

*Full Length Research Paper*

# Effect of modified atmospheres on microbiological and sensorial properties of Apulian fresh cheeses

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**Commercial samples of fresh cheeses of the Apulia region (Italy) made from cow (Giuncata cheese) and goat milk (Primosale cheese) were studied to establish their microbiological and sensorial characteristics into package in air, under vacuum or different modified atmospheres (MAP). The combination made up of 75% CO<sub>2</sub> and 25% N<sub>2</sub> allowed the best preservation for Giuncata cheese, since it was able to slow down the spoilage bacteria, without affecting the dairy microflora, more than the other entire MAP; furthermore, no negative effect on sensory quality has been reported. In contrast, no mixture was individuated to provide good microbiological and sensorial quality for Primosale cheese.**

**Key words:** Fresh cheese, modified atmosphere, shelf life.

## INTRODUCTION

The increased consumer demand for fresh and preservative-free food has led to the use of modified atmosphere packaging (MAP) as a technique to improve product image and extend the shelf life of various foods, including cheeses (Piergiovanni et al., 1993; Maniar et al., 1994; Gonzalez-Fandos et al., 2000).

Among the fresh cheeses, Giuncata and Primosale cheese, which are typical fresh cheeses from the Apulia region, can be included. The former is made from cow's milk, and it takes its name from cheese mould (or shape) called "giunco", while the second one is produced from goat's milk. They are produced with milk heated to 80°C and then cooled to 32 - 38°C and coagulated, without starter addition, in 25 - 30 min with the addition of calf or lamb liquid rennet. The curd is collected and drained into moulds. Refrigerated storage lasts only 5 days. In fresh cheeses, there is no rind and the dough is white. The texture is soft and slightly consistent. The odour of the Giuncata cheese is generally fine and delicate, but intense in the goat's cheese. The flavour is mainly acidic in

the former, but pleasant and faintly pungent in Primosale cheese.

Given the presence of oxygen, high water activity and the high pH of fresh cheese, the micro organisms responsible for spoilage can grow easily (Olarte et al., 2002). Thus, the shelf life of fresh cheese under refrigeration is only a few days. The success of modified atmosphere packaging in extending the shelf life of dairy products, including cheese, has been demonstrated (Floros et al., 2000; Papaioannou et al., 2007). These authors summarised that the achievement of cheese packaging is dependent on a number of important parameters, such as the use of starter cultures in cheese production, the type of cheese, i.e., stabilized (cream, Feta), active (semi-soft, hard) or ripened (Brie), the initial contamination and storage conditions (Papaioannou et al., 2007).

The gases normally used for MAP include carbon dioxide (CO<sub>2</sub>), oxygen (O<sub>2</sub>) and nitrogen (N<sub>2</sub>). The most important gas from a microbiological point of view is CO<sub>2</sub>, used alone or in a mixture with N<sub>2</sub> and/or O<sub>2</sub>, which inhibit the growth of many micro organisms, including spoilage bacteria (Daniels et al., 1985). Overall, it is recognized that carbon dioxide in combination with chilled storage is particularly effective in preserving spoilage by Gram-negative, aerobic bacteria and moulds (Clarck and Takacs,

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1980; Enfors and Molin, 1980). The use of MAP may reduce contamination levels, but the sensory characteristics and their evolution throughout the storage time are also important. Some authors have pointed out the adverse effect of CO<sub>2</sub> on sensory characteristics (Scott and Smith, 1971; Daniels et al., 1985). However, although there are studies on cheese packaging, only a few look at sensory aspects (Fava et al., 1993; Hong et al., 1995; Maniar et al., 1994; Olarte et al., 2001).

The objectives of the present work are to determine the microbiological, physico-chemical and sensorial changes in Giuncata and Primosale cheese during storage at 8°C under modified atmospheres.

## MATERIALS AND METHODS

### Sample preparation

Samples of fresh commercial Giuncata and Primosale cheese were purchased from dairy plants "Posta la via" (Foggia, Italy) and "La Montanara" (Monte Sant'Angelo, Italy) respectively. Cheese was produced according to the technological procedure described by Salvadori Del Prato (2004): the milk (cow or goat) was heated to 80°C and then cooled to 38°C and liquid calf rennet was added. Curd formation was achieved after 45 - 60 min and then the curd was cut and collected and drained into moulds (containers of reed, called "giunco", hence the name "Giuncata"). Samples were transported to the laboratory in polystyrene boxes containing ice and were used within 3 h from production.

