Effect of aging pit on volatile compounds and sensory attributes of traditional Italian Fossa cheese

Q1Q6

The corrections made in this section will be reviewed and approved by a journal production editor.

Diana De Santis* desdiana@unitus.it, Marcello Fidaleo

Department for Innovation in Biological, Agro-Food, and Forest Systems, University of Tuscia, Via San Camillo de Lellis, 01100, Viterbo, Italy

Q3 *Corresponding author.

Abstract

This work evaluated how the gross chemical composition and colour, the sensory characteristics, and the aromatic properties (evaluated through HS-SPME-GC), of Fossa cheese, a typical local Italian product aged in specially designed underground pits, are affected by the pit used for ripening. Four pits of different depth from 2.5 to 3.4 m were filled with sacs of cheese stacked on top of each other and used for the 90-day aging. Cheese samples taken from various positions inside each pit were collected and analysed. Results showed statistically significant differences among the cheeses aged in the different sized pits for most of the studied characteristics. The multivariate analysis of the data revealed that many cheese properties, including the overall liking, were correlated with each other and with the pit depth. By inducing different levels of whey and fat loss into the pit (presence of liquid inside the pits after the completion of the aging process), varying pressures due to the differing depths of the pits could be responsible for the different modifications of the cheese (gross composition and shape) and the pit environment (humidity) influencing directly and indirectly, by controlling microflora development, cheese sensorial properties and volatile organic compounds (VOCs) production.

Keywords:

Pit cheese, Ripening/maturation, Sensory analysis, Headspace solid-phase microextraction gas-chromatography (HS-SPME-GC), Principal component analysis (PCA)

1 Introduction

'Fossa' (Pit) cheese is a typical local Italian product, produced in a small geographical area of central Italy, Sogliano al Rubicone (Emilia Romagna region) which has been granted the denomination of protected origin (PDO) from the European Union (EU, 2009). The specific feature of this cheese concerns the 90 days of aging in special underground pits, or fossas, that are dug into soft tufa rock, during which unique, highly appreciated flavours develop. It is a hard cheese, produced in limited quantities (about 200 tons/year), and it has great economic importance as a market niche (Gioacchini, De Santi, Guescini, Brandi, & Stocchi, 2010). The production process of Fossa cheese is described in detail in Gobbetti et al. (1999). Briefly, the cheese is made from the milk of sheep, cows or from a mixture of cow and sheep milks and aged for a short period (at least 60 days) at the dairy at 6–14 °C and 75–92% humidity to achieve a consistency necessary to reduce whey losses when the curds are pressed into the pits (De Santi et al., 2010). Cheeses are then wrapped in natural cotton bags and placed in the pit for about 3 months to complete their ripening. Pits are sanitized by fire and smoke. Wooden boards are laid on the bottom of the pit forming a floor, and the pit walls are lined with a 15-20-cm-thick layer of straw before the cheeses are placed inside. The pits are then filled with the cheeses and hermetically sealed, generally from August to November (Siano et al., 2019).

The peculiar seasoning process of this product has aroused much scientific interest. Previous studies have shown that aging in the pit has a profound influence on bacterial diversity (Barbieri et al., 2012), the volatile organic compounds (Gioacchini et al., 2010) (Siano et al., 2019), the fungal microflora, and yeast biota (De Santi et al., 2010; Biagiotti, Ciani, Canonico, & Comitini, 2018), and the microbiological and biochemical characteristics (Gobbetti et al., 1999). In fact, during the 90 days of seasoning, due to the combination of pressure, humidity, and temperature, in the large pits, filled with the cheeses, a particular environment is created. This unique environment causes profound microbiological and molecular transformations, responsible for the aromatic complexity of this product, which makes it very different from a similar cheese produced from the same milk and according to the same procedure, but conventionally aged in a seasoning cell (Siano et al., 2019; Biagiotti et al., 2018; Avellini et al., 1999; Mascaro et al., 2010; Gioacchini et al., 2010).

The pits for the ripening phase have a truncated cone shape. Dimensions for the circular openings and depth of about 70–100 cm and 3–7 m, respectively, have been reported (Biagiotti et al., 2018; Gobbetti et al., 1999). As expected, differences in the environmental conditions (temperature, humidity) affect the maturation process, as has been reported for other cheeses. For example, Leclercq-Perlat et al. (2015) showed that variations in temperature and relative humidity (from 8 °C to 16 °C, and from 88% to 98% RH) have an appreciable impact on the sensory descriptors of Camembert-type cheeses, influencing the microorganism growth, pH, metabolism of the carbonaceous substrate, and moisture of the cheese, as well as microbial enzymatic activities. For this reason, several studies reported the measurement of temperature and relative humidity existing inside the pit (Mascaro et al., 2010; De Santi et al., 2010; Siano et al., 2019; Avellini et al., 1999) during pit cheese ripening.

Despite the lack of sensory homogeneity that heavily penalizes the marketability of Fossa cheese and the fact that aging in pits plays a decisive role in the development of the sharpness and flavours typical of this product, there are no studies analysing the effect of cheese aging in different pits. Many studies have reported details on this peculiar cheese, investigating microbiological and compositional aspects, but none have carried out a study on the same starting cheese lot aged in different pits, except the study of Siano et al. (2019) who considered two pits of similar shape and depth. Furthermore, previous studies on pit cheese have concerned a limited number of cheese samples (Gobbetti et al., 1999; De Santi et al., 2010; Gioacchini et al., 2010; Barbieri et al., 2012; Biagiotti et al., 2018; Siano et al., 2019) and the sensory profile of cheeses ripened in pits with different depths is still poorly understood.

Thus, the aim of this work was to investigate the contribution of the pit used for ripening on the variability of Fossa cheese chemical and sensorial characteristics. In particular, we focused on the pit pressing capacity, correlated to the depth of the pit itself, a factor that has been neglected up to now. We hypothesized that there may be a relationship with the sensory characteristics and the overall liking of the aged cheese, and therefore that pressing in the pit could affect the seasoning process and help cheese manufacturers to standardize the production.

2 Materials and methods

2.1 Cheese making and pit preparation process

The production process of Fossa cheese follows the traditional cheesemaking protocols according to the regulations imposed by the Consortium, as reported in (Gobbetti et al., 1999). Briefly, sheep milk was pasteurized (72 °C for 15–20 s), cooled to 30 °C and inoculated with the microbial starter (*Lactococcus lactis*). After approx. 30 min from powdered calf rennet addition, milk coagulation took place. The curd, cut into small grains of about 0.5–1.0 cm, was drained, inserted into cylindrical plastic sieves (height of 6–10 cm, diameter of 12–20 cm) and kept at 28 °C for ca. 8 h, with numerous inversions before being salted by immersion in brine (17–19% w/w NaCl) for 18 h. After 60 days of ripening at 6–14 °C and 75–92% relative humidity (RH), the aged cheeses, cleaned and freed from superficial moulds, were enclosed in canvas sacks, tied with natural twine, marked with the identification number of the producer and the batch, and placed in the pit for further maturation for 80–100 days. Before receiving the cheese, the pit was prepared according to the traditional method. First, the pit was sanitized with fire and smoke, burning bundles of wheat and straw. After cleaning of the ash residues, the bottom of the pit was raised with stones and boards to avoid stagnation of the whey and fat loss due to cheese pressing. Finally, the walls were covered with a layer of straw supported by vertically arranged reeds. The pit was then filled with sacks of cheese and tucked up with other products after 10 days. At the end of the filling phase, the pit was covered with sheets and/or straw to avoid transpiration, the pit was sealed, and the pit ~~phase~~ ripening phase began. Cheese was aged in the pit for about 90 days from the beginning of August to the end of October.

2.2 Sampling

Cheese samples obtained from the same production batch supplied by a local dairy producer (La Rinascita SaS, Meldola, FC, Italy) were randomly assigned for maturation to four different pits, from the same geographical location, labelled as 1–4, whose characteristics are reported in Table 1. The pits had different depths which resulted in different overpressures (the pressure in excess of the normal atmospheric pressure value of 1 bar) exerted by the cheese weight at the bottom of the pit, calculated by assuming a cheese density of 1.045 g cm^3, as 0.26, 0.26, 0.31 and 0.35 bar~~s~~ respectively for pit 1, 2, 3 and 4. During pit loading, eight labelled cheese samples were positioned in each pit at different relative positions, varying in both the axial and radial coordinate to assure a uniform sampling of the pit space: one sample at the top (center of the pit), one sample at the bottom (center of the pit) and three samples at the two intermediate vertical positions of the pit (the two positions that divide the depth of the pit into three equal intervals), one at the center and two close to the pit wall. After maturation, all labelled samples were stored at 4 °C in plastic bags in a cold room, in the dark, for no more than 72 h. Before the evaluation, each whole cheese was equilibrated at room temperature and subsequently divided into several aliquots used for sensory analysis, colorimetric dosage and HS-SPME-GC analysis. Starting from cold samples (4 °C), the aliquots for chemical analysis were shredded with a kitchen blender (HR 3652, Philips SpA, Milan, Italy) and stored at −20 °C ± 2 °C in the dark in hermetically sealed plastic containers for no more than a week before being analysed.

alt-text: Table 1

Table 1

The table layout displayed in this section is not how it will appear in the final version. The representation below is solely purposed for providing corrections to the table. To preview the actual presentation of the table, please view the Proof.

