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RESEARCH

Comparative Analysis of Chemical, Microbiological, Sensory and Volatile Compound Profiles in Manouri PDO and Artisanal Manouri Cheeses: A Preliminary Study

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Abstract
Manouri, a protected designation of origin (PDO) cheese, is one of the most popular whey cheeses produced in Greece. The objective of the current study was to investigate if there are differences between artisanal and industrial Manouri cheeses regarding microbiological quality, volatile organic compounds (VOCs) profile and other quality parameters (colour, texture), sensory attributes and spectral characteristics detected by Fourier transform infrared spectroscopy (FT-IR) that may discriminate the samples. Differences were detected in the population of the dominant microbial groups, especially for lactic acid bacteria (LAB), Enterobacteriaceae and yeast counts. No discrimination was attained from the physicochemical analyses, except for the pH values. A total of 50 VOCs were identified, including ketones, lactones, free fatty acids, aldehydes, esters, alcohols and hydrocarbons. Sensory evaluation was carried out using a quantitative descriptive analysis (QDA) panel and a consumer panel. Consumers showed a preference for the artisanal Manouri, and the QDA panel revealed significant differences in 11 out of the 17 sensory attributes. Colour and texture analyses were also performed and showed specific differences in yellowness, as well as in fracturability and hardness. FT-IR spectral analysis demonstrated potential discrimination related to the phospholipid content and profile of artisanal and industrial Manouri.

Keywords Whey cheese · Artisanal Manouri · Industrial Manouri · Volatile organic compounds · Sensory evaluation · FT-IR spectroscopy

Introduction

Whey cheeses are heat- and/or acid-coagulated cheeses produced by heating the whey at high temperatures to form a coagulum, following the denaturation of whey proteins (Papademas et al., 2018). Whey cheeses are globally produced by traditional methods either as artisanal or standardized industrial processes as a sustainable method of utilizing the whey according to the principles of circular economy (Pintado et al., 2001; Bintsis &Papademas, 2023). Whey cheeses are traditionally produced in the Mediterranean countries (Alichanidis & Polychroniadou, 2008). The Italian Ricotta is the most popular and widely studied whey cheese (Bintsis & Papademas, 2023; Mangione et al., 2023a; Pintado et al., 2001) and Manouri, Myzithra and Anthotyros, the most popular Greek whey cheeses (Bintsis & Papademas, 2023). Manouri is a Greek whey cheese, with a high fat content, possessing a minimum fat-in-dry matter (FDM) of 70% and a creamy body, delicate texture and superior sensory quality; Manouri has been registered as a protected designation of origin (PDO) cheese since 1996 (eAmbrosia, 2023).

Artisanal cheese production refers to small-scale traditional manufacture in farms or small dairies, and it is strongly linked to the local culture and natural resources of each production region (Mangione et al., 2023b). Artisanal

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Manouri is exclusively manufactured in the northern part of Greece, mainly in Western Macedonia. Cheese-making is seasonal (during June to August) using caprine milk following a special procedure in which Manouri cheese is made from the whey obtained from the manufacture of Batzos, a white-brined cheese. The milk is being 'beaten' with a wooden ladle to break the fat and bring it on the surface. In the same vat, Batzos is curdled with a small amount of rennet and removed. The remaining whey, enriched with most of the fat, is heated up to 85 °C, with continuous stirring and the floating curd which are moulded in cheesecloths and drained. Thus, Batzos is a low-fat cheese, and the fat-rich whey is used to make high-quality Manouri. With this specific manipulation, Manouri is the main product of the cheese-making process, and Batzos is the by-product (Bintsis et al., 2018; Papademas et al., 2018). Nowadays, there are only two dairies that produce artisanal Manouri following the traditional procedure, both located in Western Macedonia. Interestingly, the traditional manufacture of a special Manouri Vlastis has inscribed in the National Inventory of Intangible Cultural Heritage of Greece (Greek Ministry of Culture, 2023).

Manouri PDO is produced using the whey from ovine and caprine cheeses (e.g. Feta, Graviera, Kefalotyri), which is enriched with the cream of ovine or caprine milk to secure a fat content of at least 2.5% in the final mixture (eAmbrosia, 2023). The mixture is heated to 88–90 °C, over 40–45 min under constant stirring. At the temperature of 70–75 °C, sodium chloride is added (*ca.* 1%), together with ovine or caprine milk or cream. When the temperature reaches 88–90 °C, the curd is left for 15–30 min and is then transferred to cloth sacks where it is drained for a period of 4 to 5 h. The cheese is air-stored or packed in vacuum and kept at a temperature of 4–5 °C. Similarly to other whey cheeses, Manouri is consumed fresh, that is, without ripening; only a few whey cheeses, e.g. Ricotta forte and Xynomyzithra Kritis, undergo the ripening process (Baruzzi et al., 2000; Bintsis & Papademas, 2023; Kaminarides et al., 2013); however, fermentation may occur to some extent, as shown by the final pH of many whey cheeses. Interestingly, traditional dairy products, namely, cheeses, may serve as excellent carriers for delivering functional whey proteins (Henriques et al., 2013) and new functional ingredients, e.g. probiotics (Rudke et al., 2023).

Flavour and aroma are among the key sensorial characteristics associated with the overall cheese quality and consumer acceptability (Chen et al., 2022; Wang et al., 2023; Zheng et al., 2021). Considering that small variations in cheese manufacturing can impact significantly the four main flavour formation pathways (glycolysis, citrate utilization, proteolysis and lipolysis), it becomes evident why the numerous volatile organic compounds (VOCs) present in cheese are expected to vary significantly among whey cheeses. The analysis of these VOCs is usually carried out by gas chromatography (GC) after their extraction with or without prior concentration of the volatile fraction (Sádecká et al., 2014). Among the main extraction methods, the headspace capture method and solid-phase microextraction (SPME) are the most popular for the VOC analysis of whey cheeses (Curioni & Bosset, 2002; Dimitrellou et al., 2009, 2017; Faccia et al., 2018; Kaminarides et al., 2013; Pappa et al., 2020; Sádecká et al., 2014; Wang et al., 2023). SPME is a fast and effective flavour sampling technique that requires a small amount of sample and enables separation and pre-concentration of volatiles with relatively high reproducibility when optimally used (Sádecká et al., 2014). So far, there have been efforts to record and identify the key volatiles of several whey cheeses like Myzithra and Manouri (Kaminarides et al., 2013), May Bryndza cheese (Sádecká et al., 2014), Ricotta forte (Faccia et al., 2018) and Urda cheese (Pappa et al., 2020). To our knowledge, there are still no published studies on how industrial and artisanal processes influence the formation and concentration of the key aroma compounds in whey cheese products.

Comparison of artisanal and industrial cheese products has been reported for Maroilles (Nacef et al., 2019), for Italian Burrata (Rea et al., 2016), for Ricotta (Bergamaschi et al., 2016; Giacometti et al., 2015), Pecorino Calabrese (Campolo et al., 2013), Manchego (Ballesteros et al., 2004, 2006; Gomez-Ruiz et al., 2002) and Piacentinu Ennese (Horne et al., 2005) using different sensorial, physicochemical and microbiological approaches. In the case of artisanal Manouri, its microbiological profile has been studied by Lioliou et al. (2001). However, a comparison of physicochemical and other quality parameters of artisanal and industrial made Manouri cheeses is still not available.

