Effects of the equilibrium atmosphere on Taleggio cheese storage in micro perforated packaging

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ABSTRACT

Taleggio is an Italian smear-ripened cheese, whose complex microbiota demands the optimisation of the packaging system to avoid excessive changes during storage. Metabolic processes of the cheese rind microbiota can be usefully exploited in equilibrium modified atmosphere packaging (EMAP) by balancing microbiota respiration and film permeation. Here, we present the application of three different micro perforated EMAPs as models for smear-ripened cheese compared to two control packaging configurations. Analyses of the main microbial groups, headspace gas, textural profile, and sensory properties were performed to find the best packaging for storage. Results showed that two of the alternative micro perforated packaging systems were able to control the excessive changes during storage, thus limiting fungal overgrowth and allowing the typical development of smear microbiota with minor changes to hardness and cohesiveness. Finally, the sensory evaluation positively favoured one of the alternatively packed cheeses based on its compactness, typical dairy traits, and minor off-flavours. These findings showed that EMAP can be a valid alternative solution to control the storage of Taleggio cheese. Further studies could be conducted to evaluate this system on other smear cheeses.

1. Introduction

Traditional cheese (Montel et al., 2014) poses a great challenge to packaging design due to its sensorial characteristics and shelf life: in particular, an improper packaging system may lead to excessive spoilage or unwanted defects (Robertson, 2005), eventually resulting in food waste. Taleggio is a medium-soft, smear-ripened cheese made from raw or pasteurised milk produced in Northern Italy, which has been granted the quality label protected denomination of origin (PDO) (Gobbetti, Neviani, Fox, & Varanini, 2018). Smear-ripened cheese is also named washed-rind cheese (Carminati, Perrone, Neviani, & Mucchetti, 2000) because its rind is washed several times with brine during ripening to eliminate excess mould and favour the growth of a typical microbiota (Montel et al., 2014). According to the PDO production regulation, the cheese is ripened at 4–6 °C with environmental humidity, approximately 85–90%, for at least 35 days (Carminati et al., 2000; Gobbetti, Corsetti, Smacchi, De Angelis, & Rossi, 1997). Throughout the seasoning, a complex microbial community grows on the surface while biochemical reactions, such as proteolysis and lipolysis, take place on the surface and continue to the inside. This process is known as centripetal maturation (Cocconcelli, Fontana, Bassi, Gazzola, & Salvatore, 2013; Fontana, Cappa, Rebecchi, & Cocconcelli, 2010; Gobbetti et al., 2018), which is chiefly responsible for the cheese's texture and flavour. Chymosin, plasmin, lipoprotein lipase from raw milk and starter enzymes also contribute to this process, but to a lesser extent (Fox, Guinee, Cogan, & McSweeney, 2017; McSweeney & Sousa, 2000). The microbiota is selected during the ripening, and it is often characterised by acid and salt-tolerant moulds, yeast, and bacteria (Carminati et al., 2000).

In general, the rind of Taleggio cheese is visibly colonised by moulds from the dairy environment, mainly *Penicillium, Cladosporium, Aureobasidium* and *Mucor* (Gobbetti, Corsetti, et al., 1997; Panelli, Buffoni, Bonacina, & Feligini, 2012). Furthermore, a set of bacterial groups (such as lactic acid bacteria, micrococci, corynebacteria, staphylococci and *Pseudomonas*) and yeast genera (i.e., *Candida, Cryptococcus, Debaryomyces, Geotrichum, Pichia*) are usually present in rinds, whose dominance strongly depends on the periodic rind washing (Fontana et al.,

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2010; Gobbetti, Corsetti, et al., 1997; Panelli et al., 2012). In addition, *Listeria monocytogenes* was detected in several Taleggio rinds (De Cesare, Manfreda, Macrì, & Cantoni, 2007; Tirloni et al., 2020). These findings represent a significant risk of potential outbreaks, both because the rind is considered edible and because this microorganism is able to grow or survive in dairy products, particularly at low temperatures and high salt concentration (Carminati et al., 2000). Both the storage and packaging conditions thus become essential.

