Jimborean MA *et al.* (2021)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 49, Issue 1, Article number 12001
DOI:10.15835/nbha49112001
Research Article

Aloe vera gel microcapsules and essential oils of thyme and oregano incorporated in spreadable goat cheese: impact on its microbiological, physicochemical, and sensory characteristics during storage

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Abstract

The aim of this study was to develop a new assortment of high-quality cream cheese, with a high amount of biologically active compounds, namely a spreadable cheese from goat milk with addition of *Aloe vera* microcapsules and essential oils of thyme and oregano. The research was focused on the testing and optimization of an appropriate manufacturing recipe for this new product. Two assortments were made: one with *Aloe vera* microcapsules and thyme essential oil and the other one with *Aloe vera* microcapsules and oregano essential oil. For each assortment two different concentrations of *Aloe vera* and essential oils were tested. The obtained products were analysed for organoleptic, physico-chemical (fat, protein, total dry matter, sodium chloride and acidity) and microbiological parameters. In terms of consumer preferences, the cream cheese with 3% microcapsules of *Aloe vera* gel and 0.018% thyme essential oil was the most appreciated in terms of commercial aspect, consistency, and taste. We concluded that the addition of *Aloe vera* microcapsules improves the sensory characteristics and leads to an increase nutritional value of the finished product. Also, the addition of essential oils (thyme and oregano) ensured a stable quality during storage.

Keywords: *Aloe vera*; essential oils; goat milk; spreadable cheese

Introduction

Essential oils (EO) are extracted from aromatic plants containing a variety of natural, biologically active components with antimicrobial and antioxidant properties (Amatiste *et al.*, 2014; Hamedi *et al.*, 2014; Yousefi *et al.,* 2017, Khorshidian *et al.*, 2018). Antimicrobial properties of essential oils against various microorganisms (*L. monocytogenes, E. coli* O157:H7, *Aspergillus ochraceus* ZMPBF 318, *Penicillium spp.*) have been reported in various studies (Kotzekidou *et al.*, 2008; Yahyazadeh *et al.*, 2008; Čvek *et al.*, 2010; Jeong *et al.*, 2014).

Received: 18 Jul 2020. Received in revised form: 09 Feb 2021. Accepted: 17 Feb 2021. Published online: 24 Feb 2021.
From **Volume 49, Issue 1, 2021,** Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal will use article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

The oregano and thyme essential oils have significant antibacterial effects, due to the presence of their phenolic compounds, like carvacrol and thymol (Sakkas and Papadopoulou, 2017). In a study released by Burt and Reinders (2003), including four essential oils (oregano, thyme, bay and clove bud) the oregano and thyme oils showed the greatest antibacterial effect against *Escherichia coli* O157:H7. Various studies related to the antioxidant property of oregano essential oil, showed that it has strong antioxidant activity due to its high thymol and carvacrol content (McKay and Blumberg, 2006).

Thyme essential oil is one of the ten more important essential oils due to its proven antimicrobial, antimycotic, and antioxidant effects. Many studies have shown that thymol and carvacrol, its main constituents, are responsible for these properties. According to regulation (EC) no. 1334/2008 thyme essential oil is on the list of essential oils generally recognized as safe for ingestion.

Aloe vera is known as a perennial herb belonging to the Liliaceous family. It contains a large variety of nutrients and bioactive compounds, such as flavonoids, terpenoids, (Boudreau and Beland, 2006; Harlev *et al.*, 2012) fatty acids, mono-and polysaccharides (pectins and hemicelluloses), tannins and sterols. It also contains a variety of enzymes, minerals (calcium, chromium, copper, iron, magnesium, manganese, potassium, phosphorus, sodium, and zinc) and vitamins (A, C, E, β-carotene, B1, B2, B3, B6, choline, B12 and folic acid) (Sahu *et al.*, 2013; Rodrigues *et al.,* 2018; Marzanna *et al.*, 2019). *Aloe vera* is used in the food industry as a functional ingredient (Kazhal and Samira, 2015).

Microencapsulation may be defined as a packaging technology used for separating and storing materials in microscopic capsules, for later release under controlled conditions. The material that provides protection and controlled release of the bioactive compound is called coating material. In general, these matrices are natural or synthetic polymers (Cock and Castillo, 2013).

