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ORIGINAL PAPER

Effect of Packaging and Basil Essential Oil on the Quality Characteristics of Whey Cheese "Anthotyros"

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Abstract In this study, we compared the effect of basil essential oil (EO) and various packaging conditions on "Anthotyros," a Greek whey cheese. This cheese was stored at 4 °C under aerobic (A), vacuum (V), and modified atmosphere (M, 40%/60%; CO_2/N_2,) conditions, without or with (AB, VB, and VM) basil EO added to the cheese samples to a final concentration of 0.4% (*v*/*w*). The quality characteristics and the shelf life of both untreated and basil EO-treated cheese were assessed using microbiological, physicochemical, and sensory parameters. Microbiological results revealed that either modified atmosphere/vacuum packaging (MAP/VP) singly or in combination with basil EO delayed microbial growth as compared to the control (A) samples. The sensory and microbiological data showed that the combined use of MAP and VP with added basil EO extended the shelf life of fresh Anthotyros (4 °C) by approximately 10–12 days (treatment MB) and 6 days (treatment VB) as compared to aerobic packaging (A). Under these treatments, whey cheese samples maintained good sensory characteristics. This study has shown that the combined use of either VP or MAP, and basil EO, can extend the shelf life of whey cheese and maintain the freshness and the sensorial characteristics of the product.

Keywords Anthotyros · Packaging · Essential oils · Basil · Preservation · Quality

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Introduction

In Greece, popular traditional whey cheeses of major economic and nutritional importance are *Myzithra*, *Anthotyros*, and *Manouri*, manufactured commercially of the whey of *Feta* or hard cheeses (e.g., *Kefalotyri*, *Graviera*). Due to the high moisture content, low salt content, and an initial pH above 6.0, whey cheeses are susceptible to microbial spoilage and therefore have a limited shelf life, even under refrigeration (Pintado et al. 2001). The quality and safety of whey cheeses are affected during storage by the oxidation of fats, microbial growth, and loss of moisture and aroma.

"Anthotyros" is a traditional Greek Protected Designation of Origin whey cheese. Anthotyros is a soft cheese with a fat content of at least 18% and moisture content not greater than 70% (Anifantakis 1991).

Essential oils (EOs) are aromatic and volatile oily liquids obtained from plant material, and their constituents have been widely used as flavoring agents in foods since the earliest recorded history, and it is well established that many of them have wide spectra of antimicrobial action (Burt 2004). In recent years, several studies have shown the beneficial effects of natural antimicrobials, applied individually or in combination with packaging techniques, on food systems (Burt 2004; Valderrama Solano and de Rojas Gante 2011).

A number of studies have focused on the effects of EOs, such as oregano, thyme, and rosemary, among others, in combination with packaging techniques, on poultry meat (Giatrakou et al. 2010; Ntzimani et al. 2010), fish, and seafood (Atrea et al. 2009), but little is known with respect to these effects on whey or soft cheeses. Studies on Greek whey cheeses, such as on Anthotyros (Kalogridou-Vassiliadou et al. 1994; Tsiotsias et al. 2002; Papaioannou et al. 2007),

Myzithra (Govaris et al. 2001; Dermiki et al. 2008), and Manouri (Lioliou et al. 2001), have either examined the cheese quality or the effects of packaging techniques on shelf life. However, a few published reports have shown the effects of EOs, either singly or in combination with mild preservation techniques, such as packaging on cheese quality. Smith-Palmer et al. (2001) investigated the use of EOs, in particular, bay, clove, cinnamon, and thyme, added at concentrations of 0.1%, 0.5%, and 1% (*v*/*w*), against *Listeria monocytogenes* and *Salmonella enteritidis* in low- and full-fat soft cheese at 4 °C and 10 °C, respectively, over a 14-day period. The fat content of the cheese was shown to be an important factor in determining the effectiveness of the plant essential oils. In the low-fat cheese, all four oils at 1% reduced *L. monocytogenes* to ≤1·0 log cfu/ml. In contrast, in the full-fat cheese, oil of clove was the only oil to achieve this reduction. Oil of thyme proved to be ineffective against *S. enteritidis* in the full-fat cheese, yet was equally as effective as the other three oils in the low-fat cheese, reducing *S. enteritidis* to ≤1·0 log cfu/ml from day 4 onwards. It was concluded that selected plant essential oils can act as potent inhibitors of *L. monocytogenes* and *S. enteritidis* in a food product (Smith-Palmer et al. 2001). 'Therefore, the objective of the present work was to determine the combined effects of basil EO and packaging techniques, as a means of "natural" antimicrobial treatments, on both quality characteristics and shelf life of the Greek whey cheese Anthotyros stored under refrigeration (4 °C).

