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Effect of packaging technology on ripening events occurring during storage of portioned PDO Italian semi-hard cheese

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ABSTRACT

The aim of the present study was to assess the ability of the packaging technology (vacuum, VAC, and modified atmosphere, MAP) to preserve the microbiological, physicochemical, and sensorial properties of already ripened and portioned PDO Italian semi-hard cheese during 120 days of storage at 8 °C. Results were compared with those obtained by analyzing the original not-packed cylindrical wheel stored at 8 °C (NP). Samples packed under MAP showed yeasts' proliferation with a final count of 4.68 Log cfu g^{-1}, similar to that detected in the NP cheese wheels at 120 days of storage. In VAC samples yeasts did not proliferate, maintaining constant levels during time. Differently from the original wheels which showed a hardness increase in concomitant with a moisture loss during storage, packed samples under both VAC and MAP did not lose water preserving the original hardness of the product. At the same time, the packaging technology did not influence the proteolysis index in comparison to NP cheese. However, in MAP samples already at 30 days of storage different volatile compounds were produced in comparison with NP and VAC cheeses, suggesting that the modified atmosphere into the MAP pack promoted alternative microbial metabolisms and final products characterized by different volatile profiles. The study clearly demonstrated that the choice of packaging technology has an impact on the development of peculiar attributes of PDO cheeses, that are required by specific regulations in order to preserve their typicality and satisfy the consumers.

1. Introduction

Cheeses with Protected Designation of Origin (PDO) are traditional products manufactured following relevant production regulations to guarantee the typical sensorial properties well recognized by consumers. The PDO-labeled cheeses must be produced in a specific geographical area using local raw materials and adopting defined specifications for their production process and ripening (Lora, Zidi, Magrin, Prevedello, & Cozzi, 2020).

Cheese ripening, is a crucial step in PDO cheese production since involves specific microbiological and biochemical events. In fact, PDO cheeses are complex biological ecosystems, harboring various autochthonous microbial strains derived from local raw milk, starter, and adjunct cultures responsible for the development of distinctive cheese quality attributes (Afshari, Pillidge, Dias, Osborn, & Gill, 2020). Specifically, during ripening the breakdown of lipids, proteins, and carbohydrates, associated with moisture loss and pH changes, leads to the formation of the typical texture and flavor profile of each cheese variety (McSweeney, 2004). For semi-hard cheeses, a ripening period of about 3–6 weeks under controlled temperature and humidity is generally applied before commercialization (Darnay et al., 2019). However, cheese ripening is expected to further evolve during the distribution and before consumption (Franco, Bargiela, & Tovar, 2023). Traditionally, the whole cheese wheels are commercialized and the portioning occurs directly at the retail site upon consumer request. Today, due to the increasing demand for ready-to-eat food with high convenience (Favati, Galgano, & Pace, 2007), the portioning is frequently performed by the food industry. In this case, a proper packaging solution should be applied to guarantee the desired product stability during storage. In this context, both packaging under vacuum (VAC) or modified atmosphere packaging (MAP) can be applied in combination with the proper characteristics of packaging film. Generally, packaging materials with high-barrier properties towards gases and water vapor are used to preserve cheese safety and quality (Nájera, Nieto, Barron, & Albisu, 2021). Protection and barrier properties are guaranteed by the use of different fossil-based plastic polymers, such as polyamide (PA), polyethylene (PE), polyethylene terephthalate (PET), polyvinylidene chloride (PVDC), and polyvinyl alcohol (PVA), commonly organized in

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multilayer solutions (Zhang et al., 2022). Several researches already demonstrated the efficiency of multilayer plastic films in combination with modified atmospheres or vacuum for the preservation of cheese products (Atallah, El-Deeb, & Mohamed, 2021; Costa et al., 2016; Piscopo, Zappia, De Bruno, & Poiana, 2015). Today, the choice of the packaging technology to be used is essentially based on the film composition and barrier properties, type of product, and costs. Generally, the vacuum technology is coupled with plastic film with specific thermal and mechanical properties that allow the shrinkage and sealability of the material, assuring products with long stability. However, since the removal of the atmosphere from the pack causes the collapsing of the film which superficially adheres to the product, the final texture of the product might be negatively modified influencing consumer choice (Nájera et al., 2021). Conversely, films with high barrier properties towards gases, especially carbon dioxide, and flexibility were used applying MAP technology. The MAP technique has the advantage to preserve the original shape of the product, by substituting the oxygen inside the packaging generally with different concentrations of carbon dioxide and nitrogen (Solomakos, Govari, Botsoglou, & Pexara, 2019; Zulewska, Lobacz, Białobrzewski, Grochowina, & Kamińska, 2023). However, the use of high-barrier packaging coupled with both vacuum and MAP technologies is expected to affect the ripening events occurring during storage in comparison to the whole wheel. Generally, the moisture loss is prevented by the presence of a high-barrier film (Costa et al., 2016; Piscopo et al., 2015). At the same time changes in the microflora are expected leading to a different flavor profile of packed and unpacked products (Galli et al., 2024). Though, the proteolysis index is reported to be not influenced by either the type of plastic packaging or the packaging technology (Del Caro et al., 2016; Franco et al., 2023). Beside these data, few studies deal with the influence of the packaging solution adopted on the evolution of ripening events during cheese storage, especially in the case of protected designation of origin cheeses (Galli et al., 2024; Garabal, Rodríguez-Alonso, Franco, & Centeno, 2010). Under this circumstance, the packaging technology might affect the typicality of portioned PDO cheeses, resulting in products having final characteristics that differ from those of the cheese wheel having the same storage life. Therefore, the present study focused the attention on the impact of conventional packaging solutions on the peculiar features of a PDO cheese, in order to test the packaging suitability for preserving the typicality of the product as well as its shelf-life.