Goat milk has high nutritional properties and lower allergenic effect as compared with cow's milk, especially in non-sensitized children, which led to an increased interest in goat's milk as a functional food (Albenzio and Santillo, 2011; Hassan, 2014). Spreadable cream cheeses are fresh cheeses produced by coagulating milk, cream or whey with acid, a mixture of acid and rennet, or a combination of acid and heat (Brčina *et al.*, 2017). Fresh cheeses are characterized as slightly acid, soft, homogeneous products that are white in colour and smooth-textured (Frau *et al.*, 2014).

This study aimed to evaluate the effect of essential oils (i.e. thyme and oregano) and microcapsules with *Aloe vera* incorporation in a spreadable goat cheese product. This would enhance its nutritional value and functional properties by increasing its contents in bioactive compounds and will improve its quality and organoleptic properties.

Materials and Methods

Materials

Three successive cream cheese trials were carried out. Goat's milk was obtained from a local farm. The composition of the goat milk used was determined with an Ekomilk M apparatus (model Milkana KAM98-2A Bulteh 2000 Ltd, Stara Zagora, Bulgaria) and the mean values (±) of three replicates for all batches were as follows: 3.85 ± 0.07% fat, 3.78 ± 0.02% total protein, and 8.75 ± 0.05% non-fat dry matter. Titratable acidity was recorded as Thörner degrees (T°) according to the Thörner method (16.5±1 T°), and pH was measured with a pH meter (HI 99161, Hanna Instruments, Limena, Italia) (6.71±0.02). Dry plant material of two commercial plants (thyme and oregano) was used in this study to obtain essential oils. Starter cultures, including the mesophilic strains *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* (Lyofast MO 142), were used. Powder animal rennet was obtained from Chr-Hansen's Laboratories (Copenhagen, Denmark). Using species-specific primers, a PCR assay was performed to certify the presence of goat milk and to detect possible undeclared cow milk addition into the bulk milk used for cheese production experiments.

Preparation of microcapsules with Aloe vera gel

The matrix encapsulation or microencapsulation was accomplished using a laminar flow through a nozzle and an additional vibration of the nozzles or liquid (Cock and Castillo, 2013). To obtain the gel from *Aloe vera* leaves, they were quickly processed in order to avoid oxidation.

The *Aloe vera* gel was obtained by removing the shell, followed by a thermal treatment (72 °C for 12 sec.). Finally, the gel was chopped and homogenized. For the chemical determinations, extraction of the compounds with methanol was performed.

Microencapsulation was proposed as a successful strategy to improve the *Aloe vera* stabilization, to make easier their handling during cream cheese processing and to ensure its bioavailability when they are used as dairy food bioactive compounds. Thus, another objective of this study was to encapsulate bioactive compounds contained in *Aloe vera* gel in order to obtain microcapsules usable as natural food additives.

Extraction of thyme and oregano essential oils

For the flavouring process of the cream cheese, thyme and oregano essential oils were isolated by hydro distillation, using a Clevenger type apparatus. Fifty grams of dried and minced leaves were weighed and added into a distillation flask together with 750 ml of distilled water. The distillation process was performed during three hours. The yield was calculated as millilitres of essential oils per 100 g dry plant material (thyme or oregano). The obtained essential oils were dried using anhydrous sodium sulphate and stored in sealed vials at 6-8 °C until analysis.

PCR analysis of raw goat's milk

To identify possible undeclared addition of cow milk into goat bulk milk used for cheese production experiments, PCR assays were performed on samples collected from bulk milk under sterile conditions and transported at 4 °C. For the recovery of the somatic cells required for DNA extraction, the milk samples were centrifuged at 3000g for 10 minutes. DNA extraction from the cell pellet was performed with the DNesay Blood & Tissue kit (Qiagen), according the manufacturer instructions. Milk somatic cells recovered from the samples were digested in 90 µl of ATL solution and 10 µl of proteinase K, by incubation at 56 °C. Subsequently, 200 µl of AL solution and 200 µl of absolute alcohol were added to each tube. The lysate was transferred to the purification columns and centrifuged at 8000 rpm for 1 min. The columns were subsequently washed with AW1 and AW2 solutions respectively. After centrifugation the DNA from the column filter was eluted in 50 µl of AE solution. DNA concentration and purity were spectrophotometrically evaluated.