Materials and Methods

Sample Preparation, Packaging, and Addition of Basil EO

Samples (1.5 kg each) of commercially prepared soft whey cheese were purchased from Dodoni S.A., a local dairy plant (Ioannina, Greece). Anthotyros was processed according to the technological scheme of the company (Fig. 1). Samples were transported to the laboratory in polystyrene boxes containing flaked ice, and were used within 3 h after production. Portions (150±10 g) of cheese were packaged in low-density polyethylene/polyamide/low-density polyethylene barrier pouches (VER PACK, Thessaloniki, Greece) with thickness of 75 μm, with an oxygen permeability of 52.2 cm^3 m^{-2} day^{-1} atm^{-1} at 75% relative humidity (RH), at 25 °C, and a water vapor permeability of 2.4 g m^{-2} day^{-1} at 100% RH, at 25 °C. Six treatments were used: A: Anthotyros under aerobic packaging conditions, without basil EO added, served as the control sample, AB: Anthotyros stored under aerobic packaging conditions with basil EO added at 0.4% (*v*/*w*), V: Anthotyros stored under VP without basil EO added, VB: Anthotyros stored under VP with basil EO added at 0.4% (*v*/*w*), M: Anthotyros stored under MAP; 40%/60% (CO_2/N_2) without basil EO added, and MB: Anthotyros stored under MAP; 40%/60% (CO_2/N_2) with basil EO added at 0.4% (*v*/*w*). The gas mixture was prepared using a PBI-Dansensor model mix 9000 gas mixer (Ringsted, Denmark). Pouches were heat-sealed using a BOSS model N48 vacuum sealer (Helmut BOSS KG, Bod Homburg, Germany) connected to the gas mixer.

Pure basil EO of the genus *Ocimum basilicum* was purchased from Mane Fils Company (Le Bar-sur-Loup, France). The oil had the following composition: linalool was the most abundant component, constituting 60.6%; *epi*-α-cadinol, 11.4%; α-bergamotene, 7.9%; and γ-cadinene, 4.6%. Pure basil EO was added undiluted to the cheese samples (150±10 g) by the following procedure: a cheese sample was transferred aseptically into an open pouch, and oil was added onto the sample by using a Volar micropipette [treatments AB, VB, and MB at a final concentration of 0.4% (*v*/*w*)]. Upon addition, the oil was immediately

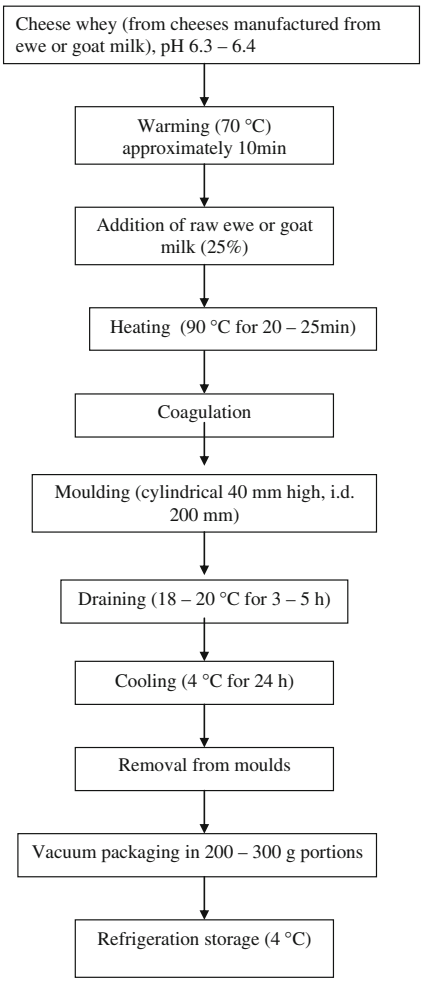

Fig. 1 Technological scheme for the manufacture of the soft whey cheese Anthotyros

massaged gently onto the cheese sample with gloved fingers to avoid cross-contamination of samples and to achieve an even distribution. After the addition of the oil, the pouches were sealed, as previously described. The optimum basil EO concentration that was subsequently used in our study was established by investigating the effects of a range of oil concentrations [0.1–1% (*v*/*w*)] on total mesophilic bacteria and the sensory (odor, taste) parameters of the cheese samples (results not shown). Cheese samples were stored in cooled incubators at 4 °C for 7 (A, AB) 15 (V, VB), and 19 (M, MB) days, respectively, and analyzed microbiologically, physicochemically, and by a sensory panel after 0, 1, 3, 5, 7 (A, AB) 11, 15 (V, VB), and 19 (M, MB) days of storage.

Microbiological Analysis

Changes in total plate counts (TPC) of lactic acid bacteria (LAB), *Enterobacteriaceae*, pseudomonads, as well as yeasts and molds, as the microbial groups mostly characterized as the natural microflora of Anthotyros cheese (Papaioannou et al. 2007) were monitored. Cheese samples (25 g) were transferred aseptically into a stomacher bag containing 225 ml of 0.1% sterile buffered peptone water (BPW; Merck, Darmstadt, Germany), and the mixture was homogenized in a stomacher (Lab Blender, Seward, London, UK) for 60 s. On each sampling day, three samples were analyzed per treatment. Appropriate dilutions of the cheese homogenates were prepared in sterile BPW (0.1%) and inoculated onto selected growth media.

