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Short communication

GC-MS metabolomics comparison of yoghurts from sheep's and goats' milk

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ABSTRACT

In this study, the polar metabolite profile of commercial yoghurt samples produced in Sardinia (Italy) from milk of local sheep and goats was studied by GC-MS and multivariate statistical data analysis (MVA). Milks underwent the same manufacturing procedures and yoghurts were analysed one day post-manufacture. Results of discriminant analysis indicated that the two yoghurt types had very different metabolite profiles, with different levels of health promoting compounds. Goats' milk yoghurt was richer in free amino acids, γ-aminobutyric acid, pyroglutamic acid and β-phenyllactic acid when compared with yoghurt produced with sheep's milk. Sheep's milk yoghurt was characterised by higher levels of *myo*-inositol, *N*-acetylgalactosamine and *N*-acetylglucosamine. Comparing yoghurt metabolites with those of the original milk, it was found that goats' milk underwent stronger metabolite changes after inoculum. The comparison between the two yoghurt types gave us a deeper insight on the effects of manufacture on different milks.

1. Introduction

Yoghurt intake has been associated to a plethora of health benefits (Aryana & Olson, 2017) and recently has been placed at the top of the wide range of carrier foods for probiotics (Song, Salam, & Saeed, 2012). Among milks suitable for yoghurt production, those from small ruminants, such as goats and sheep, are gaining an increased appeal for the consumers (Boyazoglu & Morand-Fehr, 2001). It is well known that the quality of dairy products depends on the chemical, physical and technological properties of milk. It has been clearly demonstrated that the overall composition of goats' and sheep's milk is very different (Caboni et al., 2019; Park, Juárez, Ramos, & Haenlein, 2007). Compared with other milks, thanks to its high protein and solids content, ovine milk shows good advantages for the production of yoghurt and cheese; however, goats have a longer lactation period than sheep (Scano, Ibba, Casula, Contu, & Caboni, unpublished) and the prolonged availability of milk allows an extended season of dairy production (Boyazoglu & Morand-Fehr, 2001). In comparison with yoghurts produced from cows' and sheep's milk, goats' milk yoghurt showed a looser consistency and higher acidity (Domagala, 2008) and weaker textural properties (Moschopoulou et al., 2018; Park et al., 2007).

From a social-economic point of view, the use of goats and sheep for milk and dairy production plays a crucial role in the Mediterranean area with different types of traditional and industrialised fermented milk specialties (Boyazoglu & Morand-Fehr, 2001), and Sardinia (Italy) represents one of the most prolific production regions (Scintu & Piredda, 2007). Their quality is linked to historical and cultural uniqueness, that refers to farming system, with dominant extensive grazing situations, to transformation processes, cheese-making and ripening (Boyazoglu & Morand-Fehr, 2001). Indeed, it is important to improve the scientific knowledge on small ruminant dairy products, with the aim to ameliorate the dairy industry in term of costs and quality, and to protect their uniqueness.

The polar metabolite profiles of yoghurts, produced under the same protocol, from milk of sheep and goats breed in Sardinia were characterised and compared. The yoghurt metabolite profiles were compared with those of the milk used for their production.

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2. Material and methods

2.1. Chemicals and reagents

Analytical standard grade methanol, chloroform, hexane, pyridine, methoxamine hydrochloride, potassium chloride, trichloroacetic acid, 2,2,4,4-d4 succinic acid, N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA), were purchased from Sigma Aldrich (Milano, Italy). Bi-distilled water was obtained with a MilliQ purification system (Millipore, Milan, Italy).

2.2. Collection of commercial samples

We analysed 20 ovine and 20 caprine plain yoghurts (SY and GY, respectively), and 5 ovine and 5 caprine bulk milk samples (SM and GM, respectively) before inoculum. Yoghurt obtained from whole milk underwent the same production steps; the milk samples were thermally treated and inoculated with a commercial starter culture consisting in a blend of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Food.com, Cremona, Italy). Milk and yoghurt commercial samples were kindly provided by the Azienda Chiai, Barisardo, Italy, where all the stages, from animal breeding to yoghurt production, were carried out. Yoghurt samples were produced in December 2018 and were analysed within 12 h after packaging.