Pit characteristics.

	Pit 1	Pit 2	Pit 3	Pit 4
Depth (m)	2.5	2.5	3.0	3.4
Mouth diameter (m)	1	1	1	2
Capacity (kg)	3000	3000	3000	8000
Ripening (days)	84	88	83	85

2.3 Evaluation of instrumental colour

Colour evaluation was performed directly on the sample surface of 3-cm thick slices immediately after cutting, using a portable spectrophotometer mod. CM-2500C (Konica-Minolta, Osaka, Japan). Three measurements were made on each half of each cheese sample in the internal area (the points on the surface at a distance of at least 2 cm from the edges) and averaged, obtaining the colorimetric coordinates (L*, a* and b* values), according to the CIE chromaticity diagram (ISO, 2019).

2.4 Main compositions and physicochemical characteristics

2.4.1 Dry matter

The dry matter content of the samples was determined by drying an aliquot (5 g) of the sample to a constant weight in an oven at 105 °C. The moisture content was calculated from the weight loss, corresponding to the weight of water contained in the sample, and expressed as mass fraction in percentage (AOAC, 2005).

2.4.2 Ash

Samples (5 g) previously dried in the oven at 105 °C as described above were calcined in a muffle at 550 °C for 6 h; the ash content was calculated from the net weight obtained after calcining and expressed as mass fraction in percentage (AOAC, 2005).

2.4.3 Titratable acidity

The titratable acidity of samples was determined by end point titration using 0.1 mol/L aqueous solution of NaOH and phenolphthalein. Distilled water (50 mL) previously brought to 40 °C was added to 10 g of cheese, and the mixture was vortexed for 5 min. The mixture was filled up with distilled water to 100 mL and filtered. The filtrate was then titrated.

2.4.4 Determination of the total protein content and soluble nitrogen

The total nitrogen content of the samples was determined by the Kjeldahl method (AOAC, 2005). Total protein content was calculated using a conversion factor of 6.38 and expressed as weight fraction.

For water-soluble nitrogen determination (Kuchroo & Fox, 1982), 50 mL of distilled water previously brought to 40 °C was added to 4 g of cheese and a few drops of formaldehyde, the mixture was vortexed for 5 min, filled up with distilled water to 100 mL and stored at 4 °C for 24 h before being filtered. The filtrate was digested and submitted to the Kjeldahl procedure. Proteolysis was expressed as ripening index (MI), calculated as the ratio of water-soluble nitrogen to total nitrogen and expressed as a percentage.

2.4.5 Determination of lipids

The fat content of cheese was determined gravimetrically (ISO, 2004). In short, fat was extracted from the sample (4–6 g) using petroleum ether. The solvent and fat phase were separated, and the solvent was removed by evaporation. The mass of substances extracted was determined.

2.5 Volatile compounds analysis by headspace solid-phase microextraction gas-chromatography (HS-SPME-GC)

The volatile compounds (VOCs) of cheese samples were extracted by a headspace solid-phase microextraction (HS-SPME) sampling technique, gas-chromatographic separation (GC), and identification by chemical standards and linear retention index (LRI).

An 85-μm StableFlex Carboxen/Polydimethylsiloxane fibre (CAR/PDMS, Supelco, Bellefonte, PA, USA) was used for HS-SPME (Januszkiewicz, Sabik, Azarnia, & Lee, 2008).

A 3-cm thick slice of each cheese sample was completely ground by a kitchen blender (HR 3652, Philips S.p.A., Milan, Italy) after removing 2 cm of the outer part, to avoid contamination. Ground cheese (4 g) was weighed in a 20 ml clear glass vial (22.5 mm outer diameter, 75.5 mm height; Supelco, Bellefonte, PA, USA) which, after the addition of 1 mL of ethyl vinyl ketone ethanolic solution (internal standard, 1 μL/mL), and 2 mL of distilled water, was sealed with a silicone PTFE septum cap. After 10 min of stirring at 22 °C to pre-equilibrate the sample, the fibre was inserted into the vial and exposed to the headspace for 60 min, at the same temperature. VOCs were desorbed by directly inserting the fibre into the injection port of the GC system, operating in split/splitless mode for 7 min. The split/splitless injector suitable for HS-SPME analysis was mounted on a Trace GC Ultra gas chromatograph (ThermoFinnigan Inc., San Jose, CA, USA) equipped with a fused silica capillary column (model DB-WAX; 60 m × 0.25 mm inner diameter and 0.25 μm film thickness; J&W Scientific Inc., Folsom, CA, USA). Ultra-high purity (99.999%) helium was used as carrier gas at a flow rate of 0.8 mL min^{-1}. The temperature was maintained at 40 °C for 7 min and then programmed to reach 230 °C at a rate of 3 °C per min, with a final isotherm of 7 min. A high sensitivity flame ionisation detector (FID) at 260 °C was used. The FID signal was recorded and integrated by a Mega Series integrator (Carlo Erba, Italy).

2.6 Sensory panel evaluation and consumer test

2.6.1 Preliminary phases and assessors training

During three trial sessions, eight expert sensory evaluators (2 males and 6 females, aged between 23 and 50 years), previously selected, trained and monitored according to ISO standards (ISO, 2006; ISO, 2005; ISO, 2012), received the cheese samples to develop the lexicon for Pit cheese sensory profiling. Cheese samples (1.5-cm height, 5.0–8.0-cm length, and 1.5-cm depth) were coded with three-digit randomized numbers and served in random order at room temperature (Zanoni, Pigoni, & Hunter, 2006). Meetings took place in a sensory evaluation laboratory that conformed with the international standards (ISO, 2014b), where the panellists evaluated the samples, presented in a monadic sequential order, to individually recognize the attributes that best described each sample. All the descriptors were collected into a list that was reduced by eliminating the inappropriate terms and by grouping synonymous descriptors which unequivocally referred to the same characteristics, according to ISO (2016). The list was then reduced to 27 terms, which were discussed with the judges to make sure the correct meaning of each descriptor was understood, using, for some of them, a reference standard that was anchored to a point on the intensity scale. Finally, in two separate sessions, the judges assessed the cheese samples using the 27 descriptors mentioned above, listed in a profile sheet, according to a linear intensity scale from 1 to 9, where (1) was absence and (9) was high intensity.

The results collected in a schedule were used to assess the so-called geometric mean percentage (M_j) of each j-th descriptor:

$$M_j = (F_j I_j)^{1/2}$$

where F_j is the number of j-descriptor's mentions divided by the total possible number of this descriptor's mentions expressed as percentage and I_j is the sum of intensities given by the entire panel for j-descriptor divided by the maximum possible intensity of this descriptor expressed as percentage.

The initial list of 27 descriptors was then reduced to 17, discarding those that did not reach the 35% threshold (Table S1), according to ISO (1994). Finally, the 17 main descriptors were collected in a profile sheet used for sample testing. Table 2 reports the unambiguous definition of each attribute. To train the judges in the use of the intensity scale, before the blind test, three sessions were conducted on cheeses sampled randomly from the four pits. Under the same test conditions described below, at each session, four different samples were tested collectively, under the guidance of the panel leader to attribute a precise intensity value (scale 1–9) to all profile sheet descriptors.

alt-text: Table 2

Table 2

The table layout displayed in this section is not how it will appear in the final version. The representation below is solely purposed for providing corrections to the table. To preview the actual presentation of the table, please view the Proof.

Details of sensory attributes.