Mesophilic bacteria were determined on plate count agar (Merck, Darmstadt, Germany) at 30 °C for 3 days; *Enterobacteriaceae* were determined on double-layered violet red bile glucose agar (Merck) at 37 °C for 1 day. Mesophilic LAB were enumerated on double-layered de Man Rogosa Sharpe agar (pH 6.2, Oxoid code CM 0361, Basingstoke, UK) incubated at 30 °C for 3 days. Pseudomonads were enumerated on cetrimide–fucidin–cephaloridine agar (Oxoid code CM 0559, supplemented with SR 0103) after incubation at 25 °C for 2 days. Finally, yeasts and molds were enumerated on rose bengal chloramphenicol agar (Merck) after incubation at 25 °C for 5 days. All plates were examined visually for typical colony types and morphology characteristics associated with each growth medium.

Physicochemical Analysis

The pH value of each sample was recorded using a digital pH meter (Metrohm 691, Herisau, Switzerland) equipped with a glass electrode that was immersed directly into the cheese sample for the measurement. The fat content in the aqueous phase was determined as recommended by the Association of Official Analytical Chemists (AOAC 1990). The surface color was measured using a HunterLab, model D25L optical sensor (Hunter Associates, Reston, VA, USA). The colorimeter was calibrated with a white and a black standard plate. Approximately 50 g of whey cheese was compressed into a cylindrical (base diameter 11.3 cm and height 2 cm) optical cell. Reflectance values were obtained using a 45-mm viewing aperture. The results reported are based on determination of (L^*), redness (a^*), and yellowness (b^*) values.

Sensory Analysis

On each day of sampling, sensory evaluation was carried out according to the International Dairy Federation standards (IDF 1995). Seven trained sensory descriptive panelists—members of the Laboratory of Food Microbiology—evaluated the whey cheese quality on the basis of odor and taste parameters using a 0–5 point scale (where a score of 4–5 corresponds to quality class I, no off-flavor), 3.5–3.99 corresponds to class II (initial off-flavor, but not spoiled), and a score <3.5 corresponds to quality class III (spoiled sample, unfit for human consumption). Cheese samples (30 g) were coded with randomly chosen three-digit numbers and presented in random order in 5-cm Petri dishes with lids, served at room temperature in separated individual booths with white fluorescent light. Unsalted crackers and water were used for rinsing between samples.

Statistical Analysis

Experiments were replicated twice on different occasions with different cheese samples. Triplicate samples were analyzed per replicate. Data from each replication were averaged and log transformed. These data were subjected to analysis of variance using the Statgraphics software (Statistical Graphics Corp., Rockville, MD, USA). Means and standard deviations were calculated, and, when F values were significant at the $P<0.05$ level, mean differences were separated by the least significant difference procedure.

Results and Discussion

Physicochemical Changes During Storage

Changes in physicochemical attributes (fat content and pH, Figs. 2 and 3, respectively) were monitored during storage of whey cheese samples at 4 °C, under aerobic, vacuum, or MAP conditions, both in the absence or presence of basil EO. Changes in the fat content of control and treated cheese samples under the aforementioned conditions are shown in

Fig. 2. It is apparent that the fat content of the control A and treated AB samples decreased sharply during the first week of storage, and that the presence of basil EO did not have any significant effect ($P>0.05$).

The rate of decrease in the lipid oxidation was striking since fat content would be expected to increase in cheese samples during storage, as noted by Gonzalez-Fandos et al. (2000). This reduction in cheese fat content could possibly be attributed to the absorption of moisture since water activity (a_w) values of cheese samples were not determined in our study. However, further studies are required to clarify the lipid oxidation observations.

With regard to the fat content of the V, VB, M, and MB treated samples, it is apparent that values of the fat content were significantly higher ($P<0.05$) as compared to A and AB treatments during the first week of storage. Interestingly, for the V, VB, M, and MB samples, no significant changes were recorded during the entire storage period ($P>0.05$), with the latter two treatments resulting in slightly higher values as compared to V and VB treatments. Both VP and MAP, irrespective of basil EO, reduced the degree of lipid hydrolysis after day 5 of storage, in agreement with results obtained for the whey cheese "Myzithra Kalathaki" (Dermiki et al. 2008).

Our results suggest that both packaging (VP/MAP) conditions and/or addition of basil EO inhibit lipolysis in Anthotyros, probably affecting lipase-producing microorganisms (Tomaso et al. 2011), although this hypothesis was not tested, as also noted by Pintado and Malcata (2000) for whey cheese stored under VP. Bacterial lipases may be considered lipolytic, and they can contribute to rancid flavors. These enzymes are produced by both gram-positive (e.g., LAB) and gram-negative (e.g., *Pseudomonas*, *Acinetobacter*, and *Flavobacterium*) bacteria, which are capable of surviving during pasteurization, after which they cause fat breakdown of the manufactured products (e.g., butter, cheese) during storage (Deeth and Fitz-Gerald 1976).

