Design of prebiotic cheese spreads enriched with biologically active compounds

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Abstract. The development of functional foods providing health benefits above the basic nutritional needs is of growing interest to the food industry. This research aimed to: design fresh prebiotic cheese spreads enriched with agave inulin (AI), thyme (Thymus callieri Borbás ex Velen.) and hawthorn fruit (Crataegus monogyna Jacq.) as plant sources of biologically active compounds; observe the physicochemical and microbiological changes in the cheese during storage at 4°C for 35 days; evaluate the sensory characteristics of the new functional products. Therefore, five experimental groups of fresh cheese spreads were prepared – a control; 1% AI + 0.2% thyme; 2% AI + 0.2% thyme; 1% AI + 0.4% hawthorn fruit and 2% AI + 0.4% hawthorn fruit. During the storage, pH in all experimental groups gradually decreased (reaching values between 4.17 and 4.25 on the 35th day), which corresponded to the increasing titratable acidity (reaching values from 146.30°T to 152.51°T on the 35th day). The application of thyme and hawthorn fruit did not reduce the total plate count (mesophilic aerobic and facultative anaerobic microorganisms) and yeasts; however, during the storage period the addition of agave inulin stimulated the growth of lactic acid bacteria in the treated samples compared to the control.

1 Introduction

The general concept of functional foods is related to food products enriched with specific ingredients providing health benefits above the needs of the basic human nutrition that leads to improvement of certain body functions and decreases the risk of diseases. In this respect, functional foods can be defined as natural foods of the human diet in which one of the constituents: a) has been added to provide health benefits; b) has been modified (by fortification, enhancement or enrichment); c) has been removed in order to minimize some adverse health effects. On this basis, functional foods can be classified into conventional foods, modified foods, medical foods and foods for special dietary use [1].

Milk and dairy products are essential components of the daily human diet, and can be considered functional foods which provide the necessary amounts of the basic nutrients and energy, and may also reduce the risk of some diseases. In parallel with their health benefits, dairy products represent an excellent matrix for modification and incorporation of substances supporting different metabolic functions – prebiotics, probiotics and bioactive compounds, which is of paramount importance in designing novel functional products or improving the functional properties of existing ones [2, 3].

Spreadable cheeses contain high amount of proteins and bioactive peptides, conjugated linoleic acid and lower amount of fats in comparison with other types of table spread products, such as butter (poor spreadability, high saturated fat and cholesterol content) and margarine (high levels of trans fatty acids, which increase LDL cholesterol and reduce HDL cholesterol). Besides providing certain health benefits, fresh spreadable cheeses represent suitable food matrices, which can be incorporated with biologically active compounds in order to increase their functionality [4, 5].

Prebiotics represent non-digestible compounds – oligosaccharides and polysaccharides that exert health benefits by stimulating the gastrointestinal microbiota. Inulin is a reserve plant polysaccharide, which is known as a source of dietary fibres and serves as a substrate for the growth of beneficial gut bacteria. The low sweetness of inulin and its properties similar to sucrose allow its use as a sugar substitute in some food products [6]. Incorporated into dairy products, prebiotics stimulate the lactic acid bacteria, leading to higher production of volatile compounds, which positively affects their sensory properties [7]. Agave inulin is a highly soluble prebiotic...
with potential to improve the balance of the gastrointestinal flora by stimulating the growth of bifidobacteria, thus exerting its immunomodulatory effects, controlling body weight and improving calcium absorption [8]. Besides health-promoting effects, inulin exhibits a lot of technological benefits; therefore, it is increasingly used as a bulking agent, a texture modifier and a fat replacer, thus obtaining dairy products with enhanced textural properties and low caloric value [9].