In addition to this gap, mid-infrared spectroscopic characterization that offers insight into molecular structure and/or interactions is also missing. In the last decade, non-destructive spectroscopic techniques (mid- and near-infrared, Raman, fluorescence) combined with multivariate data analysis methods have emerged as an alternative option to the classical chemical analyses of food products (Aït-Kaddour et al., 2021). Their basic advantage is that they can help to monitor the authenticity, adulteration and biochemical characteristics of the products and changes in production processes in a holistic view. In this context, FT-IR spectroscopy is often proposed as a rapid and relatively low-cost technique that can be implemented in the modern cheese industry (Woodock et al., 2008). So far, its application to Manouri or other types of whey cheeses has not received attention; only a recent publication exists about its potential to discriminate Anari cheese according to the milk species origin (bovine or caprine/ovine) encouraging further research on this topic (Tarapoulouzi & Theocharis, 2023).

The objective of the current study was to investigate whether the physicochemical and microbiological quality, VOC profile and other quality parameters (colour, texture) and sensory attributes of artisanal and industrial Manouri cheeses may aid in the discrimination according to the production process. FT-IR spectroscopy in diffuse reflectance mode was also employed, and the spectral data were chemometrically interpreted to complement the chemical analysis results and get valuable insight into the effects of different manufacturing processes of this traditional Greek cheese product.

Materials and Methods

Manouri Cheese Samples

Four samples of whey cheese products, all manufactured in Northern Greece, were examined in June 2023. Two of them represented traditional processes from dairy A (S1), located in Milochori, municipality of Eordea, and dairy B (S2), located in Mikro Sirini, municipality of Grevena. The other two represented industrial Manouri PDO samples that were manufactured in dairy B (S3) (located in Mikro Sirini) and dairy C (S4), located in Alexandria, municipality of Alexandria. For a given cheese-making process, all samples were chosen from the same batch (Nacef et al., 2019).

Microbiological Analyses

Cheese samples were transferred to the laboratory in insulated boxes for the analyses. Ten grammes of cheese was aseptically diluted in 90 ml of sterilized quarter-strength Ringer's solution in a stomacher bag and homogenized in a stomacher for 1 min at room temperature. Then, decimal dilutions were prepared in 9 ml of sterilized Ringer's solution, and the following analyses were carried out: total mesophilic count in plate count agar, incubated at 30 °C for 72 h aerobically (ISO, 2022); Enterobacteriaceae in Violet Red Bile Glucose Agar, incubated at 37 °C for 24 h (ISO, 2017); lactic acid bacteria (LAB) in Man, Rogosa & Sharpe (MRS) Agar (presumptive lactobacilli), incubated at 30 °C for 24–48 h (ISO, 1998); LAB in M17 Agar (presumptive lactococci and pediococci), incubated at 30 °C for 24–48 h; and yeasts and moulds in Dichloran-Rose Bengal Chloramphenicol (DRBC) Agar, incubated at 25 °C for 5 days (ISO, 2008). All microbiological media were purchased from Biolife Italiana S.r.I., Milan, Italy. Microbiological analyses were carried out in triplicates.

Physicochemical Analyses

The total fat, protein, lactose and total solid (TS) content of the test cheese samples was assessed using the Milko-ScanMinor™ analyser (Foss Electric, Hillerød, Denmark). Prior to analysis, 5 g of cheese was homogenized for 1 min with 25 g 1% (v/v) of MSc-Flush concentrate solution (Foss Electric, Hillerød, Denmark), using the T 25 digital ULTRA-TURRAX® (IKA-Werke GmbH & Co. KG, Germany) for 1 min and thermostated in a water bath at 40 °C. Milk pH was measured directly with a pH meter (XS Instruments, Italy). Chemical analyses were carried out in quadruplicate.

The colour of cheese samples was evaluated with a portable Hunter Lab-colour spectrophotometer (MiniScanTM XE Plus, Reston,Virginia, USA) adjusted to the CIELAB standard illuminant D65 (daylight source) and the CIELAB 1964 Standard Observer (100 visual field), according to Nacef et al. (2019). Prior to measurement, the spectrophotometer was calibrated using a white and a black standard plate. The rectangular coordinates employed by the CIELAB colour system, i.e. L^*, a^* and b^*, were determined. L^* denotes lightness from black (0) to white (100), a^* denotes chromaticity green (−)/red (+), and b^* denotes chromaticity blue (−)/yellow. Furthermore, h° (hue angle) and C^* (chroma or colour saturation) were calculated as follows: $h^\circ = 180^\circ + tan(b*/a*)^{-1}$ and $C^* = \sqrt{a*^2 + b*^2}$. The measurements were carried out at randomly selected locations on four cheese pieces for each sample (n = 10), and the results were averaged for statistical analysis.

Texture Evaluation

Instrumental texture profile analysis (TPA) of cheese samples was performed, as described by Pluta-Kubica et al. (2022) with minor modifications, using a Texture Analyser (TA.XT2i, Stable Micro-systems, Godalming, UK) equipped with a cylindrical compression plate 10 cm in diameter and 1 cm in height (P/100). Each cheese sample was cut into cubes with a side length of 2 cm, which were compressed at 50% deformation of their original height in two consecutive compression cycles. The analysis was carried out at a constant crosshead speed of 1 mm/s, and the starting distance from the sample was 5 mm. The obtained diagrams of the force dependence on time were analysed to determine the fracturability (N), hardness (N), adhesiveness (N×s), cohesiveness, springiness, gumminess (N), chewiness (N) and resilience. Independent measurements on five cheese pieces were performed for each sample ($n = 5$).

Analysis of Volatile Compounds

The extraction and analysis of VOCs from cheese samples were performed according to the procedure reported by

Ianni et al. (2020), with slight modifications. Briefly, 5 g of grated cheese was mixed with 10 mL of saturated NaCl solution (360 g/L), and the mixture was homogenized with an ULTRA-TURRAX T-25 high-speed homogenizer (IKA, Staufen, Germany). Then, 5 µL of internal standard solution (4-methyl-2-pentanol; 10 mg/kg in ethanol) was added, and the vials were sealed with a polytetrafluoroethylene-silicone septum (Supelco, Bellefonte, PA) and stirred at 60 °C upon agitation (250 rpm) in a PAL Shimadzu autosampler unit (AOC 6000, CTC Analytics, Switzerland). The VOCs were extracted from the headspace with a divinylbenzene-carboxen-polydimethylsiloxane SPME fibre (length, 2 cm; film thickness, 50/30 µm; Supelco, Germany) with an exposition time of 50 min. The extracted VOCs were analysed using a Shimadzu GCMS-QP2020 instrument, equipped with a MEGA-5 MS capillary column (30 m × 0.25 mm, 0.25 µm) (MEGA, Legnano, Italy). The VOCs were thermally desorbed into the GC injector in a splitless mode for 1 min at 250 °C. The oven temperature was set to 40 °C held for 1 min, increased at a rate of 5 °C per min up to 80 °C and held for 3 min and then increased from 80 °C to 230 °C at 8 °C per min and held for 5 min. Helium was used as a carrier gas at a flow rate of 1 mL/min. The mass spectrometer operated in electron impact ionization mode at 70 eV and data were collected in full-scan mode, with a scan time of 0.2 s over a mass range from 35 to 350. Source and interface temperature were held at 250 °C. Analyses of blanks were conducted to ensure no carryover, while pooled QC (quality control) samples ($n = 5$) were injected to monitor the instrumentation drift and analyte reproducibility. All compounds in QC presented RSD < 25% indicating satisfactory stability and reproducibility of the system during the analytical batch. The samples were analysed in triplicate and randomized order. Compounds were identified by comparing their retention times and mass spectra (over 80% match) with a commercial database NIST17 and FFNSC 3 Shimadzu.

Integration of the peak areas was conducted by LabSolutions GCMS solution (v.4.44) software. The chromatographic peak areas were used to determine relative peak area, RPA (%) = 100 × (peak area of individual VOC/total peak area of identified VOCs).

Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS)

The FT-IR spectra of the cheese samples that were collected from the different dairies were acquired using a benchtop FT-IR Shimadzu IRAffinity-1S spectrometer (Kyoto, Japan) equipped with a Michelson interferometer, a germanium-coated KBr beam splitter and a deuterated, L-alanine doped triglycine sulphate (DLaTGS) detector with a diffuse reflectance accessory (Pike Technologies, Fitchburg, USA). The cheese samples were first freeze-dried, and then approximately 50 mg per portion was ground and mixed with KBr (5% w/w). Each sample was measured in triplicate against a dried KBr background spectrum at 25 °C using micro cups (4.7 mm diameter by 1.6 mm deep). A total of 64 scans were co-added for each spectrum over the wavelength range 4000 – 400 cm^{-1}, with 4 cm^{-1} resolution. All DR spectra were obtained in triplicate using the IRsolution software (version 1.50, Shimadzu). The system performance was optimized prior to each batch of measurements via routine procedures, i.e. spectrometer aligning and fine-tuning of the DRIFTS sampling accessory.

DRIFTS Data Pre-processing and Chemometric Analysis

The raw diffuse reflectance spectral data were first transformed in Kubelka–Munk units using the IRSolution software functions to compensate for differences from their transmission equivalent. The spectra were exported and merged with the aid of the SIMCA© 16.1 software (Umetrics, Umeå, Sweden). Further pre-processing involved smoothing by seven points with the Savitzky-Golay method, normalization according to the Standard Normal Variate method and/or second-order derivatization with a Savitzky-Golay filter of 11 points. The offset among 4000 – 3700 and 650 – 400 cm^{-1} along with the low signal-to-noise region 2600–1800 cm^{-1} was excluded; the rest were mean-centred and analysed via the principal component analysis (PCA) algorithm encoded in the SIMCA© 16.1 software (Umetrics, Umeå, Sweden). The results were evaluated by assessing the covariance of the data in a new multidimensional space formed after a few significant PCs that explained > 95% of the total variance of raw spectral data. The PCA score plots were inspected for similarity/difference patterns in the spectral data. Variable loading plots were carefully explored to identify significant spectral features (absolute p loading > 0.05) that are of biochemical relevance.

Sensory Evaluation

The sensory study protocol was reviewed and approved by the Ethics and Deontology Committee of the Aristotle University of Thessaloniki (AUTH) (Doc No. 288356/2022). All participants consented to participate after reading an informed consent.

A consumer test was carried out with 50 students from the School of Veterinary Medicine, AUTH, who were previously trained on principles of cheese evaluation and familiarized with basic sensory evaluation techniques. A descriptive sensory analysis panel from six trained academic staff from the AUTH, who were familiar with quantitative descriptive analysis (QDA) and received regular training on sensory characteristics of cheese and whey cheese specifications, was used to evaluate the commercial Manouri cheese samples. The

Table 1 Microbial populations in industrial and artisanal Manouri cheese samples

Microbial group	Cheese samples[d]			
	S1	S2	S3	S4
	Mean log cfu/g ($\bar{x} \pm SD$)[e]			
Total mesophilic count	7.27 ± 0.17^{a}	7.02 ± 0.05^{b}	7.78 ± 0.09^{c}	6.43 ± 0.02^{d}
Enterobacteriaceae	3.30 ± 0.20^{a}	2.93 ± 0.22^{a}	3.77 ± 0.09^{b}	3.53 ± 0.08^{b}
Lactic acid bacteria (MRS)	6.18 ± 0.59^{a}	5.57 ± 0.09^{b}	7.87 ± 0.06^{c}	7.63 ± 0.10^{c}
Lactic acid bacteria (M17)	7.24 ± 0.14^{a}	6.90 ± 0.04^{b}	7.84 ± 0.03^{c}	6.40 ± 0.04^{d}
Yeasts and moulds	4.25 ± 0.05^{a}	4.19 ± 0.10^{a}	$<2^{b}$	$<2^{b}$

[d]*S1* artisanal Manouri from dairy A, *S2* artisanal Manouri from dairy B, *S3* industrial Manouri from dairy B, *S4* industrial Manouri from dairy C

[e]Means in the same row with different superscripts were significantly different at a level of significance of 5% of overall comparisons

sensory evaluations were carried out under controlled laboratory conditions. The samples were cut in approximately 3 g pieces, randomly coded with a three-digit number and served to the assessors in plastic plates at room temperature. The samples were presented in a randomized way to minimize any carryover effects (Muir & Hunter, 1992). Mineral water and crackers were given to clean the palate between the samples.

The QDA panelists evaluated the samples using two attributes for appearance ('yellowness' and 'appearance of surface'), four for texture ('hardness', springiness', 'crumbliness' and 'sense of moisture'), four for odour ('pleasant', 'buttery', 'cooked milk' and 'ammonia'), six for taste ('salty', 'piquant', 'bitterness', 'acid taste', 'rancid taste' and 'overall taste intensity') and one for 'overall acceptance'; the definitions are presented in Table SI1. The assessors were asked to use a 10-point unstructured intensity scale with a line of 10 cm ranged from 0 (none) to 10 (very strong). The consumer panel assessed the 'pleasant odour', 'pleasant taste' and 'overall acceptance' on a 9-point hedonic scale (Drake, 2007).

Statistical Analysis

Microbiological counts were converted to $\log_{10}$ cfu/g and together with chemical, pH, colour, textural parameter values, and volatile compounds detected from SPME were subjected to a one-way analysis of variance by using the SPSS software for Windows (IBM, 2022). Further testing was carried out by a multiple range test procedure using the Tukey's honestly significant difference (HSD) test. Sensory variables were tested for a normal distribution using the Shapiro–Wilk test, and for those that were non-normally distributed, non-parametric tests were used. The Friedman test was used for all group comparisons and Wilcoxon signed rank test for in pair comparisons (Lawless & Heymann, 2010).

Results and Discussion

Manouri cheese is manufactured after a high-temperature heat treatment which has a severe impact on the microbiological quality of the final cheese. The microflora composition of both artisanal and industrial Manouri samples was investigated and showed high numbers for total mesophilic counts and LAB. The results from the microbiological analyses are shown in Table 1.

The total mesophilic counts were variable with significant ($p < 0.05$) differences detected; the industrial sample S3 had the highest counts followed by artisanal S1 and S2, whereas the industrial sample S4 had the lowest value. The artisanal samples (S1 and S2) were found to have lower counts for all microbial groups except for the yeast counts. The slightly lower Enterobacteriaceae counts could be attributed to more efficient implementation of hygienic practices at the artisanal dairies; however, since S2 and S3 were produced in the same dairy plant, the lower Enterobacteriaceae may indicate that the specific cheese-making practice makes the artisanal Manouri less favourable for the contamination and subsequent growth of this microbial family. The high counts of yeasts in both artisanal samples show that there are some possible points of post-process contamination. On the other hand, yeasts were non-enumerable in industrial samples (< 2 log cfu/g, the enumeration limit of the method); it can be assumed that the industrial conditions of packaging and storage are more effective in preventive growth of yeasts.

Although the sampling was based on single batches, the results showed that there were differences in the counts of presumptive lactobacilli and presumptive dairy cocci between artisanal and industrial cheeses. Artisanal samples had lower numbers of presumptive lactobacilli, whereas small differences were found in LAB counted in M17 medium; industrial sample S3 had the highest LAB cocci counts, followed by S1, S2 and S4. Lactococci and pediococci are heat-resistant and may survive the

severe heat treatment of Manouri and related cheeses. On the other hand, the presence of heat-sensitive microbial groups such as Enterobacteriaceae and yeasts implies post-process contamination.