The packaging commonly used for smear-ripened cheese consists of a wrap made of an oriented polypropylene (OPP), coupled with grease-proof paper (Robertson, 2005). However, this system results in fast spoilage, providing limited protection during transport or handling. These limitations, together with the increasing tendency of consumers to prefer small to medium-sized portions, make this the ideal scenario for the development of innovative packaging systems, able to protect the food products as well as to guarantee the safety and preservation of the organoleptic properties (Favati, Galgano, & Pace, 2007). This is an intriguing way to extend the shelf life and protect the product resides when using modified atmosphere packaging (MAP) paired with more durable material, a powerful strategy for both supporting marketing needs and reducing food waste or loss (Esmer, Balkir, Seckin, & Irkin, 2009; Fadda, Palma, Azara & D'Aquino, 2020; Floros & Matsos, 2005). Conventional MAP systems used for dairy products usually consist of a high concentration of carbon dioxide or nitrogen, from 20 to 100%, and a low concentration of oxygen, usually less than 5% (Alwazeer, Tan, & Örs, 2020; Gonzalez-Fandos, Sanz, & Olarte, 2000; Mastromatteo et al., 2015; Olarte, Gonzalez-Fandos, & Sanz, 2001; Rodriguez-Aguilera, Oliveira, Montanez, & Mahajan, 2011b). Carbon dioxide can inhibit microbial growth, including the aerobic bacteria involved in the ripening processes, especially in the range of 20–60%; nevertheless, such a high value may lead to off-flavour effects and damage the maturation of the cheese (Juric, Bertelsen, Mortensen, & Petersen, 2003; Nájera, Nieto, Barron, & Albisu, 2021). On the other hand, oxygen is the principal element for microbial growth and spoilage mechanisms while being fundamental for the respiration of dairy products by preventing the insurgence of anaerobic conditions (Robertson, 2005).

Equilibrium Modified Atmosphere Packaging (EMAP), based on modulating the in-package equilibrium atmosphere through a suitable design of the mass transport processes, represents a simple yet effective strategy to obtain self-regulated headspace gas composition, achieving a significant extension of the primary shelf life (Hussein, Caleb, & Opara, 2015). The key to creating a personalised EMAP solution for fresh food with high respiration rates, such as fruits, vegetables, and dairy products, is to match the permeability of the packaging and the respiratory rates of the product, thus achieving a proper headspace atmosphere (Mangaraj, Goswami, & Mahajan, 2009; Qu, Zhang, Fan, & Guo, 2020). In the EMAP technique, micro perforations of the packaging materials can support the extension of the apparent gas permeability of the system by modulating the gas fluxes. In addition, the use of micro perforated films helps to reduce the interest in developing complex and costly solutions for fresh products, which may be an unnecessary investment for small to medium-sized food companies (Qu et al., 2020). When considering the application of EMAP technology to improve the shelf life of food, the effects on the texture and the sensory properties of the selected product must be taken into consideration: these characteristics are mainly influenced by both intrinsic factors, such as the biochemical processes involved in food ripening (Qu et al., 2020), and extrinsic factors, in particular headspace gas composition and temperature.

The aim of this study was to investigate the effect of EMAP on Taleggio cheese properties during storage, packed in trays and sealed with perforated multi-layer films with a different number of micro perforations. Three distinct equilibrium atmospheres were selected, and a mathematical model, able to predict the headspace gas concentration inside the packaging system, was employed to design the number and the diameter of the micro perforations. In addition, unperforated packaging configurations and samples packed with paper-like wraps were used as controls. To study the effects of the equilibrium atmosphere on Taleggio, a headspace gas analysis and sensory studies were conducted. The cheese rind microbial populations and texture parameters were also examined.

2. Materials and methods

2.1. Taleggio cheese

Taleggio cheese was produced and ripened by Latteria di Pandino (Pandino, Cremona, Italy) according to the guidelines supplied by the "Consorzio per la Tutela del Formaggio Taleggio" (Milan, Italy) (Carminati et al., 2000; Reps, 1993). The Taleggio cheeses were provided after 42 days of ripening: this condition represents the starting time of the following experiments (t = 0). Taleggio cheese has a parallelepiped shape, with a square base of 20–25 cm, height of 5–7 cm, and an average weight of 2 kg. In this study, 70 samples of Taleggio (14 for each packaging configuration) from the same batch of production were obtained by carefully cutting the cheese with a sharp knife, providing homogeneous pieces in terms of size and smear surface (approximately 10 cm × 3 cm x 4 cm with a weight of 175 ± 5 g), as can be seen in Fig. S1. Samples were stored at 4 ± 1 °C and collected at 0, 7, 14, 28, and 55 days of storage.

2.2. Packaging of Taleggio cheese

Taleggio slices were packed in polypropylene (PP) trays (16 cm × 13 cm x 4.6 cm, thickness 450 μm) (JPack s.r.l., Val Brembilla, BG) and sealed with a polyethylene terephthalate-based (PET) multi-layer film (MLT) (Coopbox group s.p.a., Bibbiano, RE, Italy). PP trays were heat sealed by means of a semi-automated machine (JPack model TSS115-BG, Val Brembilla, BG, Italy) using unmodified air atmosphere conditions, resulting in an average headspace of 590 cm^3. In addition, samples were also packed in recyclable paper-like packaging (PAP, Ovtene®, Arcadia S.P.A, Sedegliano, UD) as a control (Marcuzzo, Peressini, & Sensidoni, 2013). Detailed information about the materials is given in supplementary material in Table S1, and Figs. S2 and S3.