The genus *Thymus* belongs to the *Lamiaceae* family, which includes about 350 aromatic perennial species indigenous to the Mediterranean region. One of the most popular species cultivated for culinary, medicinal and ornamental purposes is *Thymus vulgaris* or common thyme. According to some studies, the number of species belonging to the genus *Thymus* in Bulgaria is 21, among which are *Thymus serpyllum* L. and *Thymus callieri* Borbás ex Velen., found in different natural locations of the country [10]. Recent studies on the chemical composition of thyme revealed that it contains phenolic compounds (flavonoids), proteins, vitamins, minerals (K, Ca, Fe, Mn, Mg, Se) and tannins [11]. The essential oil of thyme extracted from the whole plant contains aromatic and volatile compounds – thymol, carvacrol, quercol, α-terpineol, L-borneol, L-cymol, L-pinene, D-pinene, γ-terpene, caryophyllene and linalool, known to possess strong antimicrobial and antioxidative properties [12, 13]. Apart for culinary purposes, thyme is also used in medicine as an antiviral, anti-inflammatory, antiparasitic, diuretic, antirheumatic, antihypertensive, antimicrobial and sedative agent [14].

Hawthorn (*Crataegus monogyna*) is an important plant of the *Rosaceae* family, widely used in the traditional medicine and in the culinary practice of China, Europe and North America. The hawthorn fruits have been found to contain vitamin C, sugars, organic acids, phenols, flavonoids and anthocyanins that contribute to its red, purple or blue colour [15]. The hawthorn fruits are rich in minerals – Na, K, Ca, P, Mg, Fe and B [16]. Therefore, they are used for prevention and treatment of cardiovascular diseases, cancer, diabetes, asthma and nephritis, as well as for improving memory [17]. According to Tadić et al. [18], the extract of dried hawthorn berries (*C. monogyna* Jacq. and *C. oxyacantha* L.) can be used as an anti-inflammatory, gastroprotective and antimicrobial agent. Ziouche et al. [19] reported that hawthorn leaves and fruits are rich in polyphenolic compounds (rutin, quercetin and isoquercetin) that exhibit significant antioxidant activity. Despite the large number of publications on the health benefits of agave inulin, thyme and hawthorn fruit, the information regarding their application as functional components in foods is still very limited. Therefore, this research aimed: a) to design fresh prebiotic cheese spreads enriched with agave inulin, thyme and hawthorn fruit as plant sources of biologically active compounds; b) to observe the physicochemical and microbiological changes in cheese during storage at 4°C for 35 days; c) to evaluate the sensory properties of the newly obtained functional products.

## 2 Materials and methods

### 2.1 Materials

#### 2.1.1 Milk

Fresh and pasteurized (at 82-85°C/10-15 min) cow’s milk was used in the experimental procedure. The milk was delivered by the United Milk Company Ltd., Plovdiv, Bulgaria. The milk (10 ml sample) was analysed using an automatic milk analyser MilkoScope Expert (Eeton Analytical, Nigeria) at room temperature (23°C).

The obtained physicochemical parameters of the milk were as follows: pH 6.5, fat 3.6%, solids-non-fat (SNF) 9.06%, density 31.49 kg/m³, protein 3.33%, lactose 4.98%, solids 0.74%, added water 0.00%, freezing point -0.580°C, titratable acidity 18°C (determined according to the titration method).

#### 2.1.2 Starter culture

Mesophilic aromatic culture Flora Danica (CHR Hansen, Denmark), containing *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis*, *Lactococcus lactis* subsp. *lactis* and *Leuconostoc* (0.6 g/10 l milk) was used according to producer’s requirements.

#### 2.1.3 Chymosin

As a coagulant Chymosin CHY-MAX® Supreme (CHR Hansen, Denmark) (0.1 ml/10 l milk) was used according to producer’s requirements.

#### 2.1.4 Plant material

The present study used two herbs – thyme (*Thymus callieri* Borbás ex Velen.) and hawthorn fruit (*Crataegus monogyna* Jacq.). The herbs were harvested in the Western part of the Rhodope Mountains, Bulgaria, in the period June–August 2023, and identified according to the Herbarium Academiae Scientiarum Bulgariae. The herbs were air-dried and finely ground by a blender. In order to determine the total phenolic content, total flavonoid content, total caffeic acid derivatives and antioxidant activity of thyme and hawthorn fruit, 70% ethanolic extracts were prepared.

#### 2.1.5 Agave inulin

Inulin powder from Mexican agave (*Agave tequilana* Weber) was purchased from the Zoya online market (Sofia, Bulgaria). Agave inulin was characterized by the average degree of polymerization (DP) 18, Mn 2993 Da, Mw 2916 Da and 91% purity.
2.1.6 Culture media

*Plate count agar (PCA).* PCA (Scharlab S.L., Spain) was used for determining the total plate count (mesophilic aerobic and facultative anaerobic microorganisms). A quantity of 23.5 g of the PCA agar medium base was dissolved in 1 l of deionized water (pH 7.0 ± 0.2).

