SHELF-LIFE OF UNSALTED AND LIGHT "LOR" WHEY CHEESE STORED UNDER VARIOUS PACKAGING CONDITIONS: MICROBIOLOGICAL AND SENSORY ATTRIBUTES

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ABSTRACT

The shelf life of unsalted and light Turkish whey cheese "Lor" in various packaging conditions of atmospheric air, vacuum and modified atmosphere packaging (MAP) (80%CO_2/20%N_2; MAP1 and 60%CO_2/40%N_2; MAP2) under refrigeration (4C) was investigated and compared with each other for a period of 25 days. MAP1 was the most effective for inhibiting growth of total viable count, yeast and mold, and Enterobacteriaceae *microflora in Lor cheese samples until 20 days of refrigerated storage. Yeasts and molds were inhibited effectively under MAP2 packages as well. Vacuum-packaged samples gave a lower lactic acid bacteria count at a given sampling day than the other packages. MAP and vacuum packages were not found effective on psychrotrophic bacteria counts. Results show an increase in the shelf life of MAP packaged Lor whey cheese, confirming that inhibitory effects on some of the microorganism groups are responsible for the spoilage phenomena. Refrigeration condition with MAP combination of Lor cheese had good sensory characteristics for 20 days of storage, while control samples were unacceptable after 10–15 days of storage.*

PRACTICAL APPLICATIONS

The modified atmosphere packaging (MAP) of foods has considerably increased recently. MAP with CO_2 was found very effective on spoilage microorganisms because of its bacteriostatic effect. Generally, Lor cheese is packaged in atmospheric air or vacuum conditions in Turkey; there is no any

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research about the extending the shelf life of Lor cheese, and it has a very short shelf life although it is vacuum-packaged. Modified atmosphere packages of unsalted and light Lor cheese were found effective on microbial and sensory qualities of it and MAP packages could be used for Lor cheese, to give better results than vacuum packaging together with good sanitary conditions, during the packaging process.

INTRODUCTION

Whey is the main product of the cheese manufacturing industry. In the Mediterranean region, whey is used for the production of some kinds of cheeses. Whey cheeses are very popular cheeses; Ricotta (Italy), Manouri (Yugoslavia), Getost, Brunost (Norway), Ziger (Germany), Broccio (France), Anthotyro (Greece), Requeson (Spain) and Lor (Turkey) are examples of this type of cheeses. They offer a large variety of consistencies and flavors, made possible by changes in cheese-making protocols; blending of one or more cheese types to create new products and the addition of sugar, fruit purees, spices, and condiments. Their soft, ingestible consistency makes them safe for children. They are perceived as healthy by diet-conscious consumers. In general, the fat content of these cheeses is lower than that of rennet curd cheeses; also, milk serum proteins in these cheeses have high nutritional value because of containing essential amino acids in the human diet (Fox *et al.* 2000; Pintado and Malcata 2000a).

The whey from which Kashar cheese is usually manufactured is heated to 80C. The curd rises to the surface and is collected in thin filtrating clothes where it drains for 12–14 h. After the draining, "sweet Lor" is obtained, and unripening traditional fresh Lor can be consumed with or without salt (2% w/w) in Turkey (Ucuncu 2004; Ciftcioglu *et al.* 2008).

The range of these light and unsalted varieties of cheeses is growing. However, unsalted and unripening Lor cheese has a very limited shelf life, and it is very critical for the growth of pathogenic microorganisms because of the high moisture content and its critical high pH value. The short shelf life of fresh whey cheeses is mostly due to the growth of psychrotrophs, molds, yeasts and *Enterobacteriaceae*. Thus, the shelf life of fresh cheese is about 7–10 days under aerobic conditions (Gonzales-Fandos *et al.* 2000; Papaioannou *et al.* 2007). Because of these, there is an interest in extending the shelf life of dairy foods. The modified atmosphere packaging (MAP) technique is defined as the replacement of the headspace gas surrounding a food product with a gas mixture (Stiles 1991; Phillips 1996). MAP of dairy products such as cottage cheese and fluid milk has been reported to retard microbial growth (Chen and Hotchkiss 1993; Hotchkiss *et al.* 2006). Numerous studies have been carried out on the effects of MAP of hard cheeses and some soft cheeses (Favati *et al.* 2007; Jakobsen and Risbo 2009; Trobetos *et al.* 2008). However, the studies about extending the shelf life of whey cheeses (Pintado and Malcata 2000a; Papaioannou *et al.* 2007; Dermiki *et al.* 2008) are very limited, while there are no studies on the effects of MAP on the microbial and sensory qualities of “Lor” whey cheese. Lor cheese spoils easily because of its granular shape. Porous structure of Lor cheese causes increase in the oxygen contact surfaces and as the result of this aerobic mesophilic bacteria, yeast and molds can grow very fast. It was suggested that inhibition of yeasts, molds and other aerobic microorganisms in cheeses, as well as the prevention of oxidative changes can be possible in packaging under high concentrations of CO_2. Meanwhile, 20% of N_2 can prevent adhesion of Lor cheese granules in the packages (Ucuncu 2007).

