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Effect of *Origanum vulgare* essential oil used as feed supplement on the properties of organic fermented goat milk products

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Abstract: The wide availability of *Origanum vulgare* essential oil alongside its antimicrobial and antioxidant properties makes it suitable to be used as food supplement in animal nutrition for the improvement not only of the ruminant's feed efficiency but also the quality of the derived products. The present work aimed at evaluating the effect of *Origanum vulgare* essential oil used as feed supplement on the physicochemical, rheological and sensory properties of organic fermented goat milk products (kefir and spreadable-type cheese produced using the starter culture of kefir). Twenty-four milk-producing goats of Alpine breed, distributed into three groups (A, B, C), were used for the dietary treatment. The control diet was fed to the animals of group A, while in groups B and C, organic oregano essential oil of 1 mL and 2 mL, respectively, was added to the feed of each goat per day. The fermented goat milk products were analyzed for their physicochemical (pH, acidity, and dry matter, fat and protein content), rheological (kefir: apparent viscosity; spreadable cheese: firmness, consistency, cohesiveness, index of viscosity) and sensory (color, aroma, acidity, viscosity/consistency, total acceptability) properties. The introduction of *Origanum vulgare* in the goats' diet increased dry matter content, proteins, and rheological properties of the fermented goat milk products. Furthermore, the yield of the spreadable cheese samples increased, and the sensory properties of both fermented milk products were improved. Particularly, aroma, sensory viscosity/consistency and acceptability exhibited the highest values. It is concluded that the use of *Origanum vulgare* essential oil affected the properties of kefir and spreadable-type cheese derived from organic goat milk.

Keywords: organic goat milk; *Origanum vulgare* essential oil; kefir; spreadable-type cheese; physicochemical properties; rheological behavior; sensory evaluation

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1. Introduction

In the last years there has been a significant increase in the use of natural feed supplements derived from plants, such as the essential oils, in animal production to improve growth performance and the quality properties of the derived products [1]. Essential oils have been described to possess antimicrobial, antiparasitic, antioxidant, immune modulating, and anti-inflammatory properties [2] and therefore they can positively manipulate gut microbiota and rumen fermentation, inhibit pathogenic bacterial growth, stimulate blood circulation and prevent tissue oxidation [1]. Among them, *Origanum vulgare* essential oil is known for its rich natural polyphenols content, which possesses intense antimicrobial, antifungal and antioxidant properties [3]. Simultaneously, due to its easy availability, low cost, high yield and wide distribution has the potential to be used widespread in animal nutrition [2]. *Origanum vulgare* essential oil has been used by Simitzis and co-workers [3] for the supplementation of lambs feed and its effect on lamb meat characteristics was investigated. As it concerns goats' feed, the use of distilled *Rosmarinus officinalis* spp. leaves has been reported, and their effect on milk and cheese properties was evaluated [4]. Furthermore, the effect of *Rosmarinus officinalis L.* essential oil or leaves supplementation on goat's milk performances was reported by Smeti and co-workers [5]. However, the use of *Origanum vulgare* essential oil as feed supplement on goats' nutrition and its effect on the produced milk or goat milk products has not been reported in the literature, so far, to the best of our knowledge.