In this context, a semi-hard cheese that covers an important market share because of its characteristics is the PDO Italian semi-hard Montasio cheese, which was considered as a case study to evaluate the effect of packaging technologies on the ripening events. Montasio is a PDO semi-hard cheese produced in the North-East regions of Italy from raw or thermized cow's milk (Innocente, Munari, & Biasutti, 2013). In this study, two commercial high-barrier multilayer plastic films, precisely a heat-shrink film designed for the vacuum technology and one film made exclusively for MAP, were taken into consideration and physicochemical, microbiological, and sensorial properties of the Montasio packaged with these materials were assessed and compared with those of Montasio cheese without packaging.

2. Materials and methods

2.1. Cheese manufacturing

Semi-hard Montasio cheese was produced in a dairy industry located within the North-East area of Italy (Venzone, Udine, Italy). For cheesemaking, after the addition of 1% of natural milk thermophilic culture, calf rennet was added for the milk coagulation. The curd was cut, cooked at 45 °C for 15 min, and then packed into 7–8 kg circular molds as previously described by Innocente et al. (2013). After the pressing (400 kPa) and salting phases (16–18% wt/wt of sodium chloride), cheese was ripened for 60 days in a warehouse at 12 °C and 86% relative humidity (RH). For standardization, 40 kg of cheese were daily produced from the same batch of milk.

2.2. Cheese packaging and storage

Cheese portions of about 250 g were obtained from 60-d ripened wheels using an automatic cutting machine (Gelmini Food Processing Machines, Parma, Italy) and then packaged using a fully automated packaging machine (PFM Packaging Machinery S. p.a., Italy) in aseptic conditions and a room temperature set at 8 °C. Two conventional high-barrier plastic films were used: a multilayer film made up of a 40 μm layer of polyvinylidene chloride (PVDC) and a 10 μm layer of polyethylene (PE) (Amcor Plc, Victoria, Australia), characterized by oxygen transmission rate (OTR) of 19 $cm^3/m^2 \times 24$ h, carbon dioxide transmission rate (CDTR) of 28 $cm^3/m^2 \times 24$ h, and water vapor transmission rate (WVTR) of 6 $g/m^2 \times 24$ h; and a multilayer film composed of a 15 μm layer of polyamide (PA) and a 50 μm layer of polyethylene (PE) (GM Grafica s. r.l., Perugia, Italy), characterized by OTR of <3 $cm^3/m^2 \times 24$ h, CDTR of 5 $cm^3/m^2 \times 24$ h and WVTR of <7 $g/m^2 \times 24$ h. The standard methods ASTM D3985 (2017) and ASTM F2476 (2020) were used to determine OTR and CDTR of films before packaging, respectively, at 23 °C, 0% RH and 0.1 MPa partial pressure difference; while the WVTR was assessed following the ASTM F1249 (2020) standard method at 38 °C and 100% and 0% RH. Commercial conditions of packaged Montasio cheese were reached out coupling the PVDC/PE film with vacuum (VAC), while the PA/PE film with an 80% CO_2 and 20% N_2 modified atmosphere (MAP). The vacuum process involved a heat-shrink of the film under a water shower set at 90 °C for few seconds. The MAP conditions have been chosen on the basis of those currently most used in the market for portioned Montasio cheese. Five not-packaged wheels (NP) of Montasio cheese were also analyzed as control reference.

A temperature-controlled thermostat (ST 500 BM Smart, POL-EKO, Poland) set at 8 ± 1 °C was used for the storage of samples. Analyses were performed before packaging (T0) and at 30, 60, 90, 120 days of storage on three samples for each packaging solution. The RH inside the storage chamber was 48%, while in the tested environments was around 62%.

2.3. Gas composition of sample headspace

The percentage of oxygen and carbon dioxide in the headspace of cheese samples packaged with MAP was measured using a gas meter model CheckMate 3 (Dansensor, Ringsted, Denmark).

2.4. Evaluation of yeast and mold count

For microbiological analysis, about 10 g of the sample were homogenized in 90 mL of Maximum Recovery Diluent (MRD) (Oxoid, Milan). Then, suspensions were serially diluted in MRD (Oxoid, Milan), pour-plated in Rose-Bengal Chloramphenicol Agar (Oxoid, Milan), and incubated at 30 °C for 72 h.

2.5. Chemical and physicochemical properties

Firstly, from cheese samples the rind (about 2 mm) was manually removed with a knife, then the underrind (5 mm) and the inner part of the samples were ground separately using a professional chopper (1000 W, Moulinex, France) and used for the determination of moisture. The inner part was also used to determine the pH, water activity (a_w), proteolysis index (PI), and volatile compounds.

2.6. Moisture content, pH and water activity

The moisture content was determined by gravimetric method after drying the sample at 75 °C with a vacuum oven (Vuotomatic 50, Bicasa, Milan, Italy).