The aims of this study were to determine some microbiological and sensory changes in Lor cheese during storage under vacuum and two MAP conditions at 4C, as well as to optimize the suitable packaging type for it.

MATERIALS AND METHODS

Production of Lor Whey Cheese

Lor samples were produced in a local dairy plant (Kocaeli Ciftligi Milk Industry, Kocaeli, Turkey). Whey from the Kashar cheese production was heated at 50–55C. Fat was separated from whey, then was heated to 80C in a boiler tank with 2% (w/w) salt added. Serum proteins began to accumulate at the surface of the whey, and the temperature increased to 90–95C. The coagulum’s of Lor cheese were collected from the tank above the whey surface into the thin clothes and drained for 12 h at 25C.

Packaging of Cheese Samples

Lor samples were weighed ca. 250 g each, and the ratio of the volume of gas and the weight of the food product (G/P ratio) was set at 3:1 (v/w). MAP was performed by using Betapak BPZ 400 (BETA-PAK Automatic Packaging Machines Ind. and Co., Gaziosmanpasa, Istanbul, Turkey) packaging machine. Lor samples were packed in low-density polyethylene terephtalate (PET)/ethylene vinyl alcohol (EVOH)/low-density polyethylene (LDPE) trays of 750-μm thickness. A film of oriented polypropylene/LDPE/EVOH/LDPE 72 μm in thickness, an oxygen permeability of 20 cm^3/m^2/d/atm at 75% relative humidity (RH), 23C and a vapor permeability of 4 g/m^2 24 h at 90% RH, 38C was used as a sealing top.

Lor cheeses were vacuum-packed in polyamide/polyethylene (PA/PE) film 170 μm in thickness, an oxygen permeability of 20 cm^3/m^2/d/atm at 75% RH, 23C and a vapor permeability 2.5 g/m^2 24 h at 90% RH, 38C. Vacuum packaging was performed by using the VC 999/K12NA (Verpackungssysteme AG., Herisau, Switzerland) packaging machine.

Control Lor cheese groups were packed at atmospheric conditions in the same PA/PE film materials like vacuum packs.

Carbon dioxide and nitrogen concentrations in the MAP packages headspace were monitored periodically by using PBI Dansensor Check Pointer O_2/CO_2 (Ringsted, Denmark) analyzer. All samples were kept at 4C, sampling was carried out on 0, 5, 10, 15, 20 and 25 days of storage.

Proximate Analysis

Lor cheese samples were transferred to the laboratory in polystyrene boxes containing ice and they were analyzed within 4 h after production.

The pH value was recorded using Hanna Instruments model HI221 Microprocessor (Hanna Instruments Inc., Woonsocket, Rhode-Island), pH meter. It is equipped with a glass electrode that was inserted directly into the Lor samples. The moisture, protein, fat and salt contents were determined according to AOAC (1995) procedures. Total ash in the Lor samples was determined according to IDF (1964), and the lactose content was determined by subtracting the sum of fat, protein and ash contents of each cheese sample from the corresponding total solids content (Kondyli *et al.* 2008).

Microbiological Analysis

Lor cheese samples (10 g) were transferred aseptically into containing 90 mL of sterile buffered peptone water (BPW) solution (0.1%) and homogenized in a Lab Blender 400 Waring HGB 2WTS3 (Torrington, CT). For each sample, appropriate serial decimal dilutions were prepared in BPW solution (0.1%). Serial dilutions were used to determine following microbial counts:

Total viable count (TVC): Mesophilic microorganisms were determined on plate count agar by pour plate method and incubated at 31C for 72 h (Gonzales-Fandos *et al.* 2000).

Enterobacteriaceae count: *Enterobacteriaceae* count was performed on violet red bile dextrose agar, incubated at 37C for 24 h (Govaris *et al.* 2007).

Psychrotrophic count: Psychrotrophic bacteria counts were determined on plate count agar by pour plate method, incubated at 7C for 10 days (Govaris *et al.* 2007).

Lactic acid bacteria count: *Lactobacillus* count was determined using double-layer de Man Rogosa Sharpe agar at anaerobic conditions after incubation at 30C for 72 h (Whitley *et al.* 2000).