Attribute	Definition	Further Information
Colour intensity	Visual evaluation of the colour intensity	Pit cheese samples
Odour intensity	Overall olfactory perception	Pit cheese samples
Butter	Aroma associated with butter	Butter
Ewe Cheese	Aroma associated with ewe milk cheese	Pecorino 100% ewe milk
Earth/Mushroom	Aroma associated with mushroom	Dried mushrooms
Milk	Aroma associated with raw milk	Heated milk (85 °C 10 min)
Aroma intensity	Odorous retro-olfactory sensation during tasting	Pit cheese samples
Sweet	Taste sensation elicited by sugars	2% wv^{-1} sugar solution
Salty	Taste sensation elicited by salts	0.02% wv^{-1} NaCl solution
Sour	Taste sensation elicited by acids	0.05% wv^{-1} citric solution
Bitter	Taste sensation elicited by diluted aqueous solutions of various substances, such as quinine and caffeine	0.08% wv^{-1} caffeine solution
Pungent	Sensory sensation manifesting in the mouth as irritation, which could also cause pain (fine needles)	Pecorino cheese seasoned 20 months
Umami	Chemical feeling factor elicited by certain peptides and nucleotides	0.5% wv^{-1}monosodium glutamate solution
Taste Intensity	Sensorial attribute perceptible	Cheese control before pit storage
Mouldy	Aroma associated with mold	Outer layer of the Brie cheese
Hardness	Resistance to a given deformation	Pecorino cheese seasoned 20 months
Friability	Extent to which cheese fragments are increasingly perceived during mastication	Parmesan seasoned 36 months

2.6.2 Quantitative descriptive analysis

For each of the eight relative positions in the pit, four cheese samples were withdrawn from the four pits (each sample from a different pit) and evaluated simultaneously. The samples were served to each judge, at room temperature, on a disposable plastic plate, encoded with three-digit numbers, in random order, in the sensory laboratory booths (ISO, 2014a). Each assessor received medium sparkling water to cleanse the mouth during the test. Results were expressed as the average of three sensory evaluations conducted in triplicate. The CV (coefficient of variation) was always less than 3%.

2.6.3 Consumer test

A consumer test was organized to evaluate the Pit cheese acceptability of consumers. A total of 30 consumers aged 25 to 58, 18 females and 12 males, contributed to the test. Each participant received the same four samples from the quantitative descriptive analysis in random order in the sensory laboratory booths, as described above (De Santis, Equitani, & Forniti, 2009). For each cheese sample, the consumer assessed the overall preference on a 9-point hedonic scale (1: extremely unpleasant and 9: extremely pleasant (Kähkönen, Tuorila, & Rita, 1996; Kähkönen, Tuorila, & Lawless, 1997).

2.7 Statistical analysis

A completely randomized experimental design with four treatments (the four pits), eight replicates (samples placed at different positions in the pit), and different responses (gross chemical composition, sensory responses, and VOCs) was used.

All data were used for the univariate and multivariate statistical analysis. Sensory data were averaged over the panelists.

Summary statistics, one-way analysis of variance (ANOVA), Tukey multiple comparisons procedure, known also as Tukey's HSD (honestly significant difference) method and principal component analysis (PCA) of the data were carried out by using the software JMP Pro., version 16.0.0 (SAS Institute, Cary, NC, USA). The degree of significance of statistical tests (α) was set to 5%. In PCA, the correlation matrix was used to analyse the sensory and VOCs data.

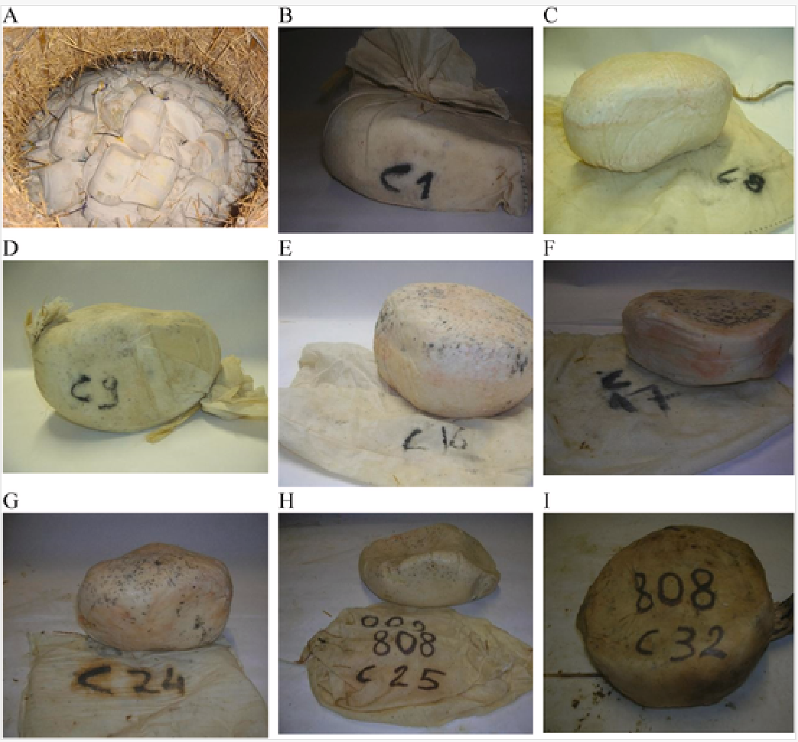
3 Results and discussion

3.1 Appearance of cheese ripened in different pits

Fig. 1 shows a few pictures of the experimental setup used for cheese aging, including cheese samples aged at different positions in the pits. After the first phase of ripening, and before pit aging, cheese samples presented a cylindrical shape. After aging in the pit, the cheese sample shape and colour changed significantly compared to the cheese before aging. In particular, the cheese aged at the bottom of the pit presented a very irregular shape compared to the samples aged at the pit top. This effect was particularly evident for cheeses aged in pits 3 and 4, subjected to a higher vertical load corresponding to the higher pit depth (see Table 1 for pit depth; pit depth is the same for pits 1 and 2 and increases with pit number). The cheese aged in pit 3 and 4 presented a superficial darker colour compared to pits 1 and 2. The change in the aspect of ripened cheese as compared to the unaged one was due to the pressure exerted by the cheese load that acted on the samples by pressing and mechanically deforming them. Cheese pressing, and consequent release of whey and fat during cheese aging in pits, is a typical phenomenon that makes the external surface of the cheese samples wet and greasy, totally modifying their appearance (Siano et al., 2019; Gobbetti et al., 1999; Papademas & Bintsis, 2018, pp. 1–496). The different compression of cheese due to the different vertical loads of cheese present in each pit may have been responsible for the observed differences of cheese aspect between pits.

alt-text: Fig. 1

Fig. 1



Picture of a typical pit loaded with cheese (top view, A) and of cheese samples after aging at the bottom or at the top of pit 1, 2, 3 and 4 (B or C, D or E, F or G, H or I for bottom or top respectively).

3.2 Chemical composition and instrumental colour

The mean and standard deviation values of moisture, protein, lipid, ash and acidity content, maturation index (MI) and colorimetric coordinates (L*, a* and b*) of cheese samples ripened in different pits are reported in Table 3. The gross chemical composition fell in the range reported for a few samples of Fossa cheese by other authors (Gobbetti et al., 1999; Mascaro et al., 2010; Di Cagno et al., 2003).

alt-text: Table 3

Table 3

The table layout displayed in this section is not how it will appear in the final version. The representation below is solely purposed for providing corrections to the table. To preview the actual presentation of the table, please view the Proof.

Gross chemical composition, maturation index and colorimetric coordinates of pit cheese ripened in different pits. Different letters in each row indicate statistically significant differences (Tukey's method with α = 5%).

Parameter	Pit 1	Pit 2	Pit 3	Pit 4
Moisture (%)	32.9 ± 0.8^{ab}	33.6 ± 1.3^{a}	31.9 ± 1.0^{b}	31.5 ± 1.6^{b}
Proteins (%)	25.4 ± 1.0^{b}	26.1 ± 1.2^{b}	29.3 ± 2.0^{a}	31.2 ± 1.7^{a}
Lipids (%)	35.1 ± 1.3^{a}	35.0 ± 0.5^{a}	32.9 ± 1.3^{b}	29.7 ± 1.8^{c}
Ash (%)	4.4 ± 0.2^{c}	4.8 ± 0.2^{c}	5.3 ± 0.2^{b}	5.8 ± 0.4^{a}
Acidity (%)	13.1 ± 1.8^{b}	14.3 ± 1.6^{ab}	15.3 ± 1.0^{a}	16.1 ± 1.1^{a}
MI (%)	62.4 ± 7.8^{a}	57.1 ± 10.7^{ab}	49.2 ± 9.6^{b}	57.5 ± 6.0^{ab}
L*	81.2 ± 1.0^{a}	79.8 ± 2.6^{ab}	80.3 ± 2.8^{ab}	77.8 ± 2.4^{b}
a*	-1.2 ± 0.4^{b}	-1.1 ± 0.4^{b}	-0.4 ± 0.7^{ab}	0.1 ± 1.4^{a}
b*	17.2 ± 2.1^{a}	17.3 ± 1.6^{a}	16.6 ± 1.9^{a}	18.1 ± 2.3^{a}

All the parameters except for b* showed statistically significant differences among the pits as assessed by one-way ANOVA (α = 5%). For each parameter, the number of distinct groups of non-statistically different parameters that was obtained using the Tukey multiple comparison procedure varied between 1 (one occurrence), 2 (6 occurrences) and 3 (one occurrence), thus showing that the pit affected the above-mentioned parameters. Each attribute followed approximately an increasing (ash, protein, acidity) or decreasing (lipid, moisture) trend moving from pit 1–2 to pit 4. As shown in Fig. 1, due to the high compression of cheese samples during pit aging, increasing from the top to the bottom of the pit, the shape of cheese curds varied during aging from cylindrical to very irregular and whey and fat loss occurred (Gobbetti et al., 1999; Mascaro et al., 2010; Siano et al., 2019; Papademas & Bintsis, 2018~~, pp. 1–496~~). Weight loss due to whey and fat losses as large as 20% during ripening of Fossa cheese have been reported (Siano et al., 2019). Such losses of course can significantly change the composition of the aging cheese, lowering the moisture and fat content and increasing the fraction of the other components as outlined by Gobbetti et al. (1999) by stating that whey losses during Fossa cheese pressing in the pit increase the cheese dry matter.

