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A rapid high-performance liquid chromatography-tandem mass spectrometry assay for unambiguous detection of different milk species employed in cheese manufacturing

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ABSTRACT

The aim of the study was to investigate the possibility to differentiate the 4 most important species in Italian dairy industry (cow, buffalo, sheep, and goat), applying a bottom-up proteomic approach to assess the milk species involved in cheese production. Selective peptides were detected in milk to use as markers in cheese products. Trypsin-digested milk samples of cow, sheep, goat, and buffalo, analyzed by HPLC-tandem mass spectrometry provided species-specific peptides, some of them recognized by Mascot software (Matrix Science Ltd., Boston, MA) as derived from well-known species specific proteins. A multianalyte multiple reaction monitoring method, built with these specific peptides, was successfully applied to cheeses with different composition, showing high specificity in detection of species involved. Neither aging nor production method seemed to affect the response, demonstrating that chosen peptides well act as species markers for dairy products.
Key words: milk, cheese, animal species, HPLC-MS/MS, trypsin, peptides

INTRODUCTION

In recent years, a general trend has been observed in the food industry to characterize manufacturing products by raw material sources, geographic origins, production processes, as well as physicochemical and dietary properties of the finished products. In dairy food production, we have noted a growing number of cheese types, both new and old, once only regionally traded and now widespread in the world market as well. Likewise, besides cow and sheep milk cheeses, it is not unusual to find goat and buffalo products in markets today.

Research of selective markers to highlight species contained in foodstuffs are a challenge for food chemists; to ensure industrial quality control and assurance, official antifraud controls, and consumer safety, useful tools to assess the authenticity of food and proper labelling are needed. For dairy products, this is emphasized by allergenic characteristics of milk products, and there is great interest in developing methods able to check the species of milk used in cheesemaking. The possibility of adding cow milk to sheep or buffalo ricotta (a typical Italian dairy product) through the HPLC determination of β-carotene was investigated by Cerquaglia et al. (2011), but the results showed difficulty detecting small additions depending on the variability of β-carotene content in the various milk species. The detection of fraudulent cow milk additions in ewe milk was carried out via a stereospecific analysis of triacylglycerols by Blasi et al. (2013); a chromatographic detection of dairy product adulteration, based on sterol fractions and on concentration of *trans*-isomers of FA, was also proposed by Chmilenko et al. (2011). It is well-known that milk of different ruminant species contains closely homologous proteins with similar structures and functionality (Wal, 2004); several analytical techniques, such as capillary electrophoresis (Cartoni et al., 1998; Herreo-Martinez et al., 2000), 2-dimensional gel electrophoresis (Yang et al., 2014), PCR (Bai et al., 2009; Drummond et al., 2013), ELISA (Hurley et al., 2004), and isobaric tags for relative and absolute quantitation (Yang et al., 2013), were used to develop methods with this purpose, but the results were partial or unsatisfactory. Better performances were achieved recently with bottom-up proteomics approaches. A matrix-assisted laser desorp-

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Table 1. Composition of cheeses made with sheep, goat, and cow milk

Item	Composition, %			
	Cheese 1	Cheese 2	Cheese 3	Cheese 4
Sheep	78	78	56	33
Goat	11	0	22	33
Cow	11	22	22	33

tion/ionization-time-of-flight mass spectrometry direct analysis of milk tryptic digestion was able to assess adulteration of sheep and goat milk (Calvano et al., 2012) and was used for milk speciation in a quaternary mixture (Cuollo et al., 2010). Liquid chromatography coupled with tandem mass spectrometry (**LC-MS/MS**; Monaci and Visconti, 2009; Guarino et al., 2010; Ansari et al., 2011) is now widely used in separating and recognizing peptides obtained by enzymatic digestion of dairy products. Guarino et al. (2010), for example, detected sheep milk in goat and cow cheeses by analyzing the tryptic digestion of isolated caseins; Ansari et al. (2011) developed a similar method able to detect milk allergens (caseins and lactoglobulins) in food products.

