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Assessing the Functional and Sensory Characteristics of Probiotic Whey Cheese with Herbs in Vacuum or Modified Atmosphere Packaging

Running title: Probiotic Whey Cheese with Herbs under Different Packaging Conditions

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SUMMARY

Research background. Some herbs are preferred to provide functional properties to foods due to especially their antibacterial and antioxidant characteristics. On the other hand, modified atmosphere packaging takes attention as an alternative to vacuum packaging in order to preserve the functional and sensory properties of foods. Since the shelf life of whey cheese is quite short, it is preferred to use different packaging methods such as modified atmosphere packaging. Besides, the addition of herbs both gives flavor to the cheese and improves its functional properties.

Experimental approach. In the present study, oregano (*Origanum onites*) or rosemary (*Rosmarinus officinalis*) were added to probiotic whey cheese (Lor) containing *Lactobacillus acidophilus* La-5 and *Bifidobacterium lactis* Bb-12 under modified atmosphere (MAP) (80 % CO_2 and 20 % N_2) or vacuum packaging. Physicochemical, microbiological and sensory properties, and antioxidant and proteolytic activities of cheese samples were determined.

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Results and conclusions. The herb addition did not negatively affect the viable counts of *B. lactis* and *L. acidophilus* and cheese samples contained both probiotic bacteria minimum 8 log CFU/g for 35 days. MAP enhanced the viability of *B. lactis* and *L. acidophilus* in rosemary added cheese in the first few weeks of storage, respectively when compared to vacuum packaging. The addition of herb significantly increased the total phenolic content and antioxidant activity both under MAP and vacuum. MAP improved the antioxidant activity more than vacuum packaging in herb added Lor cheeses on 14th and 28th days. Lor cheese containing rosemary under MAP condition exhibited the highest DPPH$^{\bullet}$ (2,2,-diphenyl-1-picrylhydrazyl) scavenging activity and also proteolytic activity throughout the storage. The sample with rosemary under MAP had the highest taste and aroma scores throughout the storage period. The fortification with herb and MAP provide advantage in the production of whey cheese. The use of rosemary and packaging under modified atmosphere allow to obtain high viability of probiotic bacteria, total phenolic content, antioxidant activity and sensory acceptance in Lor cheese.

Novelty and scientific contribution. This is the first study in which both different herbs and different packaging methods were applied to probiotic whey cheese (Lor). The study shows that the functional properties of whey cheese can be improved by using different herbs and under different packaging conditions. Among the features researched in the product, especially improving the viability of probiotic bacteria is very valuable for human health. Thus, it contributes to functional food science and enables the use of these parameters in some other foods.

Keywords: oregano; rosemary; Lor cheese; probiotic viability; antioxidant activity

INTRODUCTION

Whey is an industrial by-product of cheese obtained by coagulation of milk and elimination of the curd during cheese production. It is a good source of organic substances containing varying amounts of lactose, proteins, minerals, fat and vitamins whose amount varies depending on the cheese production processes and the milk used (*1*). Whey is proceeded to whey cheeses in many countries such as Italy (Ricotta), Yugoslavia (Manouri), Norway (Brunost), Germany (Ziger), France (Broccio), Greece (Anthotyro), Spain (Requeson) and Turkey (Lor). Whey cheese production is based on heat treatment of whey, leading to denaturation of α-lactalbumin and β–lactoglobulin (*2*). Most of the whey obtained in Turkey is used for the manufacture of fresh cheese called “Lor cheese”. The processing methods of Lor cheese varies depending on the region and consumption habits (*3*).

Functional foods “covering dairy products” or “functional dairy products” contribute benefits to human health beyond daily nutrition and so promote individuals’ well-being. They include a wide range of bioactive compounds having efficacy in health promotion and disease prevention. Probiotics are frequently used for the manufacture of functional dairy products. Products containing probiotics have the largest share in functional dairy products. Probiotics are defined by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) as "live microorganisms that provide health benefits to the host when administered in adequate amounts" (*4*). The probiotic market is growing rapidly worldwide, and today the largest share of probiotic-containing foods is dairy products. Recent estimates show that the probiotics market will grow with a compound annual growth rate (CAGR) of 8.7% from its current value of USD 63.11 billion in 2021 to USD 133.92 billion by 2030 (*5*).

In addition to having a high nutritional value, cheese can be a chance as a probiotic carrier due to providing an anaerobic medium for probiotic bacteria which is caused by protein-fat content. This complex form of cheese supports the survival of probiotic bacteria by protecting them from the acidic environment of the gastrointestinal tract (*6*). In the studies of various whey cheeses containing probiotics, an adequate number of viable bacteria were obtained (7-*10*). Moreover, cheese has been indicated to have a positive effect on some diseases. Cheese contains high levels of saturated fatty acids (SFAs), which are commonly negatively associated with increasing blood LDL-cholesterol levels, often considered an important marker for cardiovascular disease (CVD) risk. However, recent studies demonstrated that other nutrients present and the chain lengths of the SFAs in food have much more impact on this relationship. Cheese has been reported to exhibit beneficially positive to neutral effect in terms of CVD risk due to its matrix and its content of vitamin K2. Vitamin K2 has been found a potential nutrient in order to inhibit vascular calcification in some human studies (*11, 12*).

On the other hand, it has been well known that composition, water activity, acidity, storage media and packaging type affects the microbial origin spoilage and therefore durability of food (*2*). Therefore, manufacturing, packaging and storage processes of fresh cheeses should be carefully observed because of their short shelf-life under aerobic conditions (13). In recent years, natural ways have been preferred in order to protect foods and increase their shelf life. Some herbs are potential alternatives to prevent food spoilage due to its strong antimicrobial activity against food-borne pathogens. In addition, herbs draw attention in food fortification for their flavoring properties and strong antioxidant activities due to their high levels of phenolic compounds (*14*).

Origanum onites L. (oregano) and *Rosmarinus officinalis L.* (rosemary) are both aromatic plants of the Lamiaceae family which are located around the Mediterranean region. Depending on its antiseptic, antimicrobial and antioxidant activities oregano is widely used in food industry, alcoholic beverages, culinary and also perfumery due to its flavor and smell (*15*). According to Becer *et al.* (*16*) *Origanum onites L.* exhibited high total phenolic contents and the main components of its essential oil are carvacrol, γ-terpinene and *p*-cymene. *Rosmarinus officialis L.* is classified as woody and aromatic plant and can be added to foods as a flavor ingredient or to a traditional medicine because of its anti-inflammatory, antidiabetic, antimicrobial, and antiviral properties. In addition, rosemary has superior antioxidant activity because of its high phenolic acid, mainly rosmarinic acid content (*17*).

Oregano and rosemary have been used in cheese production for their potential antioxidant and antimicrobial activities in different studies (*14,17-20*). Modified atmosphere packaging (MAP) has become a preferred packaging method due to the increasing consumer demand for fresh and preservative-free food products. In this method, the food is packaged with an atmosphere-covered material containing varying levels of CO_2 and N_2 thus reducing the physicochemical changes and oxidation reactions, prolonging the shelf life and making the appearance of the food better (*21-23*). Some studies have investigated the influence of MAP on some characteristics of fresh soft cheeses such as Mozzarella, Queso Fresco, Minas Frescal and Domiati (*23-26*).