Thus, the gross composition change among pits could be explained by assuming that the degree of whey (essentially water) and fat loss increased from pits 1–2 to pit 4 with the pit depth. In fact, the pit depth increased from 2.5 to 3.4 m from pits 1–2 to pit 4. Pit depth was the only physical parameter that could influence cheese pressing because it determined the pressure acting on the cheese (the overpressure exerted at the bottom of the pit varied from 0.26 to 0.35 bar from pits 1–2 to 4) which generated the percolation flow (percolation is due to pressing). As a result, the mass fraction of the remaining components in the cheese (especially protein and ash) increased. Table 4 reports the correlation coefficient (r) and probability between the gross chemical composition variables, the colour attributes, and the pit depth. Several interesting correlations were present between composition variables and composition variables and pit depth. For example, protein (r = −0.79) and ash (r = −0.77) content were strongly negatively correlated with lipid fraction (fat decrease due to losses led to increase in remaining compounds). The last row shows the correlation coefficient between each variable and the pit depth. Proteins and ash were positively correlated (r = 0.85) with pit depth while fat (r = −0.86) and moisture (−0.55) were negatively correlated.

alt-text: Table 4

Table 4

The table layout displayed in this section is not how it will appear in the final version. The representation below is solely purposed for providing corrections to the table. To preview the actual presentation of the table, please view the Proof.

Correlation matrix of colour attributes (L*, a*, b*), gross chemical composition variables, and pit depth. Bold values indicate couple of variables that are statistically correlated (asterisks indicate the p-value level: *, **, ***, for p-value < 0.05, p-value < 0.01, p-value < 0.001, respectively).

	L*	a*	b*	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	MI (%)	Acidity (%)	Pit depth (m)
L*	1									

a*	−0.29	1.00								
b*	**−0.46*****	0.29	1.00							
Moisture (%)	0.29	−0.29	−0.07	1.00						
Protein (%)	**−0.46*****	**0.47*****	0.18	−0.29	1.00					
Lipid (%)	**0.33***	**−0.62*****	−0.12	**0.32***	**−0.79*****	1.00				
Ash (%)	**−0.43****	**0.59*****	0.22	**−0.47****	**0.74*****	**−0.77*****	1.00			
MI (%)	−0.20	−0.04	0.27	−0.19	**−0.33***	0.23	−0.16	1.00		
Acidity (%)	−0.13	**0.43****	0.02	−0.10	**0.50*****	**−0.54*****	**0.61*****	−0.27	1.00	
Pit depth (m)	**−0.40****	**0.56*****	0.12	**−0.55*****	**0.85*****	**−0.86*****	**0.85*****	−0.20	**0.60*****	1.00

The colorimetric coordinates L* and a* showed small but statistically significant differences among some groups of pits. Pit 4 showed a smaller lightness (L*) compared to pit 1 and a higher a* value compared to pit 1 and 2. Thus, the cheese surface colour difference among pits observed from the pictures of Fig. 1 reflected also in some differences in colour of the interior part of the cheese to which the colorimetric coordinates refer.

During cheese ripening, there is a decrease in lightness and a slight increase in both the redness (a*) and the yellow (b*) (Álvarez, Fresno, 2021; Pinho, Mendes, Alves, & Ferreira, 2004; Pillonel et al., 2002). Rohm and Jaros (1996) observed that the value of L*, both external and internal, decreased with maturation, a trend more evident on the surface, and that the value of the colorimetric coordinates seemed statistically correlated to the ripening time and humidity decrement. In the present study, the differences in the coordinate L* and a* of the interior as well as the observed colour of the surface of cheeses aged in different pits could reflect the different loss of whey and fat during aging (in general composition is expected to affect cheese colour).

3.3 Sensory analysis

The initial list of 27 sensorial descriptors was reduced to 17 as described in Materials and Methods. ANOVA carried out on the 17 sensorial attributes showed significant differences (p-values<0.05) for 11 descriptors. Table 5 reports all the sensory attributes, means, standard deviations, and the results of multiple comparisons. For all attributes, except for the above-mentioned ones, the pits formed two or three groups of undistinguishable means. In most cases, the cheese aged in pit 4, the one with the highest depth and pressing capacity, showed a significantly different sensorial attribute compared to the cheese aged in the other pits. Cheese aged in pit 1 and pit 2 (depth of 2.5 m) showed a similar profile. Cheese aged in pit 4 showed a different profile compared to the other products, being less sweet and presenting a much higher intensity of the salty, sour, bitter, pungency, mouldy and earth/mushroom attributes. The attributes of cheese aged in pit 3 (depth of 3 m) presented intensities in between the values obtained for pits 1 and 2 and pit 4.

alt-text: Table 5

Table 5

The table layout displayed in this section is not how it will appear in the final version. The representation below is solely purposed for providing corrections to the table. To preview the actual presentation of the table, please view the Proof.

Mean and standard deviations of sensory attributes of cheese ripened in different pits. Different letters in each row indicate statistically significant differences (Tukey's method with $\alpha = 5\%$).

Attribute	Pit 1	Pit 2	Pit 3	Pit 4
Colour intensity	1.9 ± 0.4^{c}	3.5 ± 0.5^{b}	4.1 ± 0.6^{ab}	4.8 ± 0.7^{a}
Odour intensity	4.8 ± 1.2^{b}	5.3 ± 0.7^{b}	4.9 ± 0.4^{b}	6.5 ± 0.5^{a}
Butter	5.1 ± 1.2^{a}	5.0 ± 1.1^{a}	5.1 ± 1.4^{a}	3.9 ± 1.2^{a}
Ewe cheese	3.4 ± 0.7^{a}	3.0 ± 0.0^{a}	3.1 ± 1.4^{a}	2.8 ± 0.9^{a}
Earth/mushroom	1.7 ± 1.4^{c}	2.2 ± 1.2^{bc}	3.4 ± 1.4^{b}	7.9 ± 0.6^{a}
Milk	3.9 ± 1.0^{ab}	4.6 ± 2.3^{a}	3.3 ± 1.5^{ab}	2.3 ± 0.7^{b}
Aroma intensity	6.0 ± 0.9^{a}	5.9 ± 1.2^{a}	4.4 ± 1.7^{a}	5.8 ± 1.8^{a}
Sweet	3.9 ± 1.0^{a}	3.4 ± 0.9^{a}	3.0 ± 0.9^{ab}	1.9 ± 0.4^{b}

Salty	3.9 ± 1.0^{b}	3.9 ± 0.8^{b}	4.9 ± 1.6^{b}	6.5 ± 0.9^{a}
Sour	4.5 ± 0.5^{ab}	4.1 ± 1.2^{b}	5.8 ± 1.8^{ab}	6.1 ± 1.2^{a}
Bitter	3.1 ± 2.2^{b}	2.5 ± 1.8^{b}	3.3 ± 0.9^{b}	6.0 ± 1.1^{a}
Pungent	3.8 ± 1.0^{b}	3.8 ± 1.0^{b}	4.0 ± 1.1^{b}	6.1 ± 1.6^{a}
Umami	3.7 ± 1.2^{ab}	4.9 ± 1.2^{a}	3.2 ± 1.5^{b}	2.4 ± 0.5^{b}
Taste intensity	6.0 ± 0.9^{a}	5.3 ± 1.0^{a}	5.0 ± 0.9^{a}	6.3 ± 1.2^{a}
Mouldy	2.1 ± 1.8^{bc}	1.9 ± 0.6^{c}	3.8 ± 1.5^{b}	7.1 ± 1.1^{a}
Hardness	3.5 ± 0.9^{a}	3.6 ± 0.9^{a}	3.5 ± 0.8^{a}	4.6 ± 1.2^{a}
Friability	5.3 ± 0.7^{a}	5.0 ± 1.3^{a}	4.6 ± 1.3^{a}	5.0 ± 1.2^{a}
Preference	6.6 ± 1.7^{a}	5.9 ± 1.5^{a}	3.6 ± 1.4^{b}	1.8 ± 0.7^{c}