2.3. Micro perforated packaging

The lid films were micro perforated to obtain 125 μm diameter micro perforations (Fig. S5) by means of an experimental setup designed to emulate the hot needle industrial process (Hussein et al., 2015; Qu et al., 2020). The setup was tested in trials to determine both the consistency and repeatability of the geometry and dimensions of the micro perforations, assessed by means of a laboratory stereo microscope (S9i model, Leica Microsystems, USA). A detailed description is provided in supplementary materials (Fig. S4 and Fig. S5).

A model previously developed by our research group (Florit et al., 2022) was adopted for the estimation of the headspace gas composition, considering both aerobic and anaerobic respiration processes (modelled with Michaelis-Menten-like kinetic expressions). Gas fluxes through the packaging were modelled considering both permeation through the thin film and diffusion and convection through possible micro perforations on the permeable film. The respiration kinetic data of Taleggio were provided at 8 °C (Florit et al., 2022). Therefore, the use of this model allows a first guess for the proper EMAP design for this work in which the temperature was fixed at 4 °C. The respiration parameters were adjusted using the Arrhenius law. The model details (namely, the governing equations for EMAP modelling and the kinetic expressions for the gas production/consumption rates) are reported in our previous study (Florit et al., 2022) and are briefly reported in the supplementary material together with the required parameters for this study (Table S2). By choosing the desired micro perforation diameter (125 μm) and the target conditions of oxygen and carbon dioxide, the model suggested the use of two, three and five perforations (Table 1). EMAP systems with two and

Table 1
Samples labelling, number of perforations, tray materials, and target headspace gas composition as modelled. Samples are indicated as multi-layer films (MLT) or recyclable paper-like packaging (PAP) and the number of perforations (0, 2, 3 or 5): MLT_0; MLT_2; MLT_3; MLT_5 and PAP_0.

SAMPLE ID	Number of perforations	Materials	Target $[O_2]$	Target $[CO_2]$
MLT_2	2	PP tray/PET-based film	2–4%	18–20%
MLT_3	3	PP tray/PET-based film	4–5%	15–16%
MLT_5	5	PP tray/PET-based film	10–11%	12–13%
MLT_0	0	PP tray/PET-based film	0%	28–30%
PAP_0	0	Ovtene®	/	/

three 125 μm diameter micro perforations (MLT_2 and MLT_3, respectively) were designed to obtain a low concentration of oxygen, thus avoiding a complete anaerobic condition. An EMAP system with five 125 μm diameter micro perforations (MLT_5) was selected to study the effects of a higher amount of O_2 on the Taleggio cheese microbiota. Lastly, unperforated packaging configurations (PAP_0 and MLT_0) were chosen as a control since they represent the currently used packaging and a solution close to a MAP system, respectively.

2.4. Headspace gas analysis

The packaging headspace composition was determined by means of a gas analyser (OXYBABY® M+, Witt-Gasetechnik GmbH & Co KG, Witten, Germany). The measurement of the O_2 and CO_2 concentrations was performed by puncturing the packaging films with the instrument's syringe needle through a self-adhesive rubber septum (4 measurements for each time point and each packaging configuration). The gas analysis was performed before opening the sealed film and collecting the Taleggio samples for the microbiological analysis: additional duplicates were used to monitor the headspace compositions during the storage time. Micro perforations generated through this operation were immediately sealed using an appropriate insulating tape to avoid any alteration to the internal atmosphere.

2.5. Visual evaluation

At least three specimens for each sampling time were evaluated to periodically report changes in the cheese appearance during ripening, qualitatively assessing the mould growth and texture. The mould growth was evaluated in a qualitative scale as follows: (+) little mould on the rind; (++) patches of mould on the rind; (+++) widespread mould on the rind; (++++) widespread mould on the rind and paste. Visual texture was assessed considering the softening of the paste: L (low softening), M (moderate softening underneath the rind) and E (evident softening underneath the rind and in the paste).

2.6. Microbiological analysis of Taleggio rinds and pH measurement

For microbiological analysis, a slice of rind of about 4 cm wide, 10 cm long and 2–3 mm thick (corresponding to 10–13 g in weight) was aseptically removed from cheeses by a sterile blade, diluted 1:10 with trisodium citrate 5% (w/v) and homogenised for 1.5 min at 260 rpm in a Stomacher Lab-Blender (400 Circulator; International PBI, Milan, Italy). Decimal dilutions were plated in duplicate onto the following media under the respective incubation conditions: Plate Count Agar (PCA, Oxoid, Italy) with cycloheximide 0.01% (w/v) for 72 h at 30 °C for total aerobic mesophilic bacteria; Milk Plate Count Agar (MPCA, Oxoid, Italy) containing cycloheximide 0.01% (w/v) and NaCl 5% (w/v) for the count of halotolerant aerobic bacteria for 72 h at 30 °C (Fontana et al., 2010);

Rose Bengal (Oxoid, Italy) supplemented with Chloramphenicol 0.01% (w/v) (Boehringer Ingelheim, Germany) for 5 days at 25 °C for yeast and moulds. To detect main pathogens on rinds, 25 g of rind were analysed for *Listeria monocytogenes* and *Salmonella* spp. according to ISO 11290:1–2017 and ISO 6579-1:2017, respectively. Analyses were performed in triplicate after 0, 7, 14, 28 and 55 days. After counts, averages and standard deviation were calculated, and the results were expressed in Log CFU/g. The pH values of cheese rinds were measured using the IDF method (IDF International Dairy Federation, 1989) at each sampling time.

