

The cheese used was made from pasteurized milk. Prior to being introduced into the research, it was analyzed from a microbiological point of view and the absence of any coagulase-positive *Staphylococcus* strain was verified. Then, 90 g fresh cheese was mixed with 9 ml dipotassium phosphate (pH 7.5 ± 0.1) and inoculated with 1 ml *Staphylococcus aureus* ATCC strain with turbidity of 0.5 McFarland standards 1 and 0.5, respectively. The cheese and the *Staphylococcus aureus* strain were then stirred in the stomacher until the cheese is evenly distributed (between 1 and 3 minutes). After adding the suspension and maintaining the cheese in the refrigerator, *Staphylococcus* was isolated and identified as coagulase-positive by the rabbit plasma identification method.

9 samples each contained 10 grams of fresh cheese contaminated with the *Staphylococcus aureus* strain and were transferred into sterile Petri dishes, and three different

amounts of *Satureja hortensis* oil, in concentration of 0.1%; 0.25%; and 0.5%, respectively, were added.

The levels of dry plant and essential oil were established according to the minimum inhibitory concentration [14, 15]. The antimicrobial effect was assessed after 3 and 7 days.

2.3. Chemical Composition of Fresh Cheese (FC), Satureja hortensis Plant (P), and Essential Oil (EO) and Mixtures (FC + P, FC + EO). The proximate composition of cheese and plant (moisture protein content, lipid, and ash) was done using the official methods: humidity (SR EN ISO 5534: 2004), protein (STAS 6355-89), lipids (SR ISO 3433: 2009), and ash (SR ISO 936:2009).

The chemical composition of the essential oil was determined using a gas-chromatograph with a mass spectrometer (GS/MS) Agilent Technology 7820A (Agilent Scientific, USA) coupled with MSD 5975 mass spectrometer and equipped with a capillary column with the following characteristics: DB WAX 30 m × 250 μm × 0.25 μm. The NIST database was used to identify volatile compounds.

The macro- and microelements content of cheese, *Satureja hortensis* L. plant, and mixtures (FC + P, FC + EO) was performed by atomic absorption spectrometry (AAS) using the official AOAC methods (AOAC 1997).

2.4. Sensory Evaluation. Sensory analysis of the fortified cheese samples (FC + P, FC + EO) was performed by 5-point hedonic scale method (STAS 12656-88), using a panel consisting of 10 tasters (5 women and 5 men) for this purpose. The sensory characteristics evaluated were appearance, texture, color, taste, and flavor. Sensory evaluation was performed on the day of cheese sampling (on the day of processing) and on the day of preparation of cheeses assortments with *Satureja hortensis* L. dry plant and essential oil. Samples were tested and labeled for easier identification. Tasters gave differentiate scores, between 1 and 5 for each attribute in part (1 = poor, 2 = acceptable, 3 = good, 4 = very good, and 5 = excellent) depending on the importance of that characteristic in the overall organoleptic assessment of the product. Thus the scores that were assigned for each feature were framed between 0 and 4 for appearance, 0 and 3 for consistency, 0 and 3 for color, 0 and 5 for smell, and 0 and 5 for taste. By summing up the maximum approved score for each feature, a maximum of 20 points must be obtained.

2.5. Microbiological Quality of Cheese with Satureja hortensis L. Plant, Essential Oil, and Mixtures (FC + P, FC + EO). The total plate count number (TPC) assay was used to study the effect of *Satureja hortensis* L. (plant and essential oil) on the microbiological quality of fresh cow cheese.

The total plate count number (TPC) was determined before adding the plant and the oil, but also after adding them after 3 and 7 days, respectively. For determination, we used the method based on counting colonies obtained at 30°C (SR EN ISO 4833:2003).

10 g sample with plant or oil has been weighed in the stomacher bag, to which 90 ml of diluent was added (dipotassium phosphate pH 7.5 ± 0.1) and preheated to 45°C. The samples were mixed with the help of lab mixer AES CHEMUNEX, EASY MIX STOMACHER, up to the uniform distribution of the cheese (between 1 and 3 minutes); then it has been inoculated and/or diluted according to SR EN ISO 6887-5:2011 and analyzed according to SR ISO 4833:2003 using Plate Count Agar (PCA) Oxoid CM 0325 medium.

The prepared plates were inverted and incubated in the thermostat set for 72 ± 3 hours at 30 ± 10°C. After the specified incubation period, the colonies were calculated. The number of microorganisms per milliliter or gram of product, N, was calculated with the following formula:

$$N = \frac{\sum C}{(n1 + 0{,}1n2) \cdot d}, \quad (1)$$

where

(i) $\sum C$ is the sum of the colonies counted in all retained plates;

(ii) $n1$ is the number of plates retained at first dilution;

(iii) $n2$ is the number of plates retained at the second dilution;

(iv) d is the dilution factor for the first diluted dilution.

2.6. Loss of 590 nm Absorbing Material. Loss of 590 nm absorbing material released from bacteria was measured using the dilution method (SR EN ISO 6887-5:2011) and horizontal method for the enumeration of coagulase-positive staphylococci using Bayrd-Parker agar base with Egg Yolk Tellurite Emulsion (Oxoid, CM0275) (SR EN ISO 6888-1:2002 SR EN ISO 6888-3:2002).