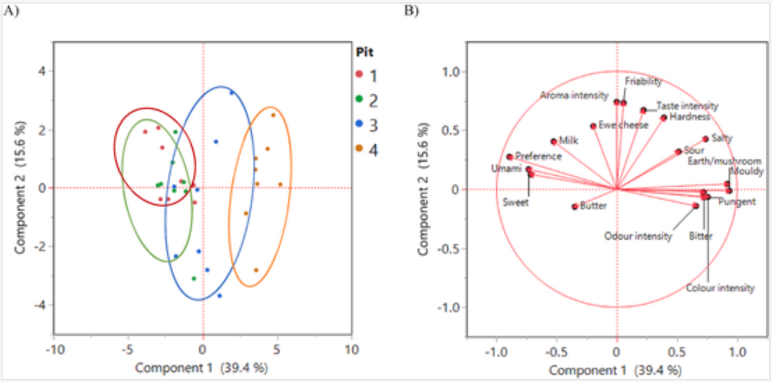
The temperature distribution inside the four pits during aging can be expected to have been approximately the same considering their common location. The humidity of the environment surrounding the cheese, on the contrary, was likely different considering the different degree of whey percolation. Cheese samples from pits 3 and 4 presented a lower moisture content compared to cheese aged in pits 1 and 2. The water lost from cheese with whey percolation likely accumulated inside the pit and increased the air humidity by evaporation. In fact, relative humidities as high as 99% have been measured inside pits (Siano et al., 2019) and this is consistent with free water in equilibrium with air (in this condition relative humidity would be 100%). Thus, it could be assumed that the higher whey percolation created a higher humidity in the environment external to the cheese. This was corroborated by the higher presence of superficial moulds in cheese ripened in pits 3 and 4 (visual inspection) and the higher intensities obtained for the mouldy sensorial attribute. Furthermore, the change of the gross chemical composition due to the different level of pressing experienced by the cheese in the different pits was likely responsible for the change in the sensorial attributes both directly (for example the salty attribute) and indirectly (attributes resulting from microflora action). In fact, Siano et al. (2019) found that Fossa cheese microflora during ripening was affected by its chemical-physical parameters change as a consequence of the considerable decrease in weight (approximately 20%). The different pressing of the cheese could also affect the sensory profile by reducing the amount of air inside the pit (reduction of the free space between the cheese samples) and, therefore, the amount of oxygen affecting the microflora pathways of the cheese (Siani et al., 2019).

Fig. 2 reports the results of the PCA of all the sensory data, including also the six attributes that did not significantly differentiate the cheeses, and the overall liking. The first two components explained about 55% of the total variance. The variables that were more correlated with the first principal component were, in order of decreasing importance, the attributes earth/mushroom, mouldy, bitter, pungent, colour intensity, salty, odour intensity, sour which were correlated positively with the first component and the attributes milk, sweet, umami which were correlated negatively with the first component. Thus, the first component seemed correlated positively with the sharpness of the cheese. The score plot shows that the cheese aged in the four pits separated (especially the cheese ripened in pit 4 compared to the others) according to the first component: mild cheese samples (from pit 1 and pit 2) fall on the II and III quadrant of the PCA plot, sharp cheese samples are on the I and IV quadrant. Furthermore, the consumer test showed that overall liking (preference) correlated negatively with the first component, i.e., the tasters did not like cheese samples with high intensities of earth/mushroom, mouldy, bitter, pungent, colour, salty, odour, and sour, preferring milder cheeses with higher sweet, milk and umami aroma.[Instruction: Fig. 2A and B should be replaced because of low quality. Please add 'A)' and 'B)' at the top, on the left of each figure.]

Images are optimised for fast web viewing. Click on the image to view the original version.

alt-text: Fig. 2

Fig. 2



PCA of sensory data collected for cheese ripened in different pits: score plot (A) and loading plot (B).

Replacement Images: FIGURE 2A-def.tiff and FIGURE 2B-def.tiff

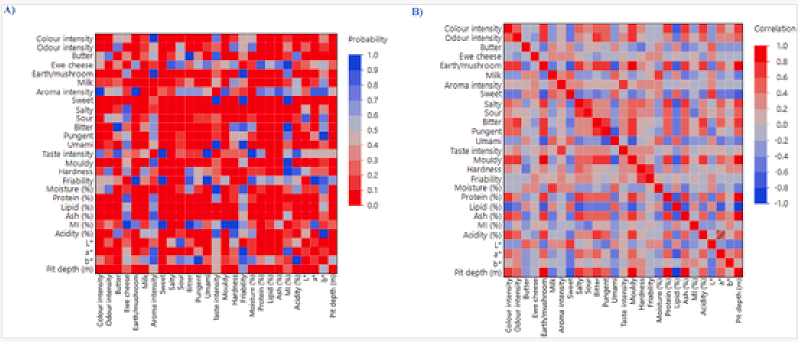
Replacement Instruction: Replace image requested

The colour map of correlations between the sensory attributes evaluated by the panel and the pit depth (Fig. 3) clearly shows that most of the attributes were correlated with the pit depth (Fig. 3A) and the sign of the correlation (Fig. 3B) followed the sign of the correlation between the attributes and the first principal axis, thus pit depth seemed to act as the first principal component. It is worth noting that the attribute salty correlated positively with the ash content of the cheese, as shown in the colour maps reported in Fig. 3A and B (correlation coefficient of 0.68, p-value of correlation <0.0001).

Images are optimised for fast web viewing. Click on the image to view the original version.

alt-text: Fig. 3

Fig. 3



[Instruction: Fig. 3A and 3B should be replaced because of low quality. Please add 'A)' and 'B)' at the top, left, of each figure.]Colour map on correlation probability (A) and coefficient (B) for sensory, consumer preference, gross chemical composition, instrumental colour coordinates, ~~gross chemical composition~~ and pit depth variables. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Replacement Images: Figure 3A-def.tiff and Figure 3B-def.tiff

Replacement Instruction: Replace image requested

3.4 VOCs analysis

The volatile profile of the cheese was studied through gas chromatographic analysis of the headspace with the main objective of finding relationships between the aromas and the seasoning pit. Table 5 reports the mean and standard deviation of peak area values for each volatile compound divided by the internal standard area.

All identified volatile compounds were detected in the cheese samples from the four different pits. The aromatic profile of Fossa cheese, as well as many other long-aged kinds of cheese, is characterized by a high concentration of some main volatile compounds, and a lot of other chemical compounds present in smaller quantities (Siano et al., 2019; Gioacchini et al., 2010), but no less important in defining the peculiar sensory characteristics, according to the perception threshold characteristic of each of them. As is well known, the concentration of a volatile compound, to contribute to the aromatic profile of a given product, must necessarily meet the minimum concentration requirements, necessary to provoke a receptor response. Having said this, although the absolute concentrations in the headspace usually do not provide exhaustive information, in addition to the fact that the analysis technique strongly influences this response, data obtained with the same procedure can optimally reveal any differences between samples.

So, we compared cheeses of the same batch aged in different pits to understand how much this aspect influenced the chemical and sensory characteristics of the samples.

From the analysis of the headspace reported in Table 6, it is evident that some compounds were influenced by the characteristics of the pit in which the seasoning was carried out. In fact, one alcohol, many acids and a few esters were more abundant in pit 3 and 4 compared to the other pits, forming two or more groups of non-statistically differing means (Tukey's method, α = 5%). In fact, according to Siano et al. (2019), the chemical family most represented in the headspace of Fossa cheese is that of acids, particularly, hexanoic acid, then butanoic, and octanoic.

alt-text: Table 6

Table 6

The table layout displayed in this section is not how it will appear in the final version. The representation below is solely purposed for providing corrections to the table. To preview the actual presentation of the table, please view the Proof.

Volatile organic compounds with relative LRI and odour description identified by SPME-GC in cheeses aged in different pits. Data re to the mean and standard deviation of the peak area ratio of each compound and the internal standard. Different letters in each row indicate statistically significant differences (Tukey's method with α = 5%).