*Chloramphenicol glucose agar (CGA).* CGA (Scharlab S.L.) is a selective medium for yeasts and fungi. A quantity of 40 g of CGA base was dissolved in 1 l of deionized water (pH 6.6 ± 0.2).

*LAPTg10 agar.* LAPTg10 (Conda lab S.A., Spain) is a selective medium for lactic acid bacteria. A quantity of 45 g of LAPTg10 broth was dissolved in 1 l of deionized water (pH 6.6 – 6.8 ± 0.2), and then 15 g agar and 1 ml Tween 80 (Sigma-Aldrich, Germany) were added.

All the culture media were prepared according to the manufacturers’ instructions and autoclaved at 121°C for 20 min before use.

2.2 Methods

2.2.1 Total phenolic content

The total phenolic content (TPC) was determined using a Folin-Ciocalteu reagent. The reaction mixture containing 1 ml of Folin-Ciocalteu reagent (Sigma-Aldrich, Merck, Germany), 0.8 ml of 7.5% sodium carbonate (Sigma-Aldrich, Merck) and 0.2 ml of the plant extract was kept at room temperature for 20 min (in darkness). The absorbance was measured by a spectrophotometer Camspec M107 (Spectronic-Camspec Ltd., UK) at 765 nm against a blank (distilled water). The results were presented as mg equivalent of gallic acid (GAE)/g of dry weight (dw) sample [20].

2.2.2 Total flavonoid content

The total flavonoid content (TFC) was evaluated according to the method described by Ivanov et al. [20]. An aliquot of 1 ml of the plant extract was added to 0.1 ml of 10% Al(NO3)3, 0.1 ml of 1 M CH3COOK (Sigma-Aldrich, Merck) and 3.8 ml of distilled water. After incubation at room temperature for 40 min, the absorbance was measured at 415 nm. Quercetin was used as a standard and the results are expressed as mg quercetin equivalents (QE)/g of dw sample.

2.2.3 Total caffeic acid derivatives

The total caffeic acid derivatives were determined by the method of Ivanov [21]. The plant extract (1 ml) was added to 2 ml 0.5 M HCl, 2 ml Arnow’s reagent (10 g sodium nitrite and 10 g sodium molybdate made up to 100 ml with distilled water), 2 ml NaOH (concentration of 2.125 M) and 3 ml of water. Each solution was compared with the same mixture without Arnow’s reagent. The absorbance was measured at 525 nm. The total caffeic acid derivatives were expressed as mg caffeic acid equivalents (CAE)/g of dw sample.

2.2.4 Antioxidant activity

*DPPH radical scavenging assay.* The reaction mixture containing 2.85 ml of DPPH reagent (2,2-diphenyl-1-picrylhydrazyl) (Sigma-Aldrich, Merck) and 0.15 ml of the tested plant extract was incubated at 37 °C for 15 min. The absorbance was measured at 517 nm against a blank (methanol). The antioxidant activity was expressed as mM Trolox equivalents (TE)/g of dw sample [20].

*Ferric-reducing antioxidant power (FRAP) assay.* The FRAP reagent was freshly prepared with 300 mM acetate buffer with pH 3.6, 10 mM 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) in 40 mM hydrochloric acid and 20 mM Iron (III) chloride hexahydrate (Sigma-Aldrich, Merck) in distilled water in a ratio of 10:1:1. The reaction mixture (3 ml of FRAP reagent and 0.1 ml of the plant extract) was incubated at 37°C for 10 min in darkness. The absorbance was measured at 593 nm against a blank (distilled water). The antioxidant activity was expressed as mM TE/g of dw sample [20].

2.2.5 Experimental procedure

The fresh cheese spreads were prepared in laboratory conditions according to the following technological scheme (Fig. 1).