For pH determination, a cheese-water dispersion was prepared by weighing 5 g of the sample in 10 mL of distilled water at room temperature. The mixture was then homogenized at 7000 rpm for 1 min using an Ultra Turrax homogenizer (IKA, T18, Staufen Im Breisgau, Germany). Measurements were carried out using a pH-meter (Basic 20, Crison, Barcelona, Spain) calibrated with standard solutions at pH 4, 7, and 9.

Water activity of samples at 25 °C was measured using the AquaLab 4 TE electronic instrument (Decagon Devices, Perugia, Italy).

2.7. Proteolysis index

The proteolysis index of cheese samples was obtained by the ratio between water soluble nitrogen (WSN) and total nitrogen (TN) contents (Todaro et al., 2017). For the determination of WSN, a cheese extract was obtained by smashing 4 g ground cheese in a mortar with distilled water at 40 °C until the formation of a homogeneous dispersion, and then diluted to 100 mL with distilled water. WSN content was determined on the dispersion, after filtration, while the TN was directly measured on 0.5 g of ground cheese using the Kjeldahl method (IDF, 2011).

2.8. Hardness of cheese

Cheese hardness was measured on discs of 1.5 cm thickness and 3.5 cm diameter obtained from the internal section of each sample. After cutting, specimens were left to rest for 1 h at room temperature inside plastic bags in order to avoid loss of moisture. The hardness of cheese samples was then determined using an Instron Universal Testing Machine model 4301 (Instron, LDT, High Wycombe, UK) equipped with a load cell of 1 kN. A uniaxial compression test (40%) was carried out using a circular probe (5.6 cm diameter) and speed test of 50 mm/min. The recorded peak force (N) was used as measure of cheese hardness.

2.9. Volatile compounds

Volatile compounds in the headspace of cheese samples were assessed during storage time through the solid phase micro-extraction technique coupled with gas chromatographic analysis and mass spectrometry (HS-SPME-GC-MS). Sealed vials containing 5 g of ground cheese were conditioned at 40 °C for 30 min in a HTA autosampler (model HT2800, HTA s. r.l., Italy). For extraction, a SPME fiber 2cm × 50/30 μm Stableflex 24Ga (Supelco, Bellefonte, PA, USA) coated with a stationary phase consisting of divinylbenzene/carboxen/polydimethylsiloxane was exposed in the headspace of the sample at a depth of 25 μm for 30 min at 40 °C. A gas chromatography-mass spectrometry (GC-MS) system (model QP2020 NX, Shimadzu Corporation, Kyoto, Japan) equipped with a DB-WAX capillary column (30 m length, 0.25 mm internal diameter, and 0.25 μm film thickness, Agilent Technologies, CA, USA) was used to separate and detect volatile compounds. Desorption into the GC injection port was at 270 °C for 3 min under splitless conditions. For separation Helium was used as carrier gas (1 mL/min flow rate), while interface, source, and quadrupole temperature were 240, 200, and 150 °C, respectively. The temperature program included three phases: 50 °C for 5 min, followed by the first ramp at 230 °C for 10 min, and a second ramp at 240 °C for 10 min. Both ramps were reached after heating 10 °C/min. The mass spectrometry worked in scan mode with a mass range from 25 to 350 m/z. Chromatographic profiles were evaluated using the GC-MS solution software (version 4.52, Shimadzu Corporation, Kyoto, Japan) and compounds were identified by spectra comparison using the NIST/EPA/NIH 20 Mass Spectral Library (John Wiley & Sons Inc., Hoboken, NJ, USA) and by comparing their Kovat's retention index with those reported in the literature (http://webbooking.nist.gov/chemestry/).

2.10. Descriptive sensory analysis

A quantitative descriptive sensory analysis (QDA) with a trained panel of 10 judges was used to evaluate the aromatic profile of packaged cheeses during storage time. Training sessions were conducted according to the method proposed by Bérodier et al. (1997) on the sensory evaluation of hard and semi-hard cheeses. Before sensory analysis, samples with no rind were cut in a 1.5 × 1.5 × 5.0 cm parallelepiped shape, placed into a three-digit coded Petri dish, and stored at 16 °C until evaluation. Mechanical and taste attributes, and flavor-odor intensities of samples were evaluated using a seven-points scale. The "SmartSensory Box2, internet of sense" version 2.2.44 software (Smart Sensory Box, Sassari, Italy) was used to collect and elaborate data. The study complied with the principles established by the Declaration of Helsinki and the protocol was approved by the Institutional Review Board of the Department of Agricultural, Food, Environmental and Animal Sciences of the University of Udine (protocol n. 0002068).

2.11. Statistical analysis

Results were expressed as mean ± standard deviation. At each sampling time, analyses were conducted at least in triplicate on three samples of each packaging solution. The *t-test* was used to identify statistical differences between mean values of control and packaged samples ($p < 0.05$). The homogeneity of variance was assessed using the Bartlett's test, while one-way analysis of variance (ANOVA) followed by the *post hoc* Tukey's HSD test were conducted to check differences between samples in the same packaging condition at different storage times. Statistical analysis was performed using the R software package version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria). Principal component analysis (PCA) was carried out using the Origin Pro 9 software (OriginLab, Northampton, MA).