LRI	Chemical compound	Pit 1				Pit 2				Pit 3				Pit 4				*Odour description*
940	Butanal. 3-methyl	0.4	±	0.6	a	1.1	±	0.7	a	0.3	±	0.1	a	0.4	±	0.4	a	*cocoa. malt. pungent*
1080	Hexanal	0.3	±	0.1	a	0.3	±	0.2	a	0.3	±	0.2	a	0.3	±	0.2	a	*green. beany*
	Aldehydes	***0.7***				***1.3***				***0.6***				***0.7***				
1030	2-Butanol	0.4	±	0.8	b	2.6	±	1.7	ab	5.8	±	5.3	a	0.9	±	1.4	b	*alchool. fruit*
1166	1-Butanol	0.3	±	0.1	a	0.2	±	0.1	a	0.2	±	0.1	a	0.4	±	0.2	a	*banana. fusel*
1253	1-Pentanol	0.3	±	0.1	a	0.3	±	0.1	a	0.3	±	0.2	a	0.4	±	0.1	a	*fused. fermente*
1344	2-Heptanol	2.9	±	2.3	b	4.9	±	8.6	b	11.6	±	8.6	b	64.2	±	46.8	a	*fresh. lemon*
1378	1-Hexanol	0.2	±	0.1	a	0.2	±	0.1	a	1.0	±	0.6	a	1.0	±	0.9	a	*green. fruit*
	Alcohols	***4.1***				***8.2***				***18.9***				***66.9***				
839	2-Propanone	0.1	±	0.0	a	0.1	±	0.0	a	0.1	±	0.0	a	0.1	±	0.1	a	*apple. pear*
926	2-Butanone	1.1	±	0.7	a	0.9	±	0.6	a	1.4	±	0.8	a	1.2	±	1.2	a	*chemica fruity*
1004	2-Pentanone	5.8	±	4.5	a	3.2	±	1.6	a	8.5	±	4.3	a	8.0	±	3.5	a	*sweet. floral*
1101	2-Hexanone	2.1	±	1.0	a	2.4	±	3.2	a	3.4	±	2.7	a	5.0	±	5.1	a	*fruity. fungal. musty*
1214	2-Heptanone	0.3	±	0.1	ab	0.1	±	0.1	b	0.2	±	0.0	ab	0.4	±	0.3	a	*blue cheese*
1303	2-Octanone	0.2	±	0.1	a	0.1	±	0.1	a	0.2	±	0.1	a	0.4	±	0.4	a	*dairy. waxy*
1380	2-Nonanone	0.2	±	0.3	ab	0.7	±	0.6	a	0.1	±	0.1	b	0.4	±	0.2	ab	*fruity. floral. musty*
	Ketones	***9.8***				***7.6***				***13.9***				***15.4***				
1493	Acetic acid	3.8	±	3.3	b	15.8	±	8.1	b	14.9	±	2.8	b	22.3	±	6.5	a	*pungent*

																		sour
1600	Propanoic acid. 2-methyl	1.6	±	0.9	b	1.6	±	0.8	b	4.5	±	2.6	b	23.1	±	11.4	a	*sour. cheesy*
1671	Butanoic acid	25.4	±	8.0	c	33.6	±	14.2	cb	57.7	±	18.5	a	46.3	±	9.7	ab	*sharp. cheese*
1760	Butanoic acid. 3-methyl	0.3	±	0.1	b	0.4	±	0.2	b	0.9	±	0.5	ab	1.5	±	0.9	a	*cheesy. dairy*
1787	Pentanoic acid	0.5	±	0.2	c	0.6	±	0.1	bc	1.0	±	0.3	a	0.9	±	0.1	ab	*sweet. rancid*
1889	Hexanoic acid	50.8	±	20.4	b	60.4	±	21.8	b	108.8	±	27.6	a	79.5	±	19.2	a	*sickenin sour*
1990	Heptanoic acid	0.2	±	0.2	b	0.3	±	0.1	b	0.9	±	0.4	a	0.8	±	0.3	ab	*rancid. cheese*
2106	Octanoic acid	11.2	±	4.0	b	12.5	±	3.9	b	23.2	±	6.1	a	21.3	±	6.6	a	*fatty. wa*
2212	Nonanoic acid	0.7	±	1.0	a	1.1	±	1.1	a	0.4	±	0.2	a	0.5	±	0.8	a	*waxy. di*
2316	Decanoic acid	3.6	±	1.7	b	6.1	±	1.9	ab	7.2	±	2.0	ab	10.7	±	5.7	a	*fatty*
	Acids	***98.2***				***132.4***				***219.4***				***206.9***				
970	Acetic acid. ethyl ester	0.6	±	0.9	a	2.1	±	1.7	a	0.7	±	0.2	a	0.7	±	0.5	a	*fruity*
1057	Butanoic acid. ethyl ester	0.4	±	0.2	a	0.7	±	0.8	a	1.0	±	0.4	a	1.2	±	0.8	a	*fruity. cheesy*
1243	Hexanoic acid. methyl ester	0.2	±	0.1	a	0.2	±	0.1	a	0.2	±	0.2	a	0.2	±	0.2	a	*fruity. pineapp apricot*
1256	Hexanoic acid. ethyl ester	3.6	±	1.0	b	2.6	±	2.8	b	4.4	±	3.2	b	26.1	±	6.0	a	*sweet. pineapp*
1459	Octanoic acid. ethyl ester	0.6	±	0.3	b	0.9	±	0.6	ab	2.5	±	1.4	a	2.3	±	1.3	a	*sweet. fruity*
1646	Decanoic acid. ethyl ester	3.2	±	1.5	c	7.3	±	2.5	bc	9.4	±	2.7	ab	14.8	±	5.7	a	*waxy. fruity*
	Esters	***8.6***				***13.8***				***18.3***				***45.3***				
2157	δ-Decalactone	0.85	±	0.97	a	2.69	±	1.85	a	1.34	±	0.46	a	2.01	±	1.85	a	*coconut. sweet*
	Lactones	***0.85***				***2.69***				***1.34***				***2.01***				

Short-chain fatty acids are the main group in the headspace of various cheeses, probably due to Propionibacteria (Wolf, Peralta, Candioti, & Perotti, 2016), known to ferment lactic acid to acetic and propionic acid. Furthermore, recent studies have shown that these bacteria play an important role in the formation of free fatty acids from lipolysis of the milk fat or branched-chain fatty acids from amino acids like leucine or isoleucine catabolism (Thierry, Maillard, & Yvon, 2002).

Free fatty acids, generally formed by the activity of lipase and esterase, strongly contribute to the aroma of cheeses, giving pungent, slightly acidic, and cheesy notes. In particular, cheese samples aged in pits 3 and 4 showed higher contents of butanoic, hexanoic, and octanoic acid, all characterized by the typical aromatic notes of oxidized, waxy, goat, and soapy fat, consistent with the sensory test.

The sensory profile of 3 and 4-pit cheeses agreed with the acid content values of the headspace, confirming that the samples from pits 1 and 2 elicited lower acidity and spiciness in the sensory test. Ketones have a unique flavor and aroma, conferring mushroom-like and 'sweet' fruit-like notes to cheese. Ketones are formed by the enzymatic oxidation of free fatty acids to keto-acids and consequent decarboxylation to methyl ketones (Siano et al., 2019). Although methyl ketones, in this study, were a fraction of the total area of the peaks not particularly high in the headspace of the cheese samples, the low olfactory perception threshold (Nagata, 2003, pp. 118–127) makes these components particularly influential in the sensory profile.

The presence of 2-pentanone and 2-hexanone mainly in the aroma of cheeses from pit 3 and 4 confirms the sensory data for the note of earth, mushroom, and mouldy.

Aldehydes were found at low levels in cheese samples, in particular 3-methyl butanal and hexanal, because they do not tend to accumulate but are rapidly converted to alcohols or oxidized to acids (Hassan, Abd El-Gawad, & Enab, 2012). The aldehyde, 3-methylbutanal, in particular, derives from Isoleucine amino acid by Strecker degradation or transamination, is responsible for dirty and heavy sensory notes (Rizzi, 2008), has a low odour threshold, and is a key-flavour of many important types of cheese such as Cheddar, Gouda, Camembert, Swiss-type (Smit, Smit, & Engels, 2005). They are not important compounds in pit cheese, as already found in previous investigations (Gioacchini et al., 2010; Siano et al., 2019), and a high concentration of aldehydes could cause unpleasant flavours.