2.3. GC-MS analysis

One hundred microlitres of thawed milk or 50 mg of yoghurt were extracted using 0.5 mL a methanol and chloroform mixture (1:1, v/v) and 10 μL of a 1% trichloroacetic acid aqueous solution. Before the extraction, 10 μL of a 1 mg mL^{-1} succinic acid 2,2,4,4-d4 aqueous solution were added in each sample as internal standard. Samples were then vortexed 4 times for 1 min every 15 min. Following the addition of 760 μL chloroform and 90 μL aqueous 0.2 M KCl, samples were vortexed again and then centrifuged for 15 min at 15,294× *g* (Eppendorf 5810R, Milan, Italy) at 4 °C. Two hundred microlitres of the hydrophilic supernatant were dried in glass vials using a gentle nitrogen stream and then derivatised using 50 μL methoxamine chloride dissolved in pyridine at 10 mg mL^{-1}. After 17 h, 100 μL MSTFA were added to the samples and then after 1 h, 800 μL hexane were added.

Milk and yoghurt derivatised samples were analysed with a Hewlett Packard 6850 gas chromatograph, 5973 mass selective detector, and 7683B series injector (Agilent Technologies, Palo Alto, CA, USA), as previously described (Caboni et al., 2019). The identification of metabolites was performed using a co-chromatography approach with analytical standards and by comparison of their mass spectra with the NIST08 library of the National Institute of Standards and Technology (Gaithersburg, MD, USA), and a library developed at the Max Planck Institute of Golm (http://gmd.mpimp-golm.mpg.de/).

2.4. Statistical data analysis

Multivariate statistical data analysis (MVA) was performed as implemented in SIMCA-P+ software (version 14.1, Umetrics, Umeå, Sweden). Variables were mean centred and scaled to unit variance. Principal component analysis (PCA) was performed to investigate sample distributions, deviating features and common trends. Classificatory and predictive powers were verified using a partial least-squares discriminant analysis (PLS-DA) on the basis of its R^2Y and Q^2. The variable importance in projection (VIP) scores, that summarise the contribution of each variable to the model, were analysed and only those metabolites having VIP values > 1 were deemed as discriminant between the classes. Using the GraphPad software (San Diego, CA, USA), the discriminant metabolites were subjected to a Mann–Whitney test.

3. Results

The polar hydrosoluble metabolites of milk and yoghurt at day one post-manufacture, as analysed by the GC-MS analysis, were represented mainly by hydroxylated carboxylic acids, straight chain dicarboxylic acids, organic acids, amino acids, polyols and saccharides (Table 1). The metabolite profile included 92

Table 1
GC-MS characteristics of metabolites.[a]