3. Results

The influence of the packaging technology on the peculiar characteristics of PDO cheese was monitored through different indexes linked to the typical aspects of the product.

3.1. Effect of packaging on yeasts and molds

The effect of high-barrier plastic packaging on the growth of spoilage microorganisms was tested by evaluating the yeasts and molds count during storage time. Moreover, the percentage of oxygen and carbon dioxide in the headspace of samples packaged with MAP was assessed over time. Microbiological results are reported in Table 1. Before packaging, the initial yeast count of Montasio cheese aged 60 days was around 2.6 Log cfu g^{-1}. The presence of yeasts in Montasio samples derives from the milk and the dairy environment, as stated in previous works on this PDO cheese (Marino, Maifreni, Bartolomeoli, & Rondinini, 2008; Marino, Maifreni, & Rondinini, 2003). Yeasts contribute to cheese ripening by releasing lipolytic and proteolytic enzymes in the cheese matrix, which are responsible for the development of aromas and flavors (Galli et al., 2024; Innocente, Renoldi, Moret, Maifreni, & Marino, 2023). The absence of oxygen in VAC samples inhibited the yeast growth during all storage times (Table 1). In contrast, in samples without packaging (NP) and packaged with MAP, after 120 days of storage, yeasts count significantly increased in comparison with the control (Table 1). NP samples were constantly exposed to the oxygen from the environment, which slowly diffused into the cheese allowing a gradual yeast proliferation. On the other hand, in MAP samples, yeast growth was initially inhibited by the high concentration of CO_2 and low levels of oxygen (Garabal et al., 2010). Values of about 0.49 vol.-% and 0.79 vol.-% of O_2, and 80.72 vol.-% and 79.45 vol.-% of CO_2 were found in the packaging with MAP at 0 and 30 days of storage, respectively. Afterward, several phenomena, including the dissolution of gases in the

Table 1
Evolution of yeasts count, pH and a_w of samples during storage time.

Sample	Storage (d)	Yeast (Log cfu g^{-1})	a_w[a]	pH
Control	0	2.65 ± 0.16	0.960 ± 0.002	5.29 ± 0.02
NP	30	2.74 ± 0.36[a]	0.933 ± 0.005[a,b]	5.30 ± 0.02[ab]
	60	2.72 ± 0.24[a]	0.931 ± 0.004[a,b]	5.32 ± 0.01[ab]
	90	2.98 ± 0.39[a]	0.922 ± 0.004[ab,b]	5.27 ± 0.04[b]
	120	3.90 ± 0.40[a,b]	0.916 ± 0.003[b,b]	5.33 ± 0.01[a,b]
VAC	30	2.50 ± 0.12[a]	0.937 ± 0.002[a,b]	5.34 ± 0.01[a,b]
	60	2.56 ± 0.36[a]	0.934 ± 0.004[ab,b]	5.23 ± 0.04[b]
	90	2.74 ± 0.19[a]	0.929 ± 0.001[b,b]	5.28 ± 0.02[ab]
	120	2.65 ± 0.01[a]	0.931 ± 0.003[ab,b]	5.35 ± 0.04[a]
MAP	30	3.10 ± 0.41[b]	0.933 ± 0.002[a,b]	5.34 ± 0.01[a,b]
	60	3.32 ± 0.59[b]	0.935 ± 0.002[a,b]	5.35 ± 0.03[a,b]
	90	4.05 ± 1.17[ab]	0.930 ± 0.002[ab,b]	5.30 ± 0.01[a]
	120	4.68 ± 0.01[a,b]	0.925 ± 0.003[b,b]	5.34 ± 0.05[a]

[a] water activity.
[b] Indicate statistically significant difference ($p < 0.05$) between control and samples. Means with different letters indicate statistically significant difference ($p < 0.05$) between storage times in the same packaging condition.

cheese, the gas consumption by microorganisms, low gas pressure inside the packaging, and the faster loss of CO_2 through the barrier film (Garabal et al., 2010), led to a gradual collapse and shrinkage of the plastic pack that adhered to the cheese surface. For this reason, gas content in the headspace of MAP packaged samples was not further measured, since it was not possible to pierce the plastic film. At the end of storage, yeasts in MAP samples were able to proliferate probably due to the loss of CO_2 from the pack.

In all samples, no molds growth was detected (mean counts <2 Log cfu g^{-1}; data not shown) during storage time, probably due to the absence of oxygen in packaged cheeses, refrigeration temperature, and cleanliness of the storage chamber in the case of NP samples (Del Caro et al., 2016; Marino et al., 2003). Moreover, in all samples, a slight decrease in water activity from the initial value of 0.96 to 0.92 (Table 1), contributed to the inhibition of molds proliferation.

During cheese ripening, fluctuations in pH values are generally caused by the conversion of lactose in lactic acids by microbes, formation of free fatty acids, use of lactic acid, and release of alkaline components due to the proteolysis (Todaro et al., 2017). Additionally, in MAP packaging, carbon dioxide might be absorbed into the cheese, causing acidification of the product (Garabal et al., 2010). In this study, all samples showed pH values similar to that of the control sample (Table 1), suggesting a balancing of these events and values stable over time.