Staphylococcus aureus ATCC 25923 was obtained from the culture collection of the Laboratory of Microbiology belonging to Interdisciplinary Research Platform of Banat's "King Michael I of Romania" University of Agricultural Science and Veterinary Medicine Timişoara. The *Staphylococcus aureus* ATCC 25923 was revived by overnight growth in Brain Heart Infusion (BHI) broth (Oxoid, CM1135), at 37°C, and passed for 24 hours at 37°C on BHI Agar. The strain was then diluted with saline solution 4,5‰, at a turbidity of 0.5 McFarland standard (DensiCHEK PLUS, Biomerieux). 150 μl of diluted suspension (1 : 30) of *Staphylococcus aureus* was added in a 96-microdilution-well plate. The extracts were tested in concentration of 10% and 1% and the EO in concentration of 0.1%; 0.25%; and 0.5%. The plates were covered and left overnight at 37°C; then the optical density (OD) was measured at 590 nm using an ELISA reader (BIORAD PR 1100). Duplicate tests were performed for all samples. The *Staphylococcus aureus* suspension was used as positive control and a water: ethanol solution (1 : 1) was used as negative control.

The plates were turned and incubated for 24 ± 2 hours and then reincubated 24 ± 2 hours in the incubator set at 37°C. The contaminated cheese was set in the refrigerator at 4°C for two days. After that each sample was analyzed using the same methods described before.

2.7. Statistical Analysis. All determinations were made in triplicate and the arithmetic mean was calculated from the obtained values ± standard deviation (SD).

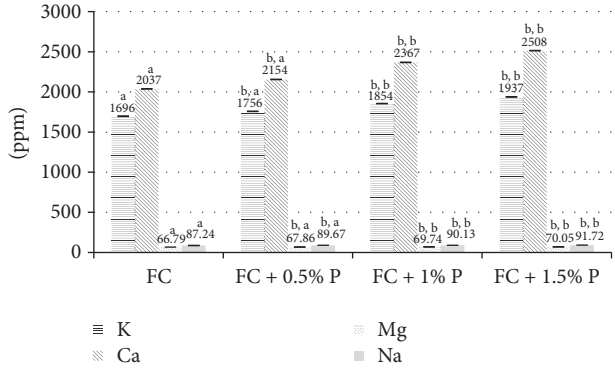

Figure 1: Macroelement composition of cheese and cheese with addition of dry plants. *For the same element distinct letters indicate significant differences according to t-test ($p \leq 0.05$). FC: fresh cheese and P: dry plant of Satureja hortensis L.*

Statistical processing data was performed using the Microsoft Excel 2010. Significant statistical differences of investigated parameters were determined by t-test: two-sample assuming equal variances at $p < 0.05$, after analysis of variance (ANOVA one-way).

3. Results and Discussions

3.1. Chemical Composition of Cheese Sample, Satureja hortensis L. Dry Plant, and Essential Oil. The results obtained regarding proximate composition of fresh cheese were in compliance with the norms provided by the legislation according to SR 3664: 2008 and recorded the values: 8% fat, 22% dry matter, and 15.5% protein content.

Proximate composition of *Satureja hortensis* L. dry plant revealed moisture content of 7%, mineral substances 7.85%, 11.83% proteins, and 0.86% lipids. Previous studies reported higher mineral substances (10%) on *Satureja hortensis* L. plant, while lipid and protein content are not specified [16]. The group of Obichi et al. 2015 [17] revealed that the leaves of different medicinal plants belonging to Lamiaceae contain carbohydrates (71.83%), proteins (10.11%), ash (9.03%), fibers (5.20%), and lipids (2.26%).

The extraction yield of *Satureja hortensis* L. essential oil was 0.7%. From the *Satureja hortensis* essential oil, 20 compounds were separated by GC-MS, three of them being in a majority rate: carvacrol (19.68%), o-cymene (30.86%), and p-cymene (28.07%), except α-pinene (2.608%), 1.3-octadiene (1.563%), β-pinene (2.238%), m-cymene (5.989%), (R)-isocarvestrene (1.142%), γ-terpinene (1.285%), and 3-carene (1.457%) proportion of other components being below 1%.

The percentage of terpenes in *Satureja hortensis* L. oil was 97.194%. Of the total terpenes the highest recorded percentage was in the case of hydrocarbons monoterpenes 78.25%, followed by oxygenated monoterpenes (20.85%). In addition, sesquiterpenes hydrocarbons (0.90%) were isolated.

Many studies reported the chemical composition of *Satureja hortensis* L. essential oil and highlight that the main components are carvacrol, γ-terpinene, p-cymene, and α-terpinene [6, 18–20] and thymol (29%). Previous studies indicated twenty-nine components in the *Satureja hortensis* essential oil including carvacrol (67%), γ-terpinene (15,3%), and p-cymene (6,73%) as the main components [6]. Farzaneh et al. (2015) [20] determined the main components as carvacrol (48%), γ-terpinene (24,2%), and p-cymene (11,7%), while Adams R.P. 2007 reported carvacrol (26,5%), γ-terpinene (22,6%), p-cymene (9,3%), and thymol (29%) as the major constituents of *Satureja hortensis* essential oil [18].

