International Dairy Journal 90 (2019) 39–44

Contents lists available at ScienceDirect

International Dairy Journal

journal homepage: www.elsevier.com/locate/idairyj

Short communication

^{1}H NMR characterisation of the lipid fraction and the metabolite profiles of Fossa (pit) cheese

Paola Scano [a, b, *], Laura R. Cagliani [b], Roberto Consonni [b]

[a] Department of Chemical and Geological Sciences, University of Cagliari, Cittadella Universitaria, SS 554 km 4.5, 09042 Monserrato, Cagliari, Italy
[b] Institute for Macromolecular Studies, National Research Council, Via Corti 12, 20133 Milan, Italy

ARTICLE INFO

Article history:
Received 12 July 2018
Received in revised form
26 October 2018
Accepted 27 October 2018
Available online 30 November 2018

ABSTRACT

Fossa cheese is a typical Italian cheese characterised by ripening in pits dug in tuffaceous rock. In this work, ^{1}H NMR spectroscopy was applied to characterise the lipid fraction and the metabolite profile of Fossa cheese produced with sheep's milk. Samples were collected before and after ripening in different pit environments. The higher levels of free amino acids (FAAs) found in samples suggest that cheeses underwent a strong proteolytic process. Differences in quantity and variety of FAAs were influenced by the two ripening environments. Diacylglycerols and butyric acid, which can be linked to lipolysis, were detected in all the samples. Longer ripened samples had higher contents of rumenic acid (18:2 *cis*-9, *trans*-11) and glycerol, and lower contents of lactate and citrate. The presence of tyramine was observed by two-dimensional NMR, while cadaverine was not detected in the samples.

1. Introduction

Nowadays, there is increased interest in ovine dairy products for their nutritional characteristics; moreover, niche products are always more appreciated by consumers for their taste and authenticity. “Formaggio di Fossa di Sogliano” (Fossa cheese) is an Italian niche cheese, entitled in 2009 by the protected designation of origin (PDO) certification (Reg. CE n. 1183 of 30.11.09). Fossa cheese is a semi-hard uncooked cheese, mainly produced using raw whole sheep's milk, aged in pits dug in the tufa ground. The ripening environment, anaerobic conditions, and the constant temperature and humidity give special characteristics to this cheese imparting a unique flavour and intense aroma (Rubino, Morand-Fehr, Renieri, Peraza, & Sarti, 1999; Toppino, Drava, Contarini, Manfredini, & Emandi, 1992). According to the PDO specifications, before being placed into the pits, a brief period (approximately 2–3 months) of maturation is necessary to achieve a certain degree of consistency, thus avoiding the risk of whey losses when the curds are pressed inside the pits (Gobbetti et al., 1999), then cheeses are ripened for 80–100 days inside the pits (Pirisi, Comunian, Urgeghe, & Scintu, 2011). A variant of this protocol comprises covering the cheese inside the pit with a thick blanket of few hundreds of kilograms of wheat-seeds and straw; the product obtained is called “Malatesta cheese”, which is characteristic of Mondaino (Italy). This unique artisanal cheese has been entitled with the Italian De.Co. designation (Council Designation, law n° 142, 1990).

Very few studies on the chemical-physical and microbiological characteristics of Fossa cheese have been conducted (Barbieri et al., 2012; De Santi et al., 2010; Gioacchini, De Santi, Guescini, Brandi, & Stocchi, 2010; Gobbetti et al., 1999; Mascaro et al., 2010; Toppino et al., 1992). In brief, Fossa cheese exhibited no hygienic risks, and contained a high degree of proteolysis with release of free amino acids (FAAs) and moderate lipolysis with release of free fatty acids (FFAs); all of these processes are responsible for the characteristic cheese flavour. Some compositional heterogeneity of this cheese has also been observed (Gobbetti et al., 1999; Pirisi et al., 2011), and therefore, to protect the uniqueness of Fossa cheese and to standardise the protocols, greater knowledge of the product is required.

To achieve this, we studied the lipid composition and the metabolite profile of Fossa cheese samples ripened under different conditions by multidimensional nuclear magnetic resonance (NMR) spectroscopy. NMR spectroscopy has the advantage of allowing an untargeted detection of all the molecules in a single experiment giving a snap-shot of the molecular profile of samples. Moreover, by performing 2D NMR experiments it is possible to unambiguously identify those molecules present in lower concentrations. Notably, most of the NMR studies on the metabolite profiles and lipid fractions of cheese have been focused on cows'

* Corresponding author. Tel.: +39 070 675 4391.
E-mail address: scano@unica.it (P. Scano).

https://doi.org/10.1016/j.idairyj.2018.10.007
0958-6946/

milk derived products, while very few studies have been dedicated to ovine cheese (Piras et al., 2013; Scano, Anedda, Melis, Lai, & Roggio, 2011).