2.7. Texture Profile Analysis

Texture Profile Analysis (TPA) was performed at room temperature 20 ± 2 °C, adapting a Universal Testing Machine MTS model 1/MH (MTS systems, Eden Prairie, MN, USA). For each packaging configuration, 3 cylindrical samples of Taleggio cheese, 2 cm in diameter and 2 cm in height, were obtained from the same slices at different time points (7, 14, and 28 days) (Gunasekaran & Ak, 2002b). The preparation was executed by accurately removing the rind with a wire cutter and then carving out a cylinder. The cylinders were collected and stored at 20 ± 2 °C for 2 h before testing.

The samples were subjected to a two-step compression (Gunasekaran & Ak, 2002a) by means of a 5 kN load cell for 70% of their height during two consecutive compression cycles (bites) at a constant crosshead speed of 0.8 mm s^{-1} (Fox et al., 2017; Muthukumarappan & Swamy, 2017). The two compression phases were divided by a waiting time of 35 s, during which the crosshead turned back to its original position. Force vs. time curves were examined to derive the following textural parameters: hardness (N), defined by the force peak on the first bite; cohesiveness, as the ratio between the first and second compression cycle positive force areas; stringiness (mm), measured as the distance travelled by the probe during the negative force area at the end of the first bite cycle (Bourne, 2002).

2.8. Sensory evaluation

The sensory evaluation of samples after 28 days of storage was carried out using the Big Sensory Test (BST) method to determine a descriptive profile of samples, mainly focusing on the aromatic profile while allowing the performance of the judges to be validated. The analysis was performed in the sensory laboratory of Good Senses S.r.l. (Brescia, Italy). The panel of tasters comprised nine trained judges, consisting of technicians, operators, producers, and scholars in the sector. The cheese portion measured approximately 1 cm on each side and the rind was removed before the sensorial evaluation. Duplicate samples were offered randomly, without any information about them. A total of 28 attributes corresponding to texture, aroma, taste, and retro-olfactory perceptions were evaluated using a nine-point quantitative scale in which nine was the highest intensity and zero was the lowest. In addition, the hedonic level and attractiveness were also evaluated from nine (maximum liking) to zero (minimum liking).

2.9. Statistical analysis

The normal distribution of data collected from the TPA were evaluated using the Kolmogorov-Smirnov method. One-way analysis of variance (ANOVA) and Tukey's test were used to evaluate the significance of the results (p-value < 0.05). Data from the microbiological analysis were submitted to both ANOVA ($p < 0.05$) to determine significant differences and to Tukey's test to compare mean values for either all samples at a defined time or for each sample during storage time, respectively, using XLSTAT (version 2020.3.1.2, Addinsoft). Data from the sensory study were validated and analysed by principal component analysis (PCA) and by Pearson's Linear Correlation using the software XLSTAT (version 2020.3.1.2, Addinsoft).

3. Results & discussion

3.1. Headspace gas analysis

Fig. 1 reports the experimental and predicted O_2 and CO_2 gas fraction evolution of the headspace of different packaging designs for Taleggio pieces (Table 1). Experimental data shows that the designed EMAP configurations effectively regulate the gas composition in the headspace (Dawange, Dash, Bal, & Panda, 2016), reaching the predicted target equilibrium conditions. As expected, a larger number of perforations enhances gas exchange through the lid, leading to a higher oxygen concentration and a lower carbon dioxide content in the headspace (Florit et al., 2022; Rodriguez-Aguilera, Oliveira, Montanez, & Mahajan, 2009).

The packaging configuration with two micro perforations (MLT_2) reached a steady state after 10 days of storage, the experimental plateau concentrations being consistent with the computed targets ([O_2] = 2.33 ± 0.79%, [CO_2] = 20.12 ± 0.84%) (Table 1). Similarly, in the 3-perforations configuration (MLT_3), after reaching the equilibrium, no deviation from steady-state conditions was observed during the first month ([O_2] = 4.37 ± 1.02%, [CO_2] = 17.74 ± 1.14%). The 5-perforations configuration (MLT_5) showed the highest oxygen fraction at steady state ([O_2] = 10–11%), as predicted by the model, while CO_2 reached a concentration of 12%. Lastly, samples with non-perforated lids (MLT_0) showed a rapid evolution within the first few days: the O_2 level reached almost 0% while CO_2 reached a stable value around 30%. Such drastic O_2 consumption and CO_2 production may be due to the intense metabolic activities of the dominant aerobic microbiota of Taleggio rind. Moreover, the divergence of the collected data for MLT_0 from the estimated trend may be explained by the significant changes undergone in the rind microbiota after 14 days of storage (Fig. 1a). Trends for O_2 and CO_2 headspace concentration were in line with similar studies. In particular, Rodriguez-Aguilera, Oliveira, Montanez, and Mahajan (2011a) investigate the effect of the MAP system on St. Killian cheese, a soft, mould-ripened cheese, which showed comparable results to those obtained from MLT_0 and MLT_2 packaging.