All portions (200 g) of Giuncata and Primosale cheese samples were packaged in commercially available bags with a thickness of 95 µm, provided by Valco (Bergamo, Italy). These were obtained by laminating a nylon layer and polyolefin layer, and have an oxygen transmission rate of 50 mL·m<sup>-2</sup>·24·h<sup>-1</sup> at 1 atm, measured at 23°C and 75% relative humidity.

The Giuncata cheese was separated into six lots and packaged as follows: Cnt (control samples: packaged under ordinary atmosphere), VP (vacuum packaged), MA (packaged in 50:50 CO<sub>2</sub>:N<sub>2</sub>), MB, (packaged in 90:10 CO<sub>2</sub>:N<sub>2</sub>), MC (packaged in 75:25 CO<sub>2</sub>:N<sub>2</sub>) and MD (packaged in 30:65:5 CO<sub>2</sub>:N<sub>2</sub>:O<sub>2</sub>). Instead, the Primosale cheese was separated into five lots and packaged as follows: Cnt (control samples: packaged under ordinary atmosphere), VP (vacuum packaged), M1 (packaged in 30:65:5 CO<sub>2</sub>:N<sub>2</sub>:O<sub>2</sub>), M2, (packaged in 10:90 CO<sub>2</sub>:N<sub>2</sub>), and M3 (packaged in 75:25 CO<sub>2</sub>:N<sub>2</sub>). All samples were stored at 8°C for 8 days.

Determinations of microbial count, pH, moisture, headspace gas composition and sensorial quality were carried out before packaging and after 1, 2, 3, 4, 7 and 8 days of storage on two samples for each batch of both cheeses investigated in this work.

### Microbiological analyses

Cheese samples (20 g) were homogenized for 1 min in 180 ml of a sterile solution of 0.1% (w/v) sodium chloride using the Stomacher (PBI, International Milan, Italy). Further, decimal dilutions were prepared with the same diluent and plated on the appropriate media Petri dishes. Analyses were carried out using the following procedures: total microbial counts were enumerated on PCA and incubated at 30°C for 48 h; lactic acid bacteria on MRS agar, supplemented with cycloheximide (100 mg·L<sup>-1</sup>, Sigma, Milan, Italy) and incubated under anaerobiosis (Anaerogen Gas Pack, Oxoid) at 37°C for 48 h; coccal-shaped lactic acid bacteria were enumerated on M17 agar (Oxoid) and incubated at 37°C for 48 h; yeast and

moulds on yeast peptone dextrose agar; total coliforms were enumerated on VRBLA and incubated at 37°C for 24 h and *Pseudomonas* spp. were enumerated on Pseudomonas Agar Base, added with SR103 selective supplement (Oxoid, Milan, Italy) and incubated at 25°C for 48 h. All media for microbiological analyses were purchased from Oxoid (Milan, Italy). Results were expressed as common logarithm colony forming units per gram of cheese. All the analyses were performed in duplicate.

### Physicochemical analyses

The pH values on each sample were determined by direct reading with pH-metre (Crison, Barcelona, Spain). The moisture (%) of the cheese samples was determined by dehydration at 105°C by using a drying oven (9000 series-RS232, ISCO, Milan, Italy). Two replicates were carried out for all samples.

### Headspace gas composition

Prior to opening the cheese bag, headspace gas composition was determined by using a checmate 9000 gas analyzer (PBI Dan-sensor, Denmark). The volume taken from the package headspace for gas analysis was about 10 cm<sup>3</sup>. To avoid modifications in the headspace gas composition due to gas sampling, each package was used only for a single determination. Two samples were used for each test.

### Sensorial analysis

The sensorial characteristics of the Giuncata and Primosale cheese were evaluated in accordance with the method described by Corradini and Innocente (2002). A panel of seven experienced judges, members of the Laboratory of Food Packaging, evaluated the sensorial attributes of colour, flavour and texture using a 1 - 7 point scale (1 = very poor; 7 = very good), according to Corradini and Innocente (2002). Panelists were also asked to score the overall quality as an average of the above-mentioned sensorial attribute values as weighted by the panelist. A score equal to 4 was used as the threshold for produce acceptability.