2. Materials and methods

2.1. Chemicals

All solvents used were of the highest available purity and were purchased from Merck (Darmstadt, Germany). Standard compounds, tyramine, cadaverine deuterium oxide (D_2O, 99.9%), deuterated chloroform ($CDCl_3$), tetramethylsilane (TMS) and sodium 3-trimethylsilyl-propionate-2,2,3,3-d_4 (TSP) were acquired from Sigma–Aldrich (Milan, Italy).

2.2. Sampling

Commercial samples of Fossa cheese, produced with spring sheep's milk, were acquired directly from the producer "Il Mulino della Porta di Sotto", Mondaino (Italy). Samples of cheese were: A, collected after 3 months of maturation at the dairy; B collected after 3 months of maturation at the dairy plus 3 months of ripening inside the pits: and C (Malatesta), collected after 3 months at the dairy of maturation plus 3 months of ripening inside the pits covered by wheat straw.

2.3. NMR experiments

For each wheel of cheese, a slice of approximately 150 g was cut and 1 cm of crust removed. Samples were freeze dried, and then pulverised in a ceramic mortar. The organic phase was obtained as follows: 100 mg of pulverised cheese were dissolved in 750 μL of $CDCl_3$; after 20 min the solution was centrifuged at 12,100 ×*g* for 15 min (Beckman J2-21 centrifuge) and an aliquot of 500 μL of supernatant collected for NMR analysis. For the aqueous phase, 100 mg of cheese was dissolved in 600 μL of D_2O; after 30 min the solution was centrifuged at 12,100 ×*g* for 20 min, an aliquot of 500 μL of supernatant was collected for NMR analysis. Measured pH values were in the range of 5.30 and 5.50.

^{1}H NMR spectra were acquired at 300 K, with a 14.09 T NMR spectrometer (Bruker Avance DRX600, Bruker Biospin GmbH Rheinstetten, Karslruhe, Germany), equipped with a 5 mm Triple Resonance Inverse (TXI) probe with z-gradient. For the aqueous extracts, ^{1}H NMR spectra were recorded with a spectral width of 7183 Hz, 32 K data points, an 8.8 μs 90° pulse, an acquisition time of 2.5 s, a relaxation delay of 2 s, and 256 transients; the residual water signal was suppressed by applying a composite pulse presaturation (CPPR) sequence as implemented in the standard Bruker experiment. For the lipid extracts, NMR spectra were recorded with a spectral width of 9260 Hz, 32 K data points, a 10.2 μs 90° pulse, an acquisition time of 1.8 s, a relaxation delay of 1.5 s, and 1024 transients. For the proton and carbon dimensions, ^{1}H and ^{13}C NMR spectra were referenced to TSP and TMS external standards at 0.00 ppm, for the aqueous and lipid fractions, respectively.

Assignment of ^{1}H NMR signals was performed with the aid of literature reports (Consonni & Cagliani, 2008; Piras et al., 2013; Scano et al., 2011; Scano, Rosa, Locci, Manzo, & Dessì, 2012), in-house database, and by performing two-dimensional experiments (^{1}H-^{1}H total correlation spectroscopy, TOCSY; ^{1}H–^{13}C heteronuclear single quantum coherence spectroscopy, HSQC). Heteronuclear experiments were acquired with 7180 Hz and 36,000 Hz of sweep width for ^{1}H and ^{13}C nuclei respectively, and 145 Hz coupling value. All the spectra were Fourier transformed with FT size of 32 k and 0.3 Hz line-broadening factor, phased and baseline corrected using TopSpin 3.5 software (Bruker BioSpin GmbH, version 3.5, Rheinstetten, Karlsruhe, Germany). Relative content of molecular components was calculated by measuring the normalised spectral areas of well baseline-resolved non-overlapping peaks, diagnostic of the different molecular components.

