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REVIEW PAPER

Evaluating the effect of modified atmosphere packaging on cheese characteristics: a review

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Abstract Dairy products are an important food group highly suggested by nutritionists. This food category is one of the most perishable, so extending their shelf life and keeping them fresh for a longer period of time is a matter of importance. Since consumers are now more aware of the possible hazards of preservatives, technologists and researchers have attempted to introduce novel preservative-free methods instead. One of these techniques is modified atmosphere packaging (MAP), which alters the natural gas surrounding the product in the package in order to delay deteriorative changes. In this paper, a review is made on the field of cheese MAP. Reported results revealed the potentiality of MAP in increasing cheese sensorial and microbial shelf life by carefully designing for an individual cheese.

气调包装对干酪性质的影响

摘要 乳制品作为重要的营养性食品,容易发生腐败变质,因此保证乳制品的品质和延长产品的货架期具有重要的意义。由于消费者非常关注食品保藏过程中防腐剂带来的食品安全问题,因此D些无防腐剂的新型保藏技术应运而生。气调包装是通过改变包装内产品周围环境中气体组成的方式来达到延缓食品腐败的方法。本文对干酪气调包装的研究和应用领域进行了综述,并论述了气调包装对干酪货架期的延长以及货架期内干酪感官品质改变和微生物变化。

Keywords Modified atmosphere packaging (MAP) \cdot Cheese \cdot Headspace gas composition evolution \cdot Physicochemical properties \cdot Microbiological analysis \cdot Sensory evaluation

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关键词 气调包装 · 干酪 · 顶空气相色谱 · 物理化学特性 · 微生物分析 · 感官评价

1 Introduction

Increasing the shelf life of food using different preservative methods has always been a major concern of humankind. Preliminary attempts in this regard include sun drying during the summer and atmospheric freezing during the winter, as well as natural fermentation, which was innovated by ancient Asians (Ooraikul and Stiles 1991). Today, several preservation techniques are available to extend the shelf storage of food products, among which packaging is the most promising. The packaging process undertakes several basic roles such as preventing microbial and chemical quality deterioration and enhancing the handling and marketing for packaged products. Now, food packaging not only targets convenience and protection properties but also presents many other applications (Han 2005).

Over the past two decades, modified atmosphere packaging (MAP) has attracted the attention of researchers as a practical technique for retaining the quality of various food products in addition to meeting the increased demand of customers for fresh and preservative-free food (Piergiovanni et al. 1993; Phillips 1999; Floros and Nielsen 2000). Furthermore, it has a number of advantages, such as quality retention of fresh products, image improvement of the product, extension of shelf life, and minimum use of additives and preservatives (Garabal et al. 2010). The atmosphere inside a package can be modified by either passive or active methods. In passive MAP, the rate of change and the final gas composition in the package depend on both the packed product and the permeability of the packaging material. This MAP technique has the main disadvantage that it may require a long storage period to achieve the optimal gas composition, which could be especially important for products with relatively short shelf life. This disadvantage can be overcome with the use of active modification of the atmosphere. Active MAP is usually accomplished by first creating a vacuum and then injecting the desired gas mixture in the package; in this case, the desired atmosphere is directly achieved at the beginning of storage and remains unchanged, provided the right packaging material is used and there is no leakage. The disadvantage of active MAP over passive MAP is the higher cost in equipment and gases (Rodriguez-Aguilera and Oliveira 2009).

MAP techniques are now used for a wide variety of products involving cakes, fresh filled pasta, potato crisps, fruits and vegetables, and meat products (Jayas and Jeyamkondan 2002; Silva et al. 2004; Guynot et al. 2004; Zardetto 2005; McMillin 2008). This paper tries to review the application of MAP in cheese by including fresh, whey, soft, semi-hard, and hard cheese along with other types which are produced and consumed all around the world.

2 Overview of basic works and important considerations in cheese MAP

Studies on the possible effectiveness of MAP started with the emergence of two works: First, Zimmerman and Kester (1960) found that hermetically sealed containers, vacuum packaging, and packaging under inert gases are successful in





delaying surface spoilage caused by aerobic organisms which were inoculated with less than ten causative organisms per gram onto Cottage cheese and were stored at $10~^{\circ}$ C. Second, Tsantilis and Kosikowski (1960) studied the effect of vacuum and N_2 or CO_2 atmospheres on the shelf life of creamed and uncreamed Cottage cheese, packed in aluminum at $5~^{\circ}$ C, reporting that CO_2 significantly reduced yeast and mold counts and maintains fresh flavor even after a prolonged storage (even after 12~weeks).

Taylor et al. (1965) reported that N₂ is insignificant in improving the shelf life of Cottage cheese. Krcal (1970) demonstrated depression in microbial damages of Cottage cheese by CO₂ and N₂ at ambient temperature, whereas the CO₂ flushing atmosphere was preferable. However, Scott and Smith (1971), who evaluated the shelf life quality of Cottage cheese (based on taste panel scoring and bacterial counts of the top centimeter of samples stored at 3-4 °C for 10-12 days in special all-glass containers with purified carbon dioxide, nitrogen, and air atmospheres), indicated that packaging Cottage cheese under neither CO₂ nor N₂ flushing could greatly improve the shelf life of samples at 3 °C. Furthermore, they proposed that CO₂ flushing could change the flavor as an acid or tart cheese, which is treated differently (as an advantage or disadvantage) with respect to local preferences. Kosikowski and Brown (1973) conducted a study evaluating the influence of carbon dioxide and nitrogen on the shelf life of creamed Cottage cheese (pH 4.5) sealed in commercial thermoplastic containers following air evacuation and CO₂ or N₂ flushing and stored at 4 °C. The authors obtained excellent qualities under CO₂ or N₂ atmospheres for 45 days at the refrigeration temperature. In addition, they pointed out that the use of carbon dioxide presents flavor-related problems, such as a fizzy flavor, suggesting that the alternation in the CO₂/N₂ ratio in favor of nitrogen can eliminate the undesired flavor while maintaining the freshness of flavor and control the microbial population.

The effectiveness of modified atmosphere packaging in extending the shelf life of dairy products, especially cheese, has been confirmed by further studies, and different gas compositions have been suggested for MAP packaging of cheese. Romani et al. (1999) evaluated storage stability of 24-month-old portioned-packed Parmigiano Reggiano cheese, packed in nylon/polyethylene bags and stored for 3 months at 4 °C. The results did not show substantial changes in the quality of differently packed products, although samples packed in 100% N_2 atmosphere showed flavor profiles quite distant from that of the freshly cut, unpacked cheese. Taniwaki et al. (2001) investigated the growth of fungi and mycotoxin production on commercial sliced cheddar cheese under modified atmospheres. Eight fungal species were incubated under conditions of decreasing concentrations of O_2 (5% to <0.5%) and increasing concentrations of O_2 (20–40%). The authors observed a reduction in the growth of fungi by 20–80% depending on species and found that the formation of aflatoxins O_1 and O_2 roquerfortin O_2 can cyclopiazonic acid were greatly decreased.

Mortensen et al. (2003) studied the impact of different MAP atmosphere on the photo-oxidation of sliced Havarti cheese and observed that for cheeses exposed to light and packed with 0.6% residual oxygen, photo-oxidation increased considerably in comparison with the ones packed with 0.01% residual oxygen.