### Statistical analysis

The values of the pH and moisture of all investigated samples were compared by one-way variance analysis (ANOVA). A Duncan's multiple range test, with the option of homogeneous groups (P < 0.05), was used to determine significance among differences. STATISTICA 7.1 for Windows (StatSoft, Inc, Tulsa, OK, USA) was used for this purpose.

## RESULTS AND DISCUSSION

### Giuncata cheese

The effect of different packaging conditions on microbiological and sensorial characteristics of Giuncata cheese is presented.

The lactic acid bacteria counts of cheese during the storage period are shown in Figure 1. All samples showed an increasing trend: the initial microbial count was about 6.00 log cfu/g becoming dominant toward the end

(a) Cocci (b) ro

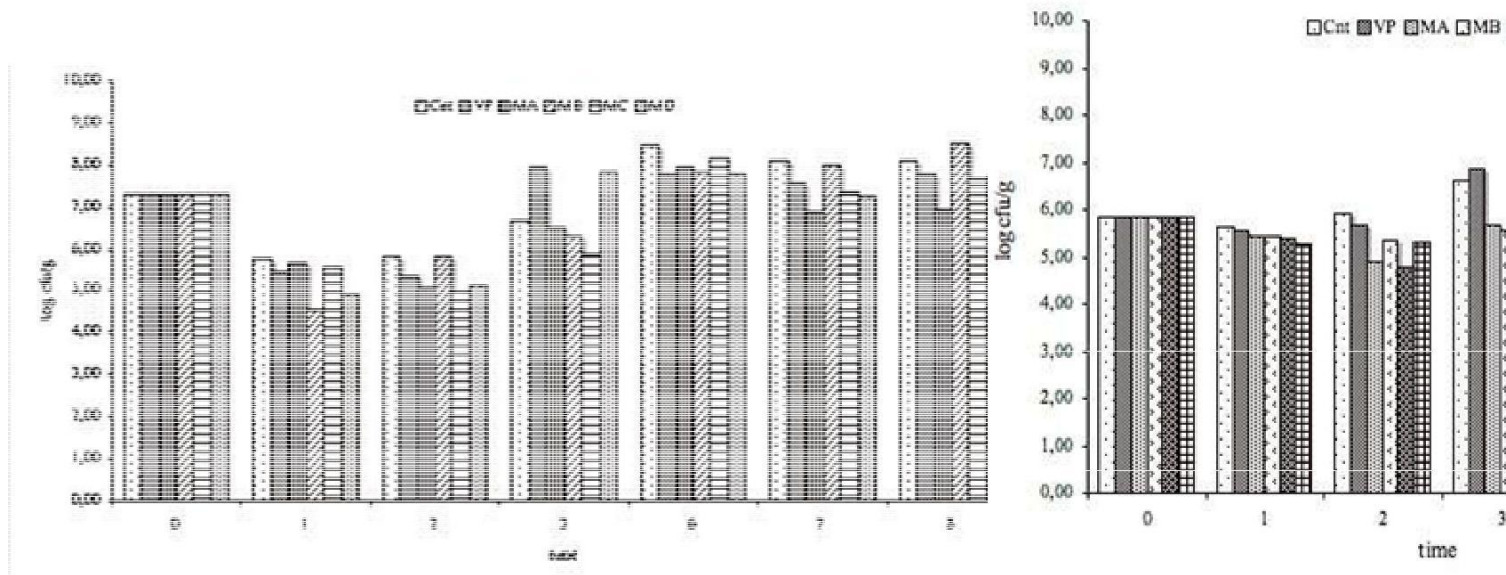


Figure 1a. Evolution of lactic acid bacteria count in Giuncata cheese during the storage period.

of the storage period, regardless of packaging conditions (about 8.50 log cfu/g). The MAP conditions did not influence the growth of typical dairy micro organisms, given the microaerophilic nature of these bacteria (Lioliou et al., 2001; Maniar et al., 1994). The inefficiency of the proposed packaging on useful dairy bacteria is particularly important for a fresh product, which is increasingly advertised as being "preservative-free and rich in viable lactic acid bacteria" (Coppola et al., 1995). Moreover, these bacteria survive the heat treatments during the process, and play an important role in food fermentations, causing the characteristic flavour changes and exercising a preservative effect on the fermented product.