3.2. Influence of packaging on cheese physicochemical properties

The moisture content of cheese samples during storage time is reported in Table 2. The 60-day-old PDO Montasio (control) was characterized by moisture values of 35% and 26% for the inner part and the underrind, respectively. During ripening, the free water entrapped in the cheese matrix is generally used by the microbial communities and partially lost due to the exchange with the environment (Chen, MacNaughtan, Jones, Yang, & Foster, 2020; Hickey, Guinee, Hou, & Wilkinson, 2013). As expected, in NP samples the moisture content progressively decreased in both external and internal parts of the cheese,

Table 2
Moisture and hardness values of samples during storage time.

Sample	Storage (d)	Moisture (%)		Hardness (N)
		Inner part	Underrind	
Control	0	35.5 ± 0.4	25.9 ± 0.8	79 ± 5
NP	30	34.9 ± 0.3[a]	21.5 ± 0.3[a,a]	103 ± 7[c,a]
	60	33.9 ± 0.6[ab,a]	18.4 ± 0.3[b,a]	124 ± 3[b,a]
	90	32.4 ± 0.7[b,a]	19.1 ± 0.9[b,a]	153 ± 5[a,a]
	120	28.9 ± 1.0[c,a]	13.1 ± 0.1[c,a]	150 ± 8[a,a]
VAC	30	35.3 ± 0.4[a]	24.4 ± 0.2[b,a]	100 ± 5[b,a]
	60	35.2 ± 0.5[a]	24.8 ± 0.5[ab,]	119 ± 6[a,a]
	90	34.2 ± 0.4[ab,a]	25.5 ± 0.3[a,]	124 ± 7[a,a]
	120	33.2 ± 1.0[b,a]	25.6 ± 0.3[a,]	118 ± 3[a,a]
MAP	30	34.4 ± 0.7[ab]	25.6 ± 0.5[b,]	91 ± 1[c,a]
	60	35.1 ± 0.5[a]	24.0 ± 0.3[c,a]	98 ± 3[bc,a]
	90	33.6 ± 1.0[ab]	24.9 ± 0.1[b,]	111 ± 4[a,a]
	120	32.9 ± 0.4[b,a]	26.6 ± 0.2[a,]	104 ± 5[ab,a]

[a] Indicate statistically significant difference ($p < 0.05$) between control and samples. Means with different letters indicate statistically significant difference ($p < 0.05$) between storage times in the same packaging condition.

reaching final values of 13% and 28%, respectively (Table 2). In both packaged solutions, the moisture content of the inner part decreased, while for the underrind moisture no apparent trends were detected over time. During storage, probably part of the water either migrated from the inner part towards the underrind regions or was lost in the headspace of the packaging, as in the case of MAP solution, generating visible small water drops on the surface of the plastic film. However, the external water drops were reabsorbed into the cheese matrix which caused an increase of the moisture content in the external parts of samples, as previously observed by Costa et al. (2016) in a semi-hard cheese packaged with plastic films with different barrier properties.

Fig. 1 showed the evolution of the proteolysis index of samples during storage time. Generally, proteolytic events are responsible for the softening of the cheese texture during ripening, since the casein matrix is gradually hydrolyzed by proteinases and peptidases (McSweeney, 2004). As depicted in Fig. 1, no significant differences were detected between samples, with PI values that progressively increased over time, and with values similar to those reported by Innocente (1997) for this kind of cheese. In this study, the packaging materials and the anaerobic conditions did not influence the PI rate. Conversely, several works

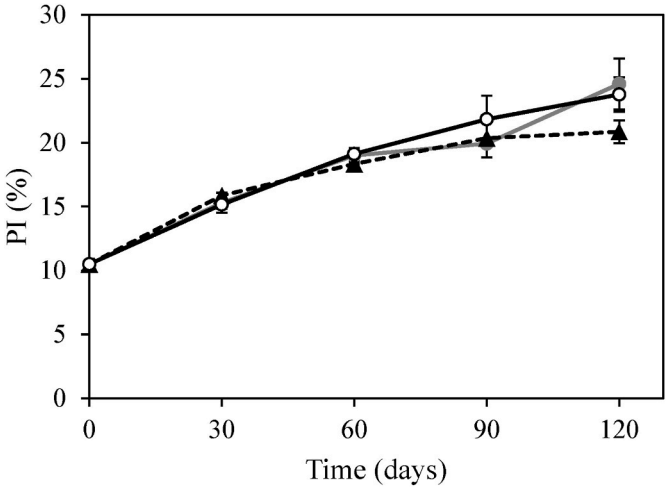

Fig. 1. Evolution of the proteolysis index (PI) of cheese samples during storage time. Samples: NP, straight grey line; VAC, dashed black line; MAP, straight black line.

reported that anaerobic conditions would be expected to affect microbial proteolytic enzymes, since with low amount of oxygen a different microbiota will prevail respect to that of unpackaged cheese (Favati et al., 2007; Galli et al., 2024; Nogueira, Lacorte, Paciulli, & Rodrigues, 2021).