3.2. Visual evaluation of the Taleggio cheese

A visual evaluation of the cheese samples was conducted during storage at 4 °C, with the purpose of assessing the effect of the packaging on the appearance of both rind and paste in terms of mould growth and visual texture. Visual evaluation was carried out since the appearance of cheese has a key role in consumers' preferences (Fox et al., 2017). Fig. 2 shows a full comparison of the samples from each packaging configuration at different time points, reporting the mould development (indicated as +; ++; +++; ++++) and the visual texture (indicated as L; M; E). In addition, Fig. S6 shows a detailed view of the cheese rind changes during storage. When the Taleggio cheeses were received (t = 0), their rind was barely populated by moulds (+) and their paste appeared very compact, slightly softer under the rind and more consistent and crumblier in the centre (L) (Fig. 2 and Fig. S6).

PAP_0 and MLT_5 continuously changed their appearance: both already showed the spread of mould patches on the rind at 28 days (+++), and after that, mould dissemination also occurred on the paste (++++) (Fig. S6). In parallel, the texture of these samples was softer underneath the rind and less crumbly on the centre at 28 days; after this time point, these samples were evidently soft in the centre due to an excessive centripetal maturation (E) (Fig. 2).

This softening could be correlated to the possible degradation of caseins and lipids due to proteolytic and lipolytic enzymes secreted by bacteria, yeasts, and moulds (Bae, Nam, Renchinkhand, Choi, & Nam, 2020; Gobbetti, Corsetti, et al., 1997). On the other hand, MLT_2 and MLT_3 remained almost unaltered in terms of mould growth (++), showing moderate and low softening respectively until 28 days. Furthermore, MLT_2 and MLT_3 configurations were not able to limit either the mould overgrowth (+++ and ++++, respectively) (Fig. S6) or the loss of compactness of the paste (M) over a longer period (55 days) (Fig. 2). It is worth mentioning that the MLT_0 samples did not show changes in mould growth (+) during the whole storage period, but moderate changes in both the paste (M) and the rind texture were noticed on the 28th day (Fig. 2). In this sample, the rind became moist and cracked, while the paste was softer and less crumbly than expected for this kind of cheese. In addition, the visual evaluation led us to consider that certain samples (PAP_0 and MLT_5) would no longer be
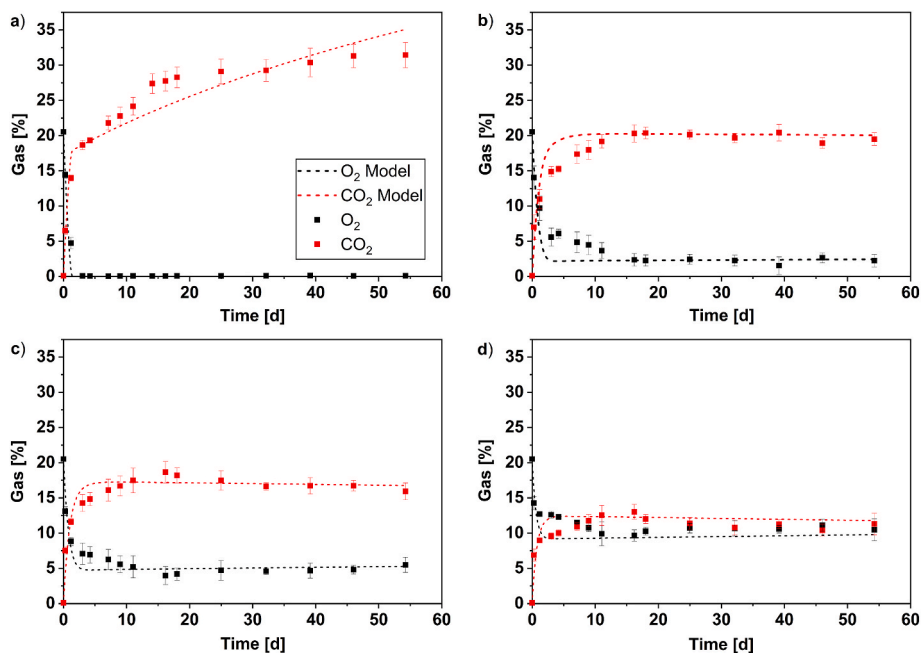