3. Results and discussion

3.1. The lipid fraction

The ^{1}H NMR spectra of the lipid fraction of samples exhibited the characteristic signals of triacylglycerols and fatty acids (FAs) of cheese (Scano et al., 2011), and as an example, the ^{1}H NMR spectrum of sample C is reported in Fig. 1. Proton NMR assignments are reported in Table 1. The quantitative evaluation of FA composition (Table 2), indicated that sample A had a lower content of monounsaturated FAs and a higher content of saturated FAs when compared with samples B and C that experienced a longer ripening time inside the pits. Ripening has typically no effects on FA composition (Addis et al., 2005), therefore differences could be due to the slightly different compositions of the raw milk.

The conjugated linoleic acid (CLA) C18:2 9-*cis*, 11-*trans* (rumenic acid) and, to a lesser extent (approximately 4% of rumenic acid), the C18:2 9-*trans*, 11-*trans* isomer (Fig. 1b) were detectable in all samples. In addition, sample A showed a lower content of rumenic acid. Studies on the effects of ripening on the CLA content are controversial (Buccioni et al., 2010; Luna, Juárez, & de la Fuente, 2007). However, the detection of both isomers in our study suggested the presence of those lactic acid bacteria (LAB) able to produce CLA from linoleic acid (Ogawa et al., 2005) with time dependent concentration increase.

Increasing the vertical scale (Fig. 1c), ^{1}H NMR signals due to 1,2 diacylglycerols were clearly detectable, as well as signals of 1,3 diacylglycerols (Table 2). Diacylglycerols in food matrices are produced by the enzymatic action of lipases and esterases that hydrolyse triacylglycerols, releasing FFAs and diacylglycerols. The great majority of lipases in dairy acts on the *sn*-3 position of triacylglycerols where the major portion of the short-chain FA are attached (Di Cagno et al., 2003). Therefore, the presence of 1,2 diacylglycerols in Fossa cheese samples is an index of lipolysis. The presence in Malatesta sample (C) of 1,3 diacylglycerols can be the result of esterases with *sn*-2 position as the preferential target (Holland et al., 2005) or due to the possible isomerisation of 1,2 diacylglycerols to the more thermodynamically stable 1,3 diacylglycerol form (Laszlo, Compton, & Vermillion, 2008).

3.2. Metabolite profile

The ^{1}H NMR spectra of the aqueous extract of samples A, B and C of Fossa cheese are shown in Fig. 2. Signals of FAAs, organic acids and other compounds, such as galactose and glycerol, were identified and the main assignments are reported in Table 3. By a visual comparison of the spectra, a different metabolite profile between samples was evident. The spectrum of sample A exhibited broad peaks centred at 7.30, 7.12, 6.82, 4.50–4.00, 3.00, 2.44–1.25 and 0.92 ppm (P in Fig. 2), assigned to residual proteins/polypeptides (Rodrigues et al., 2011). These peaks were detected in lower extent in samples B and C, which are conversely characterised by the presence of sharp peaks due to FAA. These observations indicated a more extended proteolysis process in ripened samples, in accordance with previous data (Gobbetti et al., 1999; Rodrigues et al., 2011).

This trend may be correlated with a higher rate of primary proteolysis in the first period of maturation at the dairy farm, resulting in an increased amount of peptides, and a subsequent action of aminopeptidases or exopeptidases of microbial origin in