Metabolite	RT	Mass (*m/z*)	KI	Class	Metabolite	RT	Mass (*m/z*)	KI	Class
Lactic acid	15.43	117	1037	M,Y	α-Glycerophosphoric acid	25.50	357	1668	M,Y
Alanine	16.36	116	1056	M,Y	Unk8	25.62	189	1672	Y
Histidine	16.55	155	1060	M,Y	Unk9	25.82	189	1680	Y
α-Hydroxyisobutyric acid	16.83	131	1067	Y	Azelaic acid	25.98	317	1687	M,Y
Butanoic acid	17.06	73	1072	Y	Isocitric acid	26.12	273	1692	M
Glycolic acid	17.13	133	1073	Y	Unk10	26.28	73	1801	M,Y
β-Hydroxyisobutyric acid	17.48	117	1081	M	Unk11	26.39	217	1807	M,Y
Glycine	18.31	228	1202	M,Y	Unk12	26.47	204	1811	M,Y
Valine	18.42	144	1206	M,Y	Fructose	26.60	103	1818	M,Y
α-Hydroxyisocaproic acid	18.72	159	1215	Y	Unk13	26.63	217	1820	Y
Unk1	18.95	314	1223	M,Y	Galactose	26.80	319	1829	M,Y
Urea	19.09	189	1227	M	Glucose	26.86	319	1832	M,Y
Phosphate	19.35	299	1235	M,Y	Talose	26.94	204	1837	M,Y
Isoleucine	19.65	158	1245	Y	Lysine	27.23	174	1852	Y
Proline	19.74	142	1248	M,Y	Unk14	27.34	232	1858	Y
Unk2	19.88	256	1253	M,Y	Tyrosine	27.42	218	1862	M
Succinic acid	20.02	247	1257	M,Y	Unk15	27.47	217	1865	M,Y
Glucitol	20.17	147	1262	M,Y	Gluconic acid	27.59	73	1872	M,Y
Uracil	20.35	241	1268	M,Y	Unk16	27.70	204	1877	M,Y
Itaconic acid	20.41	147	1270	M,Y	Palmitic acid	28.52	313	2023	M,Y
Serine	20.60	204	1276	M,Y	*Myo*-inositol	28.78	305	2037	M,Y
Threonine	20.95	218	1288	M,Y	N-Acetylglucosamine	29.13	319	2055	M,Y
Acetoacetic acid	22.08	231	1431	M,Y	N-Acetylgalactosamine	29.19	319	2058	M,Y
Malic acid	22.46	245	1446	M,Y	Unk17	29.32	204	2065	Y

(*continued on next page*)

Table 1 (*continued*)

Metabolite	RT	Mass (*m/z*)	KI	Class	Metabolite	RT	Mass (*m/z*)	KI	Class
Erythrose	22.50	147	1447	M,Y	Unk18	29.52	204	2076	Y
Adipic acid	22.61	111	1452	Y	Unk19	29.73	243	2087	M,Y
Aspartic acid	22.74	232	1457	M,Y	Vaccenic acid	30.09	339	2209	M,Y
Methionine	22.77	143	1458	M,Y	Stearic acid	30.31	341	2221	M,Y
Pyroglutamic acid	22.85	156	1461	M,Y	Unk20	30.71	204	2244	Y
γ-Aminobutyric acid	22.88	174	1462	Y	Unk21	30.77	387	2248	M,Y
Unk3	23.04	263	1468	Y	Unk22	32.06	204	2426	M
Creatinine	23.19	115	1474	M,Y	Unk23	32.23	204	2437	M
α-Hydroxyglutaric acid	23.39	217	1482	M,Y	Saccharide1	32.60	204	2460	M,Y
β-Phenyllactic acid	23.57	217	1489	M,Y	Saccharide2	32.86	204	2476	M,Y
Unk4	23.64	103	1491	M	Saccharide3	32.95	204	2481	M,Y
Unk5	23.74	319	1495	M,Y	Saccharide4	33.26	204	2603	Y
Pimelic acid	23.80	73	1498	Y	Saccharide5	33.49	361	2618	M,Y
Unk6	23.92	231	1604	M,Y	Unk24	33.92	204	2647	M,Y
Glutamine/Glutamic acid	23.95	246	1606	M,Y	Saccharide6	34.04	204	2655	Y
Phenylalanine	24.09	218	1611	M,Y	Unk25	34.15	204	2662	Y
Xylose	24.52	103	1628	M,Y	Saccharide7	34.42	204	2681	M
Ribitol	24.82	217	1640	M,Y	2,3-Dihydroxy-stearic acid	34.48	399	2685	M,Y
Suberic acid	24.90	73	1644	M,Y	Saccharide8	34.70	204	–	Y
Ribose	25.03	217	1649	M,Y	Saccharide9	34.75	204	–	Y
Unk7	25.07	117	1650	M	Saccharide10	34.90	204	–	Y
Ribonic acid	25.47	292	1666	M,Y	Saccharide11	34.98	204	–	Y

[a] Abbreviations are: RT, retention time; KI, Kovatz index; Class, class of samples (M, milk; Y, yoghurt) where metabolites were found; Unk, not identified; Saccharide, compounds with fragmentation pattern ascribable to mono- or disaccharides.

compounds. From the mass spectra analysis of each chromatographic peak, we were able to identify 56 metabolites, while 11 peaks shared a common fragmentation pattern ascribable to mono- or disaccharides and as such were annotated, and 25 were considered unknowns. As reported in Table 1 and depicted in the Venn diagram (Fig. 1), 6 metabolites were found solely in milk samples and 15 solely in yoghurt. The milk urea was not detected in yoghurt, as well as tyrosine and other compounds, while in yoghurt samples we found, among others, γ-aminobutyric acid (GABA), adipic acid, pimelic acid, butanoic acid and more saccharides.