To certify the presence of goat milk and an eventual undeclared cow milk addition into the bulk milk used as raw material we amplified two different size fragments from goat and cow mitochondrial DNA (cytochrome B gene).

PCR amplification was performed with a commercial kit (2X Green Master Mix, Fermentas) in 25 µl reactions, containing: 12.5 µl - 2X reaction mixture (Taq polymerase, buffer, $MgCl_2$ and dNTP), 6.5 µl sterile water, 2 µl of common-sense primer specific for cytochrome B of both species and 1 µl of each antisense primer specific for goat or cow. PCR amplification was performed in a thermocycler, under the following conditions: predenaturation – 95 °C for 3 minutes 1 cycle, followed by 35 cycles at 94 °C - 1 minute, 58 °C - 1 minute, 72 °C - 1 minute.

To differentiate the presumptive amplification products obtained in the amplification reactions, the samples were migrated in 2% concentration agarose gel, in 1X TBE buffer and 1X Gel Red Nucleic Acid Stain Gel, at 75 V for one hour. The gel image was analysed using a UV transilluminator system.

Cream cheese manufacturing

Three fresh cheese making trials were carried out during three successive weeks. In each trial, cheese was made in one vat. Goat's milk, obtained from a local farm, was pasteurized at 63-65 °C for 20-30 min. After pasteurization, the milk was cooled at 30 °C and each milk vat was inoculated with starter culture containing about 1 UC/100 l at the rate of 1% (3.5×10^6 cfu/g). After inoculation 20 g/100L of $CaCl_2$ was added. Milk was fermented in a thermostatically controlled incubator for 1 hour at 31°C. When pH reached ≤ 6.5 (6.46), commercial rennet was added under stirring conditions in all vats. The mixtures were incubated at 25-28 °C for about 12 hours. The curd was set in cheese-cloths and was pressed in a ripen chamber for draining at 16-18 °C. After drainage, the curd was cooled to 10 °C.

Cream cheese with *Aloe vera* gel microcapsules and essential oils was made by blending and cooling at 10 °C. During this procedure the cream cheese (90%) was salted (0.4% w/w) and mixed with sour cream (20% fat), 10% proportion. Two concentration of *Aloe vera* microcapsules (7% and 3%) mixed with thyme/oregano essential oil in ratios of 0.0072% and 0.018% were used. Then, cream cheese samples were packaged in plastic cups (250 g) and stored in the refrigerator at 2-6 °C for 21 days until analysis.

The control cream cheese (M) was produced without essential oils and *Aloe vera* addition. The samples were coded as follows: P1C1-7% *Aloe vera* microcapsules and 0.0072% thyme essential oil, P2C2-3% *Aloe vera* microcapsules and 0.018% thyme essential oil, P1O1-7% *Aloe vera* microcapsules and 0.0072% oregano essential oil, P2O2-3% *Aloe vera* microcapsules and 0.018% oregano essential oil.

Physicochemical and microbiological analysis

Physicochemical analysis was performed using cream cheese (two samples from each cheese making trial). The methods specified in the following standards were used to determine the fat, protein, total dry matter, and sodium chloride content of the spreadable cheeses: SR ISO 3433:2009, SR EN ISO 8968-1:2014, SR EN ISO 5534:2004, SR EN ISO 5943:2007 and SR ISO 1740:2008.

The antibacterial activity of essential oils was tested on the following bacteria: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Salmonella enteritidis* ATCC 13076. The objective was to identify the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) in accordance with the National Committee for Clinical Laboratory Standards (NCCLS, 1997). The minimum inhibitory concentration (MIC) was determined by using 96-well microtiter plates.

The microbiological determinations of the cream cheeses were performed using the following standard methods: SR ISO 16649-2: 2007 Microbiology of food and feed - Horizontal method for the enumeration of positive *Escherichia coli* beta-glucuronidase. Part 2: Colony enumeration technique at 44 °C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronate; ISO 6881-1: 1999 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) - Part 1: Technique using Baird-Parker agar medium.