Our results are in agreement with those obtained by Skočibušić and Bezić 2004 [21] that identified carvacrol (45.7%) as the major component followed by the monoterpenic hydrocarbons p-cymene (12.6%), γ-terpinene (8.1%), borneol, thymol, and thymol methyl ether.

In Figure 1 are presented the macroelements composition of *Satureja hortensis* L. plant (P), fresh cheese (FC), and mixture samples (FC + P). The experimental results show that potassium (10404 ± 0.026 ppm) was the most abundant macroelement present in *Satureja hortensis* L., followed by calcium (6621 ± 0.043 ppm), magnesium, and sodium (92.61 ppm) (Figure 1).

The potassium, calcium, and magnesium contents of *Satureja hortensis* L. samples were lower than the ones reported by Seidler-Lozykowska and Golcz, 2012 [22], and the Na content was in accordance with the results reported by the same author. Other authors reported that the levels of Mg and Ca appear in larger quantities in medicinal plants of the Lamiaceae family. Mg in medicinal plants belonging to Lamiaceae family varies between 1616 and 6405 ppm [23] and 1292 and 45460 ppm [24], while Ca is the most abundant macroelement (6763–82250 ppm) [25], (9279–48022 ppm) [23]. Our results highlight that the fortification of fresh cheese with dry *Satureja hortensis* plant leads to enrichment with macroelements. So, K content increased between 3.51 and 14.20%, Ca between 5.74 and 23.12%, and Mg between 1.60 and 4.41% depending on plant amount.

According to the literature, cheese has a high content of macroelements, especially calcium and a low content of microelements [26, 27]. Thus, the addition of *Satureja hortensis* L. in the cheese supplements can ensure the intake of microelements, especially Fe and Zn, which are found in higher quantities in *Satureja hortensis* L., resulting in a functional product.

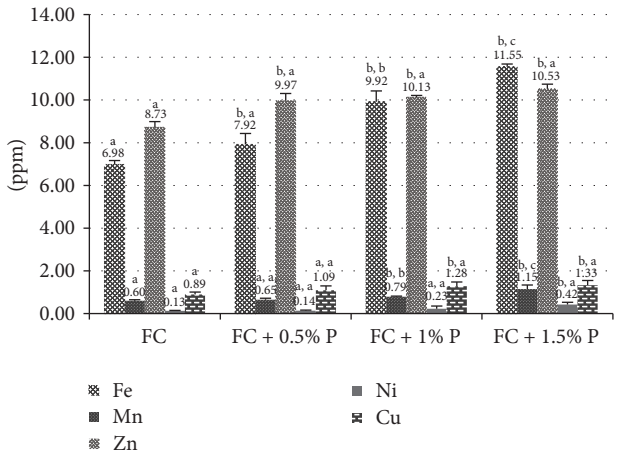

Figure 2: Microelement composition of cheese and cheese with addition of dry plants. *For the same element distinct letters indicate significant differences according to t-test ($p \leq 0.05$). FC: fresh cheese and P: dry plant of Satureja hortensis L.*

The microelement content in *Satureja hortensis* L. plant (P) was 102.8 ppm Fe, 11.69 ppm Mn, 26.24 ppm Zn, 1.69 ppm Ni, and 7.54 ppm Cu, while in fresh cheese lower values were recorded: 6.98 ppm Fe, 0.6 ppm Mn, 8.73 ppm Zn, 0.13 ppm Ni, and 0.89 ppm Cu.

The level of microelements in medicinal plants varies in wide limits depending on the culture and harvest conditions, genetic background, agrotechniques, and climatic conditions [28]. The Zn content corresponds with the data reported by other authors which detected this element in the range of 8.5–95.8 ppm [24] or 17–68 ppm [23]. On the contrary, the registered values for microelement content were lower than those reported in study of Golcz and Seidler-Lozykowska, 2009 [29].

Regarding Fe content, the plants belonging to Lamiaceae family are referred to as plants rich in iron, and the average Fe content in them is much higher than in other plants [30]. Previous studies on herbals belonging to different families indicated that the level of iron varies between 63 and 853 mg/kg [23] or 76.2 and 102.8 ppm [24]. The study of Tomescu et al. 2015 [28] highlights that the level of iron in similar medicinal plants from Romania varies between 166 and 469 ppm.

The content of microelements increases even when small amounts of *Satureja hortensis* in fresh cheese were added. Thus, the addition of *Satureja hortensis* dry plant leads to an increase in Fe content in the range of 13.46–65.54%, depending on the amount of plant added. Also, a significant increase of Mn level (8.33–88.33%) was observed when the percentage of plant varies between 0.1 and 1.5%. An important contribution of Zn (25.65% increase over the control) is provided by 1.5% dry plant supplementation (Figure 2).

3.2. Sensory Analysis. The addition of herbs, spices, and essential oils as flavoring in cheeses represents a common practice. Depending on tradition or preference, the addition may be limited to one or two plants or mixtures of several plants may be associated. An example of this is cheese made in Turkey that includes 25 different plant species. One of the most popular cheeses of Turkey is produced with the addition of 25 different herbs [3].