Fresh whey cheese can be contaminated basically after obtaining the curd; hence, packaging seems necessary and useful to constrain microbial contamination. Considering that the durability of fresh whey cheeses is less than 7 days, this method attracts a lot of attention (*13*). Several studies also researched the role of using MAP on durability of different whey cheeses such as Requeijão, Anthotryros, Myzithra Kalathaki, Ricotta and Skuta (*1,2,13,21,27*). On the other hand, a few studies investigated physicochemical, microbiological and sensory features of Lor cheese stored under different packaging conditions including MAP. Temiz *et al.* (*22*) determined that the MAP with 70 % CO_2 / 30 % N_2 is more effective in preventing food spoilage bacteria, yeast and mold than vacuum packaging and the other MAP varieties (40 % CO_2 / 60 % N_2; 60 % CO_2 / 40 % N_2) in Lor cheese. The authors also observed that cheeses under MAP containing 60 % and 70 % CO_2 provided good sensory attributes during 45 days whereas air and vacuum packaged cheese samples were not accepted after 10 days of storage. In another study, the influence of MAP1 (80 % CO_2 / 20 % N_2) and MAP2 (60 % CO_2 / 40 % N_2) in comparison to air and vacuum packaging on microbiological and sensory properties of Lor cheese was researched. MAP1 was more useful for destroying growth of yeast and mold, total Enterobacteriaceae, and viable count. Lor cheeses with MAP processes exhibited favourable organoleptic properties during 20 days whereas control cheeses showed very low sensory scores after 10–15 days of storage (*28*).

We also tried different levels of CO_2 and N_2 for the packaging of Lor cheese and the best results for shelf life were obtained for 80 % CO_2 and 20 % N_2 combination (*29*). Even though the use of MAP to preserve different kinds of cheeses covering some whey cheeses has been studied, the effect of fortification with herbs (oregano or rosemary) and probiotic bacteria (*Lactobacillus acidophilus* (La-5) and *Bifidobacterium lactis* (Bb-12)) on the functional properties of whey cheese (Lor) during storage remains unknown. An evaluation of the physicochemical, microbiological and sensory properties, and antioxidant activities of oregano or rosemary added probiotic Lor cheeses under vacuum packaging or MAP (80 % CO_2 / 20 % N_2) was aimed in the current study.

MATERIALS AND METHODS

Whey, starter cultures, herbs, packaging material and gas mixture

Kashar cheese whey, which has 6.83 % total dry matter, 0.70 % fat, 3.49 % protein and 6.29 pH value, was obtained from Sakıpaga Dairy Products Company (Menemen, Izmir, Turkey).

The freeze-dried DVS starter cultures of *Bifidobacterium lactis* (Bb-12) and *Lactobacillus acidophilus* (La-5) (Chr.Hansen A/S, Hørsholm, Denmark) were used after an activation step (30 min at 37 °C) in UHT-sterilized skim milk (Pinar Dairy Products, Izmir, Turkey).

UV-irradiated herbs of oregano (*Origanum onites*) and rosemary (*Rosmarinus officinalis*) were obtained from Omeroglu Agricultural Products Company, Kemalpasa, Izmir, Turkey.

Packaging material was originated from Polyethylene/Terephthalate/Polyamide barrier pouches of 50-210 µm thickness, with an oxygen permeability of 30 ≥ cm^3/m^2/day/atm, nitrogen permeability of 35 ≥ cm^3/m^2/day/atm, and a water vapour permeability of 5 ≥ g/m^2/day at 100 % relative humidity. The gas mixture (80 % / 20 % (CO_2 / N_2)) used in the modified atmosphere packaging of experimental cheese samples was provided by Linde Group, Izmir, Turkey.

Probiotic Lor cheese manufacture

Cheese samples were manufactured in the plant of Sakıpaga Dairy Products (Menemen, Izmir, Turkey). Kashar cheese whey was heated at 90 °C for 5 min and then salted at a ratio of 0.1 %. The curd was collected from the surface and drained for 15 min at room condition before pressing.

Afterwards, Lor cheese was inoculated with pre-activated (*Lactobacillus acidophilus* and *Bifidobacterium lactis*) such an inoculum to attain 10^8 CFU/g and then stirred before dividing into three groups. The first group was the control cheese which contain no any herb. The other two groups were enriched with oregano and rosemary at a ratio of 2 %, respectively. Each group was packaged as 100 g portion under two conditions as modified atmosphere (80 % CO_2 and 20 % N_2) and vacuum. Packaging of all experimental cheeses was completed within 1 hour and cheese samples were kept at 4 °C for 35 days for experiments.

Physicochemical analyses (total solid, fat, protein and salt) were performed at the beginning of the storage. Microbiological and sensory characteristics, and also titratable acidity and pH values were determined weekly throughout 35 days of storage. Antioxidant activity, phenolic content and proteolytic activity were determined on 1st, 14th and 28th days of storage.

Physicochemical analyses

The pH value of cheeses was stated by a pH meter (Hanna Instruments Model pH: 211; Woonsocket, RI, USA). The total solids were determined with a standard method of measuring weight loss after drying (*30*). Fat content of the cheeses was analyzed by Gerber method (20). Titratable acidity was expressed as g of lactic acid/100 g after mixing 10 g of cheese sample with 10 mL of hot distilled water and titrating with 0.1 N NaOH using 1 % phenolphthalein indicator. Salt analysis was performed by the titration method with 0.1 N AgNO3 (30). Total protein was determined by Kjeldahl method and calculated by multiplying the total nitrogen content (AOAC 920.123-1920) (*31*), by a factor of 6.38.

Microbiological analysis

The viability of probiotic bacteria in the whey cheese samples was determined according to Akalın and Ünal (*32*). 10 g of each sample was diluted with 90 mL of Ringer solution (Ringer Tablets, Merck, Darmstadt, Germany) and mixed uniformly with a vortex mixer (Heidolph Reax top Vortex, Wood Dale, North America). Subsequent serial dilutions were prepared and the bacteria were counted using the pour plate technique. MRS-NNLP (nalidixic acid, neomycin sulfate, lithium chloride, and paramomycin sulfate, Merck, Darmstadt, Germany) agar was used to determine the viable counts of *Bifidobacterium animalis* subsp. *lactis* Bb-12. The incubation was held anaerobically (using anaerobic jars, Merck, Darmstadt, Germany) at 37 °C for 72 h. The viability of *Lactobacillus acidophilus* La-5 were determined on MRS-Sorbitol (Merck, Darmstadt, Germany) agar and after an incubation at 37 °C for 48 h in anaerobic jars (Merck, Darmstadt, Germany).

Total phenolic content (TPC) and antioxidant activity

Total phenolic content of cheese samples was defined by using Folin-Ciocalteu method (*33*). 10 g of cheese sample was centrifuged (Sigma Centrifuge, Model 3-16K, SciQuip Ltd., Newtown, Wem Shropshire, England) at 9383×*g* for 25 min, filtered using Whatman no. 1 filter paper (Global Life Sciences Solutions, Buckinghamshire, UK) and the supernatant was used in the method. 0.1 mL of sample extract was mixed with 6 mL of dH2O and 0.5 mL of Folin-Ciocalteu's reactive (Folin-Ciocalteu's reagent, E463562, Carlo Erba Reagents, Val de Reuil, France) and waited for 2 min. Then, 1.5 mL of 20 % (*m*/*m*) sodium carbonate (Merck, Darmstadt, Germany) was added. After keeping the mixture at room temperature and in dark for 2 h the absorbance was read by spectrophotometer (Spekol 1300, Analytik Jena, Jena, Germany) at 760 nm. TPC of cheeses was compared to a gallic acid standard curve and the total phenolic content was expressed as milligrams gallic acid (Sigma-Aldrich Chemie, USA) equivalents (GAE) per liter of sample. The equation of the gallic acid standard curve is given below and the correlation coefficient was R^2=0.9987.

$$y=0.0014x+0.0964 \quad /1/$$

The antioxidant activity was determined using 2,2,-diphenyl-1-picrylhydrazyl (DPPH•, Sigma-Aldrich Chemie, USA) radical scavenging activity method (*34*). A 0.1 mmol/L DPPH• radical solution in 95 % ethanol was prepared. Eight milliliters of ethanolic DPPH• solution was placed in a 50-mL centrifuge tube and mixed with 2 mL of cheese sample or 95 % ethanol (as control), vortexed (Heidolph Reax top Vortex, Wood Dale, North America) well, and then incubated for 30 min at room temperature in dark. The samples were then centrifuged for 10 min at 9383×*g* at room temperature. Supernatants were filtered using Whatman no. 40 filter paper (Global Life Sciences Solutions, Buckinghamshire, UK). Absorbance of each sample was measured at 517 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Sigma-Aldrich Chemie, USA) was used as a reference antioxidant at a concentration of 0.25 mg/mL. All spectrophotometric analyses were done by using an UV-Visible spectrophotometer (Spekol 1300, Analytik Jena, Jena, Germany).