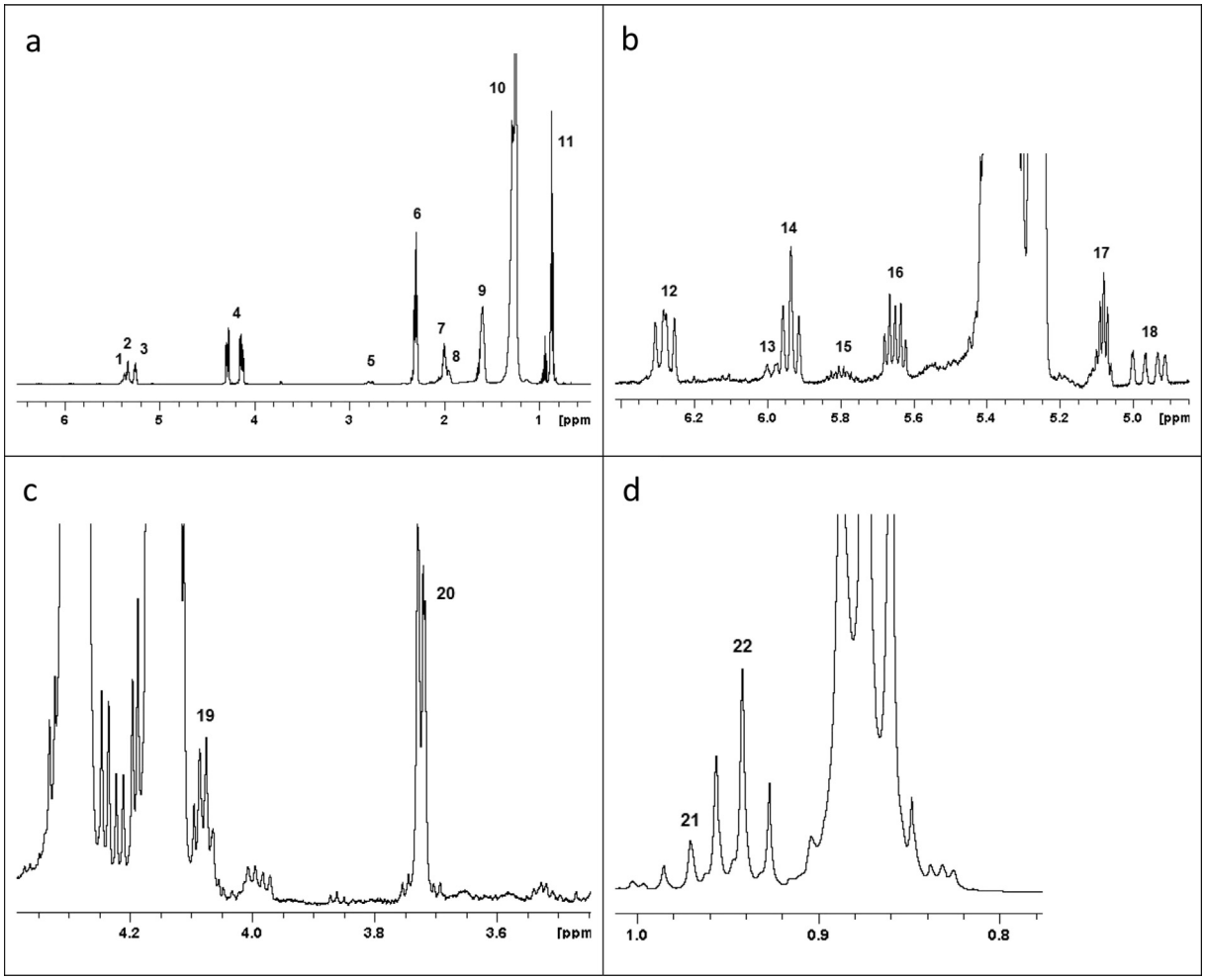

Fig. 1. ^{1}H NMR spectrum of the lipid extract in $CDCl_3$ of Fossa cheese sample C (a), and expansions (b, c, d); assignments to the functional groups of molecules are reported in Table 1.

Table 1
^{1}H NMR assignments of the lipid fraction in $CDCl_3$ of Fossa cheese samples.[a]

Peak	Compounds	Functional group	δ (ppm)
1	Unsaturated FAs *trans*	−HC═CH−	5.37
2	Unsaturated FAs *trans*	−HC═CH−	5.34
3	Glycerol in TAG	−CH−OOC−	5.26
4	Glycerol in TAG	$-CH_2-OOC-$	4.14, 4.28
5	PUFAs	$=CH-CH_2-CH=$	2.80
6	All FAs	$-OCO-CH_2-$	2.31
7	MUFAs *cis*	$CH_2-CH_2-CH=CH-$	2.01
8	MUFAs *trans*	$CH_2-CH_2-CH=CH-$	1.96
9	All FAs	$-OCO-CH_2-CH_2-$	1.62
10	All FAs	$-(CH_2)_n-$	1.26
11	All FAs, except *n*-3 and butyric acid	$-CH_3$	0.89
12	Rumenic acid (H11)	−CH═	6.28
13	CLA 9-*trans*, 11-*trans*	−CH═	6.00
14	Rumenic acid (H10)	−CH═	5.93
15	Caproleic acid (H9)	$H_2C=CH-$	5.81
16	Rumenic acid (H12)	═HC−	5.65
17	Glycerol 1,2-DAG	−CH−OOC−	5.08
18	Caproleic acid (H10,10′)	$H_2C=CH-$	4.93, 4.99
19	Glycerol in 1,3-DAG	−CH−OOC−	4.07
20	Glycerol in 1,2-DAG	$-CH_2-OH$	3.72
21	*n*-3 FA	$-CH_3$	0.97
22	Butyric acid	$-CH_3$	0.95

[a] Peaks are as reported in Fig. 1. Chemical shifts are referenced to TMS at 0 ppm. Abbreviations are: FAs, fatty acids; MUFAs, monounsaturated FAs; PUFAs, polyunsaturated FAs; CLA, conjugated linoleic acid; TAG, triacylglycerols; DAG, diacylglycerols. Caproleic acid is C10:1 *n-1*; rumenic acid is C18:2 *cis-9*, *trans-11*.