Hardness is another parameter influenced by cheese ripening, and strictly related to the proteolytic activity rate and moisture content of the product (McSweeney, 2004). In Table 2 are reported the hardness values of the inner part of samples during storage time. Hardness of NP samples significantly decreased ($p < 0.05$) over time following the

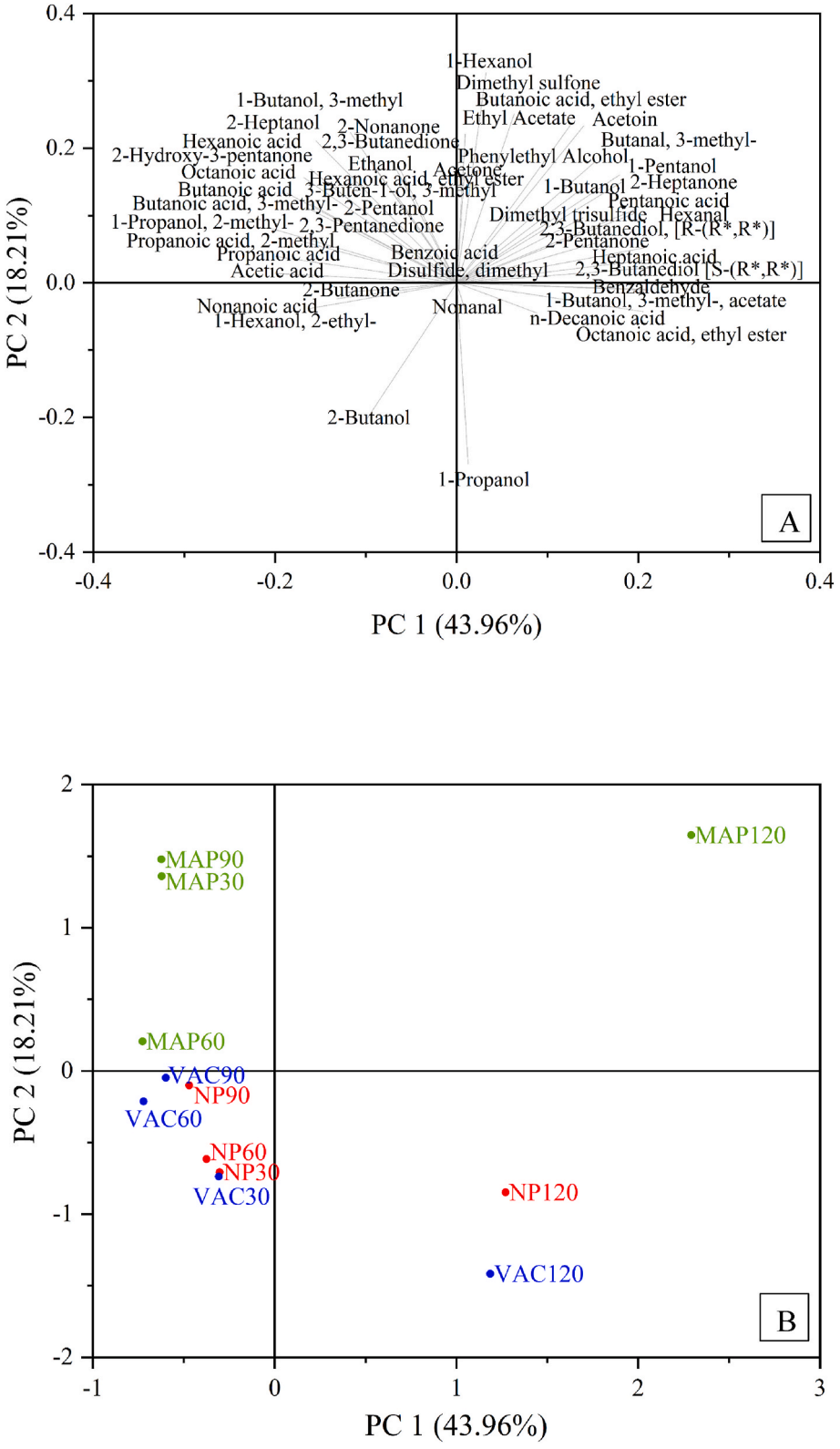

Fig. 2. Loading (A) and scoring (B) plots obtained from PCA analysis of volatile compounds generated in the headspace of samples (Red: unpackaged cheese; Blue: film coupled with vacuum; Green: film coupled with MAP) during storage time at 8 °C.

natural dehydration phenomena that occur during ripening. In this samples, the extent of moisture loss prevailed on proteolytic activities that are responsible for the softening of cheese texture, causing a gradual hardening of the product. On the contrary, in packaged products, after 30 days of storage hardness showed a lower increase as compared to NP because of the reduced moisture loss from the samples and the water migration phenomena (Table 2). As previously reported by Piscopo et al. (2015) on semi-hard Provolone cheese, hardness of unpackaged samples showed a more regular increase compared to samples with packaging, as a consequence of the different extent of water redistribution.