Goat milk is reported to be a good source of nutrients and ideal for those having problems with lactose intolerance, since it has less lactose than human and cow milk, or being sensitive (allergic) to other animals' milk [6-9]. Responsible for the allergens are assumed to be the protein fractions of cow milk [10]. Compared to cow or human milk, goat milk is reported to possess unique biologically active properties, such as high digestibility, distinct alkalinity, high buffering capacity, and certain therapeutic values in medicine and human nutrition [7,10,11]. Goat milk is easily digestible in the body due to its smaller fat globules size compared to other species' mils [7] and to its proteins that are more readily digestible as their amino acids are absorbed more efficiently than those of cow milk [7,11]. Goat milk fat contains significantly greater contents of short- and medium- chain length fatty acids (C4:0 – C12:0) than the cow milk. These acids have been shown to possess several bioactive functionalities in digestion and metabolism of lipids as well as treatment of lipid malabsorption syndromes in a variety of patients [7]. Medium chain triglycerides are able to provide energy without being deposited in the fatty tissue of the body as well as it plays a role in decreasing cholesterol levels in body [10]. Goat milk is richer than cow milk in monounsaturated fatty acids (MUFA), polyunsaturated fatty acids that are beneficial for the cardiovascular conditions [10]. Goat milk exhibits improved cholesterol mobilization and controlling its storage in the blood [9]. The balanced fatty acid profile of goat milk [6,12], helps it to prevent atherosclerosis, heart attacks, strokes and other heart complications [10]. Goat milk is rich in bioactive components that are useful in the maintenance of the proper metabolism and functioning of the human body [9,11]. Bioactive peptides present in goat milk and its products have tremendous therapeutic potential by regulating the physiological and metabolic functions of the body [9]. The lactose-derived oligosaccharides found in goat milk are thought to be beneficial to human nutrition because of their prebiotic and anti-infective properties [10]. Goat milk is also richer in vitamins B6 and A [8] and in calcium, phosphorus, potassium, chlorine [10,12], selenium, zinc and copper [10] when compared to bovine milk.

The nutritional value of goat milk can be further increased using organic goat production [13] that has been gaining popularity over the last decades, since it can improve animal welfare, protect the environment, and sustain rewarding rural lifestyles [14]. Organic milk is reported to have higher values of monounsaturated and polyunsaturated fatty acids [13], CLA [13,15], calcium content [16] and reduced saturated fatty acid content [13] when compared to conventional milk. Furthermore, organic milk is reported to possess a better microbiological profile compared to milk from conventional farms as it exhibits lower counts of the total viable count, total coliform count and somatic cell count [17]. The high nutritional value of organic milk, alongside its increased dry matter and protein content makes it a high-quality raw material to obtain products of exceptional nutritional and functional properties [16].

Fermented milk products, like kefir and spreadable-type cheese, possess various nutritional and health promoting properties. Their nutritional value is similar to that in milk, but the presence of the microorganisms and their metabolites produced during fermentation increase their functionality [18-21].

Kefir is a fermented dairy product with unique sensory properties, which is considered to be a natural probiotic that besides its nutritional value, it has been related to a variety of health benefits, such as antitumoral, anti-inflammatory antimicrobial, immunoregulatory, antiallergenic, wound healing, antidiabetic, cholesterol-lowering, anti-stress, antimutagenic and antigenotoxic properties [22-24]. The functionality of kefir is not only linked to its microflora, but also to the presence of their metabolites including kefiran the main polysaccharide found in kefir grains the traditional starter culture of kefir [22,25,26]. Kefir grains are white to yellowish, cauliflower-like, gelatinous, and irregular in shape structures, composing of a matrix of proteins-polysaccharides at which the kefir microflora (lactic acid bacteria, acetic acid bacteria and yeasts) are trapped [27,28].

Kefir microorganisms have gained researchers' interest regarding their use as starter cultures in cheese manufacturing due to its potential effect on quality and functional properties of the final product. Kefir or freeze-dried kefir culture have been used in many cheese types, such as whey-cheese similar to traditional Greek Myzithra cheese, Feta-type cheese, white pickled cheese (traditional cheese in Turkey), hard-type cheese and Camembert-type cheese resulting in the production of improved cheese products concerning preservation time, sensory and textural characteristics [28].

Spreadable-type cheeses are usually fresh and unripened cheeses, which are manufactured by the coagulation of milk and / or cream using a combination of acid and rennet gelation. Intensive heat treatment of milk (>70°C) causes denaturation of the whey proteins, that interact caseins by hydrophobic and disulphide intermolecular interactions [29], thus they remain to the final product increasing not only its yield but also its biological value. The use of kefir microorganisms as starter culture to produce spreadable-type cheese is anticipated to increase the functionality of the final product.