Table 2
Lipid composition of Fossa cheese samples.[a]

Lipid component	Sample		
	A	B	C
Fatty acids			
Saturated	80.23	72.27	72.24
Monounsaturated	16.69	23.80	23.99
Polyunsaturated	3.07	3.96	3.76
Butyric acid	11.37	10.44	12.27
Rumenic acid	0.03	0.96	1.11
Acylglycerols			
TAG	96.62	98.40	95.58
1,2 DAG	3.38	1.60	2.74
1,3 DAG			1.68

[a] Abbreviations are: TAG, triacylglycerols; DAG, diacylglycerols. Values are in mol %.

the second period of ripening inside the pit (Rodrigues et al., 2011). Interestingly, in sample C, the broad NMR signals ascribed to proteins/polypeptides (P) were almost negligible, suggesting that the covering of cheese with wheat-straw inside the pit could promote proteolytic processes, most likely favoured by the thermal maintenance played by the covering. Indeed, a very high degree of proteolysis in Fossa cheese was already reported by previous investigations (Gioacchini et al., 2010; Gobbetti et al., 1999).

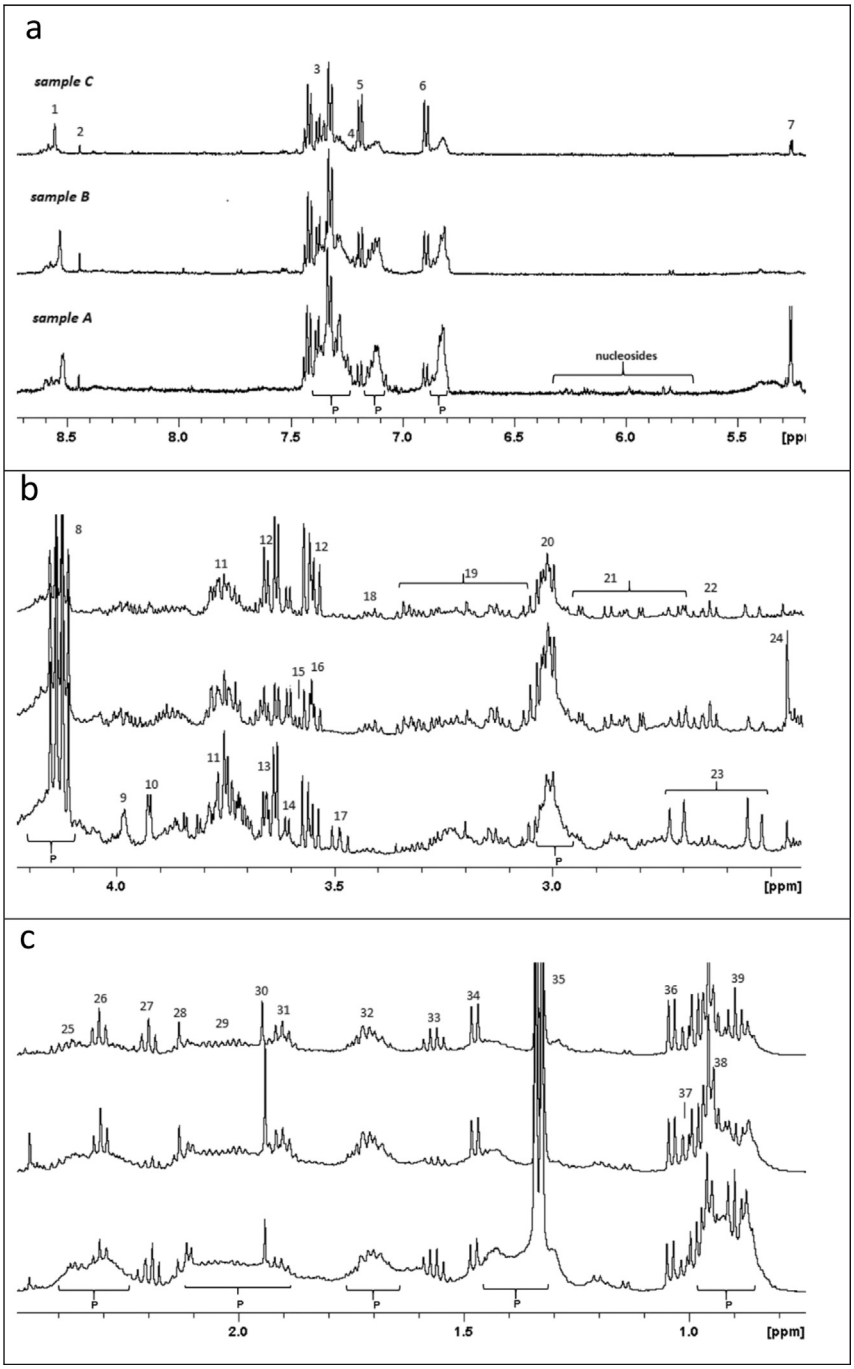

Fig. 2. Expansions (a, b, and c) of ^{1}H NMR spectra of aqueous extract in D_2O of Fossa cheese samples A, B, and C. P stands for proteins/polypeptides. Assignments to metabolites are reported in Table 3.

The relative amount of the identified metabolites in samples is reported in Table 4. Compared with sample A, samples B and C had greater FAA content, particularly in proline. Levels of GABA were quite similar, suggesting an early activity of GABA forming micro-organisms. By the comparison of samples B and C, a different FAA profile has been detected, probably as a consequence of different ripening conditions. In particular, sample B contained a greater amount of aspartate, while sample C contained a greater amount of tyrosine.

FAAs are also the precursors of biogenic amines by deamination process. Fossa cheese is rich in biogenic amines, in particular cadaverine (Mascaro et al., 2010). In this regard, although tyramine was clearly identified, cadaverine was not detectable in the NMR spectra of all investigated samples (see Supplementary material).

In sample A, the signals of galactose were detected, most likely derived from the metabolism of lactose, confirming, as generally acknowledged, that glucose was the preferred microbial substrate among the two end products of lactose hydrolysis. Although in a lower content, galactose was detected in sample C as well, and it can be hypothesised that, the ripening in wheat-straw preserved some galactose content from galactose-fermenting microorganisms.