Dankow et al. (2006) studied the effect of a packaging system on the quality and shelf life of the Rokpol-type mold cheese made from goat milk. The samples were



packed in aluminum foil and modified atmospheres (100% N₂, 30% CO₂/70% N₂, 50% CO₂/50% N₂, 70% CO₂/30% N₂, 100% CO₂, 30% CO₂/60% N₂/10% O₂, 70% CO₂/20% N₂/10% O₂ using oriented polyethylene polyamide as the packaging material) as well as in vacuum. The authors observed proliferation of the *coli* type of bacteria in the experimented cheese in aluminum foil, whereas the population of the *coli* group remained constant over the storage time in other samples, irrespective of the type of applied gas mixture.

Alam and Goyal (2007) evaluated the shelf life of Mozzarella cheese under different atmospheres (air, vacuum, 100% CO₂, 100% N₂, and 50% N₂/50% CO₂) packed in high-barrier bags and stored at -10 to -15 °C. The authors reported that Mozzarella cheese under MAP conditions compared with those in conventional air package had a significant increase in their shelf life (14–16, 90, 75, and 65 days under air, 100% CO₂, 50% N₂/50% CO₂, and 100% N₂, respectively).

The shelf life of MAP-packaged cheese is affected by some important parameters including the use of starter cultures in cheese production, type of cheese, initial microbial contamination and storage conditions (Floros and Nielsen 2000), composition of the gas atmosphere inside the package, and types of applied packaging materials (Hotchkiss 1988). Table 1 summarizes references on MAP of cheeses.

MAP design is composed of handpicking the film type and size of packaging for each product (Farber et al. 2003). Therefore, different types of packaging concepts are required for various types of cheeses. Permeability to O₂, CO₂, and water vapor transmission rates for packaging films are among the most essential factors in determining the package atmosphere composition, which may influence the product's deterioration rate (Mullan and McDowell 2003; Church 1994). The final decision on packaging film should be made based on vast assessments and in collaboration with the packaging suppliers (Sandhya 2010). Important properties of packaging materials, commonly hired for cheese packaging, are presented in Table 2.

3 Characteristics of cheese in MAP

3.1 Headspace gas composition evolution

There are three main gases used in MAP—nitrogen, oxygen, and carbon dioxide—the role and importance of each of which are related to its properties (Mullan and McDowell 2003). Other investigated gases include nitrous and nitric oxides, sulfur dioxide, ethylene, ethanol, chlorine, ozone, propylene oxide, carbon monoxide, and argon. These gases failed to enter into commercial applications due to safety, regulatory, and cost considerations (Church 1994; Sandhya 2010).

A review of the related literature showed different results for the nitrogen, oxygen, and carbon dioxide concentration changes over the storage time (Table 3). The possible reasons for the recorded changes are as follows:

CO₂ changes

 Remaining constant: The equilibrium between CO₂ dissolution in the cheese mass and CO₂ production through aerobic microbial metabolism (Eliot et al. 1998)





Table 1 References on modified atmosphere packaging of cheese

Reference	Cheese type	Packaging film	MAP conditions studied (%)	Optimal MAP (%)
Garabal et al. (2010)	San Simon da Costa (smoked semi-hard cow's milk cheese)	Multilayer polyester polyvinyl chloride and polyethylene	VP, 100 $\rm N_2,20~CO_2/80~N_2$ and 50 $\rm CO_2/50~N_2$	≥50 CO ₂
Taniwaki et al. (2001)	Commercial sliced cheddar	Polypropylene/ethylene vinyl alcohol/polypropylene	20 CO ₂ /<0.5% O ₂ , 20 CO ₂ /1 O ₂ , 20 CO ₂ /5 O ₂ , 40 CO ₂ /<0.5 O ₂ , 40 CO ₂ /1 O ₂ and 40 CO ₂ /5 O ₂	Atmospheres with $O_2 < 0.5\%$
Eliot et al. (1998)	Mozzarella	Multilayer of polyolefins with polyvinylidene chloride	100 N ₂ , 10 CO ₂ /90 N ₂ , 25 CO ₂ /75 N ₂ , 50 CO ₂ /50 N ₂ , 75 CO ₂ /25 N ₂ and 100 CO ₂	75 CO ₂ /25 N ₂
Alves et al. (1996)	Mozzarella	Thermoformed transparent laminate of polyamide and polyethylene	$100~N_2,100~CO_2$ and $50~CO_2/50~N_2$	100 CO ₂
Juric et al. (2003)	Rindless semi-hard Samøs	Thermoformed transparent laminate of polyamide and polyethylene	Thermoformed transparent laminate $100 \mathrm{N_2}, 20 \mathrm{CO_2/80 N_2}$ and $100 \mathrm{CO_2}$ of polyamide and polyethylene	$100~\text{N}_2$ and $20~\text{CO}_2/80~\text{N}_2$
Maniar et al. (1994)	Cottage	Low-density polyethylene/ biaxially oriented nylon/layer of polyvinylidene chloride	$100~\text{CO}_2,~75~\text{CO}_2/25~\text{N}_2$ and $100~\text{N}_2$	≥75 CO ₂
Mannheim and Soffer (1996)	Cottage	I	100 CO ₂	100 CO ₂
Trobetas et al. (2008)	Graviera (hard cheese)	Graviera (hard cheese) Low-density polyethylene/ polyamide/low-density polyethylene (LDPE/PA/LDPE)	$100~\text{CO}_2,~50~\text{CO}_2/50~\text{N}_2$ and $100~\text{N}_2$	$50~\text{CO}_2/50~\text{N}_2$ and $100~\text{N}_2$
Kristensen et al. (2000) Havarti	Havarti	Polyester	25 CO ₂ /75 N ₂	
Pintado and Malcata (2000)	Requeijão		$100~\mathrm{CO_2}$ and $50~\mathrm{CO_2/50~N_2}$	100 CO ₂
Olarte et al. (2002)	Cameros (fresh goat cheese)	,	$20~\text{CO}_2/80~\text{N}_2,~40~\text{CO}_2/60~\text{N}_2$ and $100~\text{CO}_2$	100 CO ₂
Papaioannou et al. (2007)	Greek whey cheese	Low-density polyethylene/ polyamide/low-density polyethylene (LDPE/PA/LDPE)	VP, 30 CO ₂ /70 N ₂ and 70 CO ₂ /30 N ₂	70 CO ₂ /30 N ₂