Proteolytic activity

The proteolytic activity of cheese samples was determined by using the o-phthaldialdehyde (OPA) method, which evaluates the liberated amino acids and peptides (*35*). In the method, OPA reagent (50 mL of 1000 mmol/L sodium tetraborat, 5 mL of 20 % (*m*/*m*) sodium-dedecyl-sulphate, 80 mg OPA dissolved in 2 mL of methanol and 200 μL of β-mercaptoethanol top up with dH2O until final volume is 100 mL) was used and the absorbance of the solutions was measured using a spectrophotometer (Spekol 1300, Analytik Jena, Jena, Germany) at 340 nm. The cheese water extract was prepared by mixing 5 g of cheese sample with 5 mL of 24 % trichloroacetic acid (Sigma-Aldrich Chemie, USA) and removed the mixture at the room temperature for 1 h. After centrifugation of the mixture at 3743×*g* at 4 ºC for 20 min, it was filtered using Whatman no. 42 filter paper (Global Life Sciences Solutions, Buckinghamshire, UK) and the supernatant was used in the method.

Sensory characteristics

Sensory properties were evaluated according to IDF (*36*). Eight experienced academicians (ages 30–55) of both genders (4 men, 4 women) from Dairy Technology Department (Ege University, Izmir, Turkey), who are experts in evaluating the organoleptic procedures of cheese, conducted the study. The evaluated sensory characteristics were appearance, consistency, odor and taste and the scores were based on 5-point hedonic scales

(1: dislike extremely; 5: like extremely). The scoring was done individually, and the cheeses, that were coded with 3 digits, were given in plastic containers to the panelists. Water was also given to the panelists in order to rinse their mouth.

Statistical analysis

The experiments, including the cheese manufacture, were performed in triplicate. The analysis was done by one-way ANOVA using the general linear model (GLM) procedure of the STATISTICA software, v. 25.0 (*37*). The means were compared with Duncan multi-comparison test at the p<0.05 level.

RESULTS AND DISCUSSION

Physicochemical properties

The contents of total solids, protein, fat, and salt are given in Table 1. As hoped, the supplementation of cheese with oregano or rosemary increased the total solids content of Lor cheese (p<0.05) whereas neither the addition of herb nor the packaging condition significantly affect the fat, protein and salt amounts (p>0.05). The obtained results for protein, total solid, and fat content of control samples kept under vacuum and MAP conditions were in parallel to the results of Temiz *et al.* (*22*). The pH and titratable acidity of the samples changed between 4.12-5.42 and 0.38-1.52 %, respectively, during storage (Fig. 1). Although there was a slight fluctuation towards the 35th day, the pH declined in all cheeses (p<0.05) throughout the storage in parallel with the rise in acidity. The increase in acidity values can be caused by the post-acidification, which is related to the degradation of sugars by non-starter lactic acid bacteria and probiotics during storage (*10*). Similar changes were observed in pH values for Lor cheese by Temiz *et al.* (*22*), Irkin (*28*) and Akpinar *et al.* (*20*), and for some other whey cheeses in the study of Papaioannou *et al.* (*13*), Cabral *et al.* (*25*) and Silva *et al.* (*10*) Compared to other studies, the lower pH values in our study can be caused by the presence of probiotic bacteria present in the cheese samples. Almedia *et al.* (*38*) reported that culture composition in fresh cheese whey affects the pH level and the acidifying rates of the product.

Table 1

The type of packaging significantly influenced the pH and acidity attributes of cheeses. The pH value of the herb-free control cheese packaged under MAP condition was lower than in vacuum packaging (p<0.05). Carbonic acid formed in the presence of CO_2 was attributed as the cause of the decrease in pH value in packaging under MAP condition (*21, 26*). In addition, the drop observed in pH values may also be caused by the free fatty acids and acidic amino acids being produced during lipolysis and proteolysis, respectively (*2*). Lower pH values during the storage period were observed in modified packaged fresh cheeses when compared to vacuum packaged ones in also some other studies (*23, 24*). In contrast, Silva *et al.* (*10*) could not observe notable differences in pH values of Minas Frescal fresh cheeses, manufactured with probiotic bacteria, packaged under vacuum packaging or MAP condition for 21 days.

Fig. 1

When Lor cheeses under MAP conditions were examined, control cheese generally had higher titratable acidity and lower pH values compared to the herb-added especially for rosemary-added samples possibly depending on its antibacterial effect. de Barros Fernandes *et al.* (*18*) detected lower pH values in control Minas Frescal cheese than rosemary essential oil added sample during 15 days of storage. The authors attributed this situation to the protective effect of rosemary essential oil on the proliferation of spoilage microorganisms. Similarly, Diniz-Silva *et al.* (*39*) observed higher lactic acid (%) and lower pH and values in herb-free control probiotic Minas Frescal fresh cheese, comprising *Lactobacillus acidophilus* La-5, when compared to sample with essential oils from oregano and rosemary in combination. Lower acidity of oregano and rosemary added probiotic cheese containing essential oils has been attributed to the subinhibitory effect of the essential oils that slowdown the *L. acidophilus* La-5 metabolism and so a lower fermentation rate of lactose and a reduced concentration of lactic acid.

On the other hand, the acidity did not differ between oregano and rosemary added Lor cheese packaged under vacuum during 35 days (p>0.05). This can be due to the similar viability of probiotic bacteria, and so their similar metabolic activity in these cheese samples.

Microbiological properties

The viable counts of both *Bifidobacterium animalis* subsp. *lactis* Bb-12 and *Lactobacillus acidophilus* La-5 (Table 2) were minimum 8 log CFU/g in all experimental cheeses during refrigerated storage, thus exceeding the minimum recommended level (6 log CFU/g) in probiotic foods to ensure potential benefits (*40*). Both probiotic bacteria were enumerated between 6-8 log CFU/g in Cottage (*41*) and more than 8 log CFU/g in Prato (*7*) cheeses. Irkin and Yalcin *(8)* detected similar viable counts of *Lactobacillus acidophilus* NRRL B 4495 and *Bifidobacterium animalis* NRRL B41410 as above 7 log CFU/g in salted Lor cheese.

Table 2

The viable counts of *B. lactis* and *L. acidophilus* did not significantly change in control and oregano added cheeses while it increased in rosemary added ones during storage ($p<0.05$). This may be due to the fact that the antimicrobial effect of rosemary initially delayed the growth of probiotic bacteria in cheese. Diniz-Silva *et al.* (*39*) observed also a slowdown on the growth of *L. acidophilus* LA-5 in Minas Frescal cheese with oregano and rosemary oils at the beginning of storage.