Concerning the organic acid content (Table 4), samples B and C contained lower levels of lactate and citrate than sample A, and sample B had a higher acetate and pyruvate content. The lower

Table 3
^{1}H NMR assignments of the metabolite fraction in D_2O of Fossa cheese samples.[a]

Peak	Compound	Group	^{1}H (ppm)	Multiplicity	Peak	Compound	Group	^{1}H (ppm)	Multiplicity
1	Histidine	C2H, ring	8.52	s	20	Lysine	εCH_2	3.00	t
2	Formiate	$HCOO^-$	8.45	s	20	GABA	γCH_2	3.00	t
3	Phenylalanine	C2,6H, ring	7.42	m	21	Aspartate	$\beta,\beta' CH_2$	2.73, 2.83	dd
3	Phenylalanine	C3,5H, ring	7.40	m	21	Asparagine	βCH_2	2.88	dd
3	Phenylalanine	C4H, ring	7.32	m	21	Tyramine	CH_2	2.94	dd
3	Histidine	C4H, ring	7.31	s	21	Asparagine	$\beta' CH_2$	2.96	dd
4	Tyramine	C3,5H, ring	7.21	d	22	Methionine	γCH_2	2.64	t
5	Tyrosine	C3,5H, ring	7.18	d	23	Citrate	$^{2,4}CH$	2.54	dd
6	Tyramine	C2,6H, ring	6.90	d	23	Citrate	$^{2',4'}CH$	2.71	dd
6	Tyrosine	C2,6H, ring	6.89	d	24	Pyruvate	CH_2	2.46	s
7	Galactose	C1Hα	5.25	d	25	Proline	βCH	2.34	m
7*	Galactose	C1Hβ	4.58	d	25	Glutamate	γCH_2	2.34	m
8	Lactate	CH	4.23	q	26	GABA	αCH_2	2.3	t
9	Galactose	C2Hα	3.98	m	27	Butyrate	αCH_2	2.19	t
10	Galactose	C3Hα, C4Hβ	3.92	m	28	Methionine	$S\text{-}CH_3$	2.13	s
11	Glycerol	CH	3.75	m	29	Glutamate	ββ′CH	2.09	m
11	Glutamate	αCH	3.76	dd	29	Proline	β′CH	2.03	m
11	Alanine	αCH	3.79	q	29	Proline	γCH_2	2.02	m
11	Lysine	αCH	3.77	t	30	Acetate	CH_3	1.94	s
11	Glutamine	αCH	3.79	d	31	GABA	βCH_2	1.91	q
12	Glycerol	CH_2	3.65, 3.55	dd	31	Lysine	βCH_2	1.91	m
13	Galactose	C3Hβ	3.64	dd	32	Leucine	βCH_2	1.7	m
14	Valine	αCH	3.60	d	32	Lysine	δCH_2	1.7	m
15	Threonine	αCH	3.58	d	33	Butyrate	βCH_2	1.57	m
16	Glycine	αCH_2	3.57	s	34	Alanine	βCH_3	1.48	d
17	Galactose	C2Hβ	3.49	dd	35	Lactic acid	CH_3	1.33	d
18	Proline	δ′CH	3.42	t	36	Valine	$\gamma' CH_3$	1.04	d
19	Phenylalanine	$\beta,\beta' CH_2$	3.26, 3.12	dd	37	Isoleucine	$\gamma' CH_3$	1.01	d
19	Proline	δCH	3.37	t	38	Leucine	$\delta CH_3,\delta' CH_3$	0.96	d
19	Tyramine	CH_2	3.24	dd	39	Butyrate	γCH_3	0.9	t
19	Tyrosine	β,β′CH	3.15, 3.04	dd					