Thus, the aim of the present work was to evaluate the effect of *Origanum vulgare* essential oil used as feed supplement on the properties of organic functional fermented goat milk products and particularly kefir and spreadable-type cheese produced using the starter culture of kefir. The use of *Origanum vulgare* essential oil used on goats feed is expected to improve the quality characteristics of the produced milk and thus physicochemical, rheological and sensory properties of the fermented products resulting in increasing their acceptance by the consumers.

2. Materials and Methods

2.1. Animals and dietary treatment

Twenty-four milk-producing goats of Alpine breed of the same age and lactating period, with similar milk yield and body weight (49 ± 1.8 kg) were selected and distributed into three groups (each consisting of 8 goats) in the Regional Unit of Evros in Greece. First group (A) was fed with the control diet (standard binary ration of roughage and concentrated organic feed), while in groups B and C, organic oregano essential oil of 1 mL and 2 mL, respectively, was added to the feed of each goat per day. The composition of the ration and chemical analysis of the organic oregano essential oil have been reported by

Kyrtsoudis et al. [30]. The milk to produce the fermented products was collected on the 60th day of the dietary treatment.

2.2. Pre-treatment of the raw milk

Immediately upon receipt, the milk was placed in a refrigerator (4°C) until usage (maximum 4 h). The milk of each group (A, B, and C) was rigorously stirred to avoid phase separation and filtered using a simple filter medium (cheese cloth). An adequate amount of each sample (groups A, B, and C) was taken to determine its physicochemical composition (pH, and moisture, fat and protein content). The milk samples were then homogenized using an ultrasonic device (Bandelin Sonopuls with UV 3400 tip, Berlin, Germany), which operated at 90% of its capacity at a frequency of 20 kHz. The time of ultrasound application to a certain amount of milk was calculated, by observing the size of fat globules in an optical microscope (Axio Observer Z1, Carl Zeiss Microscopy GmbH, Jena, Germany), which should exhibit a variation from 1 to 6 μm. During the homogenization, the temperature of the milk was controlled so that it would not exceed 60°C. The milk of each group (A, B, and C) was then divided into 2 batches for the preparation of the kefir and spreadable-type cheese samples. The fermented milk products were produced in duplicate.

2.3. Kefir production

Following homogenization, the milk was heat-treated at 95°C for 5 min, cooled down at 30°C, inoculated with the starter culture (KFA1, Micromilk, srl, Cremosano, CR, Italy) and incubated at 30°C until the pH dropped to 4.4 (approximately 20 h). Kefir was then mixed and stored at 4°C for 24 h before further analysis. The starter culture used for kefir production consisted of the following microorganisms: *Streptococcus thermophilus, Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris, Leuconostoc mesenteroides* subsp. *cremoris, Lactococcus lactis* subsp. *lactis biovar diacetylactis* and *Debaryomyces hansenii*.

2.4. Spreadable–type cheese production

The milk was heat-treated at 95°C for 5 min, cooled at 25°C and inoculated with the starter culture of kefir (KFA1, Micromilk, srl, Cremosano, CR, Italy). Rennet (Kyanous Stavros, Aristomenis Fikas & Co., Thessaloniki) was also added at a percentage of 0.1% (w/w) and the milk was let to coagulate at 25°C for about 18 h. The curd was then cut, left for 1 h at 18°C and transferred to cheese cloths for drainage for about 24 h at 4°C. The next day the samples were removed from the cheese cloths, salted at a concentration of 1% (w/w), transferred to sterilized glass containers and stored at 4°C for 24 h until analyzed.