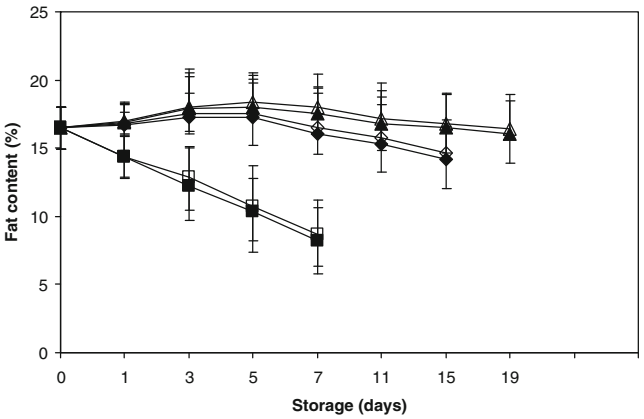

Fig. 2 Changes in fat content in Anthotyros cheese stored at 4 °C under aerobic packaging conditions without 0.4% *v/w* basil EO; A (*black square*) under aerobic packaging conditions with basil EO added at 0.4% *v/w*; AB (*white square*) stored under vacuum packaging without 0.4% *v/w* basil EO; V (*black diamond*) under vacuum packaging with basil EO added at 0.4% *v/w*; VB (*white diamond*) stored under MAP [40%/60% (CO_2/N_2)] without 0.4% *v/w* basil EO; M (*black triangle*) and under MAP [40%/60% (CO_2/N_2)] with basil EO added at 0.4% *v/w*; MB (*white triangle*). *Points* represent mean values of two replicate experiments with three samples analyzed per replicate ($n=6$). *Error bars* indicate the 95% confidence interval

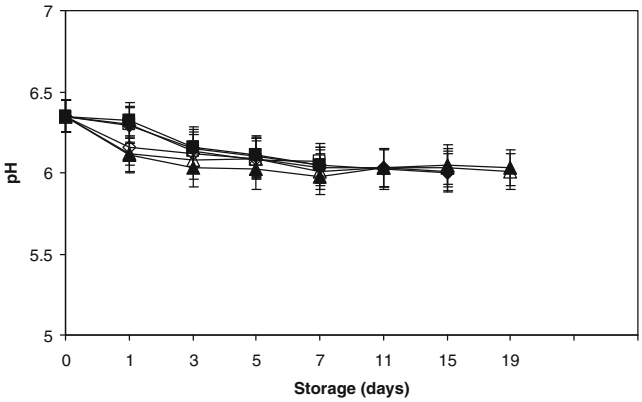

Fig. 3 Changes in pH in Anthotyros cheese stored at 4 °C under aerobic packaging conditions without 0.4% *v/w* basil EO; A (*black square*) under aerobic packaging conditions with basil EO added at 0.4% *v/w*; AB (*white square*) stored under vacuum packaging without 0.4% *v/w* basil EO; V (*black diamond*) under vacuum packaging with basil EO added at 0.4% *v/w*; VB (*white diamond*) stored under MAP [40%/60% (CO_2/N_2)] without 0.4% *v/w* basil EO; M (*black triangle*) and under MAP [40%/60% (CO_2/N_2)] with basil EO added at 0.4% *v/w*; MB (*white triangle*). *Points* represent mean values of two replicate experiments with three samples analyzed per replicate ($n=6$). *Error bars* indicate the 95% confidence interval

During the first week of storage, the pH values of the control A, and AB, V, VB, M, MB treated samples decreased, with no significant differences ($P>0.05$) between treatments or storage time periods, whereas beyond this period, pH values were practically unchanged ($P>0.05$) reaching mean values in the range of 6.05–6.01 for both control and treated samples (Fig. 3). The slight decrease in the pH values of A, AB, V, VB, M, and MB samples during the first week of storage is probably due to the formation of carbonic acid, produced by lactic acid bacteria, or intermediate acids (e.g., pyruvic or fatty acids) from the metabolism of these bacteria, as also noted by Maniar et al. (1994). Tsiotsias et al. (2002) found that the pH of Anthotyros whey cheese was not affected by VP, whereas Papaioannou et al. (2007) reported that a decrease in pH values became evident after day 22 of storage for samples packaged under modified atmosphere (70%/30%; CO_2/N_2) with final pH values in the range of 5.0–5.4. Present results suggest that the addition of basil EO appears to have a minimum effect on the pH values of cheese, irrespective of the packaging conditions.