From the GC-MS data, we created two matrices, one for the yoghurt samples and one for the milk samples, to be submitted to MVA. Initially, the two GC-MS datasets were submitted to a PCA. Both score plots showed a strong distribution along the first principal component (PC) of goats' and sheep's samples, which clearly clustered in the two sides of the plots (Fig. 2). Subsequently, a PLS-DA was performed for each set of samples to find the discriminant metabolites between sheep's and goats' yoghurt and milk. The results showed a good classificatory and predictive power for both yoghurt and milk data sets. For yoghurt samples the R^2Y was 0.99 and the Q^2 was 0.99, while for milk samples the

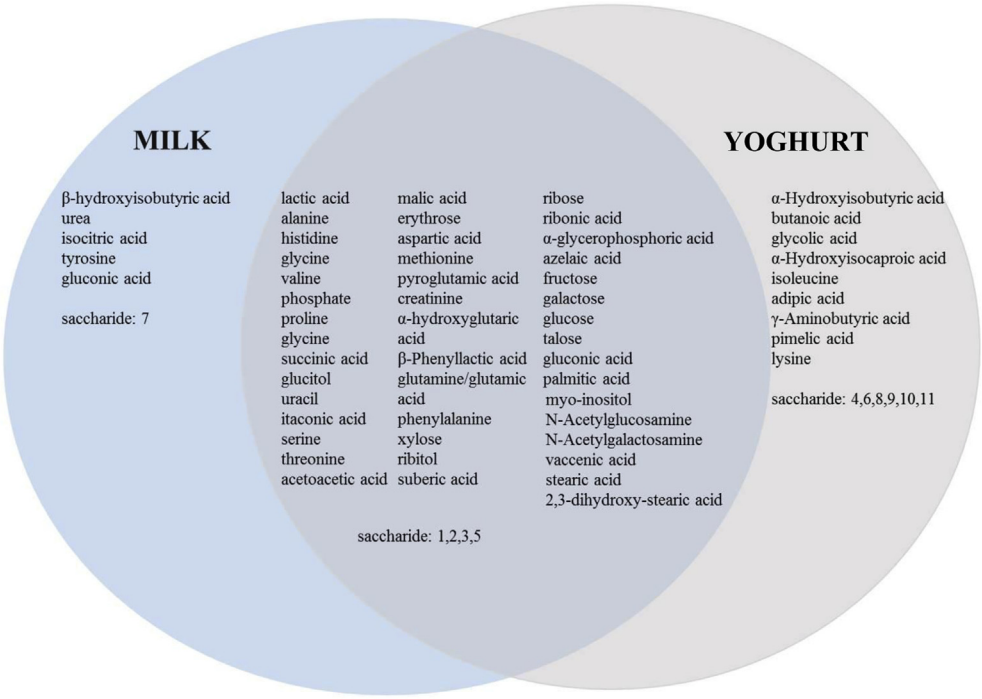

Fig. 1. Venn diagram of metabolites in milk and yoghurt samples.