In addition, packaging condition significantly affected the viable counts of *B. lactis* in cheeses with rosemary in the first 14 days of storage and in control cheese on day 1 in favor of MAP. *B. lactis* viability in oregano added sample did not differ between MAP and vacuum packaging conditions ($p>0.05$). MAP also provided higher counts of *L. acidophilus* in control and rosemary added cheeses at the beginning and in the first week of storage, respectively, when compared to vacuum packaging ($p<0.05$). The presence of high levels of carbon dioxide is a growth factor for anaerobic bacteria (*42*), so that the growth of probiotic bacteria may have been positively affected by MAP when compared to vacuum packaging especially in the first days of rosemary added ones. These results showed that the use of MAP could be a good alternative in terms of packaging in the dairy industry.

In addition, coliform group bacteria and *E. coli* could not be detected in experimental cheese samples during storage (data not shown). This can be caused by the rised acidity during storage, the protective effect of packaging conditions or the antibacterial activity of the herbs used.

Total phenolic content and antioxidant activity

The total phenolic content (TPC) and DPPH$^{\bullet}$ scavenging activity of Lor cheeses during storage are given in Table 3. TPC of Lor cheeses were detected between 133.64 and 494.36 GAE mg/L. The addition of herb significantly increased the TPC of cheese samples in both MAP and vacuum conditions. The rosemary added Lor cheese had the highest TPC at the beginning of the storage regardless of packaging type whereas the highest contents were detected in oregano or rosemary added samples under MAP condition on 14th and 28th days. Storage time significantly influenced TPC of most of the Lor cheeses. Especially herbal cheeses in MAP condition was protected better in terms of TPC during storage compared to vacuum packaging.

The antioxidant activity of the Lor cheese samples varied between 93.65 to 96.94 %. The reference antioxidant, Trolox (0.25 mg/mL), exhibited a DPPH$^•$ scavenging activity of 97.09 %. The control Lor cheeses had considerably high DPPH$^•$ scavenging activity during storage probably due to the antioxidant activity of whey proteins and proteolytic activity of probiotics releasing antioxidant peptides. Proteolytic microorganisms in cheese can generate bioactive peptides with antioxidant activity (*43*).

Table 3

The addition of oregano or rosemary significantly increased the antioxidant activity ($p<0.05$) in parallel to TPC results. MAP improved the antioxidant activity more than vacuum packaging in herb added Lor cheeses after first day. Rosemary added Lor cheese under MAP had the highest DPPH$^•$ scavenging activity throughout the storage and the similar antioxidant activities were determined in oregano added cheeses on days 14 and 28 under MAP. The antioxidant activity of all herb added Lor cheeses reduced ($p<0.05$) on day 28 when compared to 1st day except MAPO. It has been shown that fortification with oregano or rosemary enhanced TPC and DPPH$^•$ scavenging activity in different cheeses when compared to the plain samples (*14,17,19*). However, no study has been found on the effect of packaging type on antioxidant properties in herb-added cheeses. Thus, this study can be an option in the dairy industry in terms of obtaining whey cheese with functional properties.

It is known that the antioxidant activity of oregano and rosemary originates from phenolic compound content which mostly affected by the genetic factors, the subspecies, where it grows up, and the harvest step of the plant as well as processing methods (*44*).

The main phenolic compounds contained in *Origanum onites* are as follows, carvacrol, thymol, γ-terpinene and p-cymene (*15,16*). *Rosmarinus officinalis* also contain phenolic acids (mainly caffeic acid and rosmarinic acid), consequently possess high antioxidant capacity (*45*).

Proteolytic activity

The proteolytic activity of Lor cheeses is given in Fig. 2. The proteolytic activity of the experimental cheeses varied between 0.059-0.991 nm and fluctuated during storage. The proteolytic activity increased in all experimental cheeses 14th day but a decrease on day 28 ($p<0.05$). The reduction in free NH_3 groups on 28th day may have resulted probably from the utilization of free amino groups by starter culture bacteria.

Fig. 2

The proteolysis was more prominent in modified atmosphere packaged herb-added samples compared to those packaged under vacuum. Similarly, probiotic Minas Frescal cheese, containing *Bifidobacterium animalis* subsp. *lactis* Bb-12, packaged under modified atmosphere (1:1, CO_2 and N_2) was found to have higher proteolytic activity than packaged under vacuum. The absorbance indicating proteolytic activity was found lower in Minas Frescal cheese than those of our cheese samples due to the different manufacturing processes (*10*).

Lor cheese fortified with rosemary and under MAP condition had the highest proteolytic activity during the storage period, parallel to the antioxidant activity of the sample ($p<0.05$). It is known that one of the biological activities that bioactive peptides formed during proteolysis is antioxidant activity which is emerged by the hydrolysis of serum proteins (*46,47*).

The high proteolysis of the sample MAPR may also be due the proteolytic activity of the starter probiotic bacteria. It is known that several Bifidobacteria strains have low proteolytic activity whereas several *L. acidophilus* strains can secrete a great number of peptidases, amino, di and tripeptidases, and also proline-specific peptidases (*48*). Proteolysis pathways of semi-hard probiotic cheeses manufactured either with the single culture of *L. acidophilus* or the mixed probiotic culture (*Bifidobacterium lactis*, *Lactobacillus paracasei* and *Lactobacillus acidophilus*) were very analogous and *L. acidophilus* has been reported to play a great role in proteolysis because of its stronger proteolytic activity than other culture bacteria. In the same study, the level of most of the free amino acids was increased by *L. acidophilus* (*49*). This is in line with the fact that *L. acidophilus* was more dominant than *B. lactis* in terms of viable bacteria in the MAPR sample in our study.

Sensory characteristics

Sensory evaluation is given in Fig. 3. Appearance and texture scores of all samples were close to each other during storage ($p>0.05$). Similarly, Minas Frescal fresh cheese with and without oregano and rosemary essential oils also received similar scores for texture, color and appearance for 21 days (*39*). Packaging variety did not significantly affect the taste of control and oregano added cheeses which was similar to the study of Silva *et al*. (*10*). Maniar *et al.* (*50*) also stated that sensory properties of cheese were not affected by CO_2. MAP improved taste scores only in rosemary added cheese.

Fig. 3

Panelists gave the highest taste and aroma scores to rosemary added cheeses under MAP throughout the storage period. Considering that taste and aroma play a very important role in consumer acceptance of a food, this result indicates that rosemary can be used in cheese manufacture in dairy technology and thus will contribute to the functional cheese market. Supplementation with oregano positively affect taste characteristic of Lor cheese on 14th and 21st days when compared to control sample for both packaging conditions. When all experimental cheeses are compared in terms of aroma, it can be seen that they sorted as MAPR>VR>MAPO>VO>MAPC>VC (Fig. 3). Similar tendency was obtained in favor of MAP in Lor cheese (*22*), Domiati cheese (*26*), Myzithra Kalathaki cheese (*2*) and Mozzarella cheese (*24*).

In another study, Tween 80 added nanoemulsion of oregano oil-in water (O/W) did not have any effect on the aromatic profile of Anthotyros whey cheese when compared to control cheese without oregano (*14*). In contrast, Akpinar *et al.* (*20*) observed lower sensory records in rosemary fortified Lor cheese than in thyme added Lor cheese probably due to the different manufacture process and packaging method, unlike our study.

Significant reductions in taste and aroma properties of all experimental cheeses were observed on day 35 ($p<0.05$) which was similar to the study of Irkin (*28*). This may be a reflection of increased proteolysis and so higher amounts of free NH_3 groups in all cheese samples at the end of storage. It has been reported that the increased total free amino acid content released as a result of proteolysis may cause undesirable flavors such as malt and bitterness in cheese (*51*).