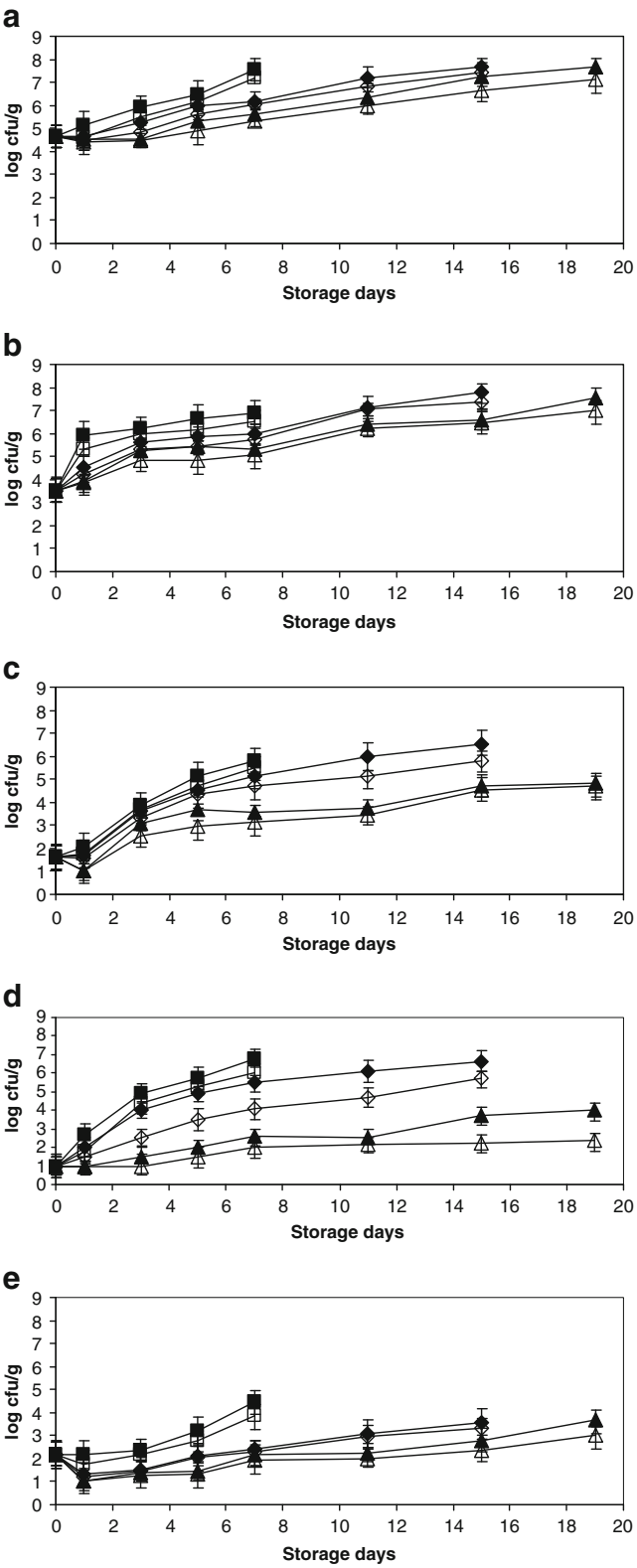

Fig. 4 Changes in **a** total viable counts, **b** lactic acid bacteria, **c** *Enterobacteriaceae*, **d** pseudomonads, and **e** yeasts and molds in Anthotyros cheese stored at 4 °C under aerobic packaging conditions without 0.4% *v*/*w* basil EO; A (*black square*) under aerobic packaging conditions with basil EO added at 0.4% *v*/*w*; AB (*white square*) stored under vacuum packaging without 0.4% *v*/*w* basil EO; V (*black diamond*) under vacuum packaging with basil EO added at 0.4% *v*/*w*; VB (*white diamond*) stored under MAP [40%/60% (CO_2/N_2)] without 0.4% *v*/*w* basil EO; M (*black triangle*) and under MAP [40%/60% (CO_2/N_2)] with basil EO added at 0.4% *v*/*w*; MB (*white triangle*). *Points* represent mean values of two replicate experiments with three samples analyzed per replicate (n=6). *Error bars* indicate the 95% confidence interval

With regard to the color of both untreated and treated samples, no significant differences ($P > 0.05$) were recorded. L^* (85.9), a^* (−3.0), and b^* (11.0) average values, both in the absence or in the presence of basil oil, irrespective of packaging conditions, were practically unchanged ($P > 0.05$) during the entire period of storage. In related studies, the color of Cottage (Maniar et al. 1994), Havarti (Kristensen et al. 2000), and *Feta* (Kneifel et al. 1992) cheese samples was also maintained, whereas Del Nobile et al. (2009) reported differences in the yellowness b^* and no differences in L^* and a^* values for modified atmosphere-packaged Ricotta whey cheese.