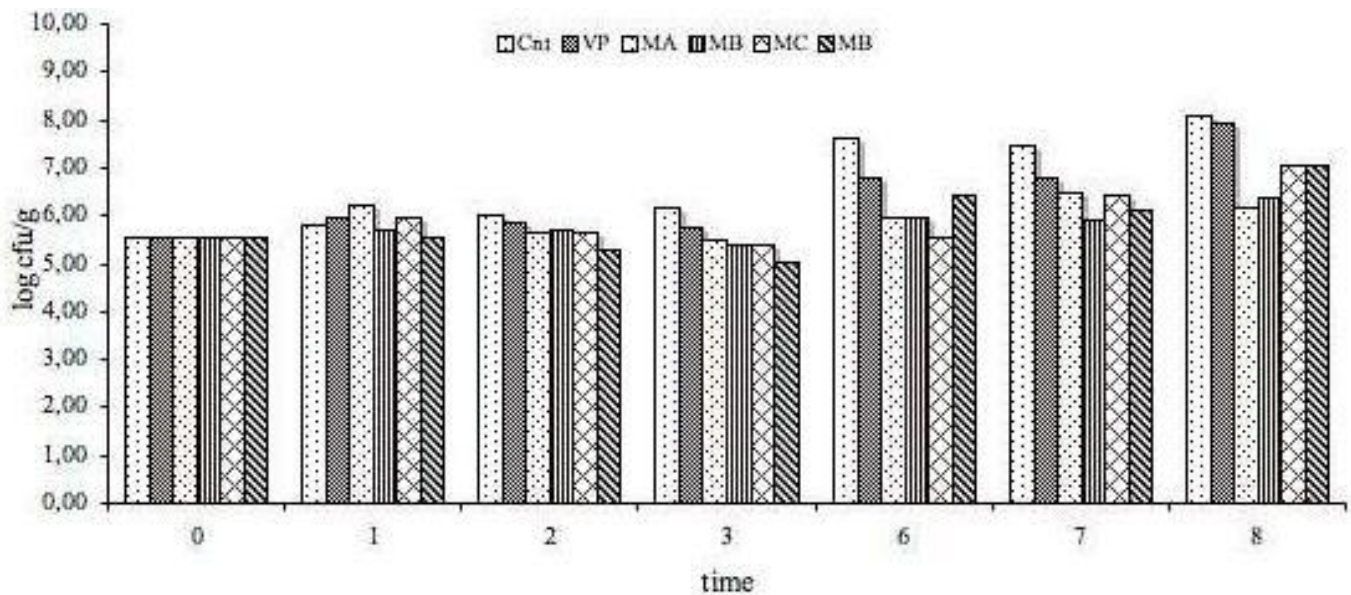
The amount of coliforms recorded during the

storage period (data not shown) explained that the viable cell concentration slight decreased until day 8 of storage for all treatments. In particular, among the MAPs, the MC was the most effective for the inhibition of these spoilage micro organism. This result was probably attributed to the inhibitory effect of a high concentration of CO<sub>2</sub> on microbial growth (Eliot et al., 1998; Maniar et al., 1994; Mannheim and Soffer, 1996). In contrast, a steady increase in counts was observed in the case of the VP and the Cnt samples that ranged between about 9.0 and 8.0 log cfu/g, respectively at the end of the storage period.

*Pseudomonas* spp. counts for the various packaging treatments are in the Figure 2. The VP and the Cnt samples showed a gradual growth of

spoilage microo samples, MA a effective in red This inhibitory e reported in simi cheeses (Faber 2000; Olarte et a

No moulds w the entire stora (data not show amounts in the e microbial load l trend was rathe of storage. The and yeast growt Sendra et al. (19



**Figure 2.** Evolution of *Pseudomonas* spp. count in Giuncata cheese during the storage period.

et al. (1998). In addition, according to Jin and Park (1995) and Johnson (1988), the increase of yeast counts at the end of storage was probably attributed to the rise of pH, which in turn can be ascribed to the yeast metabolic activities. In contrast, in the Cnt sample, the viable cell concentration decreased at the beginning, but then remained rather constant over the time. Sendra et al. (1994) and Sarais et al. (1996) found lower yeast and mould counts in cheeses packaged under vacuum than cheeses packaged in air, as occurred in our case.