The high pH and low total solid content, especially of the industrially processed samples, imply that Manouri production offers a favourable environment for the growth of different microbial groups, especially LAB. To the best of our knowledge, the use of starter cultures in Manouri has not been investigated; a starter culture could improve the functional properties (use of probiotic starter cultures), as well as microbiological safety of Manouri and similar whey cheeses, and this aspect requires further studies. Different starter cultures have been suggested for dried Myzithra (Dimitrellou et al., 2009, 2017) and Ricotta (Sameer et al., 2020). Fernández et al. (2014) suggested the combined use of nisin and Microgard™ to reduce the growth of *Listeria innocua* as a post-process contaminant microorganism and achieving a shelf life extension from 5 to 13 days for Ricotta cheese. Another strategy to increase the shelf life of Anthotyros cheese has been suggested by Tsiraki and Savvaidis (2013); the authors demonstrated that the combined use of modified atmosphere packaging and vacuum with added basil essential oil extended the shelf life of fresh Anthotyros by approximately 10–12 days stored at 4 °C. In addition, Rama et al. (2020) suggested the incorporation of *Lactobacillus buchneri* into whey protein-based films and coatings to increase the shelf life of a semi-hard cheese and Guadalupe et al. (2023) the use of whey solutions fermented by *Lactobacillus rhamnosus* and *Lactobacillus acidophilus* for the formulation of edible films for secondary packaging of Manchego-type cheese.

Total mesophilic counts and LAB were found to be similar with the results reported by Lioliou et al. (2001) for artisanal Manouri cheese stored for 20 days. However, Lioliou et al. (2001) reported higher counts of Enterobacteriaceae (7.26 log cfu/g) and yeasts (6.46 log cfu/g) on the surface of Manouri cheese, and these numbers suggested contamination from the cheesecloths and subsequent growth during draining and storage. Similar counts were reported for Anthotyros cheese (Papaioannou et al., 2007). Greek whey cheeses analysed for their microbiological profile were found to have total microbial counts at a level of 8.19 log cfu/g, LAB at a level of 8.13 log cfu/g and non-LAB at a level of 6.55 log cfu/g (Angelidis et al., 2006). Pappa et al. (2020) reported lower populations of LAB and dairy cocci for Urda cheese, another Greek artisanal whey cheese; Urda has a different composition than Manouri (higher total solids and protein, lower fat and salt can reach values of 4.1%). Kalogridou-Vassiliadou et al. (1994) reported high counts for total mesophilic counts (7.84 log cfu/g) coliforms (6.74 log cfu/g), LAB (7.18 cfu/g) and yeasts (5.48 log cfu/g) in Anthotyros cheese.

Physicochemical Characteristics of the Cheese Samples

The results from the physicochemical analyses are shown in Table 2. Artisanal Manouri S2 was found to be the richest in fat and total solids; its high fat content (67.0 g/100 g) implies that cream was added during the artisanal cheese-making process. The other artisanal sample (S1) was the richest in protein and carbohydrates. This variation in the compositional characteristics of artisanal samples probably reflects differences in the quality of the raw materials used. The pH values of the test samples ranged from 5.61 to 5.97, with the artisanal S2 sample having the lowest value and lower than the industrial sample from the same dairy plant (S3). This finding indicates that the traditional cheese-making process may enable greater acidification from the LAB microflora.

The data in Table 2 show great variability in the physicochemical parameters tested. Variability has been reported in the chemical composition of whey cheeses, reflecting differences in the origin of cheese milk and the technology used for the primary cheese product, the composition of the whey and the use of sweet or acid whey, as well as differences in the cheese-making technology of the whey cheese (artisanal or industrial method, the animal origin of milk and/or cream added, temperature of heating etc.) (Bintsis & Papademas,

Table 2 Physicochemical characteristics of industrial and artisanal Manouri cheese samples

Parameters[f]	Cheese samples[e]			
	S1	S2	S3	S4
Fat (% w/w)	45.5 ± 0.60^{a}	67.0 ± 0.70^{b}	46.7 ± 0.76^{a}	41.7 ± 0.76^{c}
Protein (% w/w)	17.0 ± 0.47^{a}	8.8 ± 0.42^{b}	9.1 ± 0.32^{b}	10.5 ± 0.35^{c}
Carbohydrates (% w/w)	2.0 ± 0.16^{a}	1.8 ± 0.25^{a}	1.6 ± 0.19^{b}	1.4 ± 0.30^{b}
Total solids (% w/w)	68.4 ± 1.28^{a}	80.6 ± 0.81^{b}	59.3 ± 0.62^{c}	56.2 ± 0.90^{d}
pH	5.74 ± 0.04^{a}	5.61 ± 0.04^{b}	5.71 ± 0.04^{a}	5.97 ± 0.01^{c}

[e] *S1* artisanal Manouri from dairy A, *S2* artisanal Manouri from dairy B, *S3* industrial Manouri from dairy B, *S4* industrial Manouri from dairy C

[f] On a fresh weight basis; means in the same row with different superscripts were significantly different at a level of significance of 5% of overall comparisons

2023; Mangione et al., 2023a). According to Bintsis and Papademas (2023), a typical composition of Manouri consists of approximately 52% total solids (37% fat, 11% protein) and 1% salt (on fresh weight basis). The addition of milk and/or cream is one of the most important factors in the development of Manouri quality characteristics. Kaminarides et al. (2013) studied the effect of the addition of milk and cream on the characteristics of Greek whey cheeses and reported that the addition of milk and cream significantly differentiated the total solids content (31.96–47.24%) and profile constituting 15.7–34.25% fat and 9.06–11.90% protein. In addition, Danezis et al. (2020) analysed several physicochemical parameters for the 21 Greek PDO cheeses and reported that Manouri had the highest fat content.

Colour and Textural Parameters of the Cheese Samples

Colour is a quality determinant in cheeses, as it directly impacts their appearance and consequently consumers' acceptance. Data in Table 3 show that artisanal cheeses were darker than the industrial ones, as it is indicated by their lower lightness (L^*) scores ($p < 0.05$). In addition, a^* coordinate values were found to be negative (green tonalities) in all samples; S1 presented the smallest ones followed by S2, S4 and S3. On the contrary, highly positive b^* coordinate values, denoting yellow tonalities, were evidenced for S1 followed by S2, S3 and S4. The green and yellow tonalities of cheese colour imply possible influence by the whey itself (Bintsis & Papademas, 2023); small differences might possibly originate from variation in the quality, animal origin and ratio of milk and/or cream used. Variation in colour saturation (C^*) displayed the same pattern as the b^* coordinate values, suggesting that the yellowness of Manouri cheese is probably the primary colour contributor and influences consumer perception of the product appearance. Regarding the overall colour hue (h°) that lied in the greenish region (9.17–102.09°) of the tristimulus colour space, it can be suggested that S3 presented the lightest green–yellow colour among the test samples.

The different cheese-making technology used in artisanal and commercial cheese samples significantly affected specific textural properties (Table 3). In particular, fracturability and hardness values were higher for S1 and S2 than those of S3 and S4, which can be attributed to their richer protein and/or total solids content (Table 2). Similar results for Greek whey cheeses were reported by Kaminarides et al. (2013). Interestingly, S2 cheese was characterized by lower adhesiveness (− 1.08 N × s) than the rest of the samples (− 0.74, − 0.63 and − 0.72 N × s for S1, S3 and S4, respectively), which is an indicator of the increased fat content (Table 2) of this sample, resulting in a smoother and less sticky texture. Similarly, artisanal Ricotta samples with high fat and low protein were associated with higher granulosity and those with low total solids with high spreadability (Mangione et al., 2023b). The secondary attribute gumminess and chewiness, derivatives of hardness, exhibited greater values for the artisanal cheeses (S1 and S2) compared to the commercial ones (S3 and S4), showing a similar trend to that observed for the primary attribute of hardness. Concerning cohesiveness, springiness and

Table 3 Comparison of colour and textural parameters of industrial and artisanal Manouri cheese samples