Yeast and mold counts: Yeasts and molds were enumerated on yeast extract glucose chloramphenicol agar plates following the surface plate method and incubated at 25C for 5–7 days (Gonzales-Fandos *et al.* 2000).

All count data were written as logarithms (log cfu/g) prior to their statistical treatment.

Sensory Analysis

The sensory characteristics were carried out according to IDF (1995) standards on each day of sampling. A panel composed of 5 experienced members from our university was used to evaluate the whey cheeses for external appearance (color), flavor, taste and texture with a point scale from 0 to 5 (0 spoiled sample and unfit for human consumption; 5, very good). Modified-atmosphere- and vacuum-packaged cheese samples were compared with fresh Lor cheese. The results were analyzed statistically as described in the next section.

Statistical Analysis

Experiments were replicated twice on different occasions with different Lor cheese samples. Analysis were run in triplicate for each replicate ($n = 2 \times 3$). The data were statistically subjected to one-way analysis of variance using SPSS 10.0. Means and standard deviations were calculated, and the least significant difference test was used at a significance level of 0.05 (Ozdamar 2004).

RESULTS AND DISCUSSION

Proximate Analysis

Proximate analysis of Lor cheese gave average moisture 71.55 ± 0.3%, protein 11.7 ± 0.2%, fat 7.4 ± 0.3%, NaCl 0.3 ± 0.05%, total ash 1.25 ± 0.03% and lactose 7.8 ± 0.02% (g/100 g moisture) for two batches, respectively.

pH

The pH values of Lor samples during the storage period are shown in Fig. 1. On the first day, the initial pH value of cheeses was 6.78, then decreased to 5.35, but it was 5.55 in the samples packaged under MAP1. The pH values of cheese samples under air-packaged samples were not significantly important ($P > 0.05$) as compared to MAP and for vacuum-packaged samples. The explanation for this is that the lactic acid bacteria count in the packages was

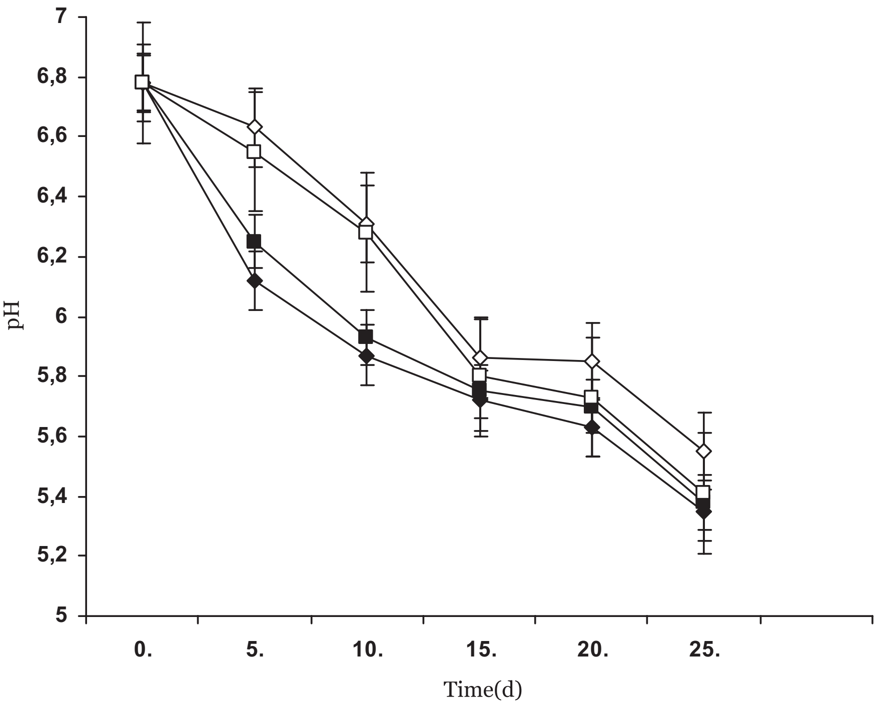

FIG. 1. EFFECT OF PACKAGING CONDITIONS ON pH IN LOR WHEY CHEESE DURING STORAGE AT 4C

(◆) A: control (air), (◇) B: 80%CO_2/20%N_2 (MAP1), (■) C: 60%CO_2/40%N_2 (MAP2), (□) D: vacuum. Data represent mean values of ($n = 2 \times 3$) measurements and error bars are indicated.

also not significant ($P > 0.05$). Similarly, Tsiotsias *et al.* (2002) found that the pH of cheese samples was not affected by vacuum-packaging. Papaioannou *et al.* (2007) noted that the decrease in pH value decreased for Anthotyros cheese samples packaged under MAP at 4C after 22 days storage. The growth of lactic acid bacteria resulting in lactic acid production was one of the major factors in pH decrease in the products (Fernandez-Lopez *et al.* 2008). However, some of the starter thermoduric lactic acid bacteria that were used in the cheese production that remained in the whey cheese can also cause the pH decrease (Hassan and Frank 2001).