2.5. Physicochemical analysis

The pH was determined by the use of a laboratory pH meter (EDT Instruments, GP353 ATC pH meter). Acidity of the samples was assessed by titration with NaOH using phenolophalein as indicator and the results were expressed as lactic acid concentration (%) [31]. Dry matter content was determined by drying at 102±1°C to a constant weight [31]. The Gerber and van Gulik methods were used for the determination of the fat content of milk and cheese samples, respectively. Total nitrogen content was assessed by the Kjeldahl method [31]. The nitrogen content multiplied by the factor 6.38 gave the protein content of the sample.

2.6. Rheological measurements

2.6.1. Apparent viscosity of kefir samples

The apparent viscosity of kefir samples was evaluated by the use of the Discovery HR-20 rheometer (TA Instruments, New Castle, DE, USA) equipped with a cone and plate geometry, where the cone had a gradient of 2°. The apparent viscosity was recorded as a function of the shear rate ranging from 1 to 100 s^{-1} and results are shown as the samples flow curves. Measurements were performed at 4°C in triplicate.

2.6.2. Rheological evaluation of spreadable-type cheese samples

The rheological properties of the spreadable-type cheese samples were evaluated at 4°C by using the Texture Analyser TA.XT.plus (Stable Microsystems, Surrey, UK). The test of back extrusion was applied and from the derived force-time curves, the following parameters were calculated: "firmness", "consistency", "cohesiveness" and "index of viscosity". Measurements were performed in triplicate.

2.7. Sensory analysis

A team of experienced panelists was used for the sensory evaluation of the samples. The characteristics examined were intensity of color, aroma, acidity and viscosity (at kefir samples)/consistency (at cheese samples), and total acceptability. An unstructured scale of 15 cm was used for the assessment of the sample's properties. The left side (0 cm) of the scale represented the zero intensity of the examined sensory characteristics, while the end (15 cm) represented their maximum intensity. The kefir samples were served in plastic glasses, while the cheese samples were served in plastic plates. A sufficient amount of sample (about 50 ml for kefir and 20 g for cheese samples) was placed in the container, (for cheese samples, each plate contained a small amount of the samples around the perimeter), while each sample bore a random three-digit number. Test subjects were given bottled water to rinse their mouth between trials.

2.8. Statistical analysis

One-way ANOVA was applied to the experimental data (group of milk used for the production of the fermented products: A, B and C), while Tukey test was used to distinguish the differences among samples (if there were any). Statistical analysis was performed using Minitab 18 software.

3. Results and Discussion

3.1. Physicochemical properties of the raw milk samples

Table 1 shows the results from the physicochemical analysis of the raw milk samples. According to statistical analysis, the pH, dry matter content and proteins of the milk samples changed significantly among the samples ($p<0.05$). Particularly, milk from group A exhibited the lowest pH, dry matter content and protein content, while the milk samples of groups B and C with *Origanum vulgare* essential oil supplementation showed the highest ones. No significant differences were found for pH values, dry matter and protein content between the samples of groups B and C, meaning that the effect of *Origanum vulgare* essential oil on goat milk quality was not affected by its concentration. The increased dry matter content of the milk samples from groups B and C can be ascribed to their increased protein content, since the fat content of the milk samples did not exhibit statistically significant variations($p>0.05$). The differences in pH values among samples can also be attributed to the variation of protein content among the samples, since milk proteins and especially caseins affect milk acidity and thus pH values [32]. Chiofalo and co-workers [33] reported an increase in both protein and fat content of sheep milk as rosemary extract supplementation increased. In contrast, Boutoial and co-workers [4] reported no differences in protein and fat content of goat milk by the introduction of rosemary in the animals' diet. Smeti and co-workers [5] also reported that rosemary incorporation into goats feed did not affect protein and fat content of the derived milk. The increase in protein content of the sheep milk with rosemary extract feed supplementation was attributed by the Authors [33] to the effect of phenolic compounds, present in rosemary extract. These compounds probably manipulate rumen fermentation through a reduction of protein degradation and an inhibition of amino acid degradation. This is also a possible explanation for the increase in protein content of goat milk by the use of *Origanum vulgare* essential oil used as feed supplement.