Sensory evaluation

A fifty-member sensory panel (aged 21-50 years, women, and men) evaluated one cream cheese of each type at the end of the manufacturing process. The cream cheeses were evaluated for appearance (creamy white), colour (white colour such as cream - degree of whiteness, low-yellow colour), consistency (Low-hard/High-easy to spread on a cracker, creamy feeling of fullness in the mouth) and taste (fresh sourness-reminding of yoghurt). A five-point hedonic scale was used for appearance, colour, consistency, smell, and taste, ranging from 0 (poor) to 5 (very good).

Data analyses

Data analysis was carried out using Minitab statistical software (version 16.1.0;LEAD Technologies, Inc., Charlotte, NC, USA) by one-way ANOVA with Tukey's post-hoc test, at a 95% confidence level ($p <$

0.05). Statistical significance of the effects was interpreted as follows: $p \geq 0.05^{NS}$, not significant; $p < 0.01^{**}$, very significant; $p < 0.001^{***}$, extremely significant.

Results and Discussion

PCR analysis of raw goat 's milk

A reference cow DNA sample was used to differentiate goat milk from cow milk (Figure 1, sample R1). In this case, a specific 274 bp fragment was obtained, which denotes the presence of mitochondrial cow DNA. In the case of DNA samples extracted and amplified from goat milk used in the preparation of the product, the presence of a single amplification product of 157 bp, specific for goat mitochondrial DNA (Figure 1, samples P1 and P2) was detected.

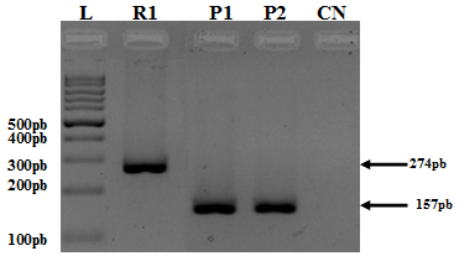

Figure 1. Specific amplification products of the cytochrome B gene from goat's and cow's milk: L-ladder 100bp; R1- cow DNA reference sample, 274 bp PCR product; P1 and P2 - DNA samples amplified from goat milk used in the preparation of the product, 157 bp PCR product; CN-negative control.

Aloe vera gel characterization

In order to characterize the *Aloe vera* samples, the following determinations were achieved: determination of the total phenolic content by the Folin-Ciocâlteu spectrophotometric method (λ = 750 nm), determination of total flavonoid content by spectrophotometric method (λ = 500 nm), determination of antioxidant capacity by DPPH method (Williams *et al.*, 1995), determination of vitamin C by titrimetric method (Deac *et al.*, 2014). Results of the total phenolic, flavonoid, antioxidant capacity and vitamin C content from the *Aloe vera* gel, are presented in Table 1.

Table 1. *Aloe vera* gel characterization

Aloe vera gel	TPC (mg GAE/100g)	Flavonoids (mg QE/100 g)	Antioxidant activity (%)	Vitamin C mg/100 g
	20.68	0.248	3.81	7.04

Determination of antimicrobial activity of essential oils and Aloe vera extract (minimum inhibitory concentration and minimum bactericidal concentration).

The concentration that resulted in complete inhibition of the bacteria (MIC) was the concentration corresponding to the culture wells where the blue colour did not turn pink. Two repetitions for each sample were performed. The minimum bactericidal concentration (MBC) was achieved by inoculating on Mueller Hinton agar from the last three culture wells where bacterial growth was completely inhibited. The results obtained can be found in Table 2.