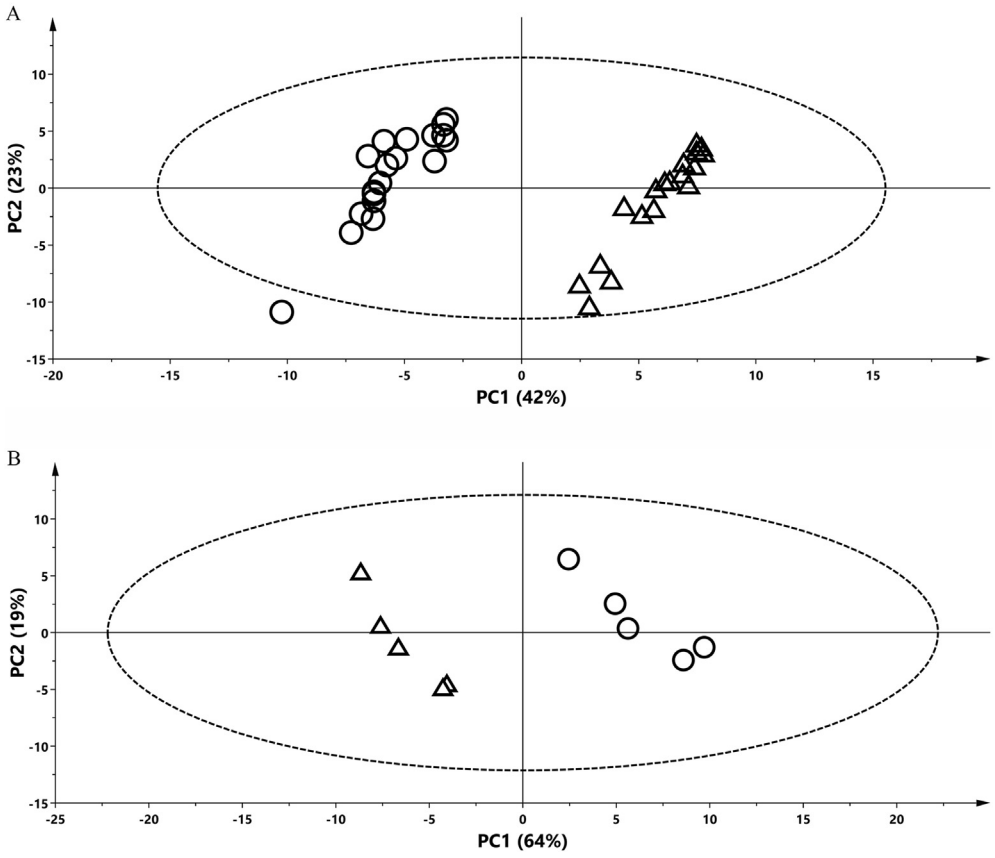

Fig. 2. PC1 and PC2 score plots of (A) PCA of SY and GY samples (5 PCs, R^2Xcum 86%); and (B) PCA of SM and GM samples (3 PCs, R^2Xcum 90%). Circles are relative to sheep samples and triangles to goat samples. The explained variance is in parentheses; the tolerance ellipse is drawn at 95% confidence interval.

R^2Y was 0.99 and the Q^2 was 0.98. Metabolites having VIP >1 were deemed as significantly discriminant (Tables 2 and 3) and subjected to a univariate analysis through the use of the Mann–Whitney U test.

According to the statistical analyses, GY samples compared with SY samples were richer in free amino acids, such as isoleucine, valine, phenylalanine, threonine, serine, methionine, lysine, glutamine/glutamic acid, proline, aspartic acid, together with GABA and pyroglutamic acid. Moreover, GY had higher levels of uracil, β-phenyllactic acid, α-hydroxyisocaproic acid, and creatinine, while SY samples were richer in *N*-acetylglucosamine, *N*-acetylgalactosamine, α-hydroxyisobutyric acid, *myo*-inositol, xylose, ribonic acid, and glucitol. Taken into consideration milk discriminants (Tables 2 and 3), both sheep's milk and yoghurt samples, compared with the

Table 2
PLS-DA discriminant metabolites between sheep yoghurt versus goat yoghurt.[a]