![Fig. 1. Technological scheme for the preparation of fresh prebiotic cheese spreads with plant sources of biologically active compounds](https://doi.org/10.1051/bioconf/202410201007)
After the whey draining and cooling, the cheese spread (yield of 2.5 kg from 10 l of milk) was divided into five equal portions (500 g). The first portion was kept as a control, while in the other four portions the agave inulin (AI) and ground herbs were added and homogenized. Inulin was added after the draining step in order to prevent its loss in the whey. Thus, five experimental groups were prepared – CS1 (untreated cheese or a control); CS2 (1% AI + 0.2% thyme); CS3 (2% AI + 0.2% thyme); CS4 (1% AI + 0.4% hawthorn fruit) and CS5 (2% AI + 0.4% hawthorn fruit) (Fig. 2). Thyme was added in a lower concentration due to its strong aroma and flavour. The samples were stored at 4°C for 35 days. The physicochemical and microbiological changes in cheese were monitored once a week.

![Whey draining through a filtering material at 4°C (A) and overall appearance of the fresh cheese spreads: CS1 or the control (B); CS2 – 1% AI + 0.2% thyme (C); CS3 – 2% AI + 0.2% thyme (D); CS4 – 1% AI + 0.4% hawthorn fruit (E) and CS5 – 2% AI + 0.4% hawthorn fruit (F)](image)

Fig. 2. Whey draining through a filtering material at 4°C (A) and overall appearance of the fresh cheese spreads: CS1 or the control (B); CS2 – 1% AI + 0.2% thyme (C); CS3 – 2% AI + 0.2% thyme (D); CS4 – 1% AI + 0.4% hawthorn fruit (E) and CS5 – 2% AI + 0.4% hawthorn fruit (F)

2.2.6 Determination of colour

The colour of cheese was determined by a portable colorimeter FRU WR-10QC (China) and recorded in L*a*b* colour system. The system CI-ELAB consists of a lightness component (L*), and two chromatic components – a* value represents green (-a) to red (+a) and b* value represents blue (-b) to yellow (+b) colours.

The colorimeter was calibrated using a standard white plate ($L^* = 96.20$, $a^* = 0.06$, $b^* = -6.20$) [22]. The values of these parameters were automatically recorded by touching the device to the sample’s surface. In the second stage of the analysis, the colorimeter was calibrated to the colour of the control cheese in order to determine the variations in the colour components ($\Delta L$, $\Delta a$, $\Delta b$) and the total colour variation index ($\Delta E$). To determine the chroma ($C^*$) and hue ($H^*$), the following equations according to Abd El-Baset and Almoselhy [23] were used:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

$$C^* = \sqrt{a^*^2 + b^*^2}$$

$$H^* = \tan^{-1}\left[\frac{b^*}{a^*}\right]$$

2.2.7 Determination of moisture

Determination of moisture was performed using a KERN DAB 100-3 moisture balance analyser (Kern & Sohn GmbH, Germany) by heating a 1 g sample of cheese at 110°C to a constant weight. The moisture content (%) was automatically recorded by the analyser based on the difference between the initial weight and the weight after drying of the sample [24].

2.2.8 Determination of pH

The pH values were determined using a pH-meter WTW pH 7110 (InoLab, Weilheim, Germany) equipped with a glass electrode [25].

2.2.9 Determination of titratable acidity

The determination of titratable acidity was implemented according to the standard titration method [25]. The titratable acidity was determined by titration of each sample with 0.1 N NaOH using phenolphthalein as an indicator until the appearance of a pale pink colour persisting over 1 min. The results were expressed as Torner degrees (°T).

2.2.10 Characteristic microorganisms

The enumeration of characteristic microorganisms (lactic acid bacteria) was implemented by the spread-plating method on LAPTg10 agar medium and incubation at 37 °C for 48 h. The results were expressed as colony-forming units/g (cfu/g) [25].

2.2.11 Total plate count

The total plate count (mesophilic aerobic and facultative anaerobic microorganisms) was determined by the pour-plating method on PCA at 30°C for 48 h. The results were expressed as cfu/g [26].
2.2.12 Yeasts and/or fungi

The yeasts and fungal counts were determined by the pour-plating method on CGA medium and incubation at 25°C for 48 h. The results were expressed as cfu/g [25].