Microbiological Changes During Storage

Initial counts of Anthotyros were ca. 4.7, 3.5, <1, <2, and 2.2 log cfu/g for TPC, LAB, *Enterobacteriaceae*, pseudomonads, and yeasts, and molds, respectively (Fig. 4a–e). Lower initial TPC of 3.3 log cfu/g were previously reported for Anthotyros cheese (Papaioannou et al. 2007), whereas higher counts in the range of 5–6 logs were recorded in an earlier study (Kalogridou-Vassiliadou et al. 1994) reflecting differences in milk quality, the extent of survival of heat-sensitive microorganisms during production of cheese, and post-processing microbial contamination.

The mean TPC of the cheese samples packaged under either VP or MAP and in the absence (V, M) or presence of basil EO (VB, MB samples) were significantly lower ($P < 0.05$) than control (A) samples during storage of 7 days at 4 °C (Fig. 4a). TPC reached a population of 7 log cfu/g after approximately 6, 7, 10, 12, 14, and 16 days of storage at 4 °C for A, AB, V, VB, M, and MB samples, respectively. Of the packaging conditions examined, MAP either singly or combined with basil EO was the most effective for the inhibition of TPC. This fact may be attributed to the combined inhibitory effect of CO_2 and basil EO on microbial growth. Because of its bacteriostatic effect, CO_2 being water and lipid soluble inhibits the growth of aerobic gram-negative bacteria, such as *Pseudomonas* spp. as a result of an extension of the lag phase of growth, and a decrease in the rate during the logarithmic phase (Farber 1991). Additionally, basil EO exerts antimicrobial and antioxidant effects, which can be attributed to its basic components—linalool and estragole (methyl-chavicol)—against both gram-positive and gram-negative bacteria, as

shown in a number of in vitro studies involving addition of EOs to culture media (Lachowicz et al. 1998; Politeo et al. 2007; Runyoro et al. 2009).

Interestingly, MAP was more effective than VP in reducing the growth of mesophilic bacteria in Anthotyros cheese. This effect is attributed to the bacteriostatic properties of CO_2 (Brody 1989), and is in agreement with results reported by Eliot et al. (1998) for Mozzarella, and by Gonzalez-Fandos et al. (2000) for Cameros cheeses, packaged under modified atmospheres. VP does not remove all the oxygen from the package, and thus, mold and yeast growth may still occur (Hocking and Faedo 1992), particularly in the region of the product–packaging interface. Therefore, MAP may be more effective than VP for extending the shelf life of these products.

Based on an upper microbiological limit (7 log cfu/g), AB, VB, and MB treatments extended the microbiological shelf life of Anthotyros cheese by approximately 1, 6, and 12 days, respectively, at 4 °C. The microbiological shelf life extension of the treated cheese samples may be due to the bacteriostatic effect of basil EO.

Counts of LAB (gram-positive, catalase-negative, small off-white colonies) followed the same pattern as TPC, with lower counts for V, VB, M, and MB samples compared to the control (A) (Fig. 4b). LAB populations, irrespective of treatment, varied little ($P>0.05$) between days 3 and 7 of storage, whereas it is noteworthy that the respective counts for V, VB, M, and MB samples on day 7 were significantly ($P<0.05$) lower (by approximately 1–2 logs) as compared to the control (A) samples. LAB, being a facultative anaerobic species, can grow under both aerobic and anaerobic packaging conditions. However, differences in LAB counts between either VB or MB and the control samples may be attributed to the bacteriostatic effect of basil EO.

The present results suggest that both VP and MAP (to a greater extent) either singly used or combined with basil EO could affect LAB growth in Anthotyros cheese. Basil EO was reported to have an effect against *Enterococcus faecalis* (Opalchenova and Obreshkova 2003), a weak action against *Leuconostoc cremoris* and *Streptococcus faecalis* (Deans and Ritchie 1987), and not effective against *Lactobacillus plantarum, Streptococcus mutans, and Streptococcus viridians (Deans and Ritchie 1987; Runyoro et al. 2009).*

With regard to the gram-negative (*Enterobacteriaceae*, *Pseudomonas* spp., Figs. 4c, d, respectively) groups enumerated in the Anthotyros microflora, it is apparent that of the packaging treatments with added basil oil (AB/VB/MB) especially the latter one produced significantly ($P<0.05$) lower counts in cheese samples, as compared to the control (approximately 4.8 log cfu/g, *Enterobacteriaceae*; Fig. 4c and 2.7 log cfu/g *Pseudomonas* spp., Fig. 4d) on day 7 of storage. It is noteworthy that MB treatment suppressed to a greater extent the *Enterobacteriaceae*, rather than pseudomonads growth throughout the entire storage period (19 days, Figs. 4c, d, respectively), and counts always remained <3 log cfu/g. With regard to the untreated (A) cheese samples, the contribution of *Enterobacteriaceae* to the cheese microbial flora equaled that of LAB (approximately 6.9 logs on day 7) followed to a lesser extent by the *Pseudomonas* spp. (5.8 log cfu/g). The slightly higher prevalence of the *Enterobacteriaceae* and LAB (ca. 1 log difference), as compared to the pseudomonads, in the control samples is difficult to account for since *Pseudomonas* spp. would be expected to prevail under aerobic conditions.