The sensory characteristics of FC, FC + P, and FC + EO samples are shown in Figure 3. The sensory analysis followed the influence of the *Satureja hortensis* L. dry plant and essential oil addition, on the cheese sensory characteristics. The cheese is presented as homogeneous cream mass, with fine consistency, buttery nature, white color, specific acid-lactic fermentation odor, cream taste, sweetness, and slight sourness. The mixture samples (FC + P, FC + EO) had the following characteristics: homogeneous mass like a smooth paste, having a white color in the case of essential oil addition or the ingredient color added in the case of dry and milled plant use, pleasant smell, and sweet flavor taste characteristic of the added spice, which was not very pronounced.

The values registered for cheese samples with *Satureja hortensis* L. dry plant and essential oil addition showed no significant differences ($p \leq 0.05$) compared to those recorded for fresh cow cheese samples without any addition. Higher score was obtained for cheese with 1% dry plant and the lower one was observed for cheese with 1.5% *Satureja hortensis* L. dry plant.

Regarding the appearance were registered significant differences ($p \leq 0.05$) particularly in the case of fresh cow cheese samples with 1% dry plant of *Satureja hortensis* L., which obtained the highest score comparative with fresh cow cheese with a 1.5% *Satureja hortensis* L. dry plant which recorded the lowest score. Moreover, no significant ($p \leq 0.05$) differences were observed in the color and consistency of all types of cheese samples studied. Thus, the addition of *Satureja hortensis* L. dried plant in a concentration of 1.5% led to a lower score compared to that obtained in the case of the other cheese samples inclusive compared with the control sample.

The addition of *Satureja hortensis* L. dry plant and essential oil significantly improves ($p \leq 0.05$) the taste and smell of fresh cow cheese samples studied.

The highest score was recorded for FC + 1% P and the lowest for FC + 1,5% P. This was justified by tasters that the fresh cheese samples with 1.5% *Satureja hortensis* L. dried plant showed too intense taste and smell.

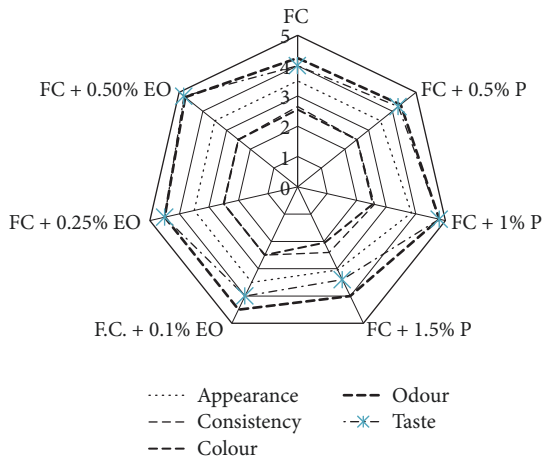

Figure 3: Sensory profile of fresh cow cheese samples without any addition (FC) and with addition of *Satureja hortensis* L. dry plant (FC + P) and essential oil (FC + EO).

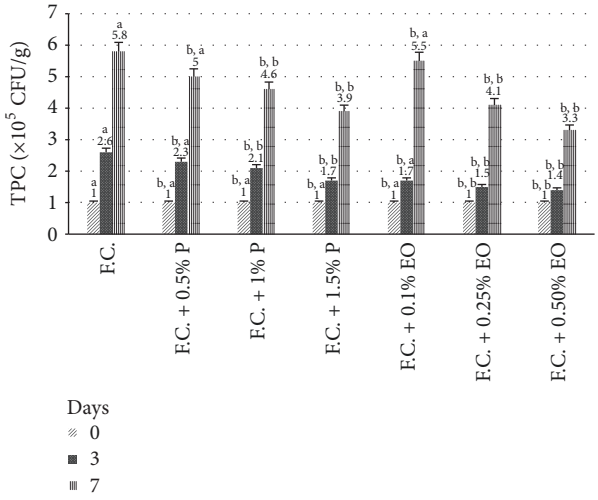

Figure 4: The antimicrobial effect of the *Satureja hortensis* L. plant and the essential oil on fresh cow cheese at 0, 3, and 7 days, respectively. *For the same day distinct letters indicate significant differences according to t-test ($p \leq 0.05$). FC: fresh cheese, P: dry plant of Satureja hortensis L., and EO: essential oil from Satureja hortensis L.*

Except the cheese sample with 1.5% *Satureja hortensis* L. dried plant, in the case of the other cheese samples, the addition of *Satureja hortensis* L. dry plant or essential oil has improved the sensory characteristics.

3.3. Antimicrobial Activity of Cheese. From a microbiological point of view in cow cheese can be found lactic bacteria from the essential microflora of milk, coagulant enzymes, germs, somatic cells, and *Staphylococcus aureus*. The total count number (TPC) includes mesophilic and aerobic organisms that develop under aerobic conditions at a moderate temperature between 20 and 45°C. These include all aerobic bacteria, yeasts, moulds, and fungi developed in the specific agar. This number also includes all pathogens and nonpathogens and is used to determine the hygienic state of foods [31]. Both the dried and milled *Satureja hortensis* L. plant as well as the essential oil had antimicrobial activity on fresh cow cheese samples, but the most increased activity could be detected when the essential oil was added (Figure 4).