The trend of total microbial count was similar in all samples (data not shown); there is a slightly increase but then remain relatively stable. However, the viable cell concentration was higher in the VP and Cnt samples compared with those packaged under MAP.

The pH values are shown in Table 1a; as can be inferred, there is a wild fluctuation in pH values, but a slight decrease can be observed for all the tested samples. The decrease at the end of storage is characteristic of fresh cheese produced without a starter culture (Martin-Hernández et al., 1990).

The average moisture decreased during storage for all cheese samples. There were no significant differences between cheese packaged in air and cheese packaged under vacuum. These results agree with those reported by Sendra et al. (1994) for fresh cheese. Similar results were also observed for the MAP samples.

The changes in pH and moisture agree with the evolution of microbial groups; however, the highest microbiological counts were detected in cheeses packaged under ordinary conditions.

Slight changes in gas concentrations in the MAP packages were detected. The initial CO<sub>2</sub> concentrations depended on the batch and the CO<sub>2</sub> concentrations

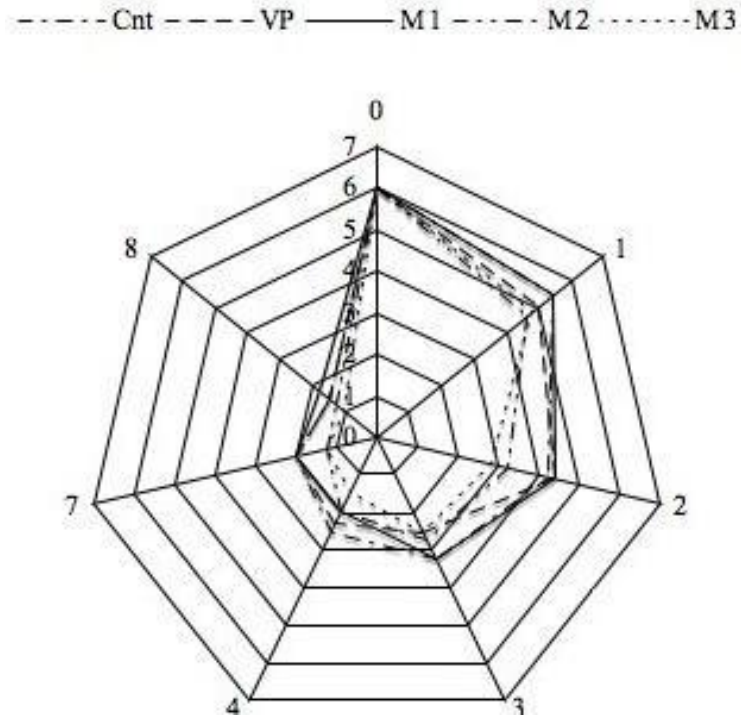
decline in all the batches packaged in MAP. Moir et al. (1993) also observed this decrease in CO<sub>2</sub> concentrations; this may be because CO<sub>2</sub> both dissolves into the cheeses and permeates out of the package. O<sub>2</sub> concentration continuously declined as CO<sub>2</sub> increased in the sample packaged under ordinary atmosphere. O<sub>2</sub> was probably consumed inside these packages as Fedio et al. (1994) has noted. This phenomenon was probably due to respiration by aerobic microflora or to oxidative and enzymatic reactions involving oxygen (Eliot et al., 1998). Generally, atmospheres with a high CO<sub>2</sub> concentration guarantee the best preservation, because CO<sub>2</sub> inhibits microbial growth; furthermore, no negative effect of CO<sub>2</sub> on product flavour has been reported in the literature (Eliot et al., 1998; Maniar et al., 1994). In fact, the overall acceptability (Figure 3) of the cheese under study packaged in CO<sub>2</sub> atmospheres received very favourable scores during the period studied. However, overall acceptability decreased throughout the storage in all the cheeses studied. On the contrary, Cnt and VP cheeses were unfavourably received by the panelists, obtaining the lowest score for odour (data no shown) and in fact they were refused on the 3<sup>rd</sup> day.