Microbiological Analysis

Microbiological results are shown in Figs 2–6. Initial microbial counts of Lor cheese were ca. 4.82, 4.23, 4.12, 1.4 and 1.5 log cfu/g for TVCs,

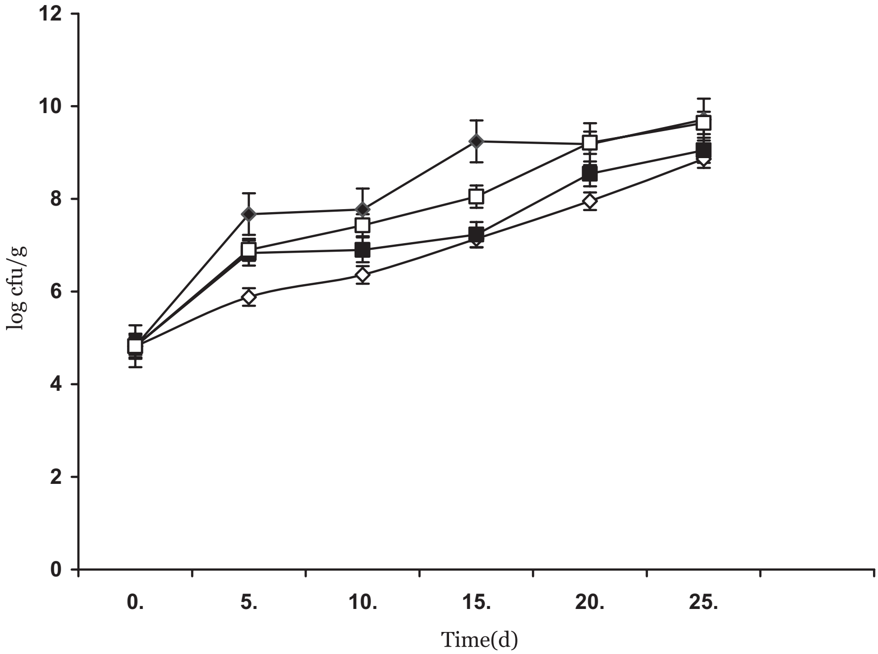

FIG. 2. EFFECT OF PACKAGING CONDITIONS ON TOTAL VIABLE COUNTS IN LOR WHEY CHEESE DURING STORAGE AT 4C

(◆) A: control (air), (◇) B: 80%CO_2/20%N_2 (MAP1), (■) C: 60%CO_2/40%N_2 (MAP2), (□) D: vacuum. Data represent mean values of ($n = 2 \times 3$) measurements and error bars are indicated.

psychrotrophic bacteria, lactic acid bacteria, yeasts and molds, and *Enterobacteriaceae*, respectively. Gonzales-Fandos *et al.* (2000) found <4, 3, 1.5 log cfu/g for mesophiles, psychrotrophs and *Enterobacteriaceae* counts in Cameros fresh cheese respectively. Dermiki *et al.* (2008) reported about 5 log cfu/g initial mesophilic bacteria, 3.5 log cfu/g psychrotrophic bacteria, 1 log cfu/g *Enterobacteriaceae* count and 4 log cfu/g lactic acid bacteria in “Myzithra Kalathaki” whey cheese. Differences in microbial quantity of raw milk, heat application parameters and post-contaminations to the cheese during process can cause different initial counts of cheese samples.

Total viable counts of the samples in air increased rapidly, and were higher than 7 log cfu/g after 5 days of storage (Fig. 2). However, Lor samples packaged under MAP1 were significantly ($P < 0.05$) lower for the growth of TVCs than the control air-packaged samples, and their counts reached above 7 log cfu/g after the 15 days of storage. The high concentration of CO_2 in MAP1 packages can be caused by the inhibition of aerobic bacteria because of its bacteriostatic effect. Similar effects of MAP have been reported in the