3.3. Production of volatile compounds

Regarding the aromatic profile, a total of 47 volatile compounds were detected in the headspace of samples and listed in Table S1. Fig. 2 reports the PCA plots and the distribution of cheese samples during storage time as a function of volatile compounds produced. The first component (PC1) explained the 43.9% of the variability, while the second component (PC2) accounted for 18.2%, thus describing 62.1% of the total variance. Based on the production of volatile compounds, samples were heterogeneously distributed in the PCA scoring plot (Fig. 2B). As described in Fig. 2, in the left negative region are positioned the NP and VAC samples until 90 days of storage, characterized by the production of 2-butanone, nonanoic acid, 1-hexanol-2-ethyl, and 2-butanol. Moreover, NP and VAC samples at 120 days of storage showed similar aromatic profiles, mainly characterized by the presence of 1-propanol, decanoic acid, ethyl octanoate, 3-methylbutyl acetate, nonanal, and benzaldehyde. Samples packaged with MAP were differentiated from the other samples, with MAP samples at 30, 60 and 90 days of storage located in the left positive quadrant (Fig. 2B). Samples placed in this PCA biplot area were characterized by the presence of acids, e.g., acetic, propanoic, butanoic and hexanoic acids, esters, and alcohols, including ethanol, 2-pentanol, and 2-heptanol (Fig. 2A). The sample packaged with MAP at 120 days was located in the right positive quadrant far from all the other samples (Fig. 2B). In this case, the sample showed to be characterized by the production of sulfur compounds, such as dimethyl sulfone and dimethyl trisulfide, ketones, and alcohols (Fig. 2A). Results demonstrated how samples packaged under vacuum maintained an aromatic profile similar to that of Montasio cheese without packaging (NP) over all storage times. On the contrary, already at 30 days and during the entire storage time, MAP samples showed differences in the production of volatile compounds compared to the control and the other samples. Garabal et al. (2010), based on correlation analysis between flavors detected with sensory analysis and different biochemical traits analyzed, showed that after 45 days of storage the unpackaged PDO semi-hard San Simon da Costa cheese was clustered together with the vacuum-packaged cheese, thus separating from those packaged under different MAP solutions. The outcomes from this work confirmed the ability of the packaging technology to influence the metabolism of the cheese microbiota and consequently the generation of volatile compounds, as previously stated (Galli et al., 2024; Nogueira et al., 2021).

3.4. Sensory analysis

A QDA test was carried out to define sensory profiles of cheese samples during storage time. The average scores of each sensory attribute evaluated by panelists are reported in Table 3. Regarding mechanical attributes, the only changes perceived by panelists over time were those related to springiness and hardness in NP samples. As expected, the high amount of water lost from the samples (Table 2) and the breakdown of the casein matrix (Fig. 1) significantly reduced elasticity and increased the hardness of the unpackaged cheese (Table 3). These sensory results were in accordance with measured hardness values showed in Table 2. On the other hand, MAP and VAC samples showed similar mechanical attributes to the control sample, with stable values

Table 3
Mechanical and taste attributes, and flavor-odor intensities of samples during storage time.