Ribeiro *et al.* (*52*) reported that emerging sensory methodologies (ESM) have been used as alternatives to conventional organoleptic analysis in recent years. The authors interpreted these methods in four groups as predefined terms, free description, global differences and similarities, and comparisons with references. Studies on cheese using these methods allowed the discrimination of the cheese samples in terms of different sensory parameters (creamy, salty, bitter taste, shiny, spreadability, matte etc.), and product type and manufacture process (maturation time, culture used, protected designation of origin, milk type, cheese composition etc.). Another innovative method called as "text highlighting technique" at which consumers are asked to mark passages indicating "likes" and "dislikes" in a text with relevant content, has been used for Minas Frescal cheese (*53*). If it is determined which of these methods to be chosen according to the product characteristics, it can be said that this will be beneficial for the dairy industry and should be taken into consideration in future studies.

CONCLUSIONS

Modified atmosphere packaging draws attention to preserve the functional properties of foods. In this study, the influence of oregano or rosemary addition on functional characteristics of probiotic whey cheese "Lor" under MAP or vacuum was investigated. High viability of both *B. lactis* and *L. acidophilus* was detected for all experimental cheeses throughout storage. TPC and antioxidant activity increased with herb addition and MAP improved the antioxidant activity more than vacuum packaging in herb added Lor cheeses.

Rosemary added cheeses under MAP generally exhibited superior properties in terms of probiotic viability, DPPH$^\bullet$ scavenging activity and proteolytic activity when compared to the sample added with oregano under same condition. Higher taste and aroma scores were also obtained in rosemary added cheeses packaged under modified atmosphere when compared to oregano added ones. As can be seen from the results obtained, packaging under modified atmosphere and the addition of rosemary at a ratio of 2 % offer an advantage to obtain a functional Lor cheese with high antioxidant activity while maintaining acceptable sensory properties and probiotic viability.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

G. Ünal and A. S. Akalın created the concept of the research experimental design, as well as reviewed and edited the manuscript. G. Ünal and D. E. Akyıl collected, analysed, and interpreted the data, and drafted the manuscript. All authors contributed equally. All authors have read and agreed to the final version of the manuscript.

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Table 1. Composition of probiotic Lor cheeses

Cheese sample	*w*(total solids)/(g/100g)	*w*(fat)/(g/100g)	*w*(protein)/(g/100g)	*w*(salt)/(g/100g)
MAPC	(31.52±0.50)^{BC}	(16.00±1.15)^{A}	(9.95±0.11)^{A}	(0.33±0.05)^{A}
VC	(30.81±0.45)^{C}	(16.00±1.15)^{A}	(10.13±0.31)^{A}	(0.30±0.00)^{A}
MAPO	(33.10±0.34)^{A}	(16.00±1.15)^{A}	(10.00±0.25)^{A}	(0.30±0.00)^{A}
VO	(32.97±0.69)^{A}	(15.00±0.00)^{A}	(10.04±0.04)^{A}	(0.30±0.00)^{A}
MAPR	(32.30±0.93)^{AB}	(16.00±1.15)^{A}	(10.17±0.33)^{A}	(0.30±0.00)^{A}
VR	(32.84±0.99)^{A}	(15.00±0.00)^{A}	(10.00±0.04)^{A}	(0.30±0.00)^{A}

$^{A–C}$Mean values±standard deviations in the same column with different superscript uppercase letters are significantly different (p<0.05). MAPC=control probiotic Lor cheese under MAP, VC=control probiotic Lor cheese under vacuum, MAPO=probiotic Lor cheese containing 2 % oregano under MAP, VO=probiotic Lor cheese containing 2 % oregano under vacuum, MAPR=probiotic Lor cheese containing 2 % rosemary under MAP, VR=probiotic Lor cheese containing 2 % rosemary under vacuum

Table 2. Changes in the viable counts of *B. lactis* and *L. acidophilus* during refrigerated storage of probiotic Lor cheeses

Product	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35
			B. lactis *N*/(log CFU/g)			
MAPC	(8.68±0.17)^{ABa}	(8.70±0.20)^{Aa}	(8.70±0.19)^{Aa}	(8.70±0.17)^{ABa}	(8.69±0.16)^{ABa}	(8.79±0.23)^{Aa}
VC	(8.47±0.06)^{Ca}	(8.76±0.20)^{Aa}	(8.60±0.34)^{Aa}	(8.60±0.23)^{Ba}	(8.58±0.21)^{Ba}	(8.68±0.26)^{Aa}
MAPO	(8.68±0.06)^{ABabc}	(8.46±0.05)^{Bc}	(8.54±0.03)^{ABbc}	(8.48±0.05)^{Bbc}	(8.86±0.06)^{Aa}	(8.77±0.36)^{Aab}
VO	(8.79±0.02)^{Aa}	(8.67±0.02)^{Ab}	(8.56±0.05)^{ABc}	(8.57±0.05)^{Bc}	(8.83±0.06)^{Aa}	(8.86±0.03)^{Aa}
MAPR	(8.60±0.06)^{BCc}	(8.78±0.01)^{Ab}	(8.75±0.07)^{Ab}	(8.89±0.02)^{Aa}	(8.74±0.02)^{ABb}	(8.71±0.06)^{Ab}
VR	(8.07±0.06)^{Dd}	(8.19±0.01)^{Cc}	(8.26±0.03)^{Bb}	(8.91±0.01)^{Aa}	(8.91±0.01)^{Aa}	(8.86±0.05)^{Aa}
			L. acidophilus *N*/(log CFU/g)			
MAPC	(8.81±0.22)^{Aa}	(8.93±0.26)^{ABa}	(8.85±0.21)^{Ba}	(8.97±0.28)^{ABa}	(8.95±0.27)^{ABa}	(9.05±0.32)^{ABa}
VC	(8.59±0.18)^{Ba}	(8.90±0.24)^{ABa}	(9.02±0.30)^{ABa}	(8.89±0.23)^{ABa}	(8.88±0.27)^{Ba}	(9.00±0.27)^{ABa}
MAPO	(8.96±0.03)^{Aab}	(8.78±0.52)^{ABb}	(8.81±0.03)^{Bb}	(8.76±0.01)^{Bb}	(9.22±0.03)^{Aa}	(8.80±0.01)^{Bb}
VO	(8.99±0.02)^{Ab}	(8.87±0.06)^{ABc}	(9.19±0.02)^{Aa}	(8.96±0.01)^{ABb}	(9.14±0.02)^{ABa}	(9.15±0.00)^{Aa}
MAPR	(8.97±0.02)^{Ae}	(9.06±0.01)^{Ac}	(8.99±0.00)^{ABd}	(9.17±0.02)^{Aa}	(9.13±0.01)^{ABb}	(9.06±0.01)^{ABc}
VR	(8.16±0.07)^{Ce}	(8.53±0.06)^{Bd}	(9.01±0.03)^{ABc}	(9.16±0.06)^{Ab}	(9.15±0.01)^{ABb}	(9.29±0.03)^{Aa}