In our trials, the antimicrobial activity of thyme essential oil against *Staphylococcus aureus* ATCC 25923, *Salmonella enteritidis* ATCC 13076 and *Escherichia coli* ATCC 25922 was found to be lower than the antimicrobial activity of oregano essential oil. Based on these results, it was found that oregano essential oil had a higher antimicrobial activity than thyme essential oil (Olmedo *et al.*, 2013; Carvalho *et al.*, 2015)

Table 2. The susceptibility profile of bacteria (1.5×10^8 CFU/ml) to thyme and oregano essential oils (µl/ml)

Samples		*Staphylococcus aureus* ATCC 25923	*Salmonella enteritidis* ATCC 13076	*Escherichia coli* ATCC 25922
Thyme essential oil	MIC	0.27±0.00	0.13±0.00	0.06±0.00
	MBC	0.27±0.00	0.13±0.00	0.06±0.00
Oregano essential oil	MIC	0.04±0.017	0.01±0.00	0.01±0.00
	MBC	0.04±0.017	0.01±0.00	0.01±0.00
Gentamicin		0.24	0.24±0.00	0.05±0.00

The susceptibility of tested bacteria (1.5×10^6 CFU/ml) to *Aloe vera* extract (mg/ml) is presented in Table 3. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the *Aloe vera* extract against microorganisms ranged from 37.79 to 56.58 mg/ml. The study revealed that the *Aloe vera* extract has a greater medicinal potential against *Staphylococcus aureus* and *Escherichia coli*. Similarly, in another study, gram-positive test organisms were found to be more susceptible to the sterile *Aloe vera* extract (Shahzad *et al*., 2009).

Table 3. The susceptibility profile of bacteria (1.5×10^6 CFU/ml) to *Aloe vera* extract (mg/ml)

Aloe vrea extract	*Staphylococcus aureus* ATCC 25923	*Salmonella enteritidis* ATCC 13076	*Escherichia coli* ATCC 25922
MIC	37.79±0.017	56.58±0.00	37.79±0.017
MBC	37.79±0.017	56.58±0.00	37.79±0.017
Gentamicin (µg/ml)	0.05±0.00	0.05±0.00	0.05±0.00

Determination of pathogenic microorganisms (coagulase-positive staphylococci and Escherichia coli) during storage highlighting the influence of essential oils and Aloe vera microcapsules on their evolution.

Table 4 presents the results of the microbiological determinations of the spreadable goat cheeses.

Table 4. Microbiological quality of the spreadable goat cheese assortments supplemented with *Aloe vera* microcapsules and essential oils

Samples	*Staphylococcus aureus*	*Escherichia coli*
P1O1	Absent	Absent
P2O2	Absent	Absent
P1C1	Absent	Absent
P2C2	Absent	Absent
Control sample	Absent	Absent

P1C1-7% *Aloe vera* microcapsules and 0.0072% thyme essential oil, P2C2-3% *Aloe vera* microcapsules and 0.018% thyme essential oil, P1O1-7% *Aloe vera* microcapsules and 0.0072% oregano essential oil, P2O2-3% *Aloe vera* microcapsules and 0.018% oregano essential oil

Physicochemical characteristics

In this work the physicochemical and the sensory properties of all cheese samples were analysed. Cheese composition is shown in Table 5.

The fat and protein content found in the present study is similar to those reported by other authors for this variety of cheese (Frau *et al*., 2014). No significant differences were found in all treatments in regards to moisture, total protein, salt, and fat content of the cheese samples during storage. The addition of thyme and oregano essential oils in cheese did not seem to affect those parameters after spreadable cheese manufacturing and during storage (Table 5).

The moisture and fat in dry matter content of all cheeses (control and *Aloe vera*/ essential oils) have been found to meet the mentioned standards (≤75% moisture and ≥ 40% FDM content) for fresh spreadable cheese (Aktypis *et al.*, 2018). The results were similar to those found in commercial goat cheeses (Gambaro *et al.*, 2017), in French goat milk cheeses (Raynal-Ljutovac *et al.*, 2011) and those found by Vieitez *et al.* (2016). Regarding the salt and total protein content no, significant differences were found among the examined cheeses. The protein content was found to be, almost, constant during their storage and ranged from 10.08 to 10.84% (Table 5).