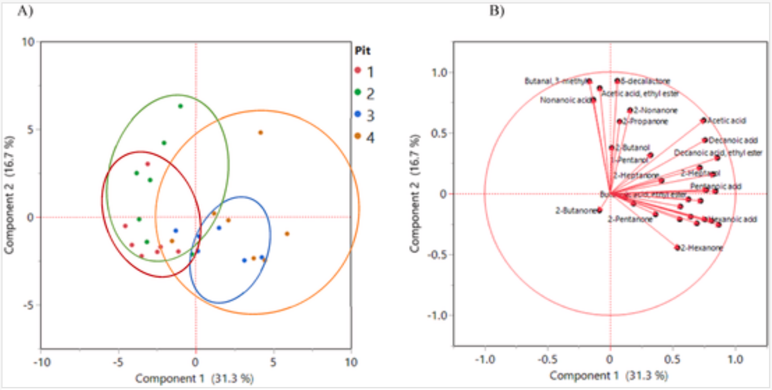
Alcohols generally derive from fermentation processes, but different metabolic pathways could contribute to their increase (Delgado, González-Crespo, Cava, García-Parra, & Ramírez, 2010). Our samples showed considerable variability even though 2-heptanol was always the most represented volatile alcohol component, reaching the highest levels in the cheeses of pit 4. 2-Heptanol could contribute to a fruity note, like that of some esters, but more lemon, herbaceous, green and fresher (Moio, Piombino, & Addeo, 2000; Moio & Addeo, 1998) emphasizing the sour perception. Secondary alcohols, such as 2-heptanol, are formed by the enzymatic reduction of the corresponding methyl ketones obtained from fatty acids by β-oxidation or from β-ketoacids (Molimard and Spinnler, 1996).

Esters are common constituents of the volatile fraction of cheese and were an abundant component of the headspace of pit cheese. They can be assigned to enzymatic activity, most frequently by lipases. We found that the more representative esters in the Fossa cheese samples were butanoic acid ethyl ester, hexanoic acid ethyl ester, octanoic acid ethyl ester and decanoic acid ethyl ester, in agreement with Siano et al. (2019), with highest content in Pit 4. The microorganisms involved in the formation of esters are mainly yeasts; however, some lactic acid bacteria, in addition to chemical reactions, may also be responsible for the formation of esters. Due to their prevalent microbial origin, ester levels increase with the ripening process (Gioacchini et al., 2010). Ethyl esters probably contribute to the overall *odour intensity* of the pit cheese, as they have low odour thresholds, without too much influence on the aromaticity, all eliciting slightly floral and fruity notes.

Fig. 4 reports the results of the PCA applied to the volatile fraction of Fossa cheese. The first component explained 31.3% of the variability in the data and allowed to separate the cheese samples aged in pit 3 and 4 (high values of the first component) from the others (low levels of the first component), as shown in the score plot (Fig. 4A). The variables that contributed more to this separation were mostly some carboxylic acids (acetic, butanoic, pentanoic, hexanoic, heptanoic, octanoic, decanoic) and esters (butanoic acid ethyl ester, hexanoic acid ethyl ester, octanoic acid ethyl ester, propanoic acid 2-methyl, decanoic acid ethyl ester, butanoic acid 3-methyl) and 2-heptanol which were more abundant in cheese aged in pit 3 and 4. By comparing the results of the PCA score plots of the sensory (Fig. 2A) and VOCs (Fig. 4A) data we see that there is some similarity in the way the points distribute with respect to the first component, thus confirming a link between the aromatic and the sensory profile.[Instruction: Figure 4A and 4B should be replaced because of low quality. Please add 'A)' and 'B)' at the top, left, of each figure.]

alt-text: Fig. 4

Fig. 4



PCA of VOC data collected for cheese ripened in different pits: score plot (A) and loading plot (B).

Replacement Images: Figure 4A-def.tiff and Figure 4B-def.tiff

Replacement Instruction: Replace image requested

4 Conclusions

Cheese aged in different pits developed different physicochemical, and sensory properties. We hypothesized that pit depth could have a profound impact on the ripening of pit cheese, affecting the pressure that generates the pressing of the cheese and the consequent loss of whey and fat. Both phenomena, influencing the physicochemical properties of cheese, were probably responsible for the observed differences in sensory attributes and VOCs. The cheese samples, despite having the same characteristics at the beginning of the pit maturation, gradually changed their gross composition according to the features of each pit likely influencing the microflora development. Furthermore, the expulsion of the whey inside the pit can change the environmental humidity and the surface water content of the cheese, thus influencing the surface growth of microorganisms (moulds) and the consequent sensory and VOC profiles. The above observations suggest that the environment developed inside each pit was unique and strongly influenced by the loaded cheese.

This is the first study that developed this hypothesis, using a large sample of cheese, and a randomized design, compared to previous predominantly observational studies. Further studies could help to understand the complex phenomena that occurs during Fossa cheese ripening, and we believe that importance should be given also to the pressing process and to its effects on cheese and pit environment.

CRediT authorship contribution statement

Diana De Santis: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **Marcello Fidaleo:** Investigation, Formal analysis, Software, Writing – original draft, Writing – review & editing.

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.

Acknowledgements

The authors wish to thank the Consortium ~~to produce~~ for the production of Pit Cheese of Sogliano al Rubicone (Sogliano al Rubicone, FC, Italy) which made it possible to collect several samples of pit cheese produced according to the production disciplinary and Dr. Cinzia Equitani for her technical support.

Appendix A Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2021.112507.

Q4 Uncited references

~~Curioni and Bosset, 2002~~.

References

The corrections made in this section will be reviewed and approved by a journal production editor. The newly added/removed references and its citations will be reordered and rearranged by the production team.

Álvarez, S., & Fresno, M. (2021). Effect of the ripening period and intravarietal comparison on chemical, textural and sensorial characteristics of palmero (PDO) goat cheese. Animals, 11, 58. doi:10.3390/ani11010058. 2021.

AOAC (2005). Official method of analysis (18th ed.). Washington DC: Association of Officiating Analytical Chemists.

Avellini, P., Clementi, F., Trabalza, M.M., Cenci, G.B., Rea, S., & Branciari, R., et al. (1999). "Pit" cheese: Compositional, microbiological and sensory characteristics. Ital. J. Food Sci. n., 4(4), 317–333.

Barbieri, E., Schiavano, G.F., De Santi, M., Vallorani, L., Casadei, L., & Guescini, M., et al. (2012). Bacterial diversity of traditional Fossa (pit) cheese and its ripening environment. International Dairy Journal, 23(1), 62–67. doi:10.1016/j.idairyj.2011.09.009.

Biagiotti, C., Ciani, M., Canonico, L., & Comitini, F. (2018). Occurrence and involvement of yeast biota in ripening of Italian Fossa cheese. European Food Research and Technology, 244(11), 1921–1931. doi:10.1007/s00217-018-3104-6.

~~Curioni, P.M.G., & Bosset, J.O. (2002). Key odorants in various cheese types as determined by gas chromatography-olfactometry. International Dairy Journal, 12, 959–984.~~

De Santis, D., Equitani, C., & Forniti, R. (2009). Influence of some technical parameters on the chemical, physical and sensory characteristics of Pit cheese. In Porretta, S. (Ed.), Ricerche ed innovazione nell'Industria alimentare: Vol. IX (pp. 451–455). Pinerolo, Italy): Chiriotti Editori.

De Santi, M., Sisti, M., Barbieri, E., Piccoli, G., Brandi, G., & Stocchi, V. (2010). A combined morphologic and molecular approach for characterizing fungal microflora from a traditional Italian cheese (Fossa cheese). International Dairy Journal, 20(7), 465–471. doi:10.1016/j.idairyj.2010.02.004.

Delgado, F.J., González-Crespo, J., Cava, R., García-Parra, J., & Ramírez, R. (2010). Characterisation by SPME-GC-MS of the volatile profile of a Spanish soft cheese P.D.O. Torta del Casar during ripening. Food Chemistry, 118(1), 182–189. doi:10.1016/j.foodchem.2009.04.081.

Di Cagno, R., Banks, J., Sheehan, L., Fox, P.F., Brechany, E.Y., & Corsetti, A., et al. (2003). Comparison of the microbiological, compositional, biochemical, volatile profile and sensory characteristics of three Italian PDO ewes' milk cheeses. International Dairy Journal, 13(12), 961–972. doi:10.1016/S0958-6946(03)00145-6.

EU. (2009). Commission Regulation (EC) No 1183/2009 of 30 November 2009 entering a name in the register of protected designations of origin and protected geographical indications [Formaggio di Fossa di Sogliano (PDO)]. Off. J. Eur. Comm. L, 317, 34–35.

Gioacchini, A.M., De Santi, M., Guescini, M., Brandi, G., & Stocchi, V. (2010). Characterization of the volatile organic compounds of Italian "Fossa" cheese by solid-phase microextraction gas chromatography/mass spectrometry. Rapid Communications in Mass Spectrometry, 24(23), 3405–3412. doi:10.1002/rcm.4782.

Gobbetti, M., Folkertsma, B., Fox, P.F., Corsetti, A., Smacchi, E., & De Angelis, M., et al. (1999). Microbiology and biochemistry of Fossa (pit) cheese. International Dairy Journal, 9.