Sample	Storage (d)	Springiness	Hardness	Deformability	Crumbliness	Adhesiveness	Spicy	Salty	Sour	Bitter	Odor intensity	Flavor intensity
Control	0	4.9 ± 0.6	3.1 ± 0.6	3.6 ± 0.7	2.7 ± 0.9	3.9 ± 0.9	1.1 ± 0.3	3.1 ± 0.7	2.9 ± 0.9	1.2 ± 0.4	3.6 ± 0.7	3.3 ± 0.7
NP	30	4.5 ± 0.9^{a}	3.4 ± 0.7^{b}	3.5 ± 0.5^{a}	2.8 ± 0.5^{a}	3.5 ± 1.1^{a}	1.3 ± 0.5^{a}	3.5 ± 0.9^{a}	2.6 ± 0.7^{a}	1.1 ± 0.4^{a}	3.6 ± 0.9^{a}	3.6 ± 0.7^{a}
	60	3.9 ± 1.4^{ab,a}	3.5 ± 1.2^{ab}	3.6 ± 0.5^{a}	2.9 ± 0.8^{a}	3.5 ± 1.2^{a}	1.9 ± 1.0^{a}	3.8 ± 0.9^{a}	3.0 ± 1.7^{a}	1.5 ± 0.5^{a}	3.4 ± 0.9^{a}	3.8 ± 0.9^{a}
	90	3.2 ± 1.6^{ab,a}	4.2 ± 0.8^{ab,a}	3.2 ± 0.4^{a}	3.2 ± 1.1^{a}	4.2 ± 1.1^{a}	1.8 ± 0.4^{a}	4.0 ± 0.7^{a,a}	3.0 ± 1.7^{a}	1.4 ± 0.5^{a}	3.4 ± 0.9^{a}	4.6 ± 1.0^{a,a}
	120	2.3 ± 1.3^{b,a}	4.8 ± 1.0^{a,a}	3.7 ± 1.1^{a}	3.4 ± 0.9^{a}	3.0 ± 0.7^{a}	1.4 ± 0.5^{a}	4.3 ± 1.0^{a,a}	3.0 ± 1.3^{a}	1.7 ± 0.7^{a}	3.8 ± 0.7^{a}	4.5 ± 0.7^{a,a}
VAC	30	4.8 ± 1.0^{a}	3.1 ± 0.8^{a}	3.8 ± 0.7^{a}	2.1 ± 0.8^{a}	3.4 ± 1.2^{a}	1.3 ± 0.5^{a}	3.4 ± 1.1^{a}	2.3 ± 1.0^{a}	1.3 ± 0.7^{a}	3.4 ± 1.2^{a}	3.8 ± 0.9^{a}
	60	3.8 ± 1.0^{a}	3.5 ± 1.1^{a}	3.1 ± 0.8^{a}	3.5 ± 1.1^{a}	3.6 ± 0.9^{a}	1.5 ± 0.9^{a}	3.8 ± 0.7^{a}	3.1 ± 1.4^{a}	1.4 ± 0.7^{a}	3.4 ± 1.1^{a}	4.0 ± 0.9^{a}
	90	5.2 ± 0.4^{a}	2.6 ± 0.5^{a}	3.4 ± 0.5^{a}	2.2 ± 0.4^{a}	4.2 ± 0.8^{a}	1.6 ± 0.9^{a}	3.8 ± 1.3^{a}	2.4 ± 1.5^{a}	2.0 ± 1.0^{a}	3.6 ± 0.9^{a}	4.0 ± 0.7^{a}
	120	4.4 ± 1.4^{a}	3.4 ± 0.7^{a}	3.8 ± 0.4^{a}	3.2 ± 0.4^{a}	3.2 ± 1.0^{a}	1.4 ± 0.7^{a}	3.6 ± 0.9^{a}	3.0 ± 1.6^{a}	1.6 ± 0.7^{a}	3.1 ± 0.9^{a}	3.6 ± 0.7^{a}
MAP	30	5.1 ± 0.6^{a}	3.1 ± 1.1^{a}	3.4 ± 0.5^{a}	3.4 ± 0.5^{a}	3.0 ± 0.8^{a}	1.5 ± 0.8^{ab}	3.5 ± 1.2^{a}	2.6 ± 1.1^{a}	1.4 ± 0.5^{a}	3.4 ± 1.4^{a}	3.8 ± 1.0^{a}
	60	4.5 ± 0.8^{a}	3.3 ± 0.7^{a}	3.3 ± 0.7^{a}	2.8 ± 0.9^{a}	3.4 ± 1.2^{a}	1.8 ± 0.7^{ab}	3.8 ± 0.7^{a}	2.8 ± 1.3^{a}	1.1 ± 0.4^{a}	4.0 ± 0.9^{a}	4.1 ± 1.2^{a}
	90	3.9 ± 1.6^{a}	3.6 ± 1.3^{a}	3.8 ± 0.4^{a}	3.4 ± 1.1^{a}	3.1 ± 0.8^{a}	1.0 ± 0.0^{b}	3.6 ± 0.9^{a}	2.6 ± 1.5^{a}	1.2 ± 0.4^{a}	3.8 ± 1.1^{a}	3.8 ± 0.4^{a}
	120	4.8 ± 1.0^{a}	3.2 ± 0.4^{a}	3.7 ± 0.5^{a}	3.1 ± 1.1^{a}	3.7 ± 0.9^{a}	2.0 ± 0.7^{a}	3.7 ± 0.5^{a}	3.0 ± 1.1^{a}	1.4 ± 0.5^{a}	3.7 ± 0.9^{a}	4.0 ± 0.8^{a}

[a] Indicate statistically significant difference ($p < 0.05$) between control and samples. Means with different lowercase letters indicate statistically significant difference ($p < 0.05$) between storage times in the same packaging condition.

for the entire storage time. In this case, the plastic films protected cheeses from excessive moisture loss and textural changes, preserving the quality of the products. Considering the basic taste attributes, only saltiness in NP samples significantly increased after 90 days of storage compared to the control sample, probably due to the concentration of sodium chloride caused by the dehydration phenomena that occurred during ripening (Table 2). Packaged samples displayed values for the taste-related attributes similar to those of the control sample. Regarding odor and flavor intensities, both parameters are strictly linked with the extent of proteolytic and lipolytic activities (Suzzi et al., 2015). In this study, only a significant increase in flavor intensity was detected in NP samples at 90 and 120 days of storage, while no differences in odor intensity were perceived among samples over storage time (Table 3). Although different volatile compounds were generated in packaged cheeses, as shown in Fig. 2, no significant differences in terms of odor and aroma intensities among packaged and control samples were perceived by trained panelists.

4. Conclusions

The results of this study show how the two packaging technologies applied to the PDO semi-hard Montasio cheese influenced the quality and sensory attributes of this product. With regard to spoilage microorganisms, only yeasts were able to multiply in NP and MAP samples during storage. The packaged cheeses resulted softer than the NP sample, due to the barrier properties of films which prevented moisture loss from the cheeses and the consequently natural hardening. Proteolytic activity was not affected by the packaging technology applied, with values similar to those obtained for the unpackaged sample. Nevertheless, during storage, the PDO Montasio cheese packaged with MAP showed different volatile profiles in comparison with NP and VAC samples, probably due to the promotion of different microbial metabolisms and generation of volatile compounds. Sensory analysis did not discriminate samples over storage time, except for structural characteristics and saltiness of NP cheese. On the basis of these results, the vacuum technology resulted the most suitable packaging to preserve the typical features of the PDO Montasio cheese.

CRediT authorship contribution statement

Niccolò Renoldi: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Anna Rossi:** Writing – review & editing, Formal analysis, Data curation. **Marilena Marino:** Writing – review & editing, Resources, Methodology, Conceptualization. **Sonia Calligaris:** Writing – review & editing, Investigation, Conceptualization. **Nadia Innocente:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization.

Data availablility statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.idairyj.2024.106109.

Data availability

Data will be made available on request.

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