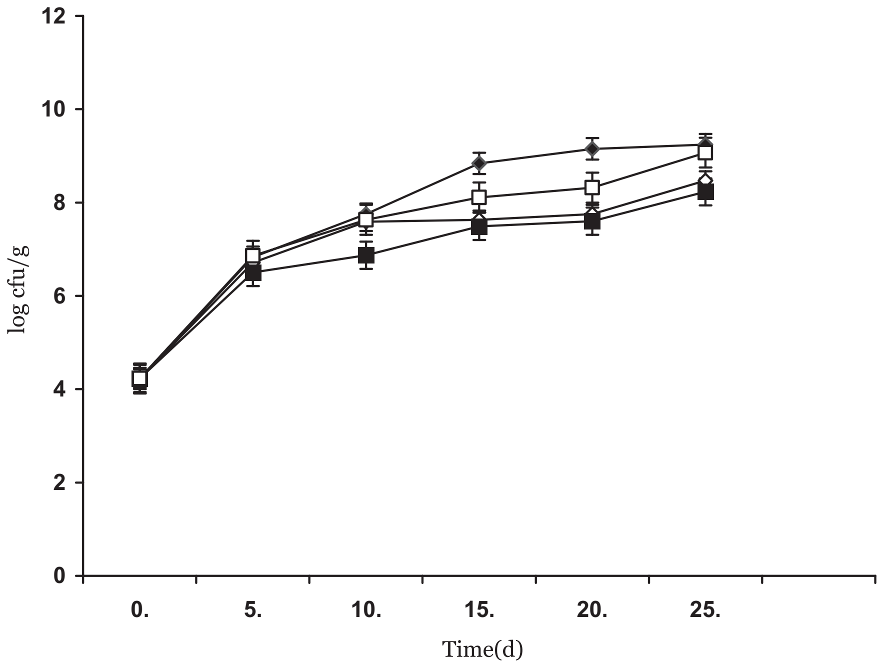

FIG. 3. EFFECT OF PACKAGING CONDITIONS ON PSYCHROTROPHIC BACTERIA IN LOR WHEY CHEESE DURING STORAGE AT 4C

(◆) A: control (air), (◇) B: 80%CO_2/20%N_2 (MAP1), (■) C: 60%CO_2/40%N_2 (MAP2), (□) D: vacuum. Data represent mean values of ($n = 2 \times 3$) measurements and error bars are indicated.

literature for some cheeses (Fedio *et al.* 1994; Gonzales-Fandos *et al.* 2000; Papaioannou *et al.* 2007). It was determined that high CO_2 concentrations were more inhibition effects on mesophilic bacteria than vacuum packages in the Dermiki *et al.* (2008) study.

Psychrotrophic counts increased by 5.01 log units between days 0 and 25 in the air-packaged samples (Fig. 3). Psychrotrophic counts in modified-atmosphere- and vacuum-packaged samples were not significantly ($P > 0.05$) different from the control air-packaged samples. It can be explained that initial psychrotrophic counts in the packages were 4.23 log cfu/g and higher than the other studies (Gonzales-Fandos *et al.* 2000; Arashisar *et al.* 2004; Dermiki *et al.* 2008) in Lor samples on day 0 and, as a result, CO_2 concentration did not retard nor inhibit psychrotrophic flora in the packages adequately. Also, it is known that the antimicrobial effect of CO_2 increases depending on the solubility in the packaged product (Arashisar *et al.* 2004). Similarly, Eliot *et al.* (1998) did not find any significant effect of CO_2 on the growth of psychrotrophs in Mozzarella cheese. Hotchkiss *et al.* (2006) stated that high CO_2