Table 5. Physicochemical characteristics of spreadable goat cheeses

Sample / Parameter	Fat (%)	Protein (%)	Moisture (%)	Total dry matter (%)	Fat in dry matter (%)	Salt (%)	Acidity (°T)
Control sample	19.68±0.042^{b}	10.84±0.028^{a}	68.20±0.325^{c}	31.80±0.325^{a}	61.90±0.481^{bc}	0.411±0.031^{c}	150±5.657^{a}
P1C1	19.07±0.156^{bc}	10.08±0.057^{b}	70.77±0.580^{a}	29.23±0.580^{c}	65.26±1.824^{ab}	0.741±0.017^{a}	158±5.657^{a}
P2C2	20.92±0.184^{a}	10.40±0.042^{b}	69.52±0.057^{c}	30.48±0.057^{b}	68.63±0.735^{a}	0.684±0.016^{ab}	155±1.414^{a}
P1O1	17.56±0.170^{d}	10.29±0.141^{b}	70.16±0.113^{ab}	29.84±0.113^{bc}	58.85±0.346^{c}	0.694±0.007^{ab}	157±1.414^{a}
P2O2	18.61±0.212^{c}	10.40±0.099^{b}	69.64±0.042^{ab}	30.06±0.042^{bc}	61.30±0.785^{c}	0.635±0.004^{b}	152±2.828^{a}
p	0.000***	0.002**	0.003**	0.003**	0.001**	0.000***	0.332^{NS}

Different letters in the same column indicate statistically significant differences at p < 0.05 (Tukey's test). Significance: $p \geq 0.05^{NS}$, not significant; $p < 0.01^{**}$, very significant; $p < 0.001^{***}$, extremely significant.

Sensory evaluation of cream cheese

Ratings for flavour and textural attributes of spreadable goat cheeses are presented in Figure 2 and Figure 3, respectively.

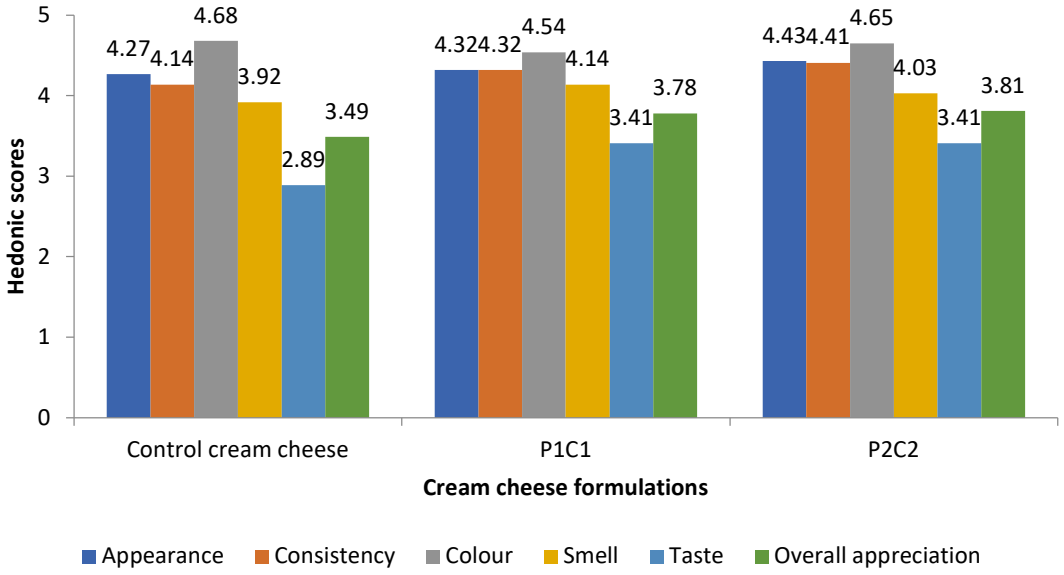

Figure 2. Sensory characteristics of control and spreadable goat cheese with thyme essential oil and *Aloe vera* gel microcapsules