Metabolite	Sheep yoghurt			Metabolite	Goat yoghurt		
	VIP	VIP cvSE	*p*		VIP	VIP cvSE	*p*
N-Acetylglucosamine	1.54	0.13	<0.0001	Isoleucine	1.54	0.13	<0.0001
N-Acetylgalactosamine	1.54	0.13	<0.0001	Valine	1.54	0.12	<0.0001
α-Hydroxyisobutyric acid	1.52	0.16	<0.0001	Phenylalanine	1.54	0.14	<0.0001
Myo-inositol	1.52	0.18	<0.0001	Threonine	1.54	0.14	<0.0001
Xylose	1.51	0.18	<0.0001	Uracil	1.54	0.10	<0.0001
Ribonic acid	1.32	0.21	<0.0001	Serine	1.54	0.14	<0.0001
Glucitol	1.08	0.48	<0.0001	Methionine	1.54	0.18	<0.0001
				Lysine	1.54	0.16	<0.0001
				β-Phenyllactic acid	1.53	0.19	<0.0001
				α-Hydroxyisocaproic acid	1.52	0.16	<0.0001
				Pyroglutamic acid	1.49	0.23	<0.0001
				Glutamine/glutamic acid	1.47	0.14	<0.0001
				Proline	1.47	0.17	<0.0001
				γ-Aminobutyric acid	1.45	0.18	<0.0001
				Aspartic acid	1.42	0.19	<0.0001
				Creatinine	1.33	0.33	<0.0001

[a] VIP cvSE, standard errors of VIP scores in cross validation; *p*-values from the Mann–Whitney U test.

Table 3
PLS-DA discriminant metabolites between sheep milk versus goat milk.[a]

Metabolite	Sheep milk			Metabolite	Goat milk		
	VIP	VIP cvSE	*p*		VIP	VIP cvSE	*p*
Glucitol	1.28	0.32	<0.01	Ribitol	1.27	0.32	<0.01
Threonine	1.27	0.27	<0.01	Glucose	1.27	0.30	<0.01
N-Acetylglucosamine	1.27	0.25	<0.01	Ribonic acid	1.27	0.34	<0.01
N-Acetylgalactosamine	1.27	0.26	<0.01	Succinic acid	1.21	0.30	<0.01
Ribose	1.27	0.26	<0.01	Caprilic acid	1.17	0.14	<0.01
Alanine	1.26	0.24	<0.01	Capric acid	1.10	0.40	<0.01
Gluconic acid	1.26	0.28	<0.01	Phosphate	1.14	0.64	<0.01
Galactose	1.25	0.24	<0.01	Histidine	1.11	0.34	<0.01
Phenylalanine	1.26	0.38	<0.01	Itaconic acid	1.04	0.69	0.03
Lysine	1.25	0.18	<0.01	Talose	1.04	0.34	0.02
Myo-inositol	1.24	0.20	<0.01	Uracil	1.03	0.66	<0.01
Urea	1.23	0.13	<0.01	Azelaic acid	1.02	0.38	0.01
Xylose	1.17	0.49	<0.01	Erythrose	1.02	0.30	0.03
Glutamine/glutamic acid	1.11	0.36	<0.01				

[a] VIP cvSE, standard errors of VIP scores in cross validation; *p*-values from the Mann–Whitney U test.

goats' counterparts, were richer in *myo*-inositol, *N*-acetylgalactosamine and *N*-acetylglucosamine.