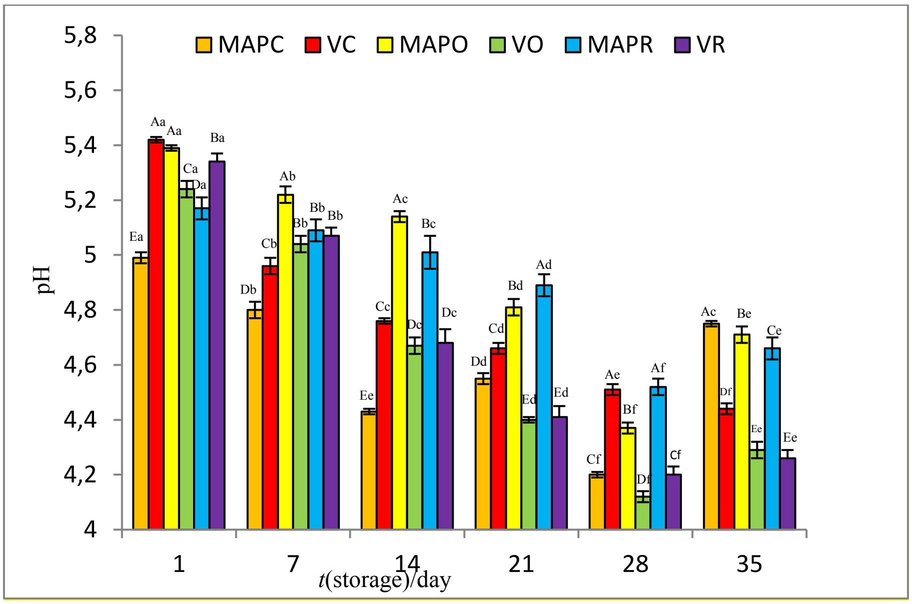
$^{a–e}$Mean values±standard deviations in the same row with different superscript lowercase letters are significantly different (p<0.05). $^{A–D}$Mean values±standard deviations in the same column with different superscript uppercase letters are significantly different (p<0.05). MAPC=control probiotic Lor cheese under MAP, VC=control probiotic Lor cheese under vacuum, MAPO=probiotic Lor cheese containing 2

% oregano under MAP, VO=probiotic Lor cheese containing 2 % oregano under vacuum, MAPR=probiotic Lor cheese containing 2 % rosemary under MAP, VR=probiotic Lor cheese containing 2 % rosemary under vacuum

Table 3. Total phenolic content and antioxidant activity of probiotic Lor cheeses during storage

Product	Day 1	Day 14	Day 28
γ(total phenols as GAE)/(mg/L)			
MAPC	$(167.57\pm6.78)^{Ca}$	$(180.97\pm19.88)^{Da}$	$(133.64\pm2.71)^{Eb}$
VC	$(168.46\pm9.00)^{Ca}$	$(175.79\pm14.10)^{Da}$	$(159.00\pm8.92)^{Da}$
MAPO	$(400.25\pm17.57)^{Ba}$	$(395.43\pm5.83)^{Aa}$	$(408.46\pm16.23)^{Aa}$
VO	$(395.79\pm17.17)^{Ba}$	$(348.47\pm9.52)^{Cb}$	$(291.50\pm7.39)^{Cc}$
MAPR	$(491.50\pm9.20)^{Aa}$	$(409.36\pm3.17)^{Ab}$	$(409.00\pm11.58)^{Ab}$
VR	$(494.36\pm10.34)^{Aa}$	$(375.86\pm16.64)^{Bb}$	$(348.46\pm16.23)^{Bc}$
$DPPH^{\bullet}$ scavenging activity/%			
MAPC	$(93.65\pm0.04)^{Ca}$	$(93.78\pm0.33)^{Ca}$	$(93.80\pm0.51)^{Da}$
VC	$(93.73\pm0.31)^{Cc}$	$(94.75\pm0.28)^{Ca}$	$(94.32\pm0.06)^{Cb}$
MAPO	$(95.64\pm1.62)^{Ba}$	$(95.80\pm0.30)^{Aa}$	$(94.67\pm1.47)^{Aa}$
VO	$(95.88\pm0.16)^{Ba}$	$(93.78\pm0.33)^{Bc}$	$(95.19\pm0.28)^{Bb}$
MAPR	$(96.94\pm0.17)^{Aa}$	$(95.83\pm0.07)^{Ab}$	$(95.60\pm0.67)^{Ab}$
VR	$(96.74\pm0.40)^{Aa}$	$(94.81\pm0.21)^{Bc}$	$(95.48\pm0.31)^{Bb}$

$^{a–c}$Mean values±standard deviations in the same row with different superscript lowercase letters are significantly different ($p<0.05$). $^{A–E}$Mean values±standard deviations in the same column with different superscript uppercase letters are significantly different ($p<0.05$). MAPC=control probiotic Lor cheese under MAP, VC=control probiotic Lor cheese under vacuum, MAPO=probiotic Lor cheese containing 2 % oregano under MAP, VO=probiotic Lor cheese containing 2 % oregano under vacuum, MAPR=probiotic Lor cheese containing 2 % rosemary under MAP, VR=probiotic Lor cheese containing 2 % rosemary under vacuum a)



b)

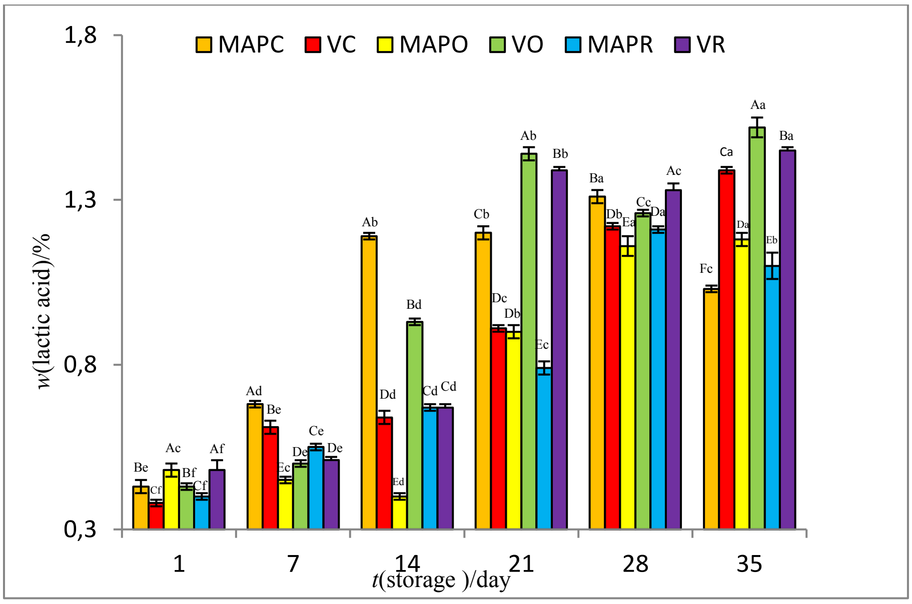


Fig. 1. pH values (a) and titratable acidity (lactic acid %) (b) of experimental cheeses during storage. MAPC=control probiotic Lor cheese under MAP (orange), VC=control probiotic Lor cheese under vacuum (red), MAPO=probiotic Lor cheese containing 2 % oregano under MAP (yellow), VO=probiotic Lor cheese containing 2 % oregano under vacuum (green), MAPR=probiotic Lor cheese containing 2 % rosemary under MAP (blue), VR=probiotic Lor cheese containing 2 % rosemary under vacuum (purple). Different superscript uppercase letters indicate significant differences among samples for the same storage period ($p<0.05$).