[a] Peaks are as reported in Fig. 2 except that marked with an asterisk. Abbreviations are: GABA, γ-aminobutyrate; s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublets; m, multiplet. Chemical shifts are reported with respect to TSP signal (0.00 ppm).

Table 4
Levels of identified metabolites as calculated from the ^{1}H NMR spectral areas[a].

Metabolite	Sample		
	A	B	C
FAA			
Leu	11.97	15.93	15.62
Ile	2.33	2.86	2.73
Val	2.64	3.62	4.77
Met	1.58	2.31	2.08
Asp	0.43	0.72	0.49
Tyr	0.32	0.50	0.77
Phe	0.96	0.98	1.19
Pro	0.46	1.10	1.05
Organic acids			
Lactate	49.87	38.51	35.94
Acetate	3.47	4.73	3.08
Pyruvate	0.49	1.20	0.44
Formate	1.11	0.89	0.76
Citrate	1.65	1.09	1.09
Others			
Butyric acid	3.47	2.06	4.18
Glycerol	0.18	0.32	1.00
GABA	6.27	7.15	6.87
Galactose	0.52	nd	0.14

[a] Abbreviations are: FAA, free amino acid (three letter system: IUPAC-IUB, 1984); GABA, γ-aminobutyric acid; nd, not detected. Values are in arbitrary units.

level of citrate in sample B could be related to the presence of citrate-positive microbial strains, which convert citrate into pyruvate and acetate (Hugenholtz, 1993), in agreement, these two latter organic acids were found at higher levels in sample B.

Signals of butyric acid (as FFA), most likely derived from lipolytic activities, were detected in all samples. In particular, a lower amount of butyric acid was detected in sample B, in agreement with the minor 1,2 diacylglycerol content. Butyric acid is responsible for the strong flavour imparted to cheese; in general carboxylic acids are not only aroma compounds by themselves but they are also precursors of other volatile compounds, such as methyl ketones, alcohols, lactones, aldehydes and esters, which were found to be abundant in Fossa cheese (Gioacchini et al., 2010).

Glycerol was detected in all the samples with a higher amount in samples B and C, which ripened inside the pits. Glycerol can be produced by filamentous fungi of which Fossa cheese is rich (De Santi et al., 2010) or could be the results of extended lipolytic processes. In sample C, where a marked lipolysis was suggested by the presence of 1,3 diacylglycerols in the lipid fraction, glycerol was found at higher levels. Finally, the spectral region 5.70–6.40 ppm (Fig. 2a), exhibited low intensity peaks assigned to nucleotides and derivatives, hardly detectable in samples B and C, conversely visible in sample A. We suggest that these molecular components were in the whey fraction of the original milk used, that in longer ripened samples was mostly drained out.

4. Conclusions

This short communication provides a snapshot of the lipid fraction and of the metabolite profile of Fossa cheese, providing complementary information on the enzymatic activities that can give rise to the final organoleptic and nutritional characteristics of Fossa cheese. Although these results need to be confirmed by a larger study, this preliminary work endorsed NMR as a suitable analytical tool in the view of valorisation of niche food products like Fossa cheese.