4. Discussion

Comparing the metabolites that characterise yoghurt and milk samples (Tables 2 and 3), we found that the metabolite profile of SY did not changed significantly from that of the original milk. On the contrary, GY showed different characterising metabolites than GM, suggesting a more pronounced action of the inoculum on GM than in SM samples. For example, the higher content of free amino acids in GY was not observed in the corresponding milk samples (GM), suggesting a stronger proteolytic activity during the fermentative process which was not observable in the SY samples. Indeed, *L. delbrueckii* subsp. *bulgaricus* hydrolyses milk casein into peptides and essential amino acids, and, in turn *S. thermophilus* uses the amino nitrogen and produces components for *L. delbrueckii* growth, including formic acid, pyruvic acid, folic acid and fatty acids (Shihata & Shah, 2000). GY samples, compared with SY samples, were richer in GABA and glutamic acid. GABA is not an essential nutrient but can participate to several metabolic pathways in humans promoting health by its anti-stress, anti-hypertensive, and anti-diabetic properties (Aryana & Olson, 2017). This production comes from an enzymatic conversion of glutamic acid, which is catalysed by glutamate decarboxylase (Tajabadi et al., 2015). Also, pyroglutamic acid and β-phenyllactic acid levels were found higher in GY compared with SY. Pyroglutamic acid is the product of cyclisation of glutamine by lactic acid bacteria enzymes (Mucchetti, Locci, Massara, Vitale, & Neviani, 2002), it has an antimicrobial activity (Ouwehand & Vesterlund, 2004) and anti-diabetic effect (Yoshinari & Igarashi, 2011). β-Phenyllactic acid is a reaction product of phenylalanine and tyrosine metabolism, with a broad spectrum of antimicrobial properties with activity against bacteria, molds and fungi including yeasts (Chaudhari & Gokhale, 2016). The ability to produce phenyllactic acids has been already reported by Valerio, Lavermicocca, Pascale, and Visconti (2004) in 29 lactobacillus strains belonging to 12 species used in the production of fermented foods. In GY, the higher levels of GABA, pyroglutamic acid and phenyllactic acid, all of them products of enzymatic transformation of amino acids, can be considered a consequence of the stronger proteolysis in GY, with release of free amino acids available to be transformed into other compounds.

In agreement with previous work (Vintila, Marcu, & Vintila, 2011), urea levels were found below the detection limit in yoghurt samples compared with milk samples, and SM was higher in urea than GM. Previous work (Caboni et al., 2019) reported that sheep's milk has higher urea content than goats' milk, moreover it was observed that decrease of time for milk clotting and yoghurt formation is related to concentration of urea in milk (Vintilia et al., 2011). Taking into account this information we can hypothesise that also milk urea levels had a role in determining the different activity of the inoculum in GM and SM, and therefore the different metabolite profiles of the yoghurt counterparts.

GY was found richer in α-hydroxyisocaproic acid and SY of α-hydroxyisobutyric acid. These two metabolites originate from the catabolism of essential dietary branched chain amino acids leucine and valine, respectively. Amino acid catabolism is a major process for flavour formation in cheese, starting from a transamination reaction and the formation of α-keto acids (Yvon & Rijnen, 2001). Furthermore, SM and SY samples, compared with the goat's counterparts, were richer in *myo*-inositol, *N*-acetylgalactosamine and *N*-acetylglucosamine. *myo*-Inositol is a health beneficial compound and, for its role in neonatal nutrition, it is often added in infant formulas to ensure against potential deficiency during early neonatal development (Woollard, Macfadzean, Indyk, McMahon, & Christiansen, 2014). *N*-Acetylgalactosamine and *N*-acetylglucosamine, that have a number of health beneficial properties, have been found in low concentrations in different milk and dairy products (Kim et al., 2015).

The higher presence of saccharides in yoghurt samples compared with milk samples (Table 1 and Fig. 1), can be due to capacity of *L. delbrueckii* and *S. thermophilus* to produce extracellular polysaccharide materials, such as the glucans, or polymers involving glucose, galactose and rhamnose as the constituent sugars (Zeidan et al., 2017).

5. Conclusion

The metabolite profiles of ovine and caprine yoghurts one day post-manufacture showed differences in the levels of amino acids and derived compounds, organic acids and saccharides, suggesting different activity of the inoculum on goats' and sheep's milk. This study confirmed the power of metabolomics for the understanding of the mechanisms that lie beneath dairy production and can help to ameliorate the manufacturing protocols.

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References

Aryana, K. J., & Olson, D. W. (2017). A 100-year review: Yogurt and other cultured dairy products. *Journal of Dairy Science, 100*, 9987–10013.

Boyazoglu, J., & Morand-Fehr, P. (2001). Mediterranean dairy sheep and goat products and their quality: A critical review. *Small Ruminant Research, 40*, 1–11.

Caboni, P., Murgia, A., Porcu, A., Manis, C., Ibba, I., Contu, M., et al. (2019). A metabolomics comparison between sheep's and goat's milk. *Food Research International, 119*, 869–875.