Fig. 1. Headspace gas composition evolution. Comparison of the model prediction and experimental data on Taleggio cheese samples at a constant temperature of 4 °C. a) MLT_0; b) MLT_2; c) MLT_3; and d) MLT_5.
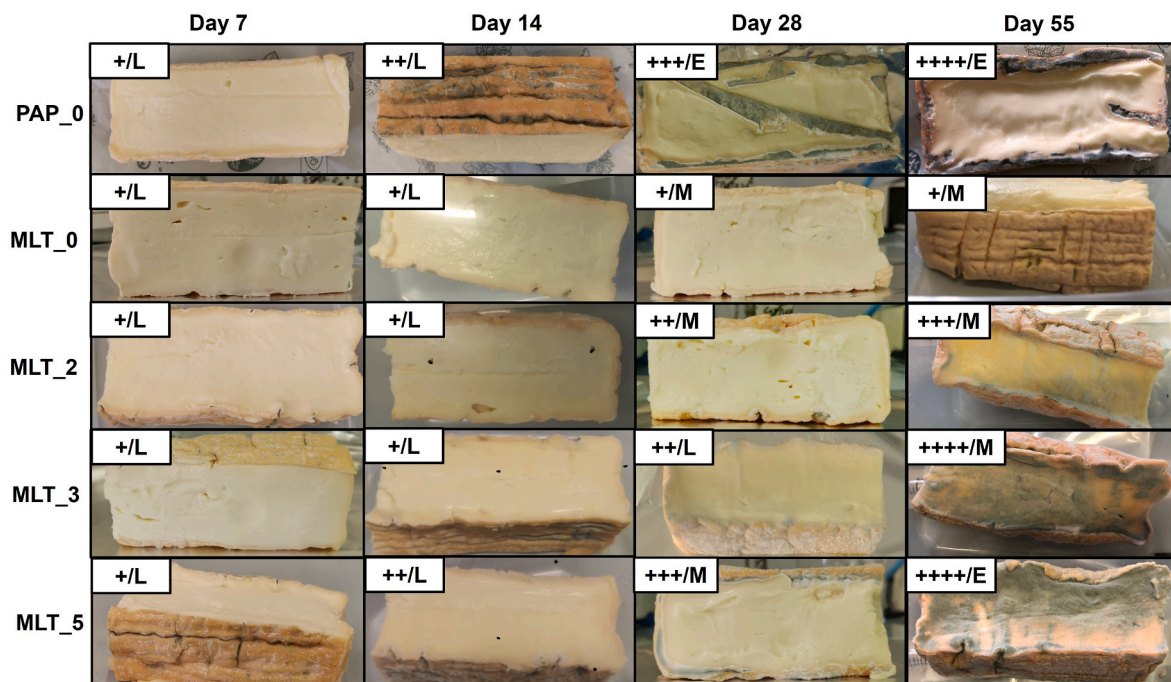
Fig. 2. Time-dependent comparison of the Taleggio cheese samples. In the upper left corner of each image is reported the qualitative scale for the mycelial growth, (+) scarce white mould on the rind; (++) patches of green mould on the rind; (+++) extended green mould on the rind; (++++) excessive green patches of mould on the rind, and the paste visual texture (L: low softening, M: moderate softening underneath the rind, and E: evident softening underneath the rind and in the paste). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

acceptable for consumption after 28 days due to an excessive mould growth, and in consequence, to perform the sensory study as described later.

3.3. Microbiological evaluation and pH measurement of Taleggio rinds

The performance of the main aerobic microorganisms in the Taleggio rinds have been studied to establish the effects of EMAP on the microbiota; additionally, it may have a relevant influence in the gas equilibrium in the EMAP system. Therefore, moulds have been assessed using two approaches: the development of mycelium by visual evaluation using a qualitative scale as well as the number of fungal spores by counts on selective media. Briefly, MLT_2 and MLT_3 did not exhibit significant changes in mycelial growth after 28 days of storage, but a remarkable mycelial growth was found for samples packed in PAP_0 and in MLT_5, likely due to the greater availability of O_2 (Fig. S6).
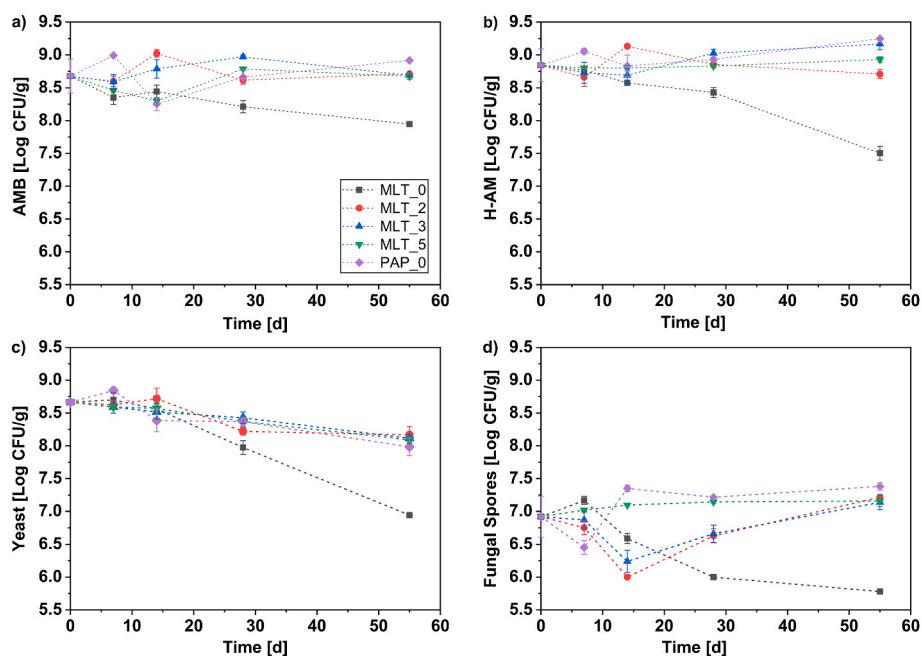
Fig. 3. Microbial growth trends on the rind of Taleggio stored in different packaging for 55 days: a) Aerobic Mesophilic Bacteria (AMB); b) Halotolerant Aerobic Bacteria (H-AM); c) Yeast and d) Fungal spores.