2.2.13 Sensory analysis

To conduct the sensory analysis, 20 unbiased testers over 18 years old were invited. The analysis was performed by testing a small amount of cheese samples spread on slices of bread. The samples were evaluated on six sensory parameters (flavour, odour/aroma, consistency, surface, colour and overall appearance) using the 9-points Hedonic scale: 9 = liked extremely, 8 = liked very much, 7 = liked moderately, 6 = liked slightly, 5 = neither liked nor disliked, 4 = disliked slightly, 3 = disliked moderately, 2 = disliked very much, 1 = disliked extremely. The results from the sensory analysis were recorded on individual questionnaire cards and then a sensory profile of each type of cheese was made using MS Excel 2010 software [25].

2.2.14 Statistical analysis

Results from triplicates were presented as mean values ± standard deviation (SD). One-way analysis of variance (ANOVA) was performed using Statgraphics Centurion statistical program version XVI, 2009 (Stat Point Technologies, Inc., Warrenton, VA, USA). Mean differences were established by Fisher’s least significant difference test for paired comparison with a significance level of p ≤ 0.05 [25].

3 Results and discussions

3.1 Total phenolic content, total flavonoid content, total caffeic acid derivatives and antioxidant activity of thyme and hawthorn fruit

The results from the phytochemical analyses of the two herbs used in the experimental design are presented in Table 1. As seen from the obtained results, the ethanolic extract (70%) of dried thyme demonstrated higher total phenolic content (TPC), total flavonoid content (TFC), total caffeic acid derivatives and antioxidant activity (determined by two independent methods – DPPH and FRAP) than the ethanolic extract of dried hawthorn fruit. The higher TPC and TFC of thyme extract corresponded to the higher antioxidant activity values.

Despite the great number of publications on Thymus species, the scientific information about Thymus callieri chemical composition and biological activities is still very limited. Georgieva et al. [27], who investigated five herbs from the same geographic region of Bulgaria (the Western Rhodopes) determined that the TPC of 70% ethanolic extract of dried thyme (T. callieri) amounted to 86.19 mg GAE/g, while the TPC of 70% ethanolic extract of a dried hawthorn fruit (C. monogyna) was 26.88 mg GAE/g. The authors stated that TFC of 70% ethanolic thyme extract amounted to 26.43 mg QE/g for a dried herb, while that of 70% ethanolic extract from a dried hawthorn fruit was 2.89 mg QE/g. The TPC and TFC values were higher in comparison with our findings for the two dried herbs. Regarding the antioxidant potential determined by the DPPH method, the same authors reported that the value for dried T. callieri was 218.97 mM TE/g (lower than our data) while for the dried hawthorn fruit was 176.23 mM TE/g (higher than our results). Higher values of TPC, TFC and antioxidant activity of 70% ethanolic extracts of thyme (Thymus vulgaris) and hawthorn fruit (C. monogyna) originating also from the Rhodope Mountains, Bulgaria were reported by Parzhanova et al. [28].

Caffeic acid (3,4-dihydroxy-cinnamic acid) is one of the most common phenolic acids in plants and occurs in the form of ester conjugates with quinic acid (caffeoylquinic acids) or saccharides. Caffeic acid derivatives are water-soluble components that determine the biological activity of the herbs in which they are presented [29]. Thyme and hawthorn fruit are good sources of caffeic acid derivatives – thyme is mainly rich in rosmarinic acid, whereas hawthorn fruit contains chlorogenic acid (our unpublished data) and their intake is associated with certain health benefits, such as antiviral, antioxidant, hepatoprotective and hypoglycaemic effects [30]. The inhibitory activity of caffeic acid derivatives against a number of human and plant fungal pathogens is also documented [31].

3.2 Moisture content of the fresh prebiotic cheese spreads

The moisture content of the fresh prebiotic cheese spreads is presented in Table 2. As seen from the results, the five experimental groups exhibited similar values of moisture content on day 0, which decreased with the prolongation of the refrigerated storage period. At the end of the storage (35th day), the moisture content was lower with 0.46% – 1.06% compared to the values at the beginning of the experiment (day 0). On the 35th day, the untreated cheese spread (CS1 or the control) had the highest moisture content compared to the other four samples, but the statistical difference between the groups was not significant.
Table 1. Total phenolic content, total flavonoid content, total caffeic acid derivatives and antioxidant activity of thyme and hawthorn fruit (70% ethanolic extracts)