In our work, we investigated the possibility of differentiating the 4 most important species in Italian dairy industry (cow, buffalo, sheep, and goat milk) by detecting selective peptides in their milk that are able to act as markers for milk identification in raw materials and mainly in cheese products. For this aim, a simple and time-saving method for sample preparation was developed, applying a tryptic digestion protocol (Harvey, 2003) directly to milk and cheese. In this way, the protocol is quick and easy: the time for sample preparation depends only on the time required by digestion, few steps are involved, and the defatting solvents are saved. Trypsin-digested milk samples were analyzed by HPLC-MS/MS and the spectra were subjected to a Mascot (www.matrixscience.com; Matrix Science Inc., Boston, MA) search to evaluate, for each milk species, the possibility of detection of compounds with good specificity and sensitivity in a multiple reaction monitoring (**MRM**) experiment, with a profile (mass, charge, and fragmentation) matching a peptide with a specific AA sequence.

The method was evaluated in our laboratory by manufacturing curd cheeses by the use of single- and mixed-species milk samples, following an experimental design based on common manufacturing practices or fraudulent purposes. Commercial samples were also analyzed.

MATERIALS AND METHODS

Materials

Milk. Samples of cow, ewe, goat, and buffalo milk were collected directly from farm bulk milk. Farms were chosen from those with at least 100 animals in lactation. For all species, the tank milk samples were collected from the evening and the following morning milking. Analyses were all performed on raw milk within 72 h of collection.

Cheese. The milk samples were 10 L/species, collected directly on the farms, in the morning, and carried to laboratory using thermostatic containers set at 4 to 5°C. Once in laboratory, milk was warmed to 38 to 49°C and rennet was added at a dose of 20 mL/100 L of milk. Following a rest of 30 min, the curd was cut and transferred in cheese hoops of approximately 300 to 350 g for fresh cheeses and 1 kg for those of 60 d aging. The aging was carried out in rooms set at 10 to 12°C and at 80 to 85% moisture. The analysis portions were collected in the cheese core.

Cheeses were produced from both milk of each individual species (buffalo, cow, sheep, or goat) and from mixed milk prepared in different proportions, and analyzed after 48 to 72 h of production. For multispecies cheese, 2 different experiments were designed: one mixing sheep, goat, and cow milk (cheeses 1–4, Table 1), and one mixing buffalo and cow milk (cheeses 5–8,

Table 2. Composition of cheeses made with buffalo and cow milk

Item	Composition, %			
	Cheese 5	Cheese 6	Cheese 7	Cheese 8
Buffalo	80	50	20	5
Cow	20	50	80	95

Table 3. Single-species products analyzed

Item	Milk	Fresh cheese	2-mo-aged cheese	Mozzarella
Buffalo (100%)	×	×		×
Cow (100%)	×	×	×	
Ewe (100%)	×	×	×	
Goat (100%)	×	×	×	

Table 2). In addition, cheeses from 100% of cow, ewe, and sheep milk were also analyzed after 2 mo of aging; for buffalo cheese this analysis wasn't performed because aged buffalo cheese has no commercial value (Table 3).

Buffalo Mozzarella. Buffalo milk is typically used to make a traditional cheese called mozzarella by a stretching manufacturing method completely different from common cheesemaking processes. Thus, the buffalo mozzarella analysis was necessary to verify buffalo markers response and to exclude interferences due to the presence of peptides eventually produced during the manufacturing method adopted.

In our laboratory, after pasteurization at 72°C for 20 s, the milk (10 L collected on farm) was cooled down to 38 to 40°C and starter fermenters usually used in cheese factories were added. After approximately half an hour, the rennet was added following the producers recommendation. Once the right consistency was reached (30–35 min after the rennet addition), the curd was cut and allowed to rest for about 2 h, until the pH reached values of 4.2 to 4.3. Afterward, the paste was stretched in boiling water (80–85°C) and the product was formed following the classic sphere shape and stored at 4 to 5°C for 2 d until analysis.

Chemicals and Reagents. Liquid chromatography-MS-grade water and acetonitrile were purchased from Fluka (St. Gallen, Switzerland). Glacial acetic acid, formic acid (≥96%), triethyl ammonium bicarbonate (**TEAB**) buffer (1.0 *M*), 1,4-dithiothreitol (**DTT**; ≥99%), and iodoacetamide (≥99%) from Sigma Aldrich (St. Louis, MO), and porcine sequencing-grade modified trypsin with its suspension dilution buffer was purchased from Promega (Madison, WI).

Methods

Enzymatic Digestion. One hundred milligrams of fresh cheese ground into small pieces or 200 mg of milk was suspended in 5 mL of TEAB 100 m*M* and stirred for 2 h at 700 rpm at room temperature with a magnetic stir bar in an uncapped 20-mL vial. Then, 0.5 mL of DTT 50 m*M* was added, mixed by stirring for 2 min, and the obtained suspension was incubated for 15 min at 90°C. The vial was cooled at room temperature for 20 min and 0.5 mL of 100 m*M* iodoacetamide was added, mixed by stirring for 2 min, and stored in a dark place for 15 min. Finally, 120 μL of dairy suspension was mixed with 20 μL of standard trypsin solution (20 μg suspended with 180 μL of their buffer) and incubated overnight at 37°C. The digestion was stopped by adding 20 μL of glacial acetic acid. After storage at −20°C for 1 h, to block any residual enzymatic activity, the suspension was centrifuged at 13,000 × *g* at 5°C for 15 min (Neofuge 13/R, Heal Force Life Science Instruments, Shanghai, China). The supernatant was separated and injected at this concentration for independent data acquisition (**IDA**) experiments or diluted 250 to 500 fold in TEAB for MRM scans.

HPLC. Tryptic-digested samples were separated by HPLC, an 1100 series equipped with a binary pump and autosampler (Agilent, Waldbronn, Germany), using a reversed phase column with a solid core: Accucore C18, 100 × 2.1 mm i.d, 3 μm particle size (Thermo Electron Corporation, Marietta, GA). Chromatographic run was carried out at 0.3 mL/min as the flow rate, with 0.1% aqueous formic acid (A) and acetonitrile (B) as eluents, and with the following gradient pump program: initial 12% B (hold for 2 min), 30% B (within 10 min), 40% B (within 2 min), 70% B (within 1 min), back to 12% B, and equilibration for 8 min. The run time was 17 min. The injection volume was 20 μL.

Electrospray Tandem Mass Spectrometry. The HPLC was coupled with a triple quadrupole-linear ion trap mass spectrometry 4000QTrap (AB Sciex, Framingham, MA), equipped with a Turbo V electrospray ionization (**ESI**) source. For each milk species, an MRM experiment was built selecting peptides with a good chromatographic response and best matching to Mascot search results. Different milk samples of a single species were digested and analyzed 3 times each to confirm the presence and the response of the chosen peptides and to verify the repeatability of the protocol. Then, comparing MRM spectra of different species, species-specific peptides were selected and used to build a multianalyte MRM experiment.

For MRM experiments, the ESI source conditions were set as follows: positive ionization mode, curtain gas, 30 psi; collision gas medium, ion spray voltage, 5,500 V; temperature, 350°C; ion source gas 1, 30 psi; ion source gas 2, 40 psi; declustering potential, 65 V; entrance potential, 10 V; and cell exit potential, 15 V.

Nitrogen was used for gas in all cases. For IDA, criteria we set to carry out in positive polarity: enhanced MS (**EMS**), enhanced resolution to confirm charge state or isotopes, and enhanced product ion (**EPI**) for the 3 most intense peaks per scan. The EMS scan was set for ion greater than 350 m/z and smaller than 1,250 m/z, with a charge state from 2 to 4, and run with a scan rate of 4,000 Da/s and a linear ion trap fill time of 20 ms; similar parameters, with 250 Da/s as scan rate, were set for enhanced resolution scan. Each EPI experiment was acquired at scan rate of 4,000 Da/s. For all experiments, source sets were: positive polarity, curtain gas at 20 psi, collision gas high, ion spray voltage at 5,500 V, temperature at 350°C, ion source gas 1 and ion source gas 2 at 30 psi, and declustering potential at 70 V (only for EMS). Data acquisition and processing were performed using Analyst 1.6 software (from AB Sciex, Concord, Canada).

Mascot Search. Trypsin digested milk samples were analyzed by HPLC-MS/MS performing an IDA experiment; then the full scan spectra were subjected to a Mascot search (Matrix Science Inc.) to check if for matching between ions from experimental MS/MS data and tabulated fragmentation of peptides expected in the database at the same experimental conditions. We made this choice manually, favoring peptides characterized by clear fragmentation and high sensitivity in EPI scan.

The Mascot engine was set specifying NCBInr (National Center for Biotechnology Information, Bethesda, MD) as the database; MS/MS Ion as type of search; trypsin as enzyme; carbamidomethyl as fixed modification; monoisotopic as mass value; unrestricted as protein mass; 0.3 Da as peptide tolerance; 0.6 Da as fragment mass tolerance; 1 as missed cleavage; and ESI-trap as instrument.

RESULTS AND DISCUSSION

Several proteomic studies were focused on detection of cow milk as adulterant of goat milk (Chen et al., 2004), buffalo milk (Czerwenka et al., 2010), sheep and buffalo milk (Cozzolino et al., 2001), and sheep and goat milk (Müller et al., 2008); in other cases was investigated the possibility of detecting sheep milk as adulterant in goat and cow cheeses (Guarino et al., 2010), or goat milk as adulterant in camel, yak and buffalo milk (Yang et al., 2014). Our project aimed to find a rapid method able to highlight the simultaneous presence of 4 most important species in Italian dairy industry—cow, buffalo, sheep, and goat milks—in milk mixture or cheeses thanks to specific markers.

At the beginning of the study, we applied a 2-step pretreatment (vacuum drying and defatting with petroleum ether) before digesting fresh milk and cheese samples with trypsin. We observed later that drying and defatting were not necessary to obtain satisfactory sensitivity and sensibility, and the digestion was not negatively affected by large amounts of water in milk or fat in cheese; thus, we developed a new digestion protocol, applying the tryptic digestion directly to milk or cheese, resulting in a procedure that was faster than those previously reported, where a preliminary protein extraction step was always performed (Czerwenka et al., 2010; Guarino et al., 2010; Ansari et al., 2011).

A multianalyte MRM experiment was built with species-specific peptides found in single-species trypsin-digested milk: 1 peptide for goat, 4 for sheep, 2 for buffalo, and 4 for cow (Table 4). The αS1-CN gi|311943 belonging to *Capra hircus* (Table 4) and recognized by Mascot matching as a precursor of tryptic peptide YLGYLEQLLK chosen as a goat marker, was previously highlighted but only used to determine camel milk adulteration (Yang et al., 2014). Chromatograms of 4 trypsin-digested milk species, shown in Figure 1, confirm the method specificity. The AA sequence was recognized, in most cases, as part of specific proteins for the species analyzed; in other cases, the peptide was unassigned to a protein and the engine only indicated some matching, shown in brackets in Table 4, compatible with an AA sequence (matching of fragmentation). This fact could depend on several reasons, such as a Mascot search parameters (database version, peptides modifications, and so on) or experimental conditions, but we did not examine this aspect in depth because our purpose was to find peptides able to be species-specific markers, not their unambiguous identification. In any case, the mass-to-charge ratio data (compatible molecular weight, polycharge), and especially the fragmentation pattern of chosen markers, give high confidence that they belong to a peptide family and that they are not generic biomolecules; in fact, the Mascot engine recognized their MS/MS spectrum as compatible with an AA and with a carbamidomethyl modification, as expected for a tryptic peptide.

Chosen peptides can run as species-specific markers not only in milk, but also in cheese, and are not affected by the cheesemaking process or ripeness. In fact, when this multianalyte MRM method was applied to produced cheeses made with single-species milk (fresh curd cheeses and 3-mo-old cheeses for cow, sheep, and goat, fresh curd cheese