Conversely, in MLT_0, the low concentration of O_2 (Fig. 1a) clearly inhibited them even at the end of the experiment (Fig. S6).

In addition, the counts of fungal spores were 6.92 ± 0.63 Log CFU/g at the beginning of the experiment (t = 0) (Fig. 3 and Table S3). Despite the varied mycelial growth, no significant differences among counts for MLT configurations and PAP_0 were registered during storage, exhibiting values in the range of 6.00 ± 0.00 and 7.38 ± 0.11 Log CFU/g. Notably, MLT_0 showed the lowest survival of spores when compared after 28 days (Fig. 3d and Table S3).

Considering yeast counts, no significant differences among samples at each sampling time were found, starting from 8.66 ± 0.07 Log CFU/g with continued stability around 8.5 Log CFU/g except for MLT_0 that showed an evident diminution of 1.72 Log CFU/g at 55 days, mainly due to the O_2 depletion (Fig. 3 and Table S3).

Halotolerant aerobic bacteria count remained almost unchanged during storage, showing values in the range of 8.53 ± 0.08–9.25 ± 0.02 Log CFU/g for most samples; only in MLT_0 samples was a significant decrease after 28 days of storage observed, a possible effect of anoxic conditions. Similarly, the aerobic mesophilic bacteria count displayed values in the range of 8.25 ± 0.21 – 9.02 ± 0.11 Log CFU/g until 28 days and then only MLT_0 exhibited a significant diminution until 7.95 ± 0.02 Log CFU/g (Fig. 3c and Table S3).

These results suggest that the analysed smear microbiota remains quite stable from the achievement of the equilibrium in all the perforated configurations (on the 10th day) until the end of the considered shelf life (on the 28th day), particularly for configurations with 2 and 3 perforations. Clear differences could instead only be assigned to mycelial growth. When dominance of moulds over other aerobic microorganisms was observed for the MLT_5 configuration, their high enzymatic activities might have caused the loss of structure of the paste.

Besides, as previously inferred, the microbiological analysis may explain the divergences between measured levels of CO_2 and O_2 and the predictions of the mathematic model in the non-perforated configuration (Fig. 1a). More specifically, the markedly increasing trends of CO_2 in the first 28 days could be related to the metabolic activities of the prevalent aerobic population. It could be hypothesised that, in the absence of moulds, these anaerobic conditions have promoted the release of enzymes from aerobic bacteria lysis, increasing the loss of the typical paste texture. Therefore, only the EMAP with 2 and 3 perforations were able to keep the microbiota on the Taleggio rind stable, contributing to the development of the typical organoleptic attributes (Agnolucci et al., 2020). Finally, *Listeria monocytogenes* and *Salmonella* spp. had not been detected on the cheese's rind at any time or in any packaging configuration, indicating no safety issues for Taleggio samples based on high-quality raw materials and good manufacturing practices. In general, counts of aerobic mesophilic bacteria and mould spores found at the initial time are according with those reported by Fontana et al. (2010) and Gobbetti, Corsetti, et al. (1997) for other Taleggio cheese obtained from market retail and after a 42-day ripening, respectively.

The pH of the cheese rinds was quite stable (around pH 7) for all the samples at each storage time point (up to 55 days) (Table S4), a possible consequence of the achievement of the pH stability after 42 days of ripening at the producer site.