<table>
<thead>
<tr>
<th>Plant material</th>
<th>Total phenolic content, mg GAE/g</th>
<th>Total flavonoid content, mg QE/g</th>
<th>Total caffeic acid derivatives, mg CAE/g</th>
<th>Antioxidant activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyme (Thymus callierti Borbás ex Velen.)</td>
<td>32.55 ± 0.50</td>
<td>10.16 ± 0.10</td>
<td>23.15 ± 1.29</td>
<td>DPPH (mM TE/g)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>325.02 ± 5.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>FRAP (mM TE/g)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>266.46 ± 8.25</td>
</tr>
<tr>
<td>Hawthorn fruit (Crataegus monogyna Jacq.)</td>
<td>14.10 ± 0.40</td>
<td>1.51 ± 0.04</td>
<td>8.48 ± 0.37</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>123.20 ± 5.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>84.50 ± 1.50</td>
</tr>
</tbody>
</table>

Table 2. Moisture content of the fresh prebiotic cheese spreads

<table>
<thead>
<tr>
<th>Fresh prebiotic cheese spreads</th>
<th>Moisture, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>CS1 (Control)</td>
<td>76.29 ± 2.09a</td>
</tr>
<tr>
<td>CS2 (1% AI + 0.2% thyme)</td>
<td>75.78 ± 0.10b</td>
</tr>
<tr>
<td>CS3 (2% AI + 0.2% thyme)</td>
<td>74.72 ± 0.47b</td>
</tr>
<tr>
<td>CS4 (1% AI + 0.4% hawthorn fruit)</td>
<td>75.61 ± 0.25b</td>
</tr>
<tr>
<td>CS5 (2% AI + 0.4% hawthorn fruit)</td>
<td>75.12 ± 0.13a</td>
</tr>
</tbody>
</table>

a,b: Means in a column without a common letter differ significantly (p ≤ 0.05).

Table 3. Physicochemical changes in the fresh prebiotic cheese spreads during the storage at 4°C for 35 days

<table>
<thead>
<tr>
<th>Day</th>
<th>CS1 (Control)</th>
<th>CS2</th>
<th>CS3</th>
<th>CS4</th>
<th>CS5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH</td>
<td>TA, °T</td>
<td>pH</td>
<td>TA, °T</td>
<td>pH</td>
</tr>
<tr>
<td>0</td>
<td>4.50 ± 0.04A</td>
<td>134.24 ± 9.45A</td>
<td>4.45 ± 0.01B</td>
<td>135.18 ± 7.06A</td>
<td>4.40 ± 0.00C</td>
</tr>
<tr>
<td>7</td>
<td>4.49 ± 0.01B</td>
<td>137.53 ± 8.80A</td>
<td>4.42 ± 0.01B</td>
<td>138.83 ± 3.33C</td>
<td>4.40 ± 0.01B</td>
</tr>
<tr>
<td>14</td>
<td>4.45 ± 0.02A</td>
<td>140.08 ± 13.19A</td>
<td>4.41 ± 0.01B</td>
<td>141.30 ± 1.33A</td>
<td>4.39 ± 0.01B</td>
</tr>
<tr>
<td>21</td>
<td>4.43 ± 0.00A</td>
<td>140.17 ± 24.00A</td>
<td>4.40 ± 0.01B</td>
<td>142.62 ± 2.93A</td>
<td>4.38 ± 0.00B</td>
</tr>
<tr>
<td>28</td>
<td>4.41 ± 0.01A</td>
<td>143.75 ± 11.46A</td>
<td>4.39 ± 0.01B</td>
<td>150.81 ± 0.92A</td>
<td>4.35 ± 0.04A</td>
</tr>
<tr>
<td>35</td>
<td>4.17 ± 0.03A</td>
<td>148.37 ± 9.99A</td>
<td>4.22 ± 0.01A</td>
<td>152.51 ± 3.86A</td>
<td>4.23 ± 0.00A</td>
</tr>
</tbody>
</table>

a,b: Means in a column without a common letter differ significantly (p ≤ 0.05); A,b: Means in a row (for pH/TA values) without a common letter differ significantly (p ≤ 0.05).