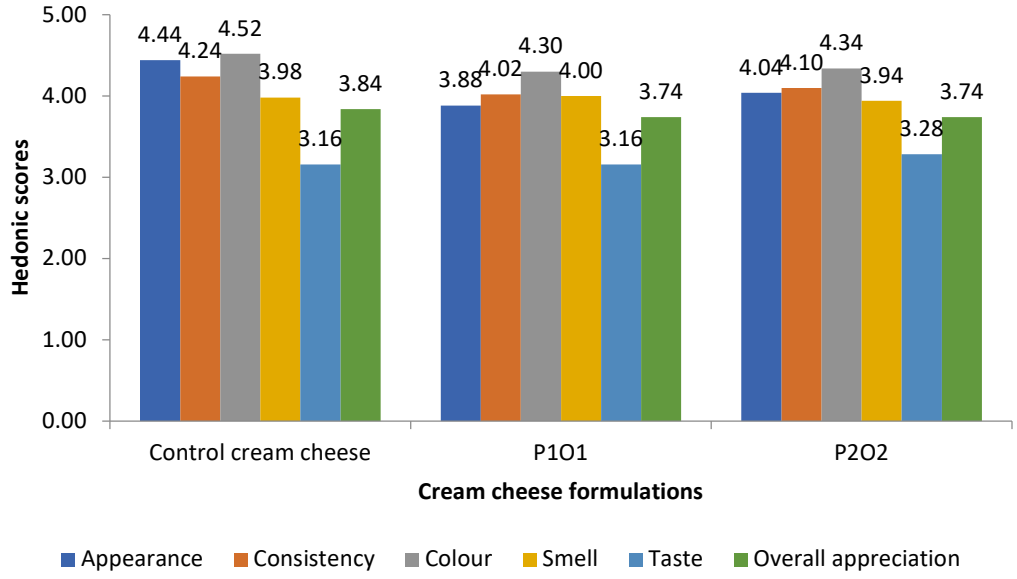

Figure 3. Sensory characteristics of control and spreadable goat cheese with oregano essential oil and *Aloe vera gel* microcapsules

As shown in Figures 2 and 3, the panellists found the differences in appearance, consistency, and colour between control, P1C1 and P2C2 cheese samples.

However, the thyme/oregano spreadable goat cheese analogue with higher content of *Aloe vera* gel microcapsules addition was poorer in consistency and appearance as reflected by their lower score. The lower score in appearance and consistency of P1C1 and P1O1 was likely due to the denser microstructure which made the sample too hard. In addition, the lower score in taste and consistency of P1C1 and P1O1 samples might be due to the too soft feeling of the samples resulting from the high moisture level of the product (Liu *et al.*, 2008).

In terms of consumer preference, the cream cheese sample with 3% *Aloe vera* gel microcapsules and 0.018% thyme essential oil was the most appreciated (P2C2). Regarding cheese samples with the addition of oregano essential oil and *Aloe vera* gel microcapsules, the sample with 3% *Aloe vera* and 0.018% oregano essential oil was the most appreciated (P2O2) as appearance, consistency, and taste, compared to the sample with more microcapsules and less oregano oil.

The cheese sample with 7% microcapsules and 0.0072% oregano essential oil, was more appreciated in terms of smell. The lower score in taste and consistency of the P1O1 sample was likely due to the denser microstructure which made the sample too hard (Jeon *et al.*, 2012).

Conclusions

In this work, the influence of Aloe vera gel microcapsules and essential oils addition on physicochemical, microbiological, and sensory properties of spreadable goat's cheese was investigated. The results highlighted that the best results, from a nutritional point of view, were obtained for the cheese samples with lower Aloe vera and higher thyme essential oil content. In addition, the spreadable goat's cheese with oregano essential oil exhibited a more intensive antimicrobial activity against *Salmonella enteritidis* and *Escherichia coli*. The sensory analysis showed that the lesser Aloe vera gel microcapsules fortification of 3% resulted to an acceptable spreadable cheese, which kept its traditional taste and aroma. Based on the above, Aloe Vera and thyme essential oil could be successfully used as natural flavours in spreadable cheese manufacture providing also antimicrobial

and functional properties. Enrichment of dairy products with polyphenols from Aloe vera plants can positively influence their oxidative stability and it may contribute to a decline in the incidence of degenerative human diseases.

Authors' Contributions

All authors contributed to the review and the editing of the article. MAJ wrote the manuscript and supervised in final reviewer the manuscript.

AMR, CRP and LCS participated in the experiment of this study. VAB performed PCR analysis. CAS supervised data analysis. DT and DM designed and conducted the research. AB in reviewer of the manuscript. All authors read and approved the final manuscript.

Acknowledgements

This work was supported by a grant of Ministry of Research and Innovation, CNCS - UEFISCDI, project number 180 CI/2018.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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