Acknowledgement

L.R.C. acknowledges support from the A. De Marco scientific foundation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.idairyj.2018.10.007.

References

Addis, M., Cabiddu, A., Pinna, G., Decandia, M., Piredda, G., Pirisi, A., et al. (2005). Milk and cheese fatty acid composition in sheep fed Mediterranean forages with reference to conjugated linoleic acid *cis*-9, *trans*-11. *Journal of Dairy Science, 88*, 3443–3454.

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*, 62–67.

Buccioni, A., Rapaccini, S., Antongiovanni, M., Minieri, S., Conte, G., & Mele, M. (2010). Conjugated linoleic acid and C18:1 isomers content in milk fat of sheep and their transfer to Pecorino Toscano cheese. *International Dairy Journal, 20*, 190–194.

Consonni, R., & Cagliani, L. R. (2008). Ripening and geographical characterization of Parmigiano Reggiano cheese by ^{1}H NMR spectroscopy. *Talanta, 76*, 200–205.

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*, 465–471.

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*, 961–972.

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*, 3405–3412.

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*, 763–773.

Holland, R., Liu, S. Q., Crow, V. L., Delabre, M. L., Lubbers, M., Bennett, M., et al. (2005). Esterases of lactic acid bacteria and cheese flavour: Milk fat hydrolysis, alcoholysis and esterification. *International Dairy Journal, 15*, 711–718.

Hugenholtz, J. (1993). Citrate metabolism in lactic acid bacteria. *FEMS Microbiology Reviews, 12*, 165–178.

IUPAC-IUB. (1984). Joint Commission on Biochemical Nomenclature (JCBN). Nomenclature and symbolism for amino acids and peptides. Recommendations 1983. *Biochemistry Journal, 219*, 345–373.

Laszlo, J. A., Compton, D. L., & Vermillion, K. E. (2008). Acyl migration kinetics of vegetable oil 1,2-diacylglycerols. *Journal of the American Oil Chemists' Society, 85*, 307–312.

Luna, P., Juárez, M., & de la Fuente, M. A. (2007). Conjugated linoleic acid content and isomer distribution during ripening in three varieties of cheeses protected with designation of origin. *Food Chemistry, 103*, 1465–1472.

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. *Italian Journal of Food Safety, 1*, 49–53.

Ogawa, J., Kishino, S., Ando, A., Sugimoto, S., Mihara, K., & Shimizu, S. (2005). Production of conjugated fatty acids by lactic acid bacteria. *Journal of Bioscience and Bioengineering, 100*, 355–364.

Piras, C., Marincola, F. C., Savorani, F., Engelsen, S. B., Cosentino, S., Viale, S., et al. (2013). A NMR metabolomics study of the ripening process of the Fiore Sardo cheese produced with autochthonous adjunct cultures. *Food Chemistry, 141*, 2137–2147.

Pirisi, A., Comunian, R., Urgeghe, P. P., & Scintu, M. F. (2011). Sheep's and goat's dairy products in Italy: Technological, chemical, microbiological, and sensory aspects. *Small Ruminant Research, 101*, 102–112.

Rodrigues, D., Santos, C. H., Rocha-Santos, T. A., Gomes, A. M., Goodfellow, B. J., & Freitas, A. C. (2011). Metabolic profiling of potential probiotic or synbiotic cheeses by nuclear magnetic resonance (NMR) spectroscopy. *Journal of Agricultural and Food Chemistry, 59*, 4955–4961.

Rubino, R., Morand-Fehr, P., Renieri, C., Peraza, C., & Sarti, F. M. (1999). Typical products of the small ruminant sector and the factors affecting their quality. *Small Ruminant Research, 34*, 289–302.

Scano, P., Anedda, R., Melis, M. P., Lai, A., & Roggio, T. (2011). ^{1}H- and ^{13}C-NMR characterization of the molecular components of the lipid fraction of Pecorino Sardo cheese. *Journal of the American Oil Chemists' Society, 88*, 1305–1316.

Scano, P., Rosa, A., Locci, E., Manzo, G., & Dessì, M. A. (2012). Modifications of the ^{1}H NMR metabolite profile of processed mullet (*Mugil cephalus*) roes under different storage conditions. *Magnetic Resonance in Chemistry, 50*, 436–442.

Toppino, P. M., Drava, G., Contarini, G., Manfredini, M., & Emandi, G. (1992). Caratteristiche di tipicità del 'formaggio di fossa'. *Rivista della Società Italiana di Scienza dell'Alimentazione, 21*, 389–414.