In the mixture samples (FC + P), the highest activity was recorded at the concentration of 1.5% ($p \leq 0.05$ compared with control) and the lowest at the concentration of 0.5% ($p \leq 0.05$).

In the case of fresh cow cheese (control) the total number of germs increased from 1.0×10^5 TPC CFU/g ($p \leq 0.05$) registered on the first day to 2.6×10^5 TPC CFU/g ($p \leq 0.05$) in the third day, so that in the seventh day the value was of 5.8×10^5 TPC CFU/g ($p \leq 0.05$).

As a result of dry *Satureja hortensis* L. plant addition after three days, there was a very small decrease, which was proportional to the amount of plant added. Thus, the highest antimicrobial activity was highlighted for the concentration of 1.5% dry plant, that is, 1.7×10^5 TPC CFU/g ($p \leq 0.05$) compared to the control which registered a value of 2.6×10^5 TPC CFU/g ($p \leq 0.05$).

After 7 days the total number of germs increased in all samples, the lower value also being recorded when a

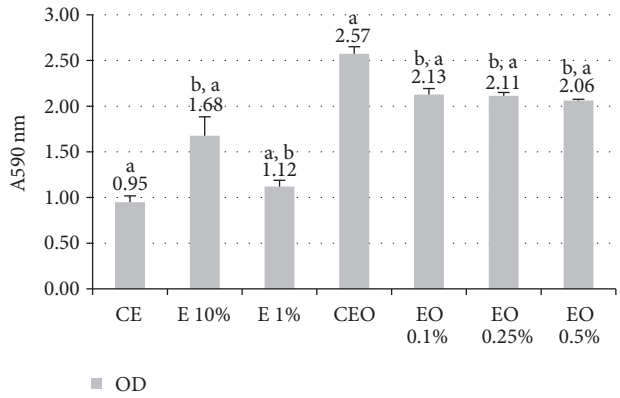

Figure 5: *In vitro* optical density (OD) of *Staphylococcus aureus* in contact with *Satureja hortensis* extracts and essential oil. *Distinct letters indicate significant differences according to t-test* ($p \leq 0.05$).

percentage of 1.5% *Satureja hortensis* L. dry plant (3.9×10^5 TPC CFU/g) ($p \leq 0.05$) was used.

Satureja hortensis L. essential oil has been found with a higher antimicrobial activity. Thus, when 0.50% *Satureja hortensis* L. EO was used, the highest antimicrobial activity was recorded and the lowest in the sample FC + 0.1% EO.

After three days, the total number of germs was 1.4×10^5 TPC CFU/g ($p \leq 0.05$) in the sample FC + 1.50% EO, 1.5×10^7 TPC CFU/g ($p \leq 0{,}05$) in the sample FC + 0.25% EO, and 1.7×10^5 TPC CFU/g ($p \leq 0.05$) in the sample FV + 0.1% EO compared with 2.6×10^5 TPC CFU/g ($p \leq 0.05$) in control.

After 7 days, the antimicrobial activity was evident in the case of the use of *Satureja hortensis* L. essential oil, and the values decreased from 5.5×10^5 TPC CFU/mL ($p \leq 0{,}05$) to 3.3×10^5 TPC CFU/g ($p \leq 0{,}05$) in the sample FC + 0.5% EO, 4.1×10^5 TPC CFU/g ($p \leq 0{,}05$) in the sample FC + 0.25% EO, and 5.5×10^5 TPC CFU/g ($p \leq 0{,}05$) to a concentration of 0.1%.

Most studies in the field focus on the determination of pathogenic microorganisms such as *Listeria monocytogenes* (LM), *Salmonella* spp. (SALM), *Escherichia coli O157:H7* (EC), and *Staphylococcus aureus* [2–4, 32, 33].

The values obtained in the present study were lower than those reported by Metwalli, 2011 [34], who conducted a comprehensive study determining both the total bacterial count and also yeast, mould, lipolytic bacteria counts, coliforms, and proteolytic bacteria. Wahba et al., 2010 [2], in their study regarding antimicrobial effects of pepper, parsley, and dill in cheese, reported higher values of total bacterial count for the control sample (cheese itself, without any addition) and lower for cheese with mixed herbs, compared with values obtained in the present study.

3.4. Loss of 590 nm Absorbing Material. In order to establish the effective concentrations of plant and essential oil that can be introduced into the cheese, the optical density (OD) of extracts (E) in concentration of $10\,\mu g{\cdot}mL^{-1}$ and $100\,\mu g{\cdot}mL^{-1}$ and three oil concentrations (0.1, 0.2, and 0.5%) were tested against the development of *Staphylococcus aureus*. The results presented in Figure 5 show that alcoholic extracts of *Satureja hortensis* do not inhibit the mycelium grown of *Staphylococcus aureus*; on the contrary, they potentiate this activity. The optical density (OD) increases with 17.8% when extract 1% was added in cheese sample and with 77.8% at 10% extract concentration. Our studies are in accordance with previous data that reported limited bacteriostatic activity of plant extracts belonging to Lamiaceae family against *Staphylococcus aureus* [35, 36].