Colour parameters[e]	Cheese samples[d]			
	S1	S2	S3	S4
L^*	59.65 ± 1.28[a]	62.19 ± 0.48[b]	65.37 ± 1.01[c]	64.46 ± 0.87[c]
a^*	− 1.60 ± 0.15[a]	− 1.39 ± 0.08[b]	− 1.04 ± 0.11[c]	− 1.13 ± 0.06[c]
b^*	8.58 ± 0.51[a]	6.49 ± 0.27[b]	6.47 ± 0.24[b]	5.65 ± 0.15[c]
C^*	8.73 ± 0.52[a]	6.63 ± 0.26[b]	6.55 ± 0.24[b]	5.76 ± 0.15[c]
h^o	100.55 ± 0.75[a]	102.09 ± 0.91[b]	99.17 ± 0.99[c]	101.27 ± 0.59[a,b]
Textural parameters[2]				
Fracturability (N)	15.03 ± 1.33[a]	15.71 ± 1.56[a]	3.18 ± 0.43[b]	5.68 ± 0.47[c]
Hardness (N)	16.34 ± 1.10[a]	16.28 ± 1.98[a]	3.20 ± 0.23[b]	5.91 ± 1.21[c]
Adhesiveness (N × s)	− 0.74 ± 0.11[a]	− 1.08 ± 0.20[b]	− 0.63 ± 0.20[a]	− 0.72 ± 0.13[a]
Cohesiveness	0.24 ± 0.02[a]	0.24 ± 0.01[a]	0.24 ± 0.03[a]	0.29 ± 0.03[b]
Springiness	0.59 ± 0.03[a]	0.63 ± 0.04[a]	0.65 ± 0.04[a,b]	0.72 ± 0.05[b]
Gumminess (N)	3.94 ± 0.63[a]	3.83 ± 0.31[a]	0.78 ± 0.12[b]	1.70 ± 0.31[c]
Chewiness (N)	2.35 ± 0.48[a]	2.43 ± 0.26[a]	0.50 ± 0.06[b]	1.22 ± 0.24[c]
Resilience	0.07 ± 0.01[a]	0.06 ± 0.02[a]	0.08 ± 0.01[a,b]	0.11 ± 0.02[b]

[d]*S1* artisanal Manouri from dairy A, *S2* artisanal Manouri from dairy B, *S3* industrial Manouri from dairy B, *S4* industrial Manouri from dairy C

[e]Means in the same row with different superscripts were significantly different at a level of significance of 5% of overall comparisons

resilience, the commercial S4 presented the highest values. Hardness, especially in the case of the artisanal cheeses, and springiness values were found to be higher and lower, respectively, than those reported previously for Greek whey cheeses by Kaminarides et al. (2013).

VOCs of Cheese Samples

The great variability in the composition of whey cheeses was also reflected in their VOC profile (Table 4). In total, at least 50 compounds were identified in each sample: 13 ketones, 11 free fatty acids (FFA), 8 aldehydes, 6 esters, 5 alcohols and 7 compounds classified as others. The most abundant class of VOCs (more than 60% RPA) for both artisanal and industrial type samples was that of FFA, especially of medium chain (6 to 12 carbon atoms) fatty acids. Apart from acids, the VOC profile was dominated by ketones, lactones and esters followed by alcohols and aldehydes, in descending order. Similar FFA profiles were reported for whey cheeses by Ianni et al. (2020) and Ricotta cheeses by Faccia et al. (2018) and were associated with the degree of lipolysis. Decanoic acid emerged as the predominant acid reaching up to 46% of the RPA, especially in industrial samples S3 and S4. Meanwhile, even distribution of various acids was evidenced in artisanal samples S1 and S2. Considering their significant contribution to the aroma of cheeses also as precursors of other aroma substances (esters, aldehydes and methyl ketones) (Wang et al., 2023), different FFA profiles of industrial compared to artisanal samples can further evolve during storage and contribute to the development of distinct flavour profiles. The artisanal sample S1 was the richest in esters (mainly ethyl decanoate), whereas the industrial sample S3 was devoid of this class. Despite the fact that their levels were much lower compared to FFAs, they can significantly alter the final cheese aroma perception as a consequence of their low odour thresholds (Faccia et al., 2018). Among the detected ketones, 2-butanone was the predominant compound in all samples, whereas acetoin (3-hydroxy-2-butanone) was present only in artisanal samples. The latter is characterized by a creamy aroma descriptor and derives from diacetyl or synthesized by LAB from pyruvate, lactose or citrate (Bintsis & Athanasoulas, 2015; Kaminarides et al., 2013). Acetoin was present in whey samples at variable amounts (Sattin et al., 2016). 2-Nonanone and 2-heptanone were the most abundant ketones found in Urda cheese (Pappa et al., 2020). The overall low levels of ketones and alcohols (less than 7% of the total RPA) in the present study signify the absence of moulds, which is a desirable factor in the manufacturing process of whey cheeses. In general, high concentrations of ketones and alcohols in cheeses are formed by moulds after lipolysis of triglycerides, oxidation of saturated FFA and decarboxylation of the respective ketoacids and reduction of the ketones (Faccia et al., 2018). The presence of γ- and δ-lactones was evidenced in all of the samples, in line with previous findings reported for dry-Myzithra cheese (Dimitrellou et al., 2009, 2017). The presence of different γ- and δ-lactones in soft cheeses might be of importance for the final aroma due to their fruity notes and their low perception thresholds (Curioni & Bosset, 2002). The very low levels of aldehydes (less than 2% of the total RPA) in all samples coincides with published findings on various types of whey cheeses (Faccia et al., 2018; Ianni et al., 2020; Kaminarides et al., 2013), whereas heptanal and nonanal were found to be abundant in Urda (Pappa et al., 2020). Heptanal, octanal and nonanal that were detected mainly in S3 and artisanal S1 and S2, as the most abundant in the aldehyde class, are expected to contribute to freshness and floral aromas (Wang et al., 2023). It should be noted that aldehydes are unstable in the cheese environment and easily reduced into alcohols or oxidized into acids (Curioni & Bosset, 2002; Wang et al., 2023). The presence of other VOCs, mainly hydrocarbons, was verified in all samples and was more evident in artisanal samples S1 and S2. These compounds are produced secondarily during lipid oxidation, and, though they do not contribute directly to the aroma of the cheese, they can act as precursors for other aroma compounds (Arora et al., 1995).

Sensorial Attributes of Cheese Samples

After the preliminary sensory sessions, seventeen attributes were selected by the QDA panel to describe the organoleptic characteristics of Manouri cheese, and the results are presented in Fig. 1. The artisanal samples S1 and S2 showed significantly higher values of yellowness than the industrial ones, in line with the results from the instrumental colour analysis. Significant differences were found in the appearance, namely, hardness, springiness and crumbliness as well as moisture in the mouth attributes. S1 had the highest scores in pleasant odour and higher, yet non-significant salty and piquant flavours than the S2 sample. It is worth noting that the QDA panel evaluation of hardness was in line with the instrumental texture analysis results. None of the samples could be discriminated in terms of buttery odour, bitter flavour, acid flavour and rancid flavour notes. In summary, the QDA panel discriminated the artisanal Manouri samples as cheeses with higher scores for yellowness, hardness, springiness, pleasant odour, salty, piquant and acid flavours and overall taste intensity. Lower scores were observed for appearance of surface, crumbliness and sense of moisture in the mouth, whereas similar scores were reported for buttery odour, cooked milk odour, ammonia odour and bitter flavour. S1 scored higher than S2, and both were higher than the industrial S3 and S4 in overall acceptance rating.