Table 1. Physicochemical properties of the raw milk samples of groups A, B and C (standard deviations are shown in parenthesis).

Milk group	pH	Dry matter (%)	Fat (%)	Proteins (%)
A	6.57±0.01^{a*}	11.62±0.07^{a}	3.50±0.01^{a}	3.04±0.02^{a}
B	6.61±0.01^{b}	11.87±0.02^{b}	3.45±0.01^{a}	3.21±0.03^{b}
C	6.60±0.01^{b}	11.97±0.01^{b}	3.40±0.14^{a}	3.35±0.05^{b}

* Means within the column with different letters differ significantly.

3.2. Physicochemical properties of fermented goat milk products

The physicochemical properties of kefir samples are shown on Table 2. According to ANOVA, there is a significant effect ($p<0.05$) on acidity, dry matter content and proteins among the samples. The kefir sample without essential oil supplementation exhibited the lowest values of the above-mentioned properties, while kefir samples with *Origanum vulgare* essential oil used as feed supplement the highest. Since all kefir samples fermented to the same final pH value (4.4), the increased acidity of the samples with essential oil supplementation is due to their increased protein content. Milk proteins possess increased buffering capacity [32], thus a higher lactic acid concentration is required to reduce pH. As it concerns chemical composition of kefir samples, they exhibited similar variation with the raw milk samples, as expected.

Table 2. Physicochemical properties of kefir samples produced with milk from groups A, B and C (standard deviations are shown in parenthesis).

Milk group	pH	Acidity (%)	Dry matter (%)	Fat (%)	Proteins (%)
A	4.38±0.01^{a*}	1.175±0.01^{a}	12.48±0.05^{a}	3.83±0.04^{a}	3.37±0.02^{a}
B	4.38±0.01^{a}	1.345±0.02^{b}	12.99±0.03^{b}	3.78±0.01^{a}	3.60±0.03^{b}
C	4.39±0.01^{a}	1.340±0.02^{b}	13.11±0.02^{b}	3.82±0.02^{a}	3.68±0.01^{b}

* Means within the column with different letters differ significantly.

The cheese yield of the spreadable-type cheese samples produced with the milk from groups A, B and C was 35.7±0.5%, 37.5±0.6% and 39.3±0.5%, respectively, showing a significant effect ($p<0.05$) among the samples. The cheese sample without essential oil supplementation showed the lowest yield value, while an increase in cheese yield was observed with increasing *Origanum vulgare* essential oil concentration. The same variation was observed for dry matter content and protein concentration (Table 3). The increase in cheese yield and dry matter content with increasing essential oil supplementation is due to the increasing protein content of the cheese samples, which in turn is affected by the phenolic compounds present in essential oils, as described above. It is worth mentioning that even though the increase in dry matter and protein contents of the raw milk sample of group C when compared to milk sample of group B were not significantly different, in the case of cheese samples the dry matter and protein content exhibited statistically significant variations among the samples with essential oil supplementation. This can be attributed to the cutting and draining process that the cheese samples underwent during production, allowing removal of whey, and thus increasing further the differences in dry matter and proteins among samples. As far as pH is concerned, it exhibited significantly lower values when essential oil was used. This might be due to the increased metabolic activity of the microorganisms of kefir. However, this change must be further studied to elucidate mechanisms that undergo this behavior.

Table 3. Physicochemical properties of spreadable-type cheese samples produced with milk from groups A, B and C (standard deviations are shown in parenthesis).

Milk group	pH	Acidity (%)	Dry matter (%)	Fat (%)	Proteins (%)
A	4.34±0.01^{a*}	1.100±0.03^{a}	28.35±0.21^{a}	18.10±0.14^{a}	15.21±0.01^{a}
B	4.29±0.01^{b}	1.215±0.01^{b}	29.30±0.14^{b}	17.65±0.21^{a}	15.49±0.02^{b}
C	4.27±0.01^{b}	1.223±0.02^{b}	30.20±0.14^{c}	17.40±0.14^{a}	15.61±0.02^{c}

* Means within the column with different letters differ significantly.