Different superscript lowercase letters indicate significant differences among different storage periods of the same sample (p<0.05)

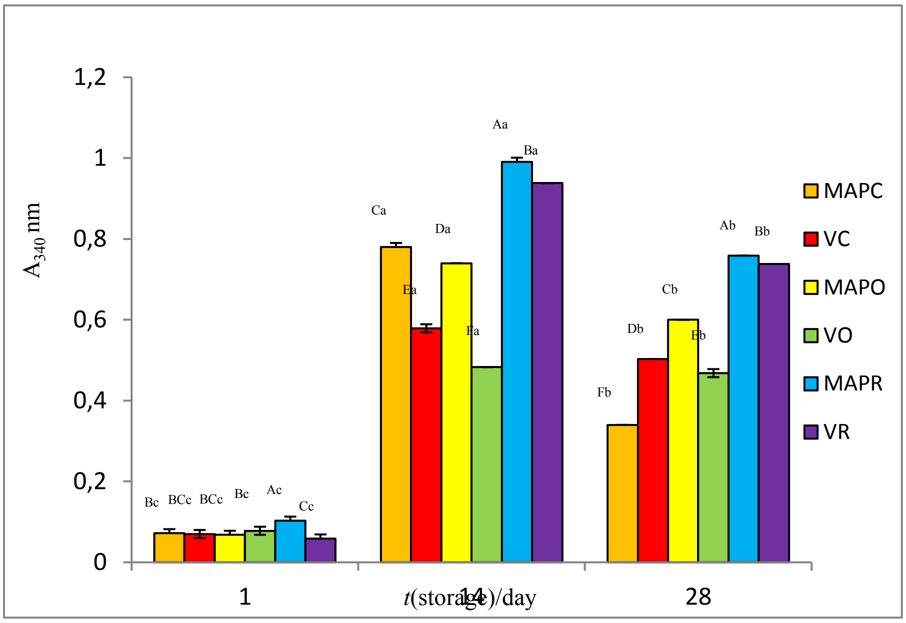
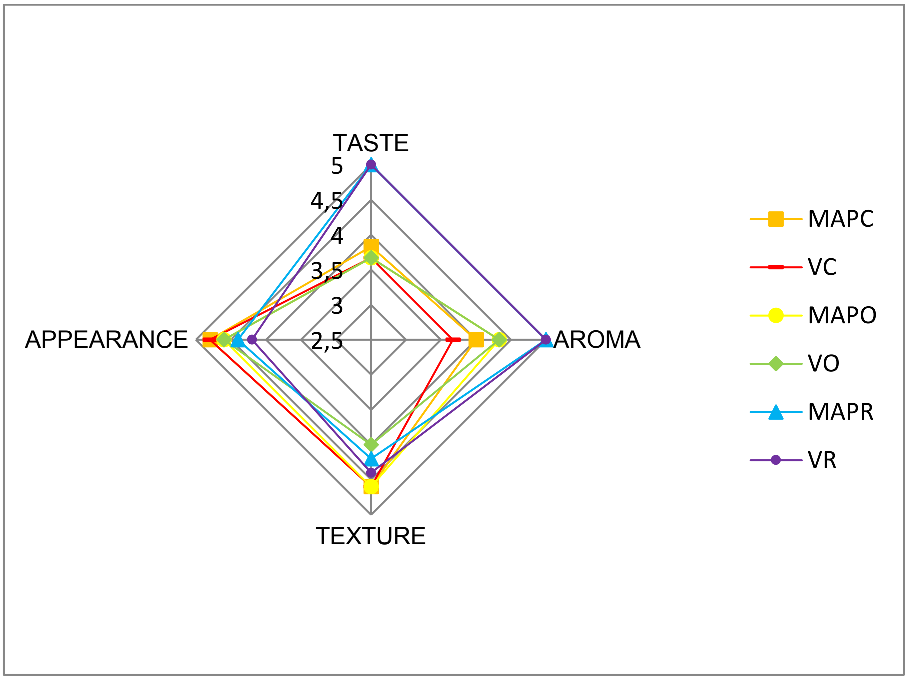
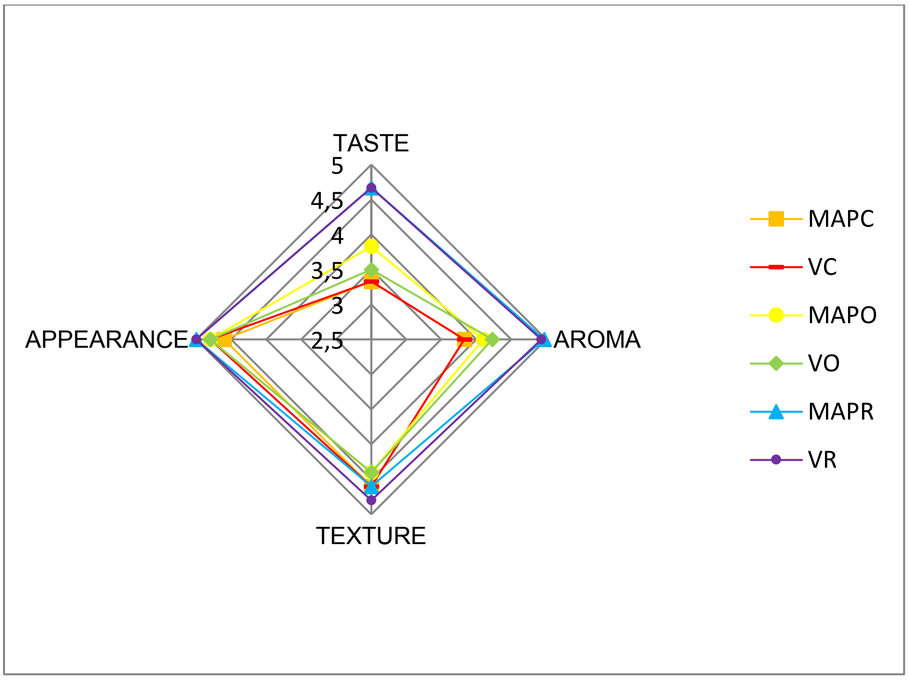


Fig. 2. Proteolysis of experimental cheeses (error bars represent SD). MAPC=control probiotic Lor cheese under MAP (orange), VC=control probiotic Lor cheese under vacuum (red), MAPO=probiotic Lor cheese containing 2 % oregano under MAP (yellow), VO=probiotic Lor cheese containing 2 % oregano under vacuum (green), MAPR=probiotic Lor cheese containing 2 % rosemary under MAP (blue), VR=probiotic Lor cheese containing 2 % rosemary under vacuum (purple). Different superscript uppercase letters indicate significant differences among samples for the same storage period ($p<0.05$). Different superscript lowercase letters indicate significant differences among different storage periods of the same sample ($p<0.05$)

a)


TASTE
5
4,5
4
3,5
3
2,5
APPEARANCE
AROMA
TEXTURE
MAPC
VC
MAPO
VO
MAPR
VR

b)


TASTE
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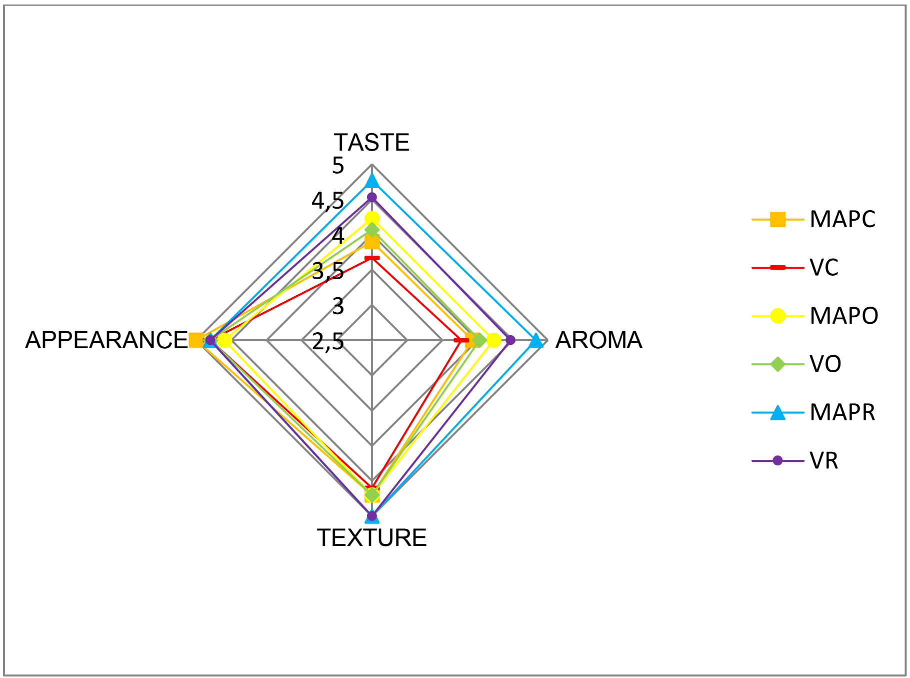
4,5
4
3,5
3
2,5
APPEARANCE
AROMA
TEXTURE
MAPC
VC
MAPO
VO
MAPR
VR

c)