Hassan, F.A.M., Abd El-Gawad, M.A.M., & Enab, A.K. (2012). Flavour compounds in cheese (review). International Journal of Academic Research in Psychology A, 4(5), 169–181. doi:10.7813/2075-4124.2012/4-5/A.20.

ISO. (1994). Sensory analysis – identification and selection of descriptors for establishing a sensory profile by a multidimensional approach ISO 11035. Geneva: International Organization for Standardization.

ISO. (2004). Cheese and processed cheese products – determination of fat content – gravimetric method (Reference method) ISO 1735. Geneva: International Organization for Standardization.

ISO. (2005). Sensory analysis – methodology – general guidance ISO 6658. Geneva: International Organization for Standardization.

ISO. (2006). Sensory analysis–Methodology–Initiation and training of assessors in the detection and recognition of odors ISO 5496. Geneva: International Organization for Standardization.

ISO. (2012). Sensory analysis - general guidance for the selection, training and monitoring of assessors - Part 2: Expert sensory assessors ISO 8586-2. Geneva: International Organization for Standardization.

ISO. (2014a). Sensory analysis - general guidance for the selection, training and monitoring of selected assessors and expert sensory assessors - Part 1: Selected assessors ISO 8586. Geneva: International Organization for Standardization.

ISO. (2014b). Sensory analysis - general guidance for the design of test rooms ISO 8589. Geneva: International Organization for Standardization.

ISO. (2016). Sensory analysis - methodology - general guidance for establishing a sensory profile ISO 13299. Geneva: International Organization for Standardization.

ISO. (2019). Colorimetry — part. 4: CIE 1976 L*A*B* colour space ISO 11664-4:2019(E). Geneva: International Organization for Standardization.

Januszkiewicz, J., Sabik, H., Azarnia, S., & Lee, B. (2008). Optimization of headspace solid phase microextraction for the analysis of specific flavors in enzyme modified and natural Cheddar cheese using factorial design and response surface methodology. Journal of Chromatography A, 1195(1–2), 16–24. doi:10.1016/j.chroma.2008.04.067.

Kähkönen, P., Tuorila, H., & Lawless, H. (1997). Lack of effect of taste and nutrition claims on sensory and hedonic responses to a fat-free yogurt. Food Quality and Preference, 8(2), 125–130. doi:10.1016/S0950-3293(96)00032-8.

Kähkönen, P., Tuorila, H., & Rita, H. (1996). How information enhances acceptability of a low-fat spread. Food Quality and Preference, 7(2), 87–94. doi:10.1016/0950-3293(95)00040-2.

Kuchroo, C.N., & Fox, P. (1982). Soluble nitrogen in cheddar cheese: Comparison of extraction procedures. Milchwissenshaft, 37, 331–335.

Leclercq-Perlat, M.N., Sicard, M., Perrot, N., Trelea, I.C., Picque, D., & Corrieu, G. (2015). Temperature and relative humidity influence the ripening descriptors of Camembert-type cheeses throughout ripening. Journal of Dairy Science, 98(2), 1325–1335. doi:10.3168/jds.2014-8916.

Mascaro, N., Stocchi, R., Ricciutelli, M., Cammertoni, N., Renzi, F., & Cecchini, S., et al. (2010). Biogenic amine content and chemical and physical features of Italian formaggio di Fossa. A.I.V.I., 8(giugno), 49–53.

Moimard, P., & Spinnler, H.E. (1996). Review: Compounds involved in the flavor of surface mold-ripened cheeses: Origins and properties. Journal of Dairy Science, 79, 169–184.

Moio, L., & Addeo, F. (1998). Grana Padano cheese aroma. Journal of Dairy Research, 65, 317–333. 1998.

Moio, L., Piombino, P., & Addeo, F. (2000). Odour-impact compounds of Gorgonzola cheese. Journal of Dairy Research, 67, 273–285.

Nagata, Y.Y.E. (2003). Measurement of odor threshold by triangular odor bag method. Odor measurement review. Japan Ministry of the Environment.

Papademas, P., & Bintsis, T. (2018). Global cheesemaking Technology: Cheese quality and characteristics (1th ed.). Hoboken: John Wiley & Sons.

Pillonel, L., Baderstscher, R., Butikofer, U., Casey, M., Dalla Torre, M., & Lavanchy, P., et al. (2002). Analytical methods for the determination of the geographic origin of Emmental cheese. European Food Research and Technology, 215, 260–267. 2002.

Pinho, O., Mendes, E., Alves, M., & Ferreira, I. (2004). Chemical, physical, and sensorial characteristics of "terrincho" Ewe cheese: Changes during ripening and intravarietal comparison. Journal of Dairy Science, 87, 249–257.

Rizzi, G.P. (2008). The strecker degradation of amino acids: Newer avenues for flavor formation. Food Reviews International, 24(4), 416–435. doi:10.1080/87559120802306058.

Rohm, H., & Jaros, D. (1996). Colour of hard cheese. 1. Description of colour properties and effects of maturation. Zeitschrift für Lebensmittel-Untersuchung und -Forschung A, 203, 241–244.

Siano, F., Fasulo, G., Giaramita, L., Sorrentino, A., Boscaino, F., & Sprovieri, M., et al. (2019). Chemical-nutritional composition, microbiological analysis and volatile compound content of Fossa cheese ripened in different pits. Italian Journal of Food Science, 31, 2019–2670.

Smit, G., Smit, B.A., & Engels, W.J.M. (2005). Flavour formation by lactic acid bacteria and biochemical flavour profiling of cheese products. FEMS Microbiology Reviews, 29(3), 591–610. doi:10.1016/j.fmrre.2005.04.002.

Thierry, A., Maillard, M.B., & Yvon, M. (2002). Conversion of L-leucine to isovaleric acid by Propionibacterium freudenreichii TL 34 and ITGP23. Applied and Environmental Microbiology, 68(2), 608–615. doi:10.1128/AEM.68.2.608-615.2002.

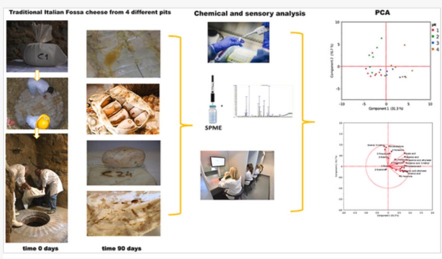
Wolf, I.V., Peralta, G.H., Candioti, M., & Perotti, M.C. (2016). The role of propionibacteria in the volatile profile of Pategrás cheeses. Dairy Science & Technology, 96(4), 551–567. doi:10.1007/s13594-016-0289-y.

Zanoni, M., Pigoni, S., & Hunter, E.A. (2006). Validazione di una scheda per l'analisi sensoriale del Parmigiano-Reggiano. Scienza e Tecnica Lattiero Casearia, 57(6), 545.

Graphical abstract

Images are optimised for fast web viewing. Click on the image to view the original version.

Q7 alt-text: Image 1



Replacement Image: graphical abstract - def.tif

Replacement Instruction: Replace image requested

Highlights

- Cheeses from the same production batch, stored in four different pits for 90 days, were analysed.

- The pit affected cheese overall liking and physicochemical, sensory, and aromatic properties.
- Pit depth caused different whey and fat loss affecting the cheese gross chemical composition.
- Different cheese composition and amount of liquid loss may affect cheese sensorial properties.

Appendix A Supplementary data

The following is the Supplementary data to this article:

Multimedia Component 1

Multimedia component 1

alt-text: Multimedia component 1

Queries and Answers

Q1

Query: Correctly acknowledging the primary **funders and grant IDs** of your research is important to ensure compliance with funder policies. Please make sure that funders are mentioned accordingly.

Answer: Reviewed

Q2

Query: Please confirm that the provided **email** "desdiana@unitus.it" is the correct address for official communication, else provide an alternate e-mail address to replace the existing one, because private e-mail addresses should not be used in articles as the address for communication.

Answer: Reviewed

Q3

Query: Note: The author's **telephone/fax number** has been removed as these are not published in journal articles.

Answer: Done

Q4

Query: The **Uncited References** section comprises references that occur in the reference list but are not available in the body of the article text. Please cite each reference in the text or, alternatively, delete it. Any reference not dealt with will be retained in this section.

Answer: Done

Q5

Query: Please confirm that **given names and surnames** have been identified correctly and are presented in the desired order and please carefully verify the spelling of all authors' names.

Answer: Reviewed

Q6

Query: Your article is registered as a regular item and is being processed for inclusion in a regular issue of the journal. If this is NOT correct and your article belongs to a Special Issue/Collection please contact H.Subramaniam@elsevier.com immediately prior to returning your corrections.

Answer: Yes

Q7

Query: For figure(s) ga1, the resolution is too low to be used. Please provide better quality figure of 300 dpi.

Answer: we added a replacement