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Table 1 (continued)				
Reference	Cheese type	Packaging film	MAP conditions studied (%)	Optimal MAP (%)
Dermiki et al. (2008)	Myzithra Kalathaki (whey cheese)	Low-density polyethylene/ polyamide/low-density polyethylene (LDPE/PA/LDPE)	20 CO ₂ /80 N ₂ , 40 CO ₂ /60 N ₂ and 60 CO ₂ /40 N ₂	40 CO ₂ /60 N2 and 60 CO ₂ /40 N ₂
Gammariello et al. (2009b)	Stracciatella	Laminate of a polyamide (nylon) and a polyolefin	VP, 50 CO ₂ /50 N ₂ , 95 CO ₂ /5 N ₂ , 75 CO ₂ /25 N ₂ and 30 CO ₂ /65 N ₂ /5 O ₂	95 CO ₂ /5 N2, 50 CO ₂ /50 N ₂
Esmer et al. (2009)	Crottin de Chavignol	Polyamide/low-density polyethylene (PA/LDPE)	VP and 50 CO ₂ /50 N_2	$20 \text{ CO}_2/80 \text{ N}_2$
Rodriguez-Aguilera et al. (2011b)	Surface mold-ripened	Acrylic cylindrical container	0 O ₂ /27±6 CO ₂ and 2±1 O ₂ /19±2 CO ₂	Low levels of O_2 (1–3%) and relatively high levels of CO_2 (17–21%)
Favati et al. (2007)	Provolone	Polyamide (PA) and polyethylene (PE)	10 CO ₂ /90 N ₂ , 20 CO ₂ /80 N ₂ , 30 CO ₂ /70 N ₂ and 100 CO ₂	$30 \text{ CO}_2/70 \text{ N}_2$
Gammariello et al. (2009a)	Giuncata (Apulian fresh cheeses)	Laminate of a polyamide (nylon) and a polyolefin	VP, 50 CO ₂ /50 N ₂ , 90 CO ₂ /10 N ₂ , 75 CO ₂ /25 N ₂ and 30 CO ₂ /65 N ₂ /5 O ₂	$75 \text{ CO}_2/25 \text{ N}_2$
	Primosale (Apulian fresh cheeses)		$\rm CO_2/10~N_2,~75~CO_2/25~N_2$ and 30 $\rm CO_2/65~N_2/5~O_2$	95 $CO_2/5$ N_2 and 50 $CO_2/50$ N_2
Temiz et al. (2009)	Lor (Turkish whey cheese)	Polyethylene	VP, 40 CO ₂ /60 N ₂ , 60 CO ₂ /40 N ₂ and 70 CO ₂ /30 N ₂ 60 CO ₂ /40 N ₂ and 70 CO ₂ /30 N ₂	$60~\text{CO}_2/40~\text{N}_2$ and $70~\text{CO}_2/30~\text{N}_2$
Temiz (2010)	Kashar	Polyethylene		40 CO ₂ /60 N ₂ and 100 CO ₂
Gonzalez-Fandos et al. (2000)	Cameros	I	VP, 50 CO ₂ /50 N ₂ , 20 CO ₂ /80 N ₂ , 40 CO ₂ /60 N ₂ and 100 CO ₂	100 CO ₂
Del Nobile et al. (2009)	Ricotta	Nylon-based high-barrier multilayer plastic bags	50 CO ₂ /50 N ₂ , 70 CO ₂ /30 N ₂ and 95 CO ₂ /5 N ₂	95 CO ₂ /5 N ₂

VP vacuum packaging





Table 2 Properties of packaging materials used for cheese

Packaging material type	Structure	Moisture permeability ^a	Oxygen permeability ^b Carbon dioxide permeability ^c	Carbon dioxide permeability ^c	Nitrogen permeability ^d	Light transmission ^e
Low-density polyethylene, LDPE	Ethylene units; density, 0.917–0.924 (g. cm ⁻³)	1–10 or 10–50 >1,000	>1,000	130–280	1.9–3.1	92
Polypropylene, PP	Propylene units	1 - 10	>1,000	92	4.4	08
Ethylvinyl acetate, EVA	Copolymerization of low-density polyethylene and 1–20% vinyl acetate	10–50	>1,000	ı	I	55-75
Polystyrene, PS	Styrene units	>50	>1,000	105	7.8	92
Polyethylene terephthalate, PET	Ethylenglycol and dimethylterephthalate or terephthalate acid	10–50	10–100 or 100–1,000	3.0	0.04-0.06	88
Polyamide (nylon), PA	Nylon 6: polymerization of caprolactam	10–50	10-100 when dry	0.4–0.8	0.95	88
Polyvinylidene chloride, PVdC (Saran)	Vinyliden units	<1 or 1–10	1–10	0.3	600.0	06
Ethylvinyl alcohol, EVOH	Ethylvinyl acetate and methanol	10–50 or >50	1–10 or below 75% RH: <1	27 mol% ethylene: 0.024 and 44 mol% ethylene: 0.012	1	06

References: McMillin (2008), Mullan and McDowell (2003), and Church (1994)

 $^{\rm b}$ Oxygen permeability (mL. $\rm m^{-2}$.day $^{-1}$.atm $^{-1}$, 20 $^{\circ}$ C, 0% RH) ^a Moisture permeability (g. m⁻² day⁻¹, 38 °C, 90% RH)

 $^{\circ}$ Carbon dioxide permeability (P×10¹¹ [mL(STP) cm. cm $^{-2}$.s $^{-1}$. (cm Hg) $^{-1}$] at 25 $^{\circ}$ C)

 $^{\rm d}$ Nitrogen permeability $(P\times10^{11}~[{\rm mL~(STP)~cm.~cm^{-2}~.s^{-1}~.(cm~Hg)^{-1}}]$ at 25 $^{\rm o}{\rm C})$

e Light transmission (%)





Table 3 Evolution of N_2 , O_2 , and CO_2 over the storage period

Reference	Cheese type	MAP conditions (%)	Change o	bserved	
			N ₂	O ₂	CO ₂
Garabal et al. (2010)	San Simon da Costa (smoked semi-hard cow's milk cheeses)	VP, 100 N ₂ , 20 CO ₂ /80 N ₂ and 50 CO ₂ /50 N ₂	NA	Constant	Increase (in 100 N ₂ , 20 CO ₂ /80 N ₂) and decrease (in 50 CO ₂ /50 N ₂)
Eliot et al. (1998)	Mozzarella	100 N ₂ , 10 CO ₂ /90 N ₂ , 25 CO ₂ /75 N ₂ and 50 CO ₂ /50 N ₂	Decrease	Decrease, except for air which had increase	Increase
		75 CO ₂ /25 N ₂	Constant	Decrease	Constant
		100 CO ₂	Increase	Decrease	Decrease
Alves et al.	Mozzarella	100 N ₂	Decrease	Decrease	Increase
(1996)		100 CO ₂	Increase	Increase	Decrease
		50 CO ₂ /50 N ₂	Constant	Decrease	Decrease
Juric et al. (2003)	Rindless semi-hard Samso	100 N ₂ , 20 CO ₂ /80 N ₂ , and 100 CO ₂	NA	Decrease	Increase
Maniar et al. (1994)	Cottage	Air, 100 CO ₂ , 75 CO ₂ / 25 N ₂ , and 100 N ₂	Constant	NA	Constant
Trobetas et al. (2008)	Graviera (hard cheese)	100 CO ₂ , 50 CO ₂ /50 N ₂ , and 100 N ₂	NA	Constant	Constant
		Air	NA	Decrease	Increase
Kristensen et al. (2000)	Havarti	25 CO ₂ /75 N ₂	NA	Constant	Constant
Pintado and Malcata (2000)	Requeijão	100 CO ₂ and 50 CO ₂ / 50 N ₂	NA	Constant	Constant
Olarte et al. (2002)	Cameros (fresh goat cheese)	20 CO ₂ /80 N ₂ , 40 CO ₂ / 60 N ₂ , and 100 CO ₂	NA	Below 2%	Decrease
Papaioannou et al. (2007)	Greek whey cheese	VP, 30 $CO_2/70$ N_2 , and 70 $CO_2/30$ N_2	Constant	Constant	Constant
Dermiki et al. (2008)	Myzithra Kalathaki (whey cheese)	20 CO ₂ /80 N ₂ , 40 CO ₂ /60 N ₂ , and 60 CO ₂ /40 N ₂	Increase	Constant	Decrease
Gammariello et al. (2009b)	Stracciatella	VP, 50 CO ₂ /50 N ₂ , 95 CO ₂ /5 N ₂ , 75 CO ₂ /25 N ₂ , and 30 CO ₂ /65 N ₂ /5 O ₂	Constant	Constant	Constant
Esmer et al. (2009)	Crottin de Chavignol	Air, VP and 50 $CO_2/50$ N_2	NA	Increase	Decrease
Rodriguez- Aguilera et al. (2011b)	Surface mold- ripened	0 $O_2/27\pm6$ CO_2 and $2\pm$ 1 $O_2/19\pm2$ CO_2	NA	Decrease	Increase