3.4. Textural profile analysis

The mean values for the different textural parameters, namely cohesiveness, hardness, and stringiness, are reported in Fig. 4 (Supplementary data are provided in Fig. S7 and Table S5). A significant decrease in cohesiveness has been observed in PAP_0 between time zero and the first two weeks of storage ($p < 0.05$), followed by an increasing trend at 28 days. A possible explanation may reside in the definition of cohesiveness as the strength of the internal bonds inside the matrix; therefore, as the paste of the Taleggio becomes more liquefied, the cohesion may increase. A similar behaviour has been found in surface
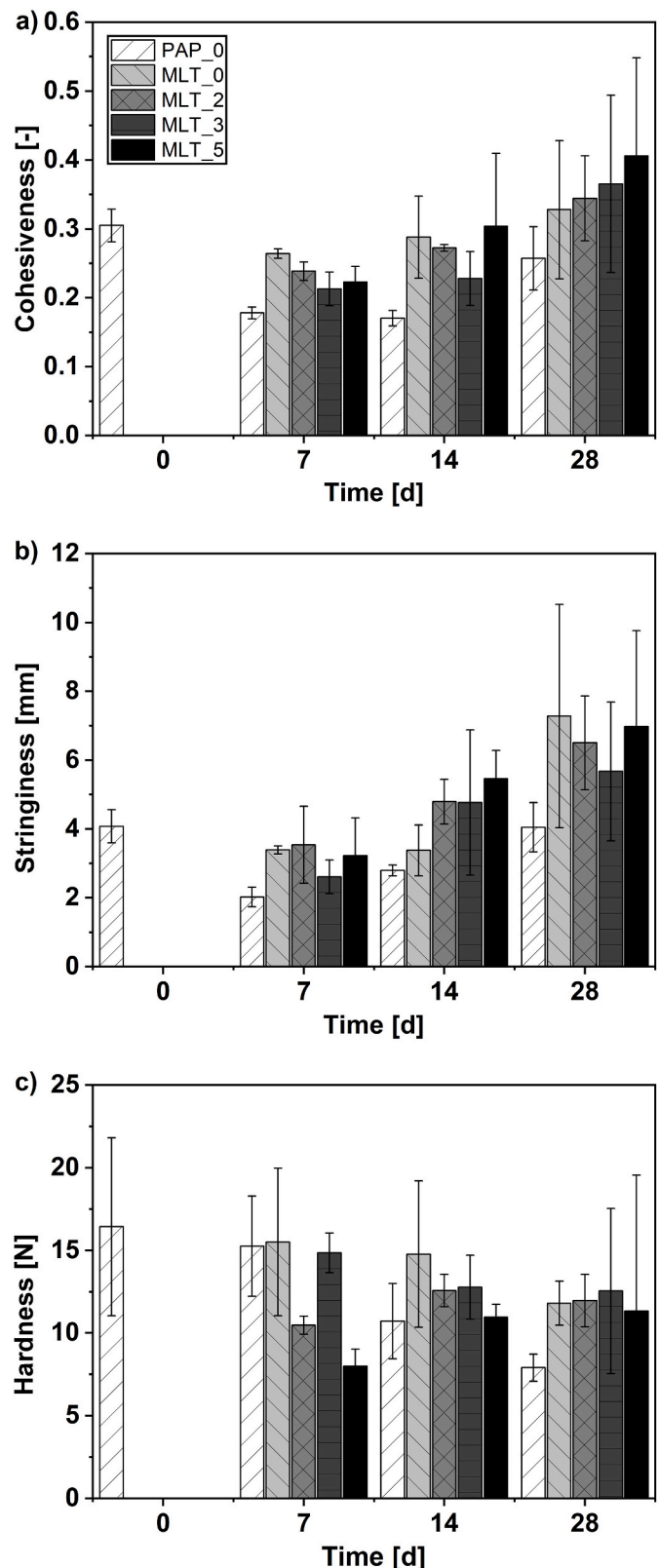

Fig. 4. Comparison of the different textural parameters (Cohesiveness; Hardness; Stringiness) obtained from the Texture Profile Analysis.

mould-ripened cheeses (Bae et al., 2020; Vázquez-García et al., 2020). As expected, MLT_0 showed almost unaltered cohesiveness during the first 28 days of observation ($p > 0.05$) since the texture remained more compact than the other configurations. Cohesiveness values for MLT_2

Limburger, Münster, Brick, and Saint-Paulin cheese. However, adjustments to the EMAP system to match the different microbiota and respiration rates of the packaged products need to be considered.

CRediT authorship contribution statement

Filippo Ghisoni: Formal analysis, Methodology, Investigation, Writing – original draft. **Andrea Fiorati:** Formal analysis, Writing – review & editing. **Federico Florit:** Formal analysis, Writing – review & editing. **Gian Paolo Braceschi:** Methodology, Writing – original draft. **Constanza Maria Lopez:** Formal analysis, Investigation, Writing – original draft. **Annalisa Rebecchi:** Conceptualization, Writing – review & editing, Supervision. **Luigi De Nardo:** Writing – original draft, Writing – review & editing, Supervision.

Declaration of competing interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2022.113464.

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