Regarding the oil activity against *Staphylococcus* spp. it can be noted that Lamiaceae family represents a viable source of active principle that inhibits this Gram-positive bacteria [6, 9]. Our results presented in Figure 4 confirm this hypothesis. It can show a 17.12% reduction of OD when 0.1% essential oil was added and this percentage increased with the quantities of essential oil at 17.89% and at 19.84%. From this point of view the essential oil of *Satureja hortensis* can be a viable antibacterial agent in the food industry and therefore has been tested further *in vivo* as a natural preservative in fresh cheese.

Starting from the premise demonstrated before that *Satureja hortensis* extracts do not inhibit the development of *Staphylococcus aureus,* for *in vivo* tests only essential oil in three concentrations was considered. The results presented in Figure 6 show that the essential oil is effective in control of *Staphylococcus aureus.*

It can be seen that the optical density decreases with the addition of essential oil, which demonstrates the *in vivo* antibacterial effect of *Satureja hortensis* against *Staphylococcus aureus*. Compared to control, the decrease of optical density varies between 55.97 and 60.55% when the inoculated concentration of Gram-positive bacteria was lower and between 15.81 and 62.21% at higher concentration of *Staphylococcus aureus.*

Previous studies highlight that the effect of spice plants and flavors on *Staphylococcus aureus in vivo* in different types of cheese is lower than that exerted *in vitro*. High concentration of Cayenne (3%) or Green Pepper (9%) inhibited *Staphylococcus aureus* (1×10^8 CFU g^{-1}) to undetectable levels within 2 days of storage at $4^{\circ}C \pm 2^{\circ}C$ [2]. This fact is due to the synergistic or antagonistic effects caused by the complex composition of the dairy matrix in combination with the active principles of the plants [37]. The protein and lipid

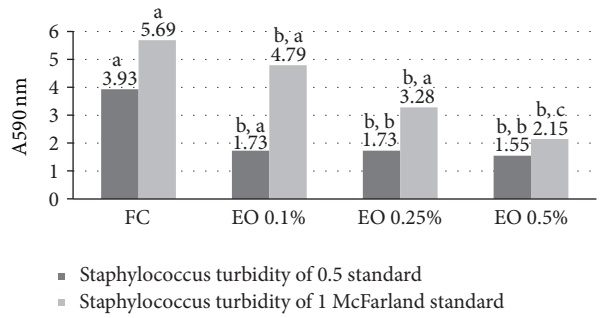

Figure 6: *In vivo* optical density (OD) of *Staphylococcus aureus* in mixed cheese. *Distinct letters indicate significant differences according to t-test ($p \leq 0.05$).*

fraction of cheese can act as a barrier that blocks the access of active principles from essential oils to microorganisms [3]. Another factor that blocks the *in vivo* antibacterial effect of essential oils is that the complex cheese matrix contains all the necessary nutrients for faster microbial growth of the culture cell. Also, the low content of water in cheese compared to the culture media prevents the transport of the natural compound to the microorganisms and allows the recovery of cells damaged [3]. However, de Carvalho et al. (2015) [38] reported lower thyme essential oil concentration ($2.5\,\mu L\,mL^{-1}$) that can be able to reduce the population of *Staphylococcus aureus*, in Cottage cheese. Our results are in agreement with these findings demonstrating that the essential oils of plants belonging to Lamiaceae family have an antibacterial potential against *Staphylococcus aureus* even at low concentrations (0.1–1%).

4. Conclusions

The present study highlights that *Satureja hortensis* plant and essential oil represent a viable solution for increasing the functionality, increasing the shelf-life period, and preventing the development of *Staphylococcus aureus* in fresh cheese.

The addition of dried and milled *Satureja hortensis* L. plant and its essential oil inhibits the development of total microbial activity in fresh cow cheese, with greater effect occurring when essential oil was used.

Besides the antimicrobial effect the addition of aromatic plants in fresh cheese gives a great flavor to the cheese and provides the enrichment with macro- and micronutrients. The level of microelements as Fe, Mn, Cu, and Zn is improved by the use of plants that increase in this way the functionality of cheese.

Regarding the antibacterial potential against *Staphylococcus aureus,* this study highlights that the essential oil of *Satureja hortensis* L., even at low concentrations, may be a natural solution to prevent the development of this Gram-positive bacteria in fresh cheese, while the ethanol extract does not prove to be effective.

The results support the future use of *Satureja hortensis* L. oil added to fresh cheese to extend the shelf-life of fresh cheese and to control the multiplication of enterotoxigenic microorganisms.

Taking into account all these considerations, we recommend the use of essential oil of *Satureja hortensis* as a natural additive in the food industry, in order to increase the functionality and microbial safety of the alimentary products.

Abbreviations

TPC: Total count number of bacteria
FC: Fresh cheese
P: Dry plant of *Satureja hortensis* L.
EO: Essential oil from *Satureja hortensis* L.
E: Extract
CE: Control sample for extracts
CEO: Control sample for essential oil
OD: Optical density.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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References

[1] D. Borda, *Technologies in the Milk Industry - High Pressure Applications*, Academică, Galaţi, Romania, 2006.

[2] N. M. Wahba, A. S. Ahmed, and Z. Z. Ebraheim, "Antimicrobial effects of pepper, parsley, and dill and their roles in the microbiological quality enhancement of traditional Egyptian Kareish cheese," *Foodborne Pathogens and Disease*, vol. 7, no. 4, pp. 411–418, 2010.