Ballestros et al. (2006) reported artisanal Manchego cheeses showed higher scores for the sensory attributes taste intensity, taste persistence, ovine milk taste, acid taste, spicy

Table 4 Volatile organic compounds of industrial and artisanal Manouri cheese samples

Compounds	Retention time (min)	Cheese samples[e]			
		S1	S2	S3	S4
		Relative peak area (%)[f]			
Free fatty acids					
Butanoic acid (C4:0)	4.11	2.17 ± 0.15[c]	0.55 ± 0.06 [b]	0.28 ± 0.02 [a]	2.60 ± 0.19[d]
Hexanoic acid (C6:0)	11.88	4.21 ± 0.30[c]	1.23 ± 0.14 [a]	2.75 ± 0.23[b]	7.15 ± 0.52[d]
Heptanoic acid (C7:0)	16.26	0.09 ± 0.03 [a]	n.d	0.24 ± 0.02 [b]	0.27 ± 0.02 [c]
Octanoic acid (C8:0)	21.42	14.28 ± 0.99 [b]	2.44 ± 0.27 [a]	16.93 ± 1.43 [c]	21.85 ± 1.60 [d]
Nonanoic acid (C9:0)	25.74	0.33 ± 0.02 [a]	1.40 ± 0.15 [c]	0.54 ± 0.05[a,b]	0.47 ± 0.03[b]
Decanoic acid (C10:0)	30.72	30.29 ± 2.12 [b]	5.98 ± 0.66 [a]	42.83 ± 3.64 [c]	46.02 ± 3.36 [d]
Dodecanoic acid (C12:0)	38.8	2.73 ± 0.19 [a]	2.70 ± 0.30 [a]	7.81 ± 0.66 [c]	4.91 ± 0.36 [b]
Tetradecanoic acid (C14:0)	46.44	0.79 ± 0.06 [a]	6.85 ± 0.75 [c]	2.64 ± 0.22 [b]	1.41 ± 0.10[a,b]
Hexadecanoic acid (C16:0)	53.52	0.74 ± 0.09 [a]	18.86 ± 2.08 [b]	1.88 ± 0.15 [a]	0.76 ± 0.06 [a]
Oleic acid (C18:1, cis-9)	58.39	3.65 ± 0.32 [a]	21.75 ± 2.59 [b]	n.d	n.d
Stearic acid (C18:0)	58.86	0.49 ± 0.14 [a]	10.90 ± 1.22 [b]	n.d	n.d
Total free fatty acids		59.86 ± 4.42 [a]	74.95 ± 8.22 [b]	75.90 ± 6.45[b]	85.45 ± 6.24 [c]
Ketones					
2-Butanone	1.84	2.91 ± 0.20[b]	2.20 ± 0.24 [a]	3.83 ± 0.32[c]	2.88 ± 0.21 [b]
Acetoin	2.85	0.26 ± 0.02 [a]	0.53 ± 0.06 [b]	n.d	n.d
2-Heptanone	7.4	0.15 ± 0.01 [a]	1.14 ± 0.12 [b]	0.15 ± 0.01 [a]	n.d
Tetrahydro-2H-Pyran-2-one	15.04	n.d	n.d	0.21 ± 0.02 [a]	0.18 ± 0.01 [a]
2-Nonanone	16.81	n.d	1.48 ± 0.16 [b]	0.10 ± 0.02 [a]	n.d
Γ-Undecalactone	34.63	n.d	n.d	0.52 ± 0.04[b]	0.28 ± 0.02 [a]
Tetrahydro-6-Pentyl-2H-Pyran-2-one	35.82	0.69 ± 0.05 [c]	0.18 ± 0.02 [a]	n.d	0.29 ± 0.02 [b]
Γ-Dodecalactone	43.33	n.d	0.11 ± 0.01 [a]	0.60 ± 0.05 [b]	0.55 ± 0.04[b]
Tridecyl methyl ketone	44	n.d	n.d	0.24 ± 0.02	n.d
2-Pentadecanone	44.01	n.d	0.92 ± 0.10	n.d	n.d
δ-Dodecalactone	44.43	0.07 ± 0.01 [a]	0.10 ± 0.01 [b]	n.d	0.51 ± 0.04[c]
Δ-Tetradecalactone	52.2	0.26 ± 0.03	n.d	n.d	n.d
Tetrahydro-6-Undecyl-2H-Pyran-2-one	52.21	n.d	n.d	1.04 ± 0.09 [a]	1.67 ± 0.12 [b]
Total ketones		4.34 ± 0.32 [a]	6.66 ± 0.73 [b]	6.70 ± 0.58 [b]	6.35 ± 0.46 [b]
Aldehydes					
Heptanal	7.92	0.11 ± 0.01 [a]	0.25 ± 0.03[b]	0.38 ± 0.03[c]	n.d
Octanal	12.52	0.16 ± 0.01 [a]	0.24 ± 0.03 [c]	0.32 ± 0.03 [b]	n.d
Nonanal	17.56	0.15 ± 0.02 [a]	0.28 ± 0.03 [b]	0.50 ± 0.04 [c]	0.26 ± 0.02 [b]
2-Nonenal	20.35	0.04 ± 0.03 [a]	0.22 ± 0.01 [c]	n.d	0.06 ± 0.01[b]
Decanal	22.6	0.10 ± 0.01 [a]	0.17 ± 0.01[a]	0.21 ± 0.02 [b]	n.d
Undecanal	27.49	0.06 ± 0.01 [a]	0.07 ± 0.01[a]	n.d	0.13 ± 0.01[c]
Dodecanal	32.14	0.10 ± 0.03 [a]	0.10 ± 0.01[a]	0.29 ± 0.02[b]	n.d
Octadecanal	40.73	0.08 ± 0.01 [a]	0.08 ± 0.01[a]	n.d	n.d
Total aldehydes		0.80 ± 0.13 [a]	1.37 ± 0.12 [c]	1.72 ± 0.15 [b]	0.45 ± 0.03 [a]
Esters					
Ethyl octanoate (Et. C8:0)	22.06	0.89 ± 0.06	n.d	n.d	n.d
Methyl decanoate (M. C12:0)	28.19	n.d	n.d	n.d	0.21 ± 0.01
Ethyl decanoate (Et. C10:0)	31.44	15.14 ± 1.06 [b]	0.20 ± 0.02 [a]	n.d	n.d
Ethyl laurate (Et. C12:0)	40.02	n.d	n.d	n.d	0.43 ± 0.07
1-Butanol, 3-methyl-, benzoate	47.35	n.d	1.18 ± 0.13	n.d	n.d
Hexanoic acid, 2,2-dimethyl propylate	47.72	n.d	n.d	n.d	0.17 ± 0.01
Total esters		16.02 ± 1.48 [b]	1.38 ± 0.35 [a]	n.d	0.82 ± 0.32 [a]
Alcohols					
Ethanol	1.85	2.73 ± 0.26 [c]	1.10 ± 0.14 [b]	3.21 ± 0.22[d]	0.68 ± 0.04 [a]

Table 4 (continued)