Chaudhari, S. S., & Gokhale, D. V. (2016). Phenyllactic acid: A potential antimicrobial compound in lactic acid bacteria. *Journal of Bacteriology and Mycology: Open Access, 2*, 37.

Domagala, J. (2008). Sensory evaluation and rheological properties of yoghurts prepared from goat, cow and sheep milk. *Electronic Journal of Polish Agricultural Universities, 11*, 4.

Kim, H. J., Bae, I. K., Jeong, M. H., Park, H. J., Jung, J. S., & Kim, J. E. (2015). A new HPLC-ELSD method for simultaneous determination of *N*-acetylglucosamine and *N*-acetylgalactosamine in dairy foods. *International Journal of Analytical Chemistry*, 892486.

Moschopoulou, E., Sakkas, L., Zoidou, E., Theodorou, G., Sgouridou, E., Kalathaki, C., et al. (2018). Effect of milk kind and storage on the biochemical, textural and biofunctional characteristics of set-type yoghurt. *International Dairy Journal, 77*, 47–55.

Mucchetti, G., Locci, F., Massara, P., Vitale, R., & Neviani, E. (2002). Production of pyroglutamic acid by thermophilic lactic acid bacteria in hard-cooked mini-cheeses. *Journal of Dairy Science, 85*, 2489–2496.

Ouwehand, A. C., & Vesterlund, S. (2004). Antimicrobial components from lactic acid bacteria. *Food Science and Technology, 139*, 375–396.

Park, Y. W., Juárez, M., Ramos, M., & Haenlein, G. F. W. (2007). Physico-chemical characteristics of goat and sheep milk. *Small Ruminant Research, 68*, 88–113.

Scintu, M. F., & Piredda, G. (2007). Typicity and biodiversity of goat and sheep milk products. *Small Ruminant Research, 68*, 221–231.

Shihata, A., & Shah, N. P. (2000). Proteolytic profiles of yogurt and probiotic bacteria. *International Dairy Journal, 10*, 401–408.

Song, D., Ibrahim, S., & Hayek, S. (2012). Recent application of probiotics in food and agricultural science. In E. C. Rigobelo (Ed.), *Probiotics*. London, UK: InTechOpen.

Tajabadi, N., Baradaran, A., Ebrahimpour, A., Rahim, R. A., Bakar, F. A., Manap, M. Y. A., et al. (2015). Overexpression and optimization of glutamate decarboxylase in *Lactobacillus plantarum* Taj-Apis362 for high gamma-aminobutyric acid production. *Microbial Biotechnology, 8*, 623–632.

Valerio, F., Lavermicocca, P., Pascale, M., & Visconti, A. (2004). Production of phenyllactic acid by lactic acid bacteria: An approach to the selection of strains contributing to food quality and preservation. *FEMS Microbiology Letters, 233*, 289–295.

Vintila, C., Marcu, A., & Vintila, T. (2011). Milk urea dynamics during its transformation into yogurt. *Scientific Papers Animal Science and Biotechnologies, 44*, 458–460.

Woollard, D. C., Macfadzean, C., Indyk, H. E., McMahon, A., & Christiansen, S. (2014). Determination of myo-inositol in infant formulae and milk powders using capillary gas chromatography with flame ionisation detection. *International Dairy Journal, 37*, 74–81.

Yoshinari, O., & Igarashi, K. (2011). Anti-diabetic effect of pyroglutamic acid in type 2 diabetic Goto-Kakizaki rats and KK-A y mice. *British Journal of Nutrition, 106*, 995–1004.

Yvon, M., & Rijnen, L. (2001). Cheese flavour formation by amino acid catabolism. *International Dairy Journal, 11*, 185–201.

Zeidan, A. A., Poulsen, V. K., Janzen, T., Buldo, P., Derkx, P. M., Øregaard, G., et al. (2017). Polysaccharide production by lactic acid bacteria: From genes to industrial applications. *FEMS Microbiology Reviews, 41*, S168–S200.