Previous in vitro studies (mostly on laboratory growth media) report that basil EO may act against a wide range of *Enterobacteriaceae* spp. including: *Escherichia coli*, *Salmonella typhimurium*, *Salmonella enteriditis*, *Proteus vulgaris*, *E. faecalis*, *Enterococcus faecium*, *Enterobacter cloacae*, and *Klebsiella pneumonia* (Fyfe et al. 1998; Bagamboula et al. 2004; Moreira et al. 2005; Gutierrez et al. 2008; Nedorostova et al. 2009; Runyoro et al. 2009).

The higher degree of resistance of the pseudomonads, as compared to the enterobacteria, to the action of basil EO may be attributed to a number of factors. For instance, *Pseudomonas* spp. are known to antagonize other bacterial (gram-positive or gram-negative) groups for nutrients by forming siderophores, which may inhibit the growth of both spoilage microorganisms or pathogens (Freedman et al. 1989; Wei et al. 2006). However, further research is needed to verify whether this mechanism was involved in our findings.

Of the psychrotrophic microorganisms, yeasts and molds are an important contaminant in the dairy industry (Beresford et al. 2001) and have been implicated in the spoilage of whey cheeses, considering that these fresh whey cheeses exhibit a high pH, a high moisture, and low salt contents, usually stored under refrigeration with a limited shelf life (Pintado et al. 2001). Yeasts in the control (A) samples showed a completely different pattern of growth compared to the LAB, *Enterobacteriaceae*, and pseudomonads microflora populations, reaching in general lower counts ($P<0.05$) compared to the aforementioned bacterial groups (Fig. 4e). The mean yeast counts of the cheese samples packaged under either VP or MAP and in the absence (V, M) or presence of basil EO (VB, MB) were significantly lower ($P<0.05$) than in the control (A) samples during storage of first 7 days at 4 °C (Fig. 4e). Yeast populations of approximately 1.9, 2.2, 2.3, 2.4, 3.8, and 4.4 log cfu/g for MB, M, VB, V, AB, and A cheese samples, respectively, were recorded on day 7 of storage (Fig. 4e). It is apparent that the use of MAP/VP (M, V) either singly or in combination with added basil EO (MB,

VB) is efficient against yeast growth maintaining low counts (<2.4 log cfu/g) during the first 7 days of storage (4 °C). CO_2 acts directly on yeasts and molds (retarding growth rate, thus extending shelf life) whereas under VP growth of these species may still occur, particularly as a result of package wrinkling in regions of the food product–packaging interface (Hocking and Faedo 1992). Similar results have been shown for the inhibition of yeasts in Requeijão cheese by VP and MAP (Pintado and Malcata 2000).

Of the treatments with added basil, EO, MB, and VB had a significant effect ($P<0.05$) mostly on pseudomonads (Fig. 4d), whereas no significant changes ($P>0.05$) were noted in the absence or presence of basil EO in the case of the total mesophilic bacteria, LAB, *Enterobacteriaceae*, and yeasts. Studies describing the effects of EOs, including basil EO, with packaging as an antimicrobial treatment combination on cheeses, in general, are limited.

Sensory Changes During Storage

The results of the sensory evaluation (odor, taste, and overall acceptability parameters) of A, AB, V, VB, M, and MB treatments are shown in Fig. 5a–c. Individual odor and taste scores, irrespective of packaging conditions, with or without basil EO, showed a similar pattern of decreasing acceptability (Fig. 5a, b, respectively). As previously noted for pH, fat content, and most of the bacterial groups examined, the addition of basil EO did not have a significant effect ($P>0.05$) on the sensory parameters of the cheese samples. Generally, odor was a more sensitive attribute than taste; therefore, in the present study, odor attribute was used for the determination of the sensorial shelf life of untreated and treated cheese samples.

During the first 5 days of storage, all samples received odor scores between 4 and 5.0, and, it is noteworthy that, the presence of basil EO imparted a pleasant odor to the cheese samples. The observed shelf life of Anthotyros, based on odor, was the longest for MB cheese samples (18 days) followed by M (14–15 days), VB (12 days), V (9–10 days), AB (7 days), and control samples A (6 days), respectively (Fig. 5a). The results of the sensory evaluation (odor) correlated relatively well with those of the microbiological analyses (TPC).

In the present study, Anthotyros was better preserved under MAP or VP and in the presence of basil EO. Addition of basil EO to the whey cheese improves the odor of the product (as judged by sensory attributes), whereas when combined with either MAP or VP, it may increase its shelf life. In a related study, Anthotryros, stored under MAP 30%/70% and 70%/30% CO_2/N_2, was acceptable for 22 and 27 days, respectively (Papaioannou et al. 2007). EOs interact with food components, and if used as the only preservatives may often convey a strong flavor to the foodstuffs, imparting unpleasant sensorial attributes such as bitter taste and strong odor.