3.3. Rheological properties fermented goat milk products

Figure 1 shows the flow curves of kefir samples. As can be seen, the increase in essential oil concentration resulted in increasing the apparent viscosity of the samples. However, in the case of the sample with the highest essential oil concentration there is a significant increase in its pseudoplastic behavior. A pheudoplastic material at the application of low values of stress (low shear rates) exhibits increased values of apparent viscosity, however, with increasing applied stress (increased shear rates) the apparent viscosity starts to reduce. The more the decrease in the apparent viscosity with increasing shear rate, the more the increase in the pseudoplastic behavior [34]. A product that shows increased pseudoplasticity, at low shear rates, such when it is inside the mouth, it exhibits increased apparent viscosity giving the impression of a favorable mouth feel, while when subjected in increased shear rates (while swallowing) the material will exhibit viscosity decrease and will be easily swallowed. The increase in the apparent viscosity of kefir samples with increasing protein content is due to the increased number of molecules resulting in increased resistance to the application of flow and thus apparent viscosity. The increase in pseudoplastic behavior at high protein concentrations is attributed to the increased interactions of the molecules owing to the reduction in the distance between them that is caused by their molecules increment. However, due to the increased flexibility of the milk protein molecules [32], at the application of high values of applied force the molecules orientate themselves in the direction of flow resulting in decreased viscosity values.

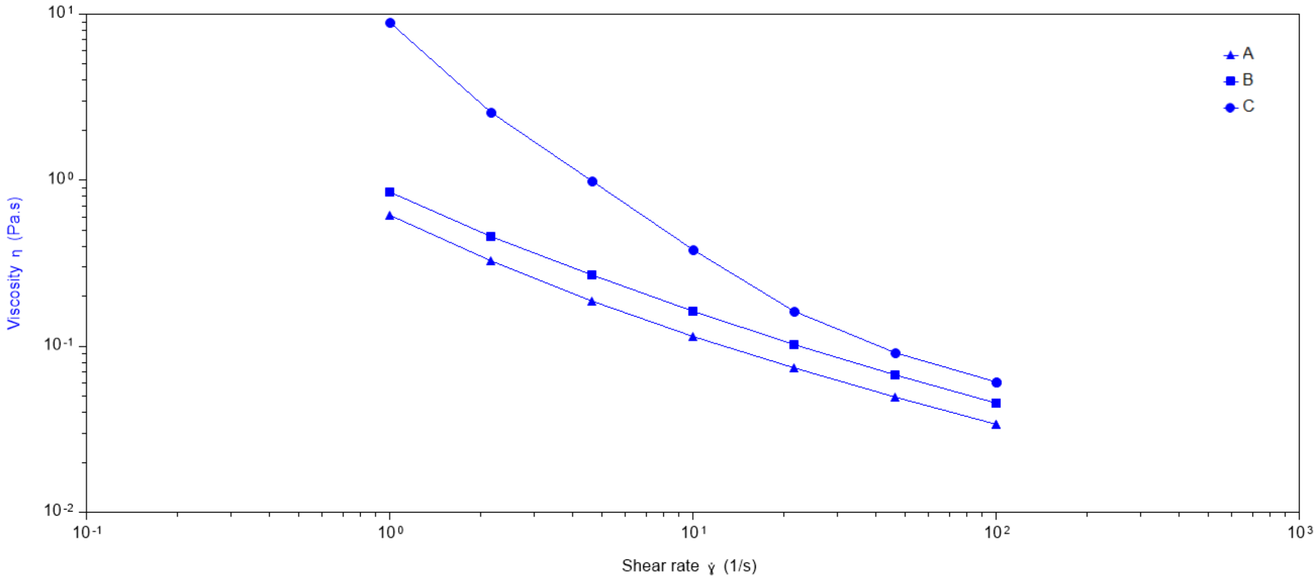

Figure 1. The flow curves of kefir samples produced with milk from groups A, B and C.