### Primosale cheese

The results obtained for the Giuncata cheese clearly showed that the mixture 50:50 CO<sub>2</sub>:N<sub>2</sub> had the same behavior as the mixture 30:65:5 CO<sub>2</sub>:N<sub>2</sub>:O<sub>2</sub>. The gas composition 90:10 CO<sub>2</sub>:N<sub>2</sub> had a negative effect on sensorial quality. Therefore, for Primosale the following gas combinations were studied: 10:90 CO<sub>2</sub>:N<sub>2</sub>, 30:65:5 CO<sub>2</sub>:N<sub>2</sub>:O<sub>2</sub> and 75:25 CO<sub>2</sub>:N<sub>2</sub>.

**Table 1a:** Physico-chemical indices monitored in fresh cheese samples during the period of observation (Giuncata cheese: Cnt (control), VP (v MB (90:10 CO<sub>2</sub>:N<sub>2</sub>), MC (75:25 CO<sub>2</sub>:N<sub>2</sub>), MD (30:65:5 CO<sub>2</sub>:N<sub>2</sub>:O<sub>2</sub>).

Time (day)	Cnt		VP		MA		MB		MC	
	pH	Moisture	pH	Moisture	pH	Moisture	pH	Moisture	pH	Moistur
0	6.57 ± 0.01cA	62.11± 0.75cA	6.57 ± 0.01cA	62.11± 0.75dA	6.57 ± 0.01dA	62.11 ± 0.75cA	6.57 ± 0.01dA	62.11 ± 0.75cA	6.57 ± 0.01aA	62.11 ± 0.75bA
1	6.59 ± 0.01dE	59.53 ± 0.89bA	6.67 ± 0.01dF	59.56 ± 0.06 cA	6.41 ± 0.01cA	67.98 ± 0.61dD	6.43 ± 0.01cB	55.71 ± 0.60abB	6.56 ± 0.01aC	57.24 ± 0.95aC
6	5.42 ± 0.01aD	55.68 ± 0.18aA	5.55 ± 0.01aB	54.94 ± 0.75 bA	5.53 ± 0.01aA	57.98 ± 0.83bCD	5.39 ± 0.01aC	55.99 ± 0.90bAB	5.52 ± 0.01bA	57.09 ± 0.31aB
8	6.01 ± 0.01bC	54.74 ± 0.08aA	5.73 ± 0.01bA	56.92 ± 0.94 aB	6.10 ± 0.01bE	56.42 ± 0.61aB	5.91 ± 0.01bB	54.49 ± 0.56aA	6.07 ± 0.01cD	56.93 ± 0.11aB



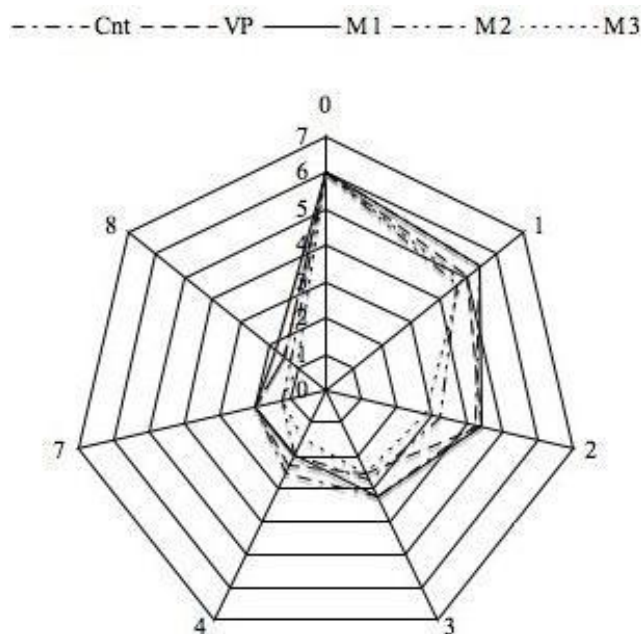
**Figure 3.** Giuncata cheese sensorial quality during the storage period.



**Table 1b:** Physico-chemical indices monitored in fresh cheese samples during the period of observation (Primosale cheese: Cnt (control), VP (vacuum), M1 (30:65:5 CO<sub>2</sub>:N<sub>2</sub>:O<sub>2</sub>), M2 (10:90 CO<sub>2</sub>:N<sub>2</sub>), M3 (75:25 CO<sub>2</sub>:N<sub>2</sub>).