TASTE
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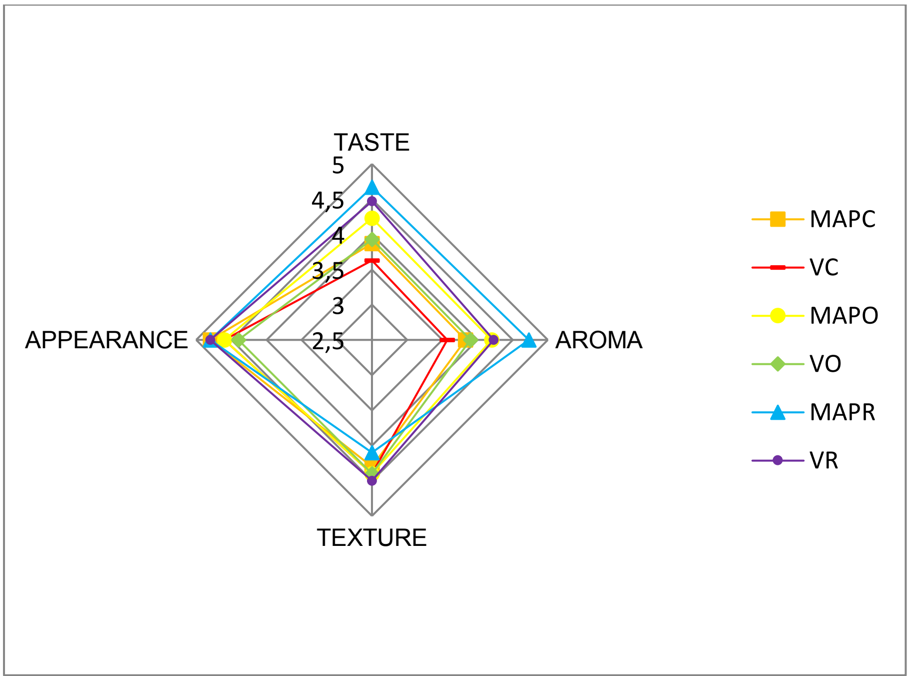
2,5
APPEARANCE
AROMA
TEXTURE
MAPC
VC
MAPO
VO
MAPR
VR

d)


TASTE
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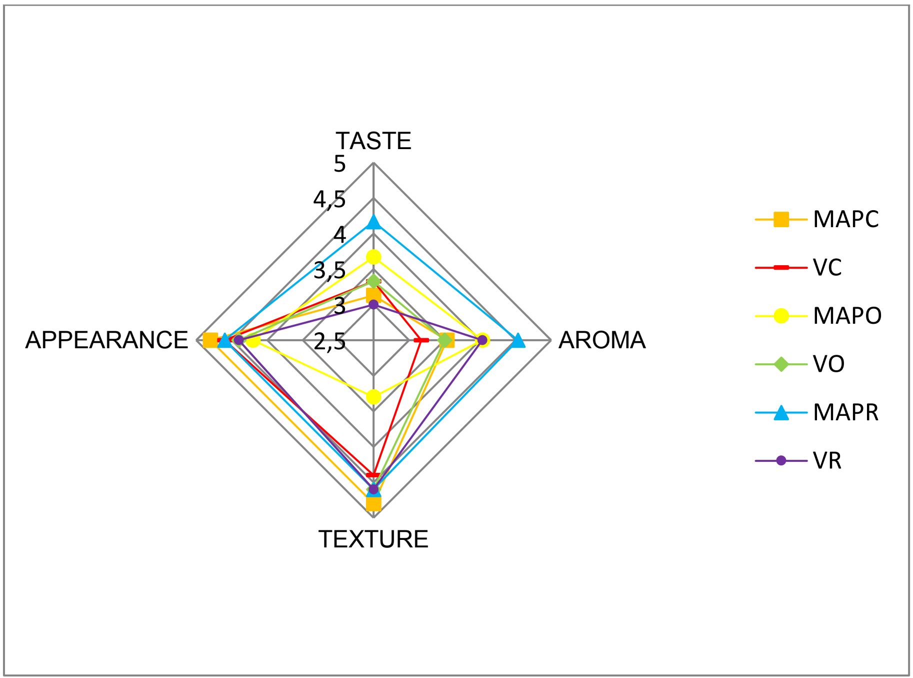
3
2,5
APPEARANCE
AROMA
TEXTURE
MAPC
VC
MAPO
VO
MAPR
VR

e)



f)

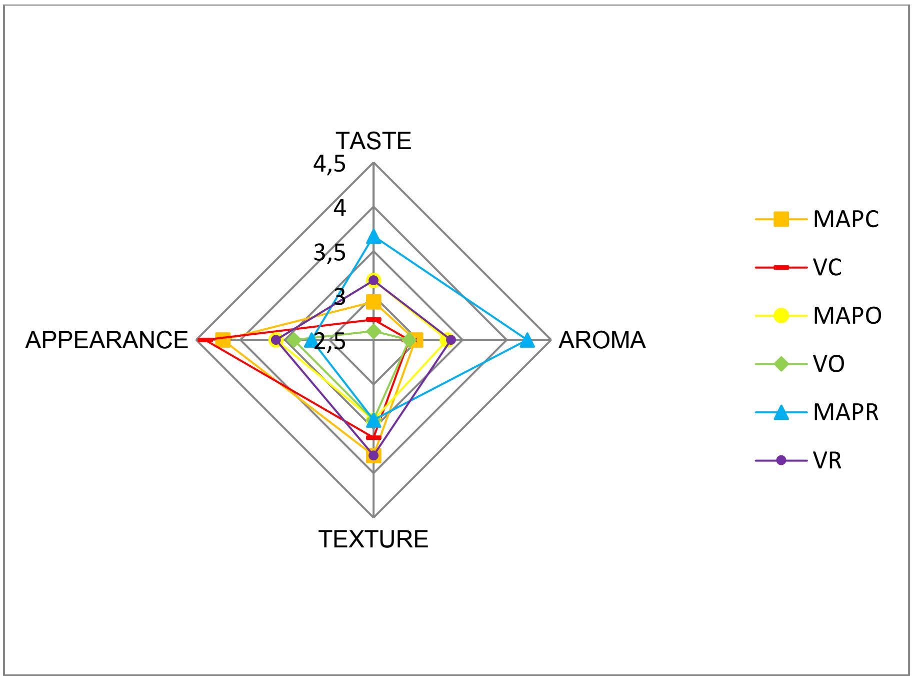


Fig. 3. Sensory characteristics of experimental cheeses on day 1 (a), day 7 (b), day 14 (c), day 21 (d), day 28 (e) and day 35 (f). MAPC=control probiotic Lor cheese under MAP, VC=control

probiotic Lor cheese under vacuum, MAPO=probiotic Lor cheese containing 2 % oregano under MAP, VO=probiotic Lor cheese containing 2 % oregano under vacuum, MAPR=probiotic Lor cheese containing 2 % rosemary under MAP, VR=probiotic Lor cheese containing 2 % rosemary under vacuum