Compounds	Retention time (min)	Cheese samples[e]			
		S1	S2	S3	S4
		Relative peak area (%)[f]			
2,3-Butanediol	4.44	n.d	n.d	0.28 ± 0.02	n.d
1-Hexanol	6.69	0.04 ± 0.01 [a]	0.01 ± 0.00 [a]	0.16 ± 0.06 [b]	0.03 ± 0.01 [a]
1-Heptanol	10.96	n.d	0.05 ± 0.01 [a]	0.06 ± 0.03 [a]	n.d
1-Octanol	15.89	n.d	n.d	0.12 ± 0.01 [a]	0.10 ± 0.01 [a]
Total alcohols		2.78 ± 0.26 [b]	1.15 ± 0.15 [a]	3.83 ± 0.34 [c]	0.81 ± 0.06 [a]
Miscellaneous					
Hexadecane	44	0.50 ± 0.04	n.d	n.d	n.d
Octadecane	47.71	1.50 ± 0.10 [a]	0.88 ± 0.10 [b]	n.d	n.d
1-Eicosene	49.21	0.28 ± 0.02 [a]	3.46 ± 0.38 [d]	0.46 ± 0.04[c]	0.35 ± 0.03[b]
Nonadecane	51.29	3.39 ± 0.24 [c]	1.13 ± 0.13 [b]	0.30 ± 0.02 [a]	n.d
Eicosane	54.72	4.30 ± 0.33 [c]	4.83 ± 0.53 [c]	3.66 ± 0.48 [b]	2.14 ± 0.08 [a]
Hexacosane	57.01	3.55 ± 0.25 [a]	n.d	4.91 ± 0.42[b]	3.63 ± 0.26 [a]
Docosane	59.45	2.69 ± 0.19 [a]	6.53 ± 0.72 [b]	2.52 ± 0.21[a]	n.d
Total miscellaneous		16.21 ± 1.16 [c]	16.82 ± 1.85 [d]	11.86 ± 1.18 [b]	6.12 ± 0.37 [a]

n.d. not detected

[e]*S1* artisanal Manouri from dairy A, *S2* artisanal Manouri from dairy B, *S3* industrial Manouri from dairy B, *S4* industrial Manouri from dairy C

[f]Mean values ($n = 3$) of identified VOCs in cheese samples; values were expressed as relative peak area (%) = 100 × (peak area of individual VOC/total peak area of identified VOCs); data in the same row with different superscripts were significantly different at a level of significance of 5% ($p < 0.05$) of overall comparisons

taste and bitter taste compared to the industrial Manchego cheeses, and Gomez-Ruiz et al. (2002) found that the artisanal Manchego cheeses obtained higher scores for odour intensity and pungent odour than the industrial cheeses.

The consumer panel consisted of 50 semi-trained panelists, 22–25 years old, 29 (58%) of them consumed cheese 4–5 times a week and 21 (42%) 2–3 times a week; 26 (52%) were male and 24 (48%) female, 21 (42%) had a

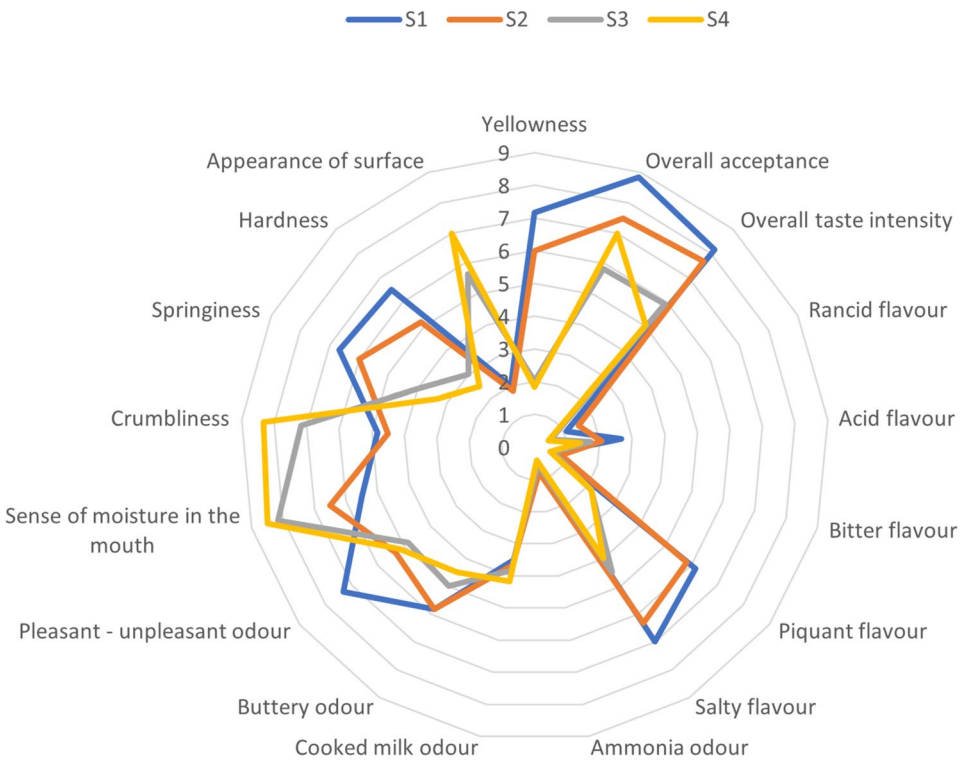

Fig. 1 Sensory evaluation results from the quantitative descriptive analysis of industrial and artisanal Manouri cheese samples (S1, artisanal Manouri from dairy A; S2, artisanal Manouri from dairy B; S3, industrial Manouri from dairy B; S4, industrial Manouri from dairy C)

preference for piquant cheeses and 29 (58%) a preference for mild cheeses; 20 (40%) preferred artisanal cheeses, and 30 (60%) preferred industrial cheeses. The results from the sensory evaluation of the consumer panel are shown in Table 5.

In general, the consumer panel judged the sensory attributes similar to the QDA panel, for the limited number of sensory attributes that were asked to evaluate. Both panels overall liked more the S1 artisanal Manouri; however, while the QDA panel judged as second the S2 artisanal Manouri with non-significant difference and the S3 as third choice, the consumer panel ranked first the S1, followed by the other three cheeses, which were not judged as significantly different in terms of overall acceptance (Table 5).

Evaluation of Overall Compositional Differences Between Manouri PDO Cheese and Artisanal Whey Cheese Samples Through FT-IR Spectral Analysis

Figure 2a displays the overlaid DRIFT spectra (3600–650 cm^{-1}) of Manouri cheese samples S1–S4 after K–M transformation and pre-processing (smoothing and normalization). Subtle differences, primarily in the intensity, shape and position of bands among 3600–2800 and 1800–900 cm^{-1}, were visually observed. As most of the water molecules were removed from the test samples, the remaining ones probably formed inter or intra-molecular OH bonds, as evidenced by the sharp -OH band at 3200 cm^{-1}. Our DRIFT spectra are very similar to the transmission ones presented by Pax et al. (2019) for high lipid areas of thin Mozzarella cheese slices after freeze-drying and FT-IR microspectroscopy. It is emphasized that the intensity of lipid-related peaks at 1745, 1461 and 1162 cm^{-1} relative to the amide-related ones at 1645 and 1537 cm^{-1} is much higher in the Manouri cheese samples under our study.

Table 5 Mean ranks of certain attributes of industrial and artisanal Manouri cheese samples from the consumer panel

Attributes[e]	Cheese samples[d]			
	S1	S2	S3	S4
Pleasant odour	3.14[a]	2.06[b]	2.25[c]	2.55[b, c]
Pleasant taste	3.49[a]	3.40[a]	1.65[b]	1.46[b]
Overall acceptance	3.17[a]	2.34[b]	1.97[b, c]	2.52[b]

[d]*S1* artisanal Manouri from dairy A, *S2* artisanal Manouri from dairy B, *S3* industrial Manouri from dairy B, *S4* industrial Manouri from dairy C

[e]Means in the same row with different superscripts were significantly different at a level of significance of 5% of overall comparisons