Table 4 shows the rheological properties of the spreadable-type cheese samples. According to ANOVA, rheological properties changed significantly among the samples ($p<0.05$). As can be seen the increment in essential oil supplementation caused an increase in the rheological properties of cheese samples. This is also related with the protein content of the samples, causing an increase in the intensity and number of interactions of the system resulting in increased resistance to the application of stress and thus increased firmness, consistency, cohesiveness and index of viscosity.

Table 4. Rheological properties of spreadable-type cheese samples produced with milk from groups A, B and C (standard deviations are shown in parenthesis).

Milk group	Firmness (N)	Consistency (N × s)	Cohesiveness (N)	Index of Viscosity (N × s)
A	24.85±0.50[a*]	324.5±1.8[a]	57.0±1.2[a]	19.5±0.4[a]
B	27.25±0.34[b]	396.9±1.6[b]	63.2±1.5[ab]	24.9±0.5[b]
C	31.75±0.35[c]	478.2±4.2[c]	67.2±1.4[bc]	27.9±0.4[c]

* Means within the column with different letters differ significantly.

3.4. Sensory evaluation fermented goat milk products

The sensory properties of kefir and spreadable-type cheese samples are shown in Tables 5 and 6, respectively. According to statistical analysis, the color and acidity of both fermented goat milk products did not exhibit significant differences among samples ($p>0.05$). Panelists could not distinguish the differences in cheese samples acidity. However, they did differentiate their rheological characteristics, alongside their aroma and total acceptability. Particularly, the samples (kefir and spreadable-type cheese) of milk groups B and C exhibited the highest values of aroma, viscosity/consistency and acceptability. Results from rheological evaluation of fermented milk samples are in agreement with those of sensory analysis. The increased acceptability of the fermented samples with essential oil supplementation might be attributed to their aroma and textural characteristics. Further experiments must be performed to elucidate the compounds responsible for the increased aroma intensity of these samples.

Table 5. Sensory properties of kefir samples produced with milk from groups A, B and C (standard deviations are shown in parenthesis).

Milk group	Color	Aroma	Acidity	Viscocity	Total acceptability
A	13.3±0.6[a*]	10.7±0.3[a]	7.5±0.7[a]	8.8±0.4[a]	9.9±0.5[a]
B	13.2±0.9[a]	13.2±0.4[b]	7.8±0.4[a]	10.9±0.5[b]	13.1±0.1[b]
C	13.4±0.5[a]	12.6±0.6[b]	7.4±1.2[a]	12.5±0.3[c]	13.8±0.4[b]

Table 6. Sensory properties of spreadable-type cheese samples produced with milk from groups A, B and C (standard deviations are shown in parenthesis).

Milk group	Color	Aroma	Acidity	Consistency	Total acceptability
A	11.1±0.4[a*]	11.4±0.5[a]	6.9±0.3[a]	11.9±0.3[a]	10.4±0.4[a]
B	11.4±0.7[a]	13.9±0.2[b]	7.1±0.7[a]	13.5±0.4[b]	13.7±0.7[b]
C	11.2±0.6[a]	13.8±0.4[b]	6.8±0.9[a]	14.9±0.2[c]	14.1±0.9[b]

* Means within the column with different letters differ significantly.

4. Conclusions

The inclusion of *Origanum vulgare* essential oil in goats' nutrition can positively affect the quality characteristics of fermented milk products such as kefir and spreadable-type cheese prepared with the kefir microorganisms as starter culture. The use of *Origanum vulgare* essential oil as feed supplement resulted in increasing the protein and thus dry matter content of the derived milk and the corresponding fermented milk products. As a result, an increase in the rheological properties of the fermented products and cheese yield was assessed. The incorporation of *Origanum vulgare* essential oil in goats' nutrition improved sensory properties of the fermented products by increasing their aroma and total acceptability.

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