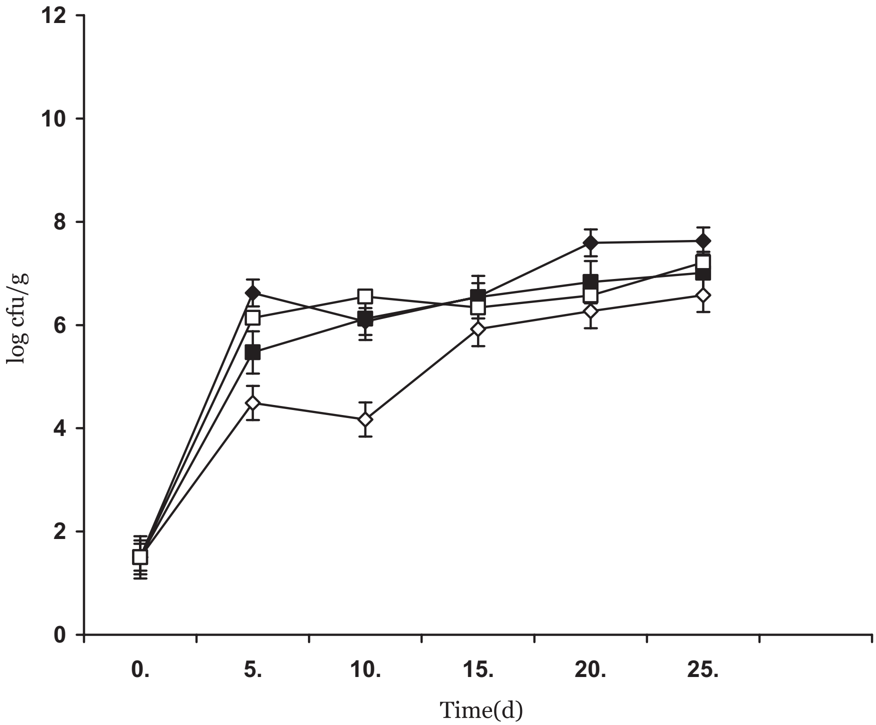

FIG. 4. EFFECT OF PACKAGING CONDITIONS ON *ENTEROBACTERIACEAE* IN LOR WHEY CHEESE DURING STORAGE AT 4C

(◆) A: control (air), (◇) B: 80%CO_2/20%N_2 (MAP1), (■) C: 60%CO_2/40%N_2 (MAP2), (□) D: vacuum. Data represent mean values of ($n = 2 \times 3$) measurements and error bars are indicated.

concentration has the largest inhibition against Gram-negative psychrotrophs, particularly for *Pseudomonas* spp., and the least inhibition effect generally observed with Gram-positive psychrotrophs and lactic acid bacteria. Contrary to our results, Pintado and Malcata (2000a) found an inhibitory effect of high CO_2 concentration against *Pseudomonas* growth under modified-atmosphere-packaged "Requeijao" whey cheese samples, and Dermiki *et al.* (2008) observed important inhibitory effects of CO_2 on the growth of psychrotrophs in "Myzithra Kalathaki" whey cheese. Different results may be due to the fact that psychrotrophs flora behaviors are complex and the susceptibility to CO_2 inhibition could be different.

Initial counts of *Enterobacteriaceae* (<2.0 log cfu/g) indicate adequate sanitary production of Lor cheese (Fig. 4). Especially, survival of heat-sensitive microorganisms in cheese shows post-contaminations from cheese clothes to the cheese surface during draining process. *Enterobacteriaceae* counts under

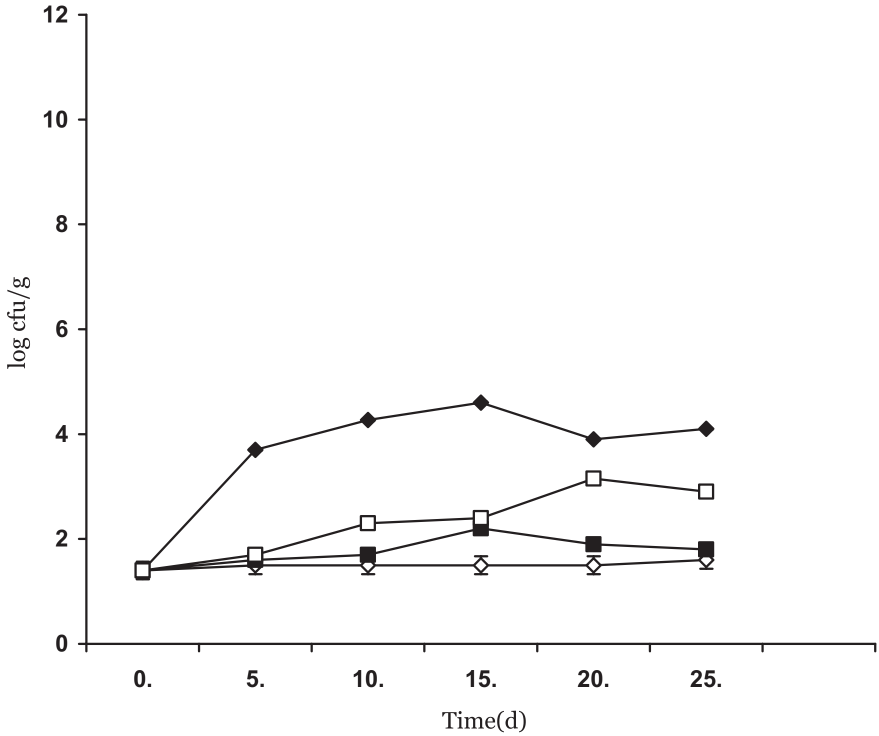

FIG. 5. EFFECT OF PACKAGING CONDITIONS ON YEASTS AND MOULDS IN LOR WHEY CHEESE DURING STORAGE AT 4C

(◆) A: control (air), (◇) B: 80%CO_2/20%N_2 (MAP1), (■) C: 60%CO_2/40%N_2 (MAP2), (□) D: vacuum. Data represent mean values of ($n = 2 \times 3$) measurements and error bars are indicated.