[3] F. D. S. Gouvea, A. Rosenthal, and E. H. D. R. Ferreira, "Plant extract and essential oils added as antimicrobials to cheeses: A

review," *Ciência Rural*, vol. 47, no. 8, Article ID e20160908, 2017.

[4] B. Shan, Y.-Z. Cai, J. D. Brooks, and H. Corke, "Spice and herb extracts as natural preservatives in cheese," in *Handbook of cheese in health: Production, nutrition and medical sciences*, V. R. Preedy, R. R. Watson, and V. B. Patel, Eds., Wageningen Academic Publishers, 2013.

[5] J. Hadian, S. N. Ebrahimi, and P. Salehi, "Variability of morphological and phytochemical characteristics among Satureja hortensis L. accessions of Iran," *Industrial Crops and Products*, vol. 32, no. 1, pp. 62–69, 2010.

[6] T. Mihajilov-Krstev, D. Radnović, D. Kitić, B. Zlatković, M. Ristić, and S. Branković, "Chemical composition and antimicrobial activity of Satureja hortensis L. essential oil," *Central European Journal of Biology*, vol. 4, no. 3, pp. 411–416, 2009.

[7] L. S. Muntean, M. Tămaş, S. Muntean et al., *Cultivated and Spontaneous Medicinal Plants*, Risoprint, Cluj-Napoca, Romania, 2nd edition, 2017.

[8] M. Boskovic, N. Zdravkovic, J. Ivanovic et al., "Antimicrobial Activity of Thyme (Tymus vulgaris) and Oregano (Origanum vulgare) Essential Oils against Some Food-borne Microorganisms," *Procedia Food Science*, vol. 5, pp. 18–21, 2015.

[9] M. Mahboubi and N. Kazempour, "Chemical composition and antimicrobial activity of Satureja hortensis and Trachyspermum copticum essential oil," *Iranian Journal of Microbiology*, vol. 3, no. 4, pp. 194–200, 2011.

[10] D. Gammariello, S. Di Giulio, A. Conte, and M. A. Del Nobile, "Effects of natural compounds on microbial safety and sensory quality of Fior di Latte cheese, a typical Italian cheese," *Journal of Dairy Science*, vol. 91, no. 11, pp. 4138–4146, 2008.

[11] N. Dikbasa, K. Karagözb, F. Dadasogluc, and R. Kotanb, "Determination of relationship between Satureja hortensisL. essential oil susceptibility of Bacillus cereus strains and their fatty acid methyl ester profiles," *Romanian Biotechnological Letters*, vol. 17, no. 5, pp. 7564–7569, 2012.

[12] D. Djenane, K. Lefsih, J. Yangüela, and P. Roncalés, "Chemical composition and anti-Salmonella enteritidis CECT 4300 activity of Eucalyptus globulus, Lavandula angustifolia and Satureja hortensis essential oils. Tests in vitro and efficacy in liquid whole eggs stored at 7 ± 1◦C," *Phytotherapy Research*, vol. 9, no. 6, pp. 343–353, 2011.

[13] H. Hosseini and R. Khaksar, "Application of Zataria multiflora, Satureja hortensis, essential oil and their combination against total viable bacteria and Listeria monocytogenes in minced beef packaging," in *Proceedings of the International Congress of Meat Science and Technology*, vol. 54, Cape Town, South Africa, 2008.

[14] D. Bukvički, D. Stojković, M. Soković et al., "Satureja horvatii essential oil: In vitro antimicrobial and antiradical properties and in situ control of Listeria monocytogenes in pork meat," *Meat Science*, vol. 96, no. 3, pp. 1355–1360, 2014.

[15] D. Tsimogiannis, E. Choulitoudi, A. Bimpilas, G. Mitropoulou, Y. Kourkoutas, and V. Oreopoulou, "Exploitation of the biological potential of Satureja thymbra essential oil and distillation by-products," *Journal of Applied Research on Medicinal and Aromatic Plants*, vol. 4, pp. 12–20, 2017.

[16] P. N. Ravindran, G. S. Pillai, and M. Divakaran, "Other herbs and spices: Mango ginger to wasabi," in *Handbook of Herbs and Spices*, K. V. Peter, Ed., vol. 2, pp. 557–582, 2nd edition, 2012.

[17] E. A. Obichi, C. C. Monago, and D. C. Belonwu, "Nutritional qualities and phytochemical compositions of solenostemon monostachyus (family lamiaceae)," *Journal of Environment and Earth Science*, vol. 5, no. 3, pp. 105–111, 2015.

[18] R. P. Adams, *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*, Allured Publishing Corporation, Carol Stream, Ill, USA, 4th edition, 2007.

[19] M. K. Hassanzadeh, Z. T. Najaran, M. Nasery, and S. A. Emami, "Summer Satureja hortensis (Satureja hortensis L.) Oils," in *Essential Oils in Food Preservation, Flavor and Safety*, V. Preedy, Ed., pp. 757–764, Academic Press, 2016.

[20] M. Farzaneh, H. Kiani, R. Sharifi, M. Reisi, and J. Hadian, "Chemical composition and antifungal effects of three species of Satureja (S. hortensis, S. spicigera, and S. khuzistanica) essential oils on the main pathogens of strawberry fruit," *Postharvest Biology and Technology*, vol. 109, pp. 145–151, 2015.