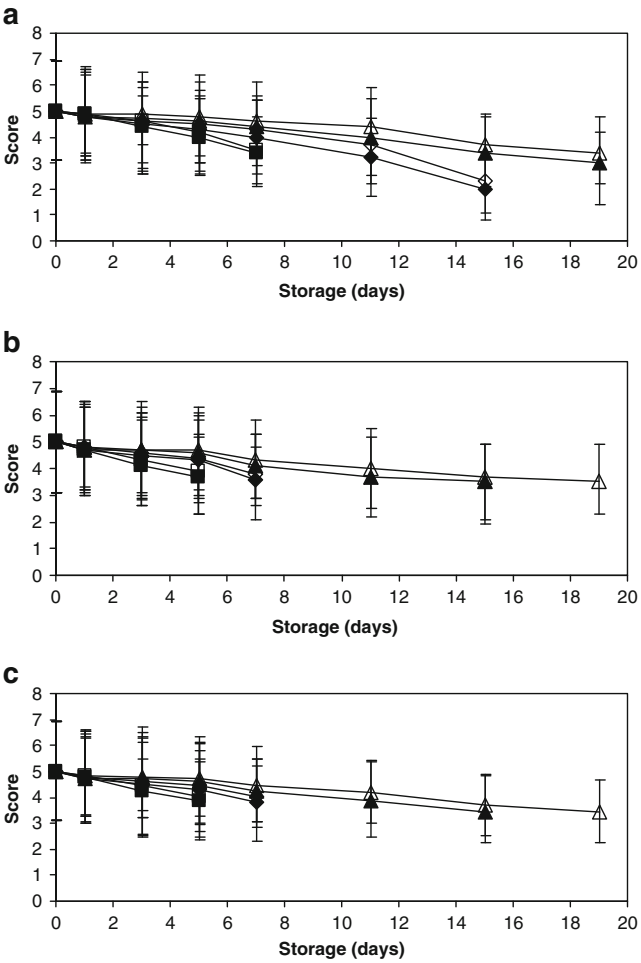

Fig. 5 Changes in **a** odor, **b** taste, and **c** overall acceptability scores in Anthotyros cheese stored at 4 °C under aerobic packaging conditions without 0.4% *v*/*w* basil EO; A (*black square*) under aerobic packaging conditions with basil EO added at 0.4% *v*/*w*; AB (*white square*) stored under vacuum packaging without 0.4% *v*/*w* basil EO; V (*black diamond*) under vacuum packaging with basil EO added at 0.4% *v*/*w*; VB (*white diamond*) stored under MAP [40%/60% (CO_2/N_2)] without 0.4% *v*/*w* basil EO; M (*black triangle*) and under MAP [40%/60% (CO_2/N_2)] with basil EO added at 0.4% *v*/*w*; MB (*white triangle*). *Points* represent mean values of two replicate experiments with three samples analyzed per replicate ($n=6$). *Error bars* indicate the 95% confidence interval

It is noteworthy that potential use of EOs such basil, oregano, thyme, etc. as preservatives in foods needs to be carefully evaluated in terms of sensorial acceptability (Atrea et al. 2009). However, it should be stressed that consumers should take extra care with regard to the quality and safety of cheese, especially in the case of new products such as goat's cheese with added herbs (basil), which are served before meals, both at home or in restaurants.

This study, in combination with the current, limited knowledge of the potential use of basil EO as a "natural" preservative in whey or soft cheeses, suggests that the application of basil EO can be expanded to the extension of the shelf life of whey cheeses by (1) delaying the growth of aerobic spoilage microorganisms and (2) by imparting a pleasant and possibly a more appealing flavor to the cheese. The latter application may be extended to other dry herbs or their EOs, such as mint, which, when added to cheeses such as goat cheese, Mozzarella, *Halloumi*, etc., may be more favorably experienced by consumers.

Conclusions

The sensory evaluation and microbiological data (TPC) primarily showed that the combined use of MAP or VP with added basil oil extended the shelf life of fresh Anthotyros stored at (4 °C) by approximately 10–12 days (MB treatment) and 6 days (VB treatment) compared to aerobic packaging (A) with cheese maintaining good sensory characteristics. What is important to stress as a final point is that present recommendations on use of MAP/VP and basil EO for soft whey cheese Anthotyros correspond to samples from one dairy plant, and thus, their general application is yet to be verified. Further studies are needed with regard to preservation of whey cheeses, involving "hurdle" technologies and in particular effects of essential oils on quality characteristics (sensory parameters), establishing optimum concentrations of EOs that may extend the shelf life, and most importantly, maintain product's original quality.

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