MAP1 Lor cheeses were significantly ($P < 0.05$) different from the control and vacuum-packaged cheese. However, *Enterobacteriaceae* counts in MAP2 and vacuum were not found significantly ($P > 0.05$) different from the control cheeses. Similarly, in the Dermiki *et al.* (2008) study, it was observed that *Enterobacteriaceae* counts in vacuum packages exceeded control sample counts in Myzithra whey cheese after 23 days. Similarly, Gonzales-Fandos *et al.* (2000) and Papaioannoou *et al.* (2007) found lower Enterobacteriaceae counts which were packaged in high CO_2 concentration than air and vacuum-packaged groups in their researches. Pintado and Malcata (2000b) stated that *Enterobacteriaceae* counts were severely inhibited by the vacuum-packaged samples of “Requeijao” whey cheese. In Pintado and Malcata (2000a) study, it was shown that *Enterobacteriaceae* counts did not increase within 15 days under 100% CO_2 conditions in “Requeijao” whey cheese samples. It is clearly

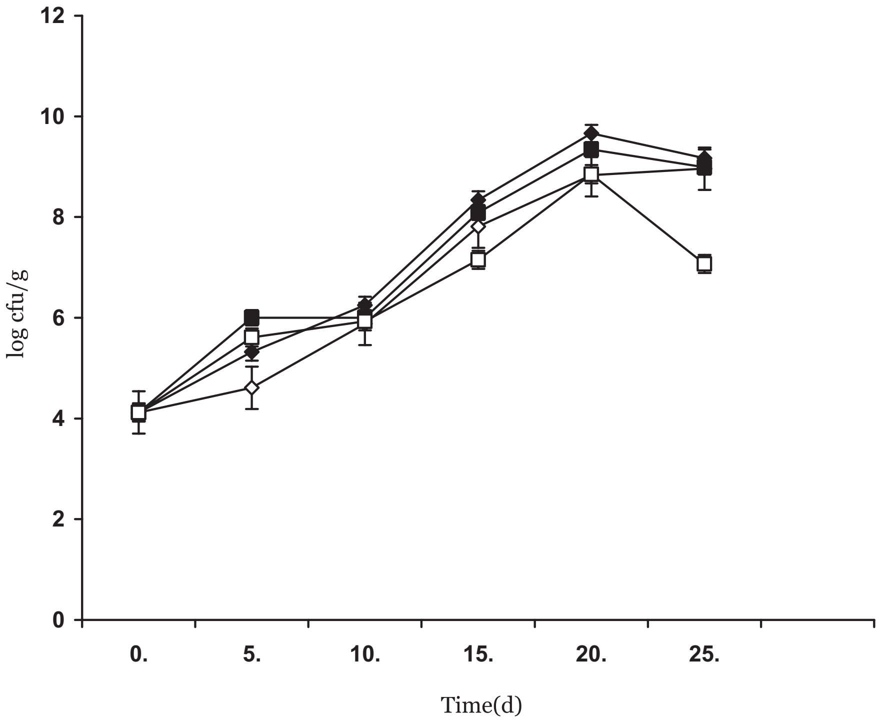

FIG. 6. EFFECT OF PACKAGING CONDITIONS ON LACTIC ACID BACTERIA IN LOR WHEY CHEESE DURING STORAGE AT 4C

(◆) A: control (air), (◇) B: 80%CO_2/20%N_2 (MAP1), (■) C: 60%CO_2/40%N_2 (MAP2), (□) D: vacuum. Data represent mean values of ($n = 2 \times 3$) measurements and error bars are indicated.

stated that the growth of facultative anaerobic bacteria can be inhibited in the presence of CO_2 or they can also grow in the absence of CO_2 (Jones 1989).

Yeasts and molds are important microbial contaminants in the dairy industry. Yeasts and molds for MAP1, MAP2 and vacuum whey cheese samples were significantly ($P < 0.05$) different from the control Lor samples (Fig. 5). Also, there was no significant ($P > 0.05$) difference between the yeast and mould counts of MAP1 and MAP2 samples. Air-packaged samples reached a count of 4.27 log cfu/g after 10 days. Yeasts and molds for MAP1 samples remained nearly constant during the storage period. It is obvious that high CO_2 concentration was very effective for the inhibition of the growth of yeasts and molds. Similar results were found by Fedio *et al.* (1994) for cottage cheese, Gonzales-Fandos *et al.* (2000) for Cameros cheese, Pintado and Malcata (2000b) for Requeijao whey cheese, Dermiki *et al.* (2008) for Myzithra Kalathaki and Papaioannoou *et al.* (2007) for Anthotryros cheese.