NA not analyzed

VP vacuum packaging





• *Increasing*: CO₂ production by the growth of aerobic and anaerobic microorganisms (Alves et al. 1996), hetero-fermentative *Lactobacilli* (Lee et al. 1990; Bellengier et al. 1993), yeasts activities (Belin 1990), and cheese respiration (Juric et al. 2003)

 Decreasing: CO₂ dissolution into the cheese matrix, CO₂ consumption by anaerobic microorganisms, and CO₂ spreading out of the package (Moir et al. 1993; Ucuncu 2007)

O2 changes

- Remaining constant: Steady-state conditions between microbial respiration rate and O₂ permeation through packaging materials (Maniar et al. 1994)
- Increasing: Coming from the trapped air between the slices (in sliced cheeses)
 and absorption to the EPS tray (Alves et al. 1996) and permeability of packaging
 film to O₂ (Maniar et al. 1994)
- *Decreasing*: (1) Aerobic consumption by microorganisms, oxidative and enzymatic reactions involving oxygen (Fedio et al. 1994; Eliot et al. 1998) and (2) cheese respiration (Juric et al. 2003)

As far as N_2 is concerned, its concentration inside the package is affected by N_2 penetration into the package when its level becomes <78% and a balanced relationship between N_2 and CO_2 levels [as $N_2=100\%$ (% $CO_2+\%$ O_2)] (Alves et al. 1996; Eliot et al. 1998).

In addition to the used gas mixtures, there are some other factors affecting the inpack gas composition, including (1) permeability of package materials—retaining appropriate CO_2 concentrations in headspace requires the use of high-barrier packaging materials (Mannheim and Soffer 1996); (2) light exposure—there are significant differences in O_2 between cheese kept in the dark and cheese exposed to the light, which could be explained by oxidative reactions induced in the samples under the light leading to the increased O_2 consumption (Juric et al. 2003; Trobetas et al. 2008); and (3) storage temperature—an increase in storage temperature is followed by the rise in CO_2 and fall in O_2 levels inside the package which, consequently, is evidence for the great impact of temperature on the gas exchange rate (Rodriguez-Aguilera et al. 2011a).

3.2 Physicochemical properties

Studies have introduced different gas mixtures for retarding the physicochemical deterioration such as oxidation, lipolysis, and proteolysis, which are summarized in Table 4.

3.2.1 pH changes

The presence of CO₂ is expected to result in a pH drop, which is thought to be associated with the formation of carbonic acid, acidic amino acids, and free fatty acid production during proteolysis and lipolysis, respectively (Dermiki et al. 2008). pH reduction, caused by CO₂ atmosphere, was reported in the literature (Farber 1991; Mannheim and Soffer 1996; Koskeli 1998) and was presumed as one of the



Table 4 Changes in physicochemical properties, including pH, oxidation, lipolysis, and proteolysis

Reference	Cheese type	Gas mixture (%)			
		pH ^a	Oxidation ^b	Lipolysis ^b	Proteolysis ^b
Maniar et al. (1994)	Cottage	100 CO ₂	NA	NA	NA
Pintado and Malcata (2000)	Requeijão	NA	NA	100 CO $_2$ for all temperature treatments; 50 CO $_2$ /50 N $_2$ and 100 N $_2$ at 4 °C	NA
Olarte et al. (2002)	Cameros (fresh goat cheese)	100 CO ₂	NA	NA	NA
Dermiki et al. (2008)	Myzithra Kalathaki (whey cheese)	60 CO ₂ /40 N ₂	40 CO ₂ /60 N ₂	40 CO ₂ /60 N ₂	40 CO ₂ /60 N ₂
Gammariello et al. (2009b)	Stracciatella	30 CO ₂ /65 N ₂ /5 O ₂	NA	NA	NA
Esmer et al. (2009)	Crottin de Chavignol	20 CO ₂ /80 N ₂	NA	NA	NA
Rodriguez-Aguilera et al. (2011b)	Surface mold- ripened	$0 O_2/27 \pm 6 CO_2$	NA	NA	NA
Favati et al. (2007)	Provolone	100 CO ₂ and 20 CO ₂ /80 N ₂	NA	30 CO ₂ /70 N ₂	VP
Gammariello et al. (2009a)	Giuncata (Apulian fresh cheeses)	VP	NA	NA	NA
	Primosale (Apulian fresh cheeses)	90 CO ₂ /10 N ₂	NA	NA	NA
Temiz et al. (2009)	Lor (Turkish whey cheese)	70 CO ₂ /30 N ₂	70 CO ₂ /30 N ₂	40 CO ₂ /60 N ₂	NA
Temiz (2010)	Kashar	100 CO ₂	NA	NA	NA

^a Final lowest pH value obtained

NA not analyzed, VP vacuum packaging

mechanisms for microbial growth inhibition by CO₂ (King and Nagel 1967; Daniels et al. 1985). Mannheim and Soffer (1996), in their study evaluating the shelf life extension of Cottage cheese by MAP (pure CO₂ at 8 °C storage), observed a drop in the value. Despite the expected depression in pH values, there are various reports in the literature.

The comparison between samples under vacuum, air, and MAP atmosphere at 4 °C showed higher pH values in MAP-packaged whey cheeses (Myzithra Kalathaki), which lowered as a portion of the CO_2 increase in the CO_2/N_2 ratio (pH values order for CO_2/N_2 , 20/80 > 40/60 > 60/40; Dermiki et al. 2008). This higher value was attributed to the higher lactic acid bacteria (LAB) counts of air and vacuum packaging treatments, which resulted in high lactic acid production with a consequent drop in pH value. However, Moir et al. (1993) found that the presence of CO_2 had no effect on the pH values of Cottage cheese. Possibly, CO_2 absorption occurred mainly on the surface of the samples rather





^b Optimal gas mixture in retarding the given physicochemical property

than into the total matrix, the result of which was the acidification of just some spots. Increase in pH values was also reported in a study conducted by Maniar et al. (1994) on direct-set Cottage cheese stored at 4 °C and was justified as the absorption of the produced acid by the curd from the cream. Garabal et al. (2010) associated their observation, about the pH increase, in industrially made smoked semi-hard cheese (San Simón da Costa, from cow's milk), which increased from 5.25 in ripened cheese to 5.45, 5.46, and 5.40 for gas mixtures of 100% N₂, 20% CO₂/80% N₂, and 50% CO₂/50% N₂, respectively, after 45 days of storage, to proteolysis and associated formation of amines and ammonium. Temiz (2010) observed an increase in the pH values of sliced Kashar cheese, which was followed by a subsequent depression as the lowest final pH value (5.70) was determined in cheese under 100% CO₂. The pH increase was thought to be associated with the consumption of lactic acid by molds and yeast, proteolytic events, which would release large amounts of alkaline components (Prieto et al. 2000; Awad 2006), and the next reduction related to CO₂ presence, which was expected to cause a decrease in pH because of the formation of carbonic acid (Dermiki et al. 2008).

Additionally, cheese type and storage temperature are the parameters which should be considered significant in pH changes. For instance, the increase in the pH level of MAP-packaged surface mold-ripened soft cheeses (Rodriguez-Aguilera et al. 2011b), well known for this kind of cheese, was considered to be related to the metabolism of lactic acid, deamination of amino acids, and the release of NH₃ caused by activity of the mold on the surface of the cheese (Sousa and McSweeney 2001). Monitoring the storage temperature, especially at the last stages of storage, is another critical parameter since Pintado and Malcata (2000), in their study on the optimization of MAP (100% CO₂ and 50% CO₂/50% N₂) for Requeijao cheese, reported that storing cheese above refrigeration temperatures (i.e., 12 or 18 °C) results in great pH drop.

3.2.2 Oxidation

Oxidative damage, for all various kinds of cheese, is a well-known destructive event which emerges as undesirable modifications in flavor, odor, and appearance. To restrict the damage caused by oxidation, the contributors such as oxygen availability and light exposure should be eliminated or reduced.

With respect to retard oxidation, vacuum packaging is one of the most recommended techniques, whereas in the aspect of cheese as a target, to shield it against oxygen-related deterioration is not most appropriate considering possible undesirable changes in the structure and appearance characteristics of some kinds of cheese encountered. Instead, MAP has been suggested as a reliable technique to minimize any undesirable changes in sensory characteristics, along with extending the product shelf life (Romani et al. 2002).

Researchers have reported contradictory results on the influence of light exposure to the oxidation of cheese samples packaged with MAP. Trobetas et al. (2008) studied light-induced changes in grated Graviera hard cheese packaged under modified atmospheres (100% CO_2 , 100% N_2 , and 50% CO_2 /50% N_2) and showed that the degree of oxidation for samples kept away from light compared



with light-exposed samples was significantly lower. Absorbance values for the former were >0.04% while for the latter between 0.06% and 1.0%.

However, Kristensen et al. (2000), in their study on light-induced oxidation in sliced Havarti cheese under modified atmosphere (25% $\rm CO_2/75\%~N_2$ with initial 0.4% $\rm O_2$) exposed to light (1,000 lx) and stored at 5 °C, found no significant difference between all treatments, either under light or in the dark.

Temiz et al. (2009) observed a peak point in the lipid oxidation figure of Turkish whey cheeses (Lor) stored at 4 °C, in all treatments on day 31 of storage, so that gaseous atmosphere of 60% and 70% CO₂ exhibited lower oxidation. Thiobarbituric acid values, after approaching the maximum value on the 31st day of storage, declined with prolonged storage time, which could be explained by the interaction between malonaldehyde and the decomposition products of proteins in order to produce tertiary degradation products (Nawar 1996). At the end of storage (45 days), samples in package containing 70% CO₂ presented the lowest oxidation value.

3.2.3 Lipolysis

The degree of lipolysis is expressed as milliliters of 0.01 N KOH based on the procedure described by the International Dairy Federation (IDF 1989). Lipolysis is also a significant biochemical event in creating the final flavor of cheese (Georgala et al. 2005; Guler 2005). Lipolytic enzymes originated in milk, microorganisms, and probably the rennet (Poveda et al. 2000). Temiz et al. (2009) observed an increase in the acidity index (AI) of Turkish whey cheese for all the samples which was followed by a decrease after reaching the peak value on day 31 of storage. At the end of storage, air- and vacuum-packaged samples, when compared with the MAP ones (under 40%, 60%, and 70% CO₂), had a higher AI value, and out of the three modified atmospheres, the highest amounts of AI were determined in the 40% CO₂/60% N₂ gas mixture. The authors related this to the decomposition of free fatty acids, principally via the oxidation of methyl ketones (Prieto et al. 2000). However, Gonzalez-Fandos et al. (2000) found no significant differences among the combinations of CO₂/N₂ (20% CO₂/ $80\% N_2$, $40\% CO_2/60\% N_2$, $50\% CO_2/50\% N_2$, and $100\% CO_2$) stored at 3–4 °C studied in retarding the lipolysis of Cameros cheese.

3.2.4 Proteolysis

The degree of proteolysis is expressed in terms of the free amino acids present in the sample. Gonzalez-Fandos et al. (2000) found that the highest degree of proteolysis was detected in the control and vacuum-packaged Cameros cheese. They explained it with the observed higher counts of microorganisms in them in comparison with MA-packed samples. Although no significant difference was reported between applied modified atmospheres (20%, 40%, 50%, and 100% CO₂). Dermiki et al. (2008), as well, reported that proteolysis of packaged Myzithra Kalathaki cheeses under MAP was significantly lower than the rest of the samples. However, they demonstrated that the applied gaseous combinations (20%, 40%, and 60% CO₂)





were not effective in preventing the proteolysis; the observed reduction was considered to be related to the resistance of albumin and whey proteins (α -lactalbumin and β -lactoglobulin) present in the cheese against the action of psychrotrophic proteases rather than the effect of CO_2 concentration. Moreover, fluctuation was observed in the degree of proteolysis, which might be attributed to the consumption of proteolysis products as nutrients by increasing the microbial population (Lioliou et al. 2001).

However, in the study conducted on whey cheese (Manouri) by Dermiki et al. (2008), there was no significant increase in the degree of proteolysis in the cheese samples despite the presence of proteolytic as the main spoilage bacteria (Enterobacteriaceae and *Staphylococci*). This was attributed to the failure of caseinolytic bacteria present in Manouri cheese, degrading whey proteins (Lioliou et al. 2001).

3.2.5 Moisture content and weight loss

Moisture content is an important factor in cheese packaging from the economic point of view and for keeping the cheese fresh. Rodriguez-Aguilera et al. (2011b) demonstrated that surface mold-ripened cheese packed under MAP condition of 0% $O_2/27\pm6\%$ CO_2 exhibited the lowest values of moisture content. There was no significant difference between the control and the gas mixture of $2\pm1\%$ $O_2/19\pm2\%$ CO_2 . However, the latter resulted in almost no weight loss. Others have indicated that different gas mixtures did not significantly influence the moisture content or weight loss in the cheese when packaged under modified atmospheres. However, MAP conditions were reported as slightly efficient in keeping the moisture content of cheese stable (San Simón da Costa smoked semi-hard: Garabal et al. 2010; Greek whey: Papaioannou et al. 2007; Provolone: Favati et al. 2007; Primosale and Giuncata: Gammariello et al. 2009a; Lor Turkish whey cheese: Temiz et al. 2009; Parmigiano-Reggiano cheese: Romani et al. 2002).

There are, though, observations reporting a decrease in moisture content during storage for all cheese samples, and the statistically significant differences were found between the Cameros cheese packaged in MAP and the rest of package treatments in which MAP was preferable. However, these studies, as well as Sendra et al. (1994), in a study on goat fresh cheese, reported that the highest weight losses were found in samples packaged under 100% CO₂.

3.2.6 Dry matter, protein, fat, and salt (%)

Papaioannou et al. (2007) (Greek whey cheese) and Favati et al. (2007) (Provolone cheese) obtained no significant difference between MAP and control samples from dry matter, protein, fat, and salt percentage aspects. However, other authors reported an increase in the mentioned properties (Kristensen et al. 2000 for Havarti cheese: dry matter—from 61.7 ± 0.6 to 65.6 ± 0.8 in light and 64.3 ± 1.3 in dark; Temiz et al. 2009 for Turkish whey cheese: dry matter—from 34.56 to 35.587, protein—from 10.51 to 10.69, fat—from 15.5 to 16, and salt—from 3.65 to 3.79; Trobetas et al. 2008 for Gravira hard cheese: fat—from 30.2 to 31.8).



3.3 Microbiological analyses

Variables such as milk quality, survival of heat-sensitive microorganisms during cheese making, and post-processing microbial contamination all influence the microbiology of the cheese (Del Nobile et al. 2009). Table 5 presents the studied microorganisms and optimal gas mixtures for each given microorganism in inhibiting the microbial growth for the cheese packed under a modified atmosphere. Other studies have pointed out the significant effect of MAP containing CO₂ in preventing the cheese from microbial spoilage and prolonging their shelf life, which shows the destructive impact of CO₂ on the spoilage microbes. The inhibitory effect of CO₂ on microbial spoilage could be explained by the increase in the lag phase and the decrease in the growth rate over a logarithmic phase (Farber 1991) because microorganisms have to adapt to the new atmospheric conditions (Olarte et al. 2002). There are some theories regarding the antimicrobial properties of carbon dioxide (Farber 1991; Pintado and Malcata 2000), which include:

- Malfunctions in cell membrane, such as glucose and free amino acid uptake/ absorption
- · Direct physicochemical changes of membrane-located proteins and lipids;
- Inhibition of enzyme systems directly or depression in the rate of enzymatic reactions
- Intracellular pH change
- Inhibiting cell division and altering cell morphology

3.3.1 Lactic acid bacteria

Articles show that LAB were slightly affected under the modified atmospheres and were able to grow well (Mozzarella cheese: Eliot et al. 1998; Cottage cheese: Maniar et al. 1994; Greek whey cheese: Papaioannou et al. 2007; whey cheese: Dermiki et al. 2008; Apulian fresh cheese: Gammariello et al. 2009a; Turkish whey cheese: Temiz et al. 2009) since LAB are facultative anaerobic Gram-positive in nature. The inefficiency of the MAP packaging on these useful dairy bacteria has become a suitable advertising tool to increasingly market dairy products with MAP as being "preservative-free and rich in viable lactic acid bacteria" (Coppola et al. 1995). However, Maniar et al. (1994) showed that LAB counts of Cottage cheese (with gas mixture of 100% CO₂, 75% CO₂/25% N₂, and 100% N₂) remained constant and independent from MAP effect, which was possibly due to the low storage temperature (4 °C) applied in their study. These findings agree with the results in a study conducted by Papaioannou et al. (2007) on Greek whey cheese in which the higher LAB counts were measured at 12 °C rather than 4 °C, for all the cheese samples.

3.3.2 Psychrotrophic bacteria

Maniar et al. (1994) reported that psychrotrophic bacteria counts remained unchanged for Cottage cheese with modified atmospheres (100% $\rm CO_2$, 75% $\rm CO_2$ / 25% $\rm N_2$, and 100% $\rm N_2$). The absence of $\rm O_2$ from the package headspace, because psychrotrophic bacteria are aerobes, and the bacteriostatic properties of $\rm CO_2$ may





Table 5 Studied microorganisms and optimal gas mixture in inhibiting microbial growth

Reference	Cheese type	Optimal gas mixture (%) ^a	xture (%) ^a					
		Lactic acid bacteria (LAB)	Psychrotrophic bacteria	Psychrotrophic Mesophilic bacteria bacteria	Pseudomonas spp.	Yeasts	Molds	Enterobacteriaceace
Garabal et al. (2010)	San Simon da Costa	NA	NA	50 CO ₂ /50 N ₂	NA	≥50 CO ₂	≥50 CO ₂	NA
Eliot et al. (1998)	Mozzarella	75 CO ₂ /25 N ₂	75 CO ₂ /25 N ₂ 75 CO ₂ /25 N ₂ 75 CO ₂ /25 N ₂	75 CO ₂ /25 N ₂	NA	$50 \text{ CO}_2/50 \text{ N}_2,$ $100 \text{ N}_2,$ 100 CO_2	10–100% CO ₂	NA
Alves et al. (1996)	Mozzarella	NA	100 CO ₂	NA	NA	100 CO_2	100 CO_2	NA
Maniar et al. (1994)	Cottage	$75 \text{ CO}_2/25 \text{ N}_2$	75 CO ₂ /25 N ₂ 75 CO ₂ /25 N ₂ NA	NA	NA	NA	NA	NA
Mannheim and Soffer (1996)	Cottage	NA	NA	NA	NA	100 CO ₂	100 CO ₂	NA
Olarte et al. (2002)	Cameros (fresh goat cheese)	NA	100 CO ₂	100 CO ₂	NA	NA	NA	NA
Papaioannou et al. (2007)	Greek whey cheese	$70 \text{ CO}_2/30 \text{ N}_2$ NA	NA	70 CO ₂ /30 N ₂	70 CO ₂ /30 N ₂	70 CO ₂ /30 N ₂	$70 \text{ CO}_2/30 \text{ N}_2$	$70 \text{ CO}_2/30 \text{ N}_2$
Dermiki et al. (2008)	Myzithra Kalathaki (whey cheese)	40 CO ₂ /60 N ₂	$40 \text{ CO}_2/60 \text{ N}_2 + 40 \text{ CO}_2/60 \text{ N}_2 + 40 \text{ CO}_2/60 \text{ N}_2$	40 CO ₂ /60 N ₂	NA	40 CO ₂ /60 N ₂ , 60 40 CO ₂ /60 N ₂ , CO ₂ /40 N ₂ 60 CO ₂ /40 N ₂	40 CO ₂ /60 N ₂ , 60 CO ₂ /40 N ₂	40 CO ₂ /60 N ₂ , 60 CO ₂ /40 N ₂
Gammariello et al. (2009b)	Stracciatella	DN	DN	95 CO ₂ /5 N ₂	75 CO ₂ /25 N ₂	DN	DN	95 CO ₂ /5 N ₂
Esmer et al. (2009)	Crottin de Chavignol	NA	NA	$20 \text{ CO}_2/80 \text{ N}_2$	NA	$20 \text{ CO}_2/80 \text{ N}_2$	$20 \text{ CO}_2/80 \text{ N}_2$	NA



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Reference	Cheese type	Optimal gas mixture (%) ^a	xture (%) ^a					
		Lactic acid bacteria (LAB)	Psychrotrophic bacteria	Psychrotrophic Mesophilic bacteria bacteria	Pseudomonas spp.	Yeasts	Molds	Enterobacteriaceace
Favati et al. (2007)	Provolone	30 CO ₂ /70 N ₂ 100 CO ₂	100 CO ₂	100 CO_2 (for aerobic) NA and VP (for anaerobic)	NA	100 CO ₂	20 CO ₂ /80 N ₂ NA	NA
Gammariello et Apulian fresh al. (2009a) cheeses	Apulian fresh cheeses	50 CO ₂ /50 N ₂ NA		DN	50 $CO_2/50 N_2$, 90 $CO_2/10 N_2$	DN	ND	NA
Temiz et al. (2009)	Lor (Turkish whey cheese)	60 CO ₂ /40 N ₂	$60 \ \text{CO}_2/40 \ \text{N}_2 40 \ \text{CO}_2/60 \ \text{N}_2 60 \ \text{CO}_2/40 \ \text{N}_2, \\ 70 \ \text{CO}_2/30 \ \text{N}$	60 CO ₂ /40 N ₂ , 70 CO ₂ /30 N ₂	NA	60 CO ₂ /40 N ₂ , 70 CO ₂ /30 N ₂	60 $CO_2/40 N_2$, 70 $CO_2/30 N_2$	$70 \text{ CO}_2/30 \text{ N}_2$
Temiz (2010)	Kashar	NA	NA	100 CO ₂	NA	100 CO_2	100 CO_2	NA
Gonzalez- Fandos et al. (2000)	Cameros	NA	100 CO ₂	100 CO ₂	NA	ND	N Q	100 CO ₂
Del Nobile et al. Ricotta (2009)	Ricotta	95 CO ₂ /5 N ₂	95 CO ₂ /5 N ₂ 95 CO ₂ /5 N ₂ 95 CO ₂ /5 N ₂	95 CO ₂ /5 N ₂	95 CO ₂ /5 N ₂	95 CO ₂ /5 N ₂ , 70 CO ₂ /30 N ₂	ND	$70 \text{ CO}_2/30 \text{ N}_2$

 $^{\rm a}$ Optimal gas mixture for best preservation against given microbial target. M not analyzed, $N\!D$ not detected, $D\!N$ data not shown, $P\!P$ vacuum packaging





explain this observation (Brody 1989). Alves et al. (1996) demonstrated that MAP (100% CO₂) was able to retard just the beginning of the psychrotrophic bacteria growth in Mozzarella cheese, which was confirmed by others (Pintado and Malcata 2000 on Requeijão cheese; Gammariello et al. 2009a on Apulian fresh cheeses). Also, Eliot et al. (1998) found that there was psychrotroph growth during the first weeks of storage for those with the modified atmospheres under study (10%, 25%, 50%, 75%, 100% N₂) since psychrophiles are a complex population and species in Mozzarella cheeses are resistant to CO₂ inhibitory effect.

In addition, different storage temperatures (10 °C by Moir et al. 1993 on Cottage cheese; 7 °C by Alves et al. 1996 on Mozzarella cheese; 10 °C by Eliot et al. 1998 on Cameros cheese; 4 °C by Gonzalez-Fandos et al. 2000 on Cameros cheese) were evaluated as a possible contributor means for MAP in controlling psychrotrophic bacteria growth, and applying low temperatures was found to be a fruitful hurdle in combination with MAP. Due to lower temperatures, the higher CO₂ solubility leads to a higher inhibitory effect of CO₂.

3.3.3 Other studied microorganisms

In addition to the investigated microorganisms, presented in Table 5, a number of articles have studied the effect of MAP on some other bacteria with health-related concerns and found it to be an efficient means to control or even inhibit their growth.

3.3.4 Staphylococci

Eliot et al. (1998) studied the growth of staphylococci under different MAP conditions (10%, 25%, 50%, 100% CO_2 , and 100% N_2 on Mozzarella cheese). Its growth was inhibited in all studied treatments; the counts of staphylococci were 1 log CFU.g⁻¹ after 8 weeks storage and did not meet the suggested microbial threshold of 10^7 CFU.g⁻¹ (ICMSF 1978).

3.3.5 Coliforms

Gammariello et al. (2009a, b) explained that the viable cell concentration of coliforms slightly declined up to day 8 of storage for all treatments. In particular, among the MAPs studied, the gas mixtures of 95% CO₂/5% N₂ for Apulian fresh cheeses and 75% CO₂/25% N₂ for Stracciatella, respectively, were the most effective for the inhibition of these spoilage microorganisms, supporting the results obtained in Gonzalez-Fandos et al. (2000) which showed that CO₂ had an inhibitory effect on coliforms on Cameros cheese; later, they introduced plain CO₂ as an optimum gas combination. Moreover, Mannheim and Soffer (1996) reported that coliform counts were about 1 log cycle higher in control Cottage cheese when compared with the samples flushed with pure CO₂.

3.3.6 Listeria monocytogenes

Since the 1970s, *Listeria monocytogenes* has been introduced as a food-borne pathogen. Not only vegetables and meat but also certain kinds of cheeses were



proven to be ideal media for *Listeria* growth (Genigeorgis et al. 1991; Mossel et al. 1995). The reported outbreaks of listeriosis associated with the consumption of Mexican-style cheese from California (James et al. 1985), Mexican soft cheeses (Linnan et al. 1988), and Vacherin Mont D'Or (Bille 1990) have caused great concern toward L. monocytogenes. Previously, Chen and Hotchkiss (1991) pointed out the inhibitory effect of CO₂ on L. monocytogenes, especially in a hurdle manner with low-temperature storage (4 °C) and pH in Cottage cheese. For preventing Listeria growth, Whitley et al. (2000) found that an increase in the CO₂ level to 20% (20% CO₂/80% N₂) was more efficient than a simple reduction in the O₂ concentration (80% N₂/10% CO₂/10% O₂) of the package headspace surrounding ripened mold cheese (Stilton) at refrigeration temperatures (2–8 °C). However, they concluded that MAP in either 10% or 20% CO₂ did not have the potential for controlling the growth of L. monocytogenes in a mold-ripened cheese. Olarte et al. (2002) incubated Cameros cheese inoculated with this pathogen and examined the effectiveness of MAP (20%, 40%, and 100% CO₂) on its growth inhibition and found pure CO_2 effective in declining the growth rate of L. monocytogenes, but not inhibition. They even showed that L. monocytogenes was able to grow in the cheese samples stored at low temperature (4 °C) during the storage period. The availability of suitable growth conditions in cheese composition (no starter culture, near-neutral pH, low salt content, and high moisture content), wide range of growth temperature of this bacterium (from -1 to 45 °C), and overlapping the pH levels (6-6.7) of Cameros cheeses with pH range for the growth of *Listeria* (pH range of 4.1–9.6 with an optimum in the region of pH 6) might cause the growth of the microorganisms in this type of cheese (Whitley et al. 2000).

In contrast, Moir et al. (1993) did not observe growth of L. monocytogenes inoculated into Cottage cheese packaged in air or 40% $\rm CO_2/60\%~N_2$. The ability of L. monocytogenes to grow in Cottage cheese probably resulted from the low pH of this type of cheese (Bahk and Marth 1990). It is worth noting that since the organism is not generally known as thermoduric, most thermal processes such as pasteurization should be able to easily eliminate Listeria species from foods (Donnelly and Briggs 1986).

3.3.7 Clostridium sporogenes

To the best of our knowledge, Chen and Hotchkiss (1991) were the only investigators who monitored the growth of *Clostridium sporogenes* in inoculated Cottage cheese, applying dissolved carbon dioxide, in which they did not observe a significant effect of CO₂ on *C. sporogenes* growth. No growth was observed in the Cottage cheese packaged in the atmosphere containing/not containing CO₂. They concluded that the lack of growth in *C. sporogenes* was possibly associated with the effect of suboptimal pH and temperature in the combination action, not with the CO₂ influence.

3.4 Sensory evaluation

3.4.1 Color

HunterLab is the most common equipment used to monitor the color (Hunter L^* , a^* , and b^*) values of cheese samples over the storage time. Temiz et al. (2009) reported





that the highest L^* value (lightness) and the greatest negative a^* value (greenness), among all packaging treatments, belonged to the atmospheres consisting of 70% $CO_2/30\%$ N_2 and 40% $CO_2/60\%$ N_2 , respectively. The b^* values (yellowness), despite a significant effect of storage time and packaging technique, remained unchanged between days 17 and 38 in the Turkish whey cheese.

However, Maniar et al. (1994) showed no changes in the L^* , a^* , and b^* values of Cottage cheeses stored at 4 °C for treatments (marked samples, packed samples in air, 100% CO₂, 100% N₂, and 75% CO₂/25% N₂) and observed only minor differences, which were considered to be instrument-related noise and variations in sample presentation (Clydesdale 1969; Dixon and Kelly 1988). The findings by Favati et al. (2007) were in line with these results, in which the authors proposed that storage conditions (i.e., gas composition, storage duration, and storage temperature) were not able to have a significant influence on the color of Provolone cheese. However, regarding b^* (yellowness), Del Nobile et al. (2009) noted significant differences between the Ricotta cheese stored under ordinary and modified atmospheres with the gas mixtures of 50% CO2/50% N2, 70% CO2/30% N2, and 95% CO₂/5% N₂. Esmer et al. (2009) also indicated no significant effect of gaseous atmosphere of 20% $CO_2/80\%$ N_2 on the L*, a*, and b* values of Crottin de Chavignol cheese stored at 4 °C. Temiz (2010) observed the relative decline in color values $(L^*, a^*, \text{ and } b^*)$ in Kashar cheese, but no statistical effect was recorded for MAP (20%, 40%, and 100% CO₂) treatments. The best gas mixture, to limit the color deterioration, was found to be pure CO₂ flushing, except for the b* value which received the worst and the highest score given to packaging under air atmosphere.

Trobetas et al. (2008) observed the gradual discoloration only in the Graviera hard cheese packed under MAP atmospheres (100% CO₂, 100% N₂, and 50% CO₂/50% N₂) and exposed to light at 4 °C. The changes in color were as follows: *L** value decreased, which was related to carotenoid degradation induced by light, as an inner filter (Hansen and Skibsted 2000), and the riboflavin degradation, as a photosensitizer in the photo-oxidation reaction (Bosset et al. 1995). With regard to the *a** and *b** values, these experienced an increase and decrease, respectively, while the values of the samples stored in the dark remained constant. These observations agree with Juric et al. (2003) who found similar trends in the color values of semi-hard cheese (Samøs) packed in different gas mixtures (100% CO₂, 100% N₂, and 20% CO₂/80% N₂) for the first 4 days of storage. The authors also showed that the packaged cheese flushed with pure CO₂ under the light was darker in comparison with the samples in atmospheres with/without 20% CO₂ stored in the dark, perhaps due to the drying effect of CO₂ and light exposure.

However, Kristensen et al. (2000) reported no significant differences in L^* and b^* values between Havarti cheese packed in 25% $\rm CO_2/75\%$ $\rm N_2$ stored either under the light or in the dark at 4 °C, although a trend of decreasing yellowness was notable in samples and an increase in a^* values of samples was detected. Moreover, Colchin et al. (2001) observed that shredded cheddar cheeses packaged under pure $\rm CO_2$ and fluorescent light (1,000 lx), among treatment groups ($\rm CO_2$ +light, $\rm CO_2$ +dark, $\rm N_2$ +light, and $\rm N_2$ +dark), exhibited significantly higher L^* values, whereas the a^* and b^* values detected were considerably lower. On the other hand, the shredded cheese color shifted from the traditional



orange color to a definite white hue. Light intensity is well known to be fatal to the color stability of stored cheese, which should be taken into consideration while designing MAP by paying attention to the fact that light intensities ≥1,614 lx may help cheese color fade (Deger and Ashoor 1987; Hong et al. 1995).

3.4.2 Odor and taste

The lowest acceptability score for odor and taste is 3.5 [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)] (Papaioannou et al. 2007) or 4.0 [a 9-point scale (where a score of 9 corresponds to extremely good, 8 very good, 7 quite good, 6 moderately good, 5 neither good nor bad, 4 moderately bad, 3 quite bad, 2 very bad, and 1 extremely bad)] (Temiz et al. 2009). The majority of studies reported the effectiveness of MAP in prolonging the sensory acceptability of cheeses in terms of taste and odor (Maniar et al. 1994: \geq 75% CO₂ for taste; Papaioannou et al. 2007: 70% CO₂/30% N₂ for both odor and taste; Dermiki et al. 2008: 60% CO₂/40% N₂ for both odor and taste; Temiz et al. 2009: 40% CO₂/60% N₂ and 70% CO₂/30% N₂ for odor and taste; Temiz 2010: 40% CO₂/60% N₂ and 100% CO₂ for taste as an effective gas mixture for Cottage cheese, Greek whey cheese, whey cheese, Turkish whey cheese, and Kashar cheese, respectively).

Although Gonzalez-Fandos et al. (2000) demonstrated that the atmosphere containing 100% CO₂ had a very negative effect on the sensory quality of Cameros cheese, the worst scores for taste were obtained in pure CO₂-packaged cheeses, and panelists rejected them after 14 days of storage. Similarly, Garabal et al. (2010) showed that vacuum packaging is the best method for retaining the sensory quality of San Simón da Costa smoked semi-hard cheeses.

Despite these results, Alves et al. (1996) and Maniar et al. (1994) reported that plain CO_2 was the best at maintaining the sensorial characteristics of Mozzarella and Cottage cheeses, respectively. Mannheim and Soffer (1996), as well, showed that Cottage cheese samples do not suffer from packaging with 100% CO_2 with regard to the sensorial characteristics, and no changes in taste or even texture were recorded. However, Esmer et al. (2009) noted that the highest score was given to the Crottin de Charignol cheeses with a 20% $CO_2/80\%$ N_2 gas composition.

4 Conclusion

Dairy products, especially cheese, are attractive and nutritional products. Therefore, keeping them fresh and extending their shelf life is important and favorable for both producers and consumers. Increased awareness of consumers from fresh and preservative-free food has led scientists to investigate new alternative approaches to the traditional preservation methods, one of which is to use the modified





atmosphere packaging (MAP) as a technique for improving the product image and extending the shelf life of various foods.

The MAP technique was proven to be useful in prolonging the shelf life of cheese samples in terms of microbiological and sensorial aspects, in which the recommended gas mixtures depend on cheese type, cheese manufacturing conditions, initial microbial load of cheese, packaging materials, and storage circumstances, as well as post-processing activities.

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