Similar changes in physicochemical parameters of low-fat soft cheese enriched with inulin (1, 3, 5 and 7%) were observed by El-Baz [32]. The author reported a decrease in pH values and a concomitant increase in titratable acidity in all cheese samples (including the control) during the refrigerated storage (7°C) for 30 days. Giri et al. [5] developed a functional processed cheese spread with addition of inulin (0%, 4%, 6% and 8%) from a chicory root and monitored its physicochemical, sensory and fatty acid profiles, and microstructural quality. As the
concentration of inulin addition increased moisture content, water activity (\(a_w\)) and titratable acidity, decreased. Other authors [33] designed a Mozzarella cheese with addition of agave inulin as a functional ingredient. The results showed that the application of inulin (0.7% - 3.3%) improved the sensory, functional and physicochemical properties of the cheese. In addition, the authors observed lowering values of the moisture content and pH, as well as changes in the textural profile during the storage period for 31 days.

Santanatoglia et al. [34] developed an Italian fresh cheese Giuncata enriched with inulin from a chicory root (\(Cichorium intybus\)) (10 g/500 ml and 15 g/500 ml of milk) and found that the addition of inulin had no remarkable effect on the pH values of cheese and did not significantly influence its textural properties, colour and the total fat content compared with the control.

### 3.4 Microbiological changes in the fresh prebiotic cheese spreads

During the 35-day period of storage at 4°C, three microbiological parameters – mesophilic aerobic and facultative anaerobic microorganisms (total plate count – TPC), characteristic microorganisms (lactic acid bacteria – LAB) and yeasts and/or fungi in the five cheese spread samples (the control or CS1, CS2, CS3, CS4 and CS5) – were monitored (Table 4). Taking into account that no chemical preservatives were added in the fresh prebiotic cheese spreads, during the first week of the storage period all samples retained low number of mesophilic aerobic and facultative anaerobic microorganisms (TPC), and yeasts.

From the second week of the storage, the TPC and yeast counts in all experimental groups began to increase gradually, reaching highest values at the end of the monitoring period (35th day). Fungi in all experimental conditions were detected only at the end of the monitoring period.

**Table 4.** Microbiological changes in the fresh prebiotic cheese spreads during the storage at 4°C for 35 days.

<table>
<thead>
<tr>
<th>Day</th>
<th>Parameter</th>
<th>CS1 (Control)</th>
<th>CS2</th>
<th>CS3</th>
<th>CS4</th>
<th>CS5</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>TPC, cfu/g</td>
<td>1.0 × 10^2</td>
<td>1.5 × 10^2</td>
<td>2.0 × 10^2</td>
<td>1.0 × 10^2</td>
<td>2.0 × 10^2</td>
</tr>
<tr>
<td></td>
<td>Yeasts, cfu/g</td>
<td>2.0 × 10^2</td>
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</table>

*-TPC - total plate count; **-LAB - lactic acid bacteria.
groups until the end of the storage were not detected. Throughout the entire storage period, the application of thyme (0.2%) and hawthorn fruit (0.4%) did not reduce the TPC and yeasts counts; consequently, the two herbs applied in a dried form cannot serve as biopreservatives in the treated fresh cheese spreads. However, the application of agave inulin in both concentrations (1 and 2%) promoted the growth of LAB in treated fresh cheese spreads (CS2, CS3, CS4 and CS5), maintaining their viable counts higher with 1 log unit during the first week and with 2 log units between the second and the fourth week of the storage compared to the control (CS1). At the end of the storage period (35th day or fifth week) a decrease in LAB counts with 1 log unit in all experimental groups was observed (Table 4). As reported earlier, the addition of inulin or other prebiotics in the food products exerts a protective effect on the LAB survival by providing a substrate for their growth and stimulating their metabolic activity, thus enhancing their therapeutic effects on the organism [35]. An increase in the number of viable LAB cells in fermented milk products (which exhibited LAB counts of 8 log units or higher) enriched with inulin was reported also by Zuleta et al. [36].

### Table 5. Colour changes in the fresh prebiotic cheese spreads

<table>
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<tr>
<th>Fresh prebiotic cheese spreads</th>
<th>Colour index</th>
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<tr>
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<td>L*</td>
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<tr>
<td>CS1 (Control)</td>
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<td>CS3</td>
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<td>CS5</td>
<td>68.13 ± 0.15d</td>
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*a–d*: Means in a column without a common letter differ significantly (p ≤ 0.05).