Lactic acid bacteria count was not significantly ($P > 0.05$) different for all packages, but vacuum-packaged samples gave the lowest counts and reached 7.07 log cfu/g after 25 days of storage (Fig. 6). The effects of CO_2 on the growth of lactic acid bacteria were not definite because of the microaerophilic nature of these bacteria, and they can grow under the CO_2 atmosphere. Oyugi and Buys (2007) reported that under the CO_2 packaging conditions, the lactic acid bacteria in shredded Cheddar cheese can be inhibited slightly. Eliot *et al.* (1998) determined that there was no significant effect in the growth of lactic acid bacteria under CO_2 atmosphere or under vacuum-packaged Mozzarella samples. Papaioannou *et al.* (2007) reported lower counts of lactic acid bacteria for MAP gas mixtures compared with vacuum-packaged samples.

Sensory Evaluation

Odor and taste scores for the Lor cheese are given in Table 1. There were no significant differences ($P > 0.05$) in odor and taste scores of cheeses for all packaging treatments until 5 days of storage. After 5 days, however, significant difference ($P < 0.05$) was recorded among the control and other samples. The worst scores for odor and taste were reached in cheeses packaged in vacuum after 25 days. The odor of control cheeses were very poor and their taste was unacceptable after 15 days of storage. The odor and taste scores of cheeses under the MAP packages were similar. In general, odor and taste scores were

TABLE 1.
ODOR AND TASTE EVALUATIONS OF LOR WHEY CHEESE PACKAGED UNDER VACUUM AND MAP DURING STORAGE AT 4C

Sample	Storage day					
	0	5	10	15	20	25
Odor evaluation						
Control	5*	5.0 ± 0.2†	4.0 ± 0.3	1 ± 0.1	0.5 ± 0.2	0.5 ± 0.7
Vacuum	5	5.0 ± 0.4	4.6 ± 0.2	3.6 ± 0.3	3.5 ± 0.5	3.1 ± 0.5
MAP1(80%CO_2/20%N_2)	5	4.7 ± 0.2	4.8 ± 0.5	3.8 ± 0.3	3.5 ± 0.4	3.3 ± 0.4
MAP2(60%CO_2/40%N_2)	5	4.8 ± 0.5	4.5 ± 0.3	3.7 ± 0.4	3.4 ± 0.4	3.2 ± 0.2
Taste evaluation						
Control	5	4.5 ± 0.3	2.2 ± 0.4	0	0	0
Vacuum	5	4.6 ± 0.6	4.0 ± 0.5	3.5 ± 0.4	3.3 ± 0.6	3.2 ± 0.5
MAP1(80%CO_2/20%N_2)	5	4.9 ± 0.3	4.6 ± 0.3	4.5 ± 0.6	3.6 ± 0.5	3.4 ± 0.8
MAP2(60%CO_2/40%N_2)	5	4.7 ± 0.4	4.5 ± 0.3	4.2 ± 0.7	3.5 ± 0.5	3.4 ± 0.4

Scoring scale: very good = 5, good = 4, fair = 3, poor = 2, very poor = 1, unfit for human consumption = 0.

* Values reported are the mean values of two different experiments run on different occasions.

† ±SD values.

higher for samples packaged under a CO_2 atmosphere. Similarly, it was stated that the presence of high concentrations of CO_2 in the packaging systems result with the inhibition of spoilage bacteria and off-flavors in the products (Dermiki *et al.* 2008). However, Maniar *et al.* (1994) determined that CO_2 did not have any effect on the sensory characteristics on cheeses in their research.

CONCLUSIONS

Based on the results, it can be concluded that MAP1 was the most effective condition for extending the shelf life of unsalted and light Lor whey cheese. In MAP1 packages, the microorganism numbers of total viable bacteria, Enterobacteriaceae, yeast and mold were lower than the air packaged control groups. Also, CO_2 concentration in MAP2 packages was sufficient to inhibit yeast and molds; however, under vacuum packaging conditions, lactic acid bacteria was inhibited more effectively than the other groups of packages. Unfortunately, psychrotrophic counts were not affected from the tested packaging systems. In MAP technology under high CO_2 concentrations, especially above 60% with refrigeration condition, this can reduce some of the microbial growth and, therefore, may extend the shelf life of the product; but absence of oxygen may lead to the growth of anaerobic pathogens. Thus, further research should be carried out to determine the effect of various modified atmospheres on the growth of some post-contamination pathogens in Lor cheeses.

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