[21] M. Skočibušić and N. Bezić, "Phytochemical analysis and In vitro antimicrobial activity of two Satureja species essential oils," *Phytotherapy Research*, vol. 18, no. 12, pp. 967–970, 2004.

[22] K. Seidler-Lozykowska and A. Golcz, "The effect of organic cultivation on the content of macro- and micro-elements studied in polish cultivars of three medicinal plants," *Medicinal and Aromatic Plant Science and Biotechnology*, vol. 6, no. 1, pp. 111–114, 2012.

[23] I. Queralt, M. Ovejero, M. L. Carvalho, A. F. Marques, and J. M. Llabrés, "Quantitative determination of essential and trace element content of medicinal plants and their infusions by XRF and ICF techniques," *X-Ray Spectrometry*, vol. 34, no. 3, pp. 213–217, 2005.

[24] Z. Ullah, M. K. Baloch, I. B. Baloch, and F. Bibi, "Proximate and nutrient analysis of selected medicinal plants of tank and South Waziristan area of Pakistan," *Middle East Journal of Scientific Research*, vol. 13, no. 10, pp. 1345–1350, 2013.

[25] S. T. Magili, H. M. Maina, J. T. Barminas, O. N. Maitera, and A. I. Onen, "Study of some trace and macroelements in selected anti diabetic medicinal plants used in Adamawa State, Nigeria by neutron activation analysis (NAA)," *Peak Journal of Medicinal Plant Research*, vol. 2, no. 2, pp. 13–22, 2014.

[26] A. Lante, G. Lomolino, M. Cagnin, and P. Spettoli, "Content and characterisation of minerals in milk and in Crescenza and Squacquerone Italian fresh cheeses by ICP-OES," *Food Control*, vol. 17, no. 3, pp. 229–233, 2006.

[27] N. Bilandžić, M. Sedak, M. Dokić, D. Božić, and A. Vrbić, "Content of macro- and microelements and evaluation of the intake of different dairy products consumed in Croatia," *Journal of Food Composition and Analysis*, vol. 40, pp. 143–147, 2015.

[28] A. Tomescu, C. Rus, G. Pop et al., "Researches regarding proximate and selected elements Composition of some medicinal plants belonging to the Lamiaceae family," *Lucrări ştiinţifice. Seria Agronomie*, vol. 58, 2015.

[29] A. Golcz and K. Seidler-Lozykowska, "Microelements content in raw materials of basil (Ocimum basilicum L.), Satureja hortensis (Satureja hortensis L.) and marjoram (Origanum majorana L.) collected in the different stages of plant development," *Nauka Przyroda Technologie*, vol. 3, no. 3, p. 74, 2009.

[30] A. Arceusz, I. Radecka, and M. Wesolowski, "Identification of diversity in elements content in medicinal plants belonging to different plant families," *Food Chemistry*, vol. 120, no. 1, pp. 52–58, 2010.

[31] L. H. Ledenbach and R. T. Marshall, "Compendium of the Microbiological Spoilage of Foods and Beverages," in *Food Microbiology and Food Safety*, W. H. Sperber and M. P. Doyle, Eds., Springer Science + Business Media, LLC, 2009.

[32] A. Lobacz, J. Zulewska, and J. Kowalik, "The analysis of the behaviour of listeria monocytogenes in fresh cheeses with

various spices during storage," *Procedia Food Science*, vol. 7, pp. 80–84, 2016.

[33] M. J. Mendoza-Yepes, L. E. Sanchez-Hidalgo, G. Maertens, and F. Marin-Iniesta, "Inhibition of Listeria monocytogenes and other bacteria by a plant essential oil (DMC) in Spanish soft cheese," *Journal of Food Safety*, vol. 17, no. 1, pp. 47–55, 1997.

[34] S. A. H. Metwalli, "Extended shelf life of kareish cheese by natural preservatives, Egypt," *Journal of Agriculture Research*, vol. 89, no. 2, pp. 639–649, 2011.

[35] E. Alexa, C. Danciu, I. Radulov et al., "Phytochemical screening and biological activity of Mentha x piperita L. and Lavandula angustifolia Mill," *Extracts, Analytical Cellular Pathology*, vol. 2018, 7 pages, 2018.

[36] C. L. Quave, L. R. W. Plano, T. Pantuso, and B. C. Bennett, "Effects of extracts from Italian medicinal plants on planktonic growth, biofilm formation and adherence of methicillin-resistant *Staphylococcus aureus*," *Journal of Ethnopharmacology*, vol. 118, no. 3, pp. 418–428, 2008.

[37] J. R. Calo, P. G. Crandall, C. A. O'Bryan, and S. C. Ricke, "Essential oils as antimicrobials in food systems—a review," *Food Control*, vol. 54, pp. 111–119, 2015.

[38] R. J. de Carvalho, G. T. de Souza, V. G. Honório et al., "Comparative inhibitory effects of Thymus vulgaris L. essential oil against Staphylococcus aureus, Listeria monocytogenes and mesophilic starter co-culture in cheese-mimicking models," *Food Microbiology*, vol. 52, pp. 59–65, 2015.