#### 3.5 Colour changes in the fresh prebiotic cheese spreads

As seen from the results in Table 5, the lightness component (L*) of the four treated experimental groups (CS2, CS3, CS4 and CS5) was statistically different from those of the control (CS1). In terms of parameter a*, CS1 or the control, CS2 and CS3 did not show statistically significant difference in the results obtained. The same applies to CS4 and CS5. Samples CS5 and CS3 demonstrated the highest results in relation to the chroma (C*) and hue (H*) parameters. The value of the parameter ΔE showed that the addition of 2% AI + 0.4% hawthorn fruit changed the colour of the cheese spread CS5 (ΔE > 2.0) compared to the other four experimental groups, which did not significantly reflect on the sensory profile and overall acceptance of this product.

Consequently, the addition of inulin, thyme and hawthorn fruit increased the consumers’ acceptance of the overall appearance, colour, odour/aroma, flavour and surface parameters of the treated cheese compared to the control (in which the scores for these parameters were 8.40, 8.50, 7.56, 7.56 and 8.38, respectively). Based on the results from the sensory analysis, the five experimental groups can be arranged as follows: CS3 (total score 8.48) > CS2 (total score 8.38) > CS5 (total score 8.34) > CS4 (total score 8.33) > the control (total score 8.17) from maximum 9 points.

#### 3.6 Sensory evaluation

The results from sensory evaluation (Fig. 3) showed that sample CS1 (untreated sample or the control) kept the highest score (8.63) only for the consistency parameter; sample CS3 (2% AI + 0.2% thyme) kept the highest scores for the overall appearance (8.47), odour/aroma (8.41), flavour (8.38), consistency (8.63) and surface (8.44) parameters; sample CS5 (2% AI + 0.4% hawthorn fruit) kept the highest score (8.63) only for the colour parameter.

![Fig. 3. Sensory profile of the fresh prebiotic cheese spreads](image)

Karthikeyan et al. [37] investigated the effects of the application of inulin in concentrations of 1%, 1.5% and 2% on the organoleptic properties of Cheddar cheese. The authors stated that the addition of 1.5% inulin in cheddar cheese resulted in the best flavour, body and texture, finish and overall acceptability compared to the control and the other inulin concentrations, without adversely affecting the taste and colour of the product. Rafiq and Ghosh [38] determined that addition of inulin up to 4% had no significant effect on the textural and organoleptic properties of processed Cheddar cheese. The authors stated that the higher concentrations of inulin (6%)
increased the hardness and adhesiveness, while the moisture content, cohesiveness, gumminess, springiness and chewiness significantly decreased. These alterations resulted in lower scores from the sensory evaluation for body/texture and overall acceptability parameters in comparison with the other experimental groups (2% and 4% inulin) and the control. Khalil et al. [39] included inulin as a fat replacer combined with probiotics to improve the physicochemical, organoleptic and microbiological properties of synbiotic Ras cheese during the ripening period for 90 days. It was observed that the addition of inulin (3% and 5%) significantly enhanced the textural profile, yield, probiotic population and sensory characteristics of the Ras cheese. Moreover, cheese supplemented with 3% or 5% of inulin exhibited the equivalent flavour and textural properties as full-fat cheese (control). Similar findings were reported by El-Assar [40], who investigated the influence of inulin on the physicochemical, rheological and sensory properties of low-fat processed cheese spread, and concluded that addition of 5% inulin reduced the hardness and improved the textural and sensory characteristics of the product.

4 Conclusion

The design of functional foods providing health benefits above the basic nutritional needs is of growing interest to the food sector. To the best of our knowledge, this is the first research to investigate the application of agave inulin (as a prebiotic) and thyme and hawthorn fruit (as plant sources of biologically active compounds) in fresh cheese spread in order to obtain a dairy product with enhanced functional and sensory properties. The addition of agave inulin, thyme and hawthorn fruit did not affect the physicochemical characteristics of the samples during the storage period, however promoted the growth of lactic acid bacteria and improved the sensory properties of the treated fresh cheese spreads. The future efforts in the design of such kind of products will be focused on the reduction of unwanted microflora by natural agents, thus extending the shelf life of the certain functional food, which is of particular interest to the dairy industry.

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References