Valuable insights into the spectra collected from different dairy plants (A, B, C) were derived after PCA of the data. In particular, Fig. 2b illustrates the distribution of cheese samples according to their manufacturer and different production processes on a PC score plot that captures 96.3% of the total variance of spectral data. Sample allocation across the 1st PC indicates that the milk type or the processes inside each processing plant probably contributed the most to the observed variation. This could be the reason why samples S2 and S3, representing two different production systems within the same plant, tended to form a distinct group from those manufactured at dairies A and C. A closer look at the t1/t2 score plot, however, revealed that samples originating from artisanal production processes (S1, S2) were located in the upper part of the axis ($t2 > 0$), separated from S3 and S4 (industrially produced samples). The most significant bands for the distribution across both axes were exposed after careful evaluation of p1/p2 loading values. Figure 2c and d displays two spectral regions (2970–2800 and 1270–1080 cm^{-1}, respectively) disposing of those discriminating features. The two broad regions are highly associated with vibrations from fatty acids and their esters, implying the significance of the lipid composition of the tested Manouri cheeses for their overall physicochemical characteristics. In particular, the shifts between 2970–2940 cm^{-1} and at 2854–2866 cm^{-1} that are related to variation in -CH_3 asymmetric and -CH_2 symmetric stretching vibrations, respectively, were found most specific for distribution along PC1. Other researchers propose that the FT-IR spectral shifts around 2855 cm^{-1} may reveal changes in the fat physical state in cheese (e.g. melting of membrane lipids) during heating (Boubellouta & Dufour, 2012) which could be of relevance to the thermal processes applied by different manufacturers. Intensity variation in the small band at 2954 cm^{-1} is also attributed to differences in the acyl chain length of the predominant fatty acid esters (see also Table 4). On the other hand, shifts and intensity variation of the small bands at 1190–1140 and 1116–1101 cm^{-1} (Fig. 2d) were found more specific for differential distribution along PC2. These bands are assigned to P = O or P-O-C stretching modes arising from $> PO^{2-}$ moieties in phospholipids, as suggested also in the relevant literature about cheese production (Boubellouta & Dufour, 2012; Sakkas et al., 2021). Caprine and ovine milks are considered a rich source of phospholipids due to their high content in milk fat globule membrane (Verardo et al., 2017). Variations in the relevant proportion or in the thermal processes applied to produce Manouri cheese may thus be critical for the total content and profile of those biomolecules (Bintsis & Papademas, 2023).

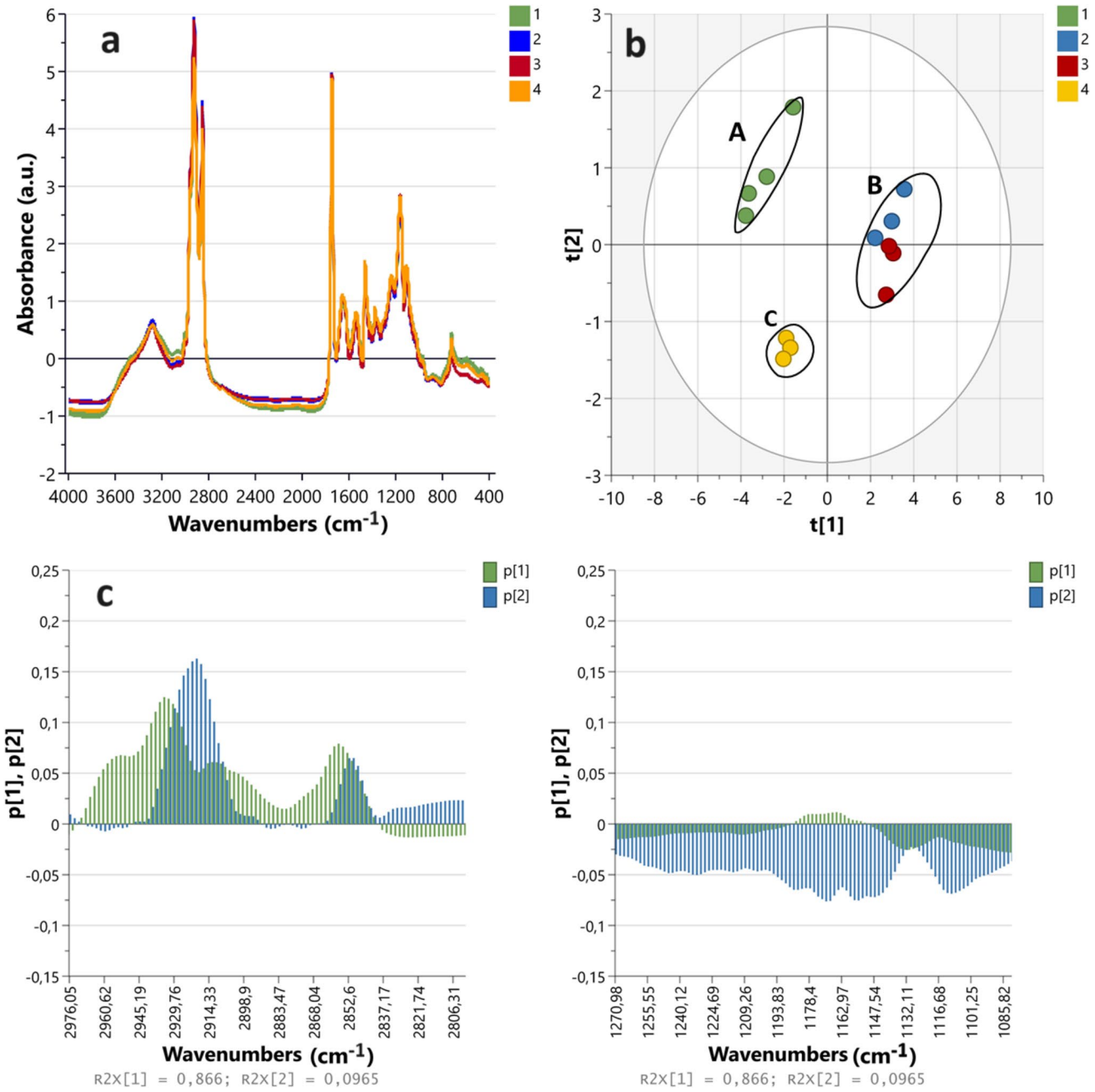

Fig. 2 **a** Normalized DRIFT spectra of the Manouri PDO cheese and artisanal whey cheese samples S1–S4 (S1, artisanal Manouri from dairy A; S2, artisanal Manouri from dairy B; S3, industrial Manouri from dairy B; S4, industrial Manouri from dairy C). **b** PCA score plot of the spectral data (PC1, $R^2(X) = 0.819$; PC2, $R^2(X) = 0.132$), coloured according to the sample code; The p1 (green) and p2 (blue) loading values that contribute highly ($|p| > 0.05$) to PC1 and PC2, respectively, are shown at **c** 2970–2800 and **d** 1270–1080 cm^{-1}

Conclusions

Artisanal and industrial Manouri samples showed variation in the microbiological, physicochemical, textural and organoleptic characteristics as well as in volatile compounds. The severe heat treatment used during the cheese-making process results in the elimination of the autochthonous microorganisms. However, the chemical composition and the high pH make Manouri a favourable medium for the proliferation of any post-process contamination.

Artisanal Manouri samples showed a higher acceptability by the consumer and the QDA panels. FFA, few ketones and lactones, several aldehydes and alcohols together with some hydrocarbons were found to be the most abundant volatile compounds that characterized artisanal and industrial Manouri. Results from the FT-IR spectral analysis showed that variation in the phospholipid content and profile of the Manouri cheese samples that may affect the fat melting temperature and phase transition properties is probably the most important attribute for FT-IR-based discrimination. To our knowledge, this is the first report about the evaluation of FT-IR spectral characteristics of Manouri cheeses, produced via artisanal or industrial manufacturing processes.

The special cheese-making practice that is followed in the manufacture of Manouri cheese and the selection and dosing of raw materials, namely, whey with (or without) the addition of milk and cream along with the high heating temperature, are critical factors for the development of products with distinct organoleptic characteristics. Future studies are needed to understand further the role of critical parameters throughout the cheese-making processes of artisanal and industrial Manouri. To this direction, FT-IR spectroscopy could be a complementary monitoring tool for optimal Manouri cheese production processes.

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Data Availability The authors confirm that the data supporting the findings of this study are available within the article and its Supplementary materials.

Declarations

Conflict of Interest The authors declare no competing interests.

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