Time(day)	Cnt		VP		M1		M2		M3	
	pH	Moisture	pH	Moisture	pH	Moisture	pH	Moisture	pH	Moisture
0	6.46 ± 0.02dA	58.99 ± 0.48aA	6.46 ± 0.02dA	58.99 ± 0.48aA	6.46 ± 0.02cA	58.99 ± 0.48aA	6.46 ± 0.02dA	58.99 ± 0.48cA	6.46 ± 0.02dA	58.99 ± 0.48cA
1	5.24 ± 0.00cE	58.21 ± 2.19abB	5.11 ± 0.01cD	59.47 ± 0.05aB	4.96 ± 0.00bB	53.96 ± 1.55bA	5.02 ± 0.01cC	55.05 ± 0.32bA	4.81 ± 0.00cA	54.01 ± 0.04aA
7	4.61 ± 0.01bC	57.2 ± 0.05abB	4.53 ± 0.01aA	53.81 ± 2.56bAB	4.59 ± 0.01aB	55.22 ± 3.73abAB	4.66 ± 0.01bE	52.29 ± 1.57aA	4.63 ± 0.01bD	52.15 ± 0.38bA
8	4.56 ± 0.01aA	55.99 ± 1.83aBC	4.57 ± 0.01bA	57.09 ± 0.93aC	4.59 ± 0.01aB	53.80 ± 1.23aA	4.57 ± 0.01aA	52.84 ± 0.19aA	4.60 ± 0.01aB	54.12 ± 0.22aAB

Data are presented ± standard error. Data in column with different small letters are significantly different (p < 0.05). Data in line with different capital letters are significantly different (p < 0.05).



**Figure 5.** Primosale cheese sensorial quality during the storage period.

growth, even if the growth in the Cnt was very limited. The major effect of CO<sub>2</sub> on cheeses is the inhibition of surface mould growth (Maniar et al., 1994), although high CO<sub>2</sub> MAP atmospheres have been shown to inhibit growth of moulds and yeasts on shredded mozzarella cheese (Eliot et al., 1998).

Finally, the mesophilic total count was similar in all samples (data not shown); there was a slight increase in populations over the observation period. Conclusions about a specific effect of CO<sub>2</sub> on mesophilic bacteria may not be reliable because this class contains many different populations, as stated by Eliot et al. (1998).

The physicochemical results are shown in Table 1b. As can be seen from the Table, the pH steadily decreases

during storage. Moreover, the differences among the tested samples are small, and, in some cases, not statistically significant. The strong decrease, on the first day, is probably due to the manufacturing of fresh cheese without a starter culture (Martin-Hernandez et al., 1990). As can be inferred from the table, the average moisture decreased during storage for all cheese samples. The headspace atmosphere did not undergo changes in composition throughout the storage period (data not shown).

The results of the sensorial evaluation (overall acceptability) of all systems are presented in Figure 5. It should be noted that the highest score was awarded to the control batch, although these were refused by day 3. A similar trend was observed for VP and M1, whereas the

remaining MAPs were unacceptable on the second day. Authors have reported that CO<sub>2</sub> has some negative effects on milk products in general, with respect to the colour and aroma (Scott and Smith, 1971). Maniar et al. (1994) found that CO<sub>2</sub> did not affect the sensorial characteristics of cottage cheese. These different results can be explained in term of CO<sub>2</sub> concentrations used and types of products studied. In contrast, in the present study, colour and odour were not affected in Primosale cheese packaged in 75% CO<sub>2</sub> (M3), but taste and texture were very negatively affected (data not reported).

Structural losses at high CO<sub>2</sub> concentrations could be explained by the high solubility of CO<sub>2</sub> in cheese aqueous phase. On the other hand, high concentrations of CO<sub>2</sub> are known for their antimicrobial effect, in fact M3 is the most effective for the inhibition of investigated spoilage bacteria.

To sum up, among the proposed MAPs, the gas mixture made up of 75% CO<sub>2</sub> and 25% N<sub>2</sub> allowed the best preservation for Giuncata cheese; in contrast, no gas mixture was individuated to provide good microbiological and sensorial quality for Primosale cheese. Further studies are needed with regard to preservation on the use of MAP of these fresh cheeses, and more specifically on establishing optimum CO<sub>2</sub> concentrations to ensure the microbiological quality, and at the same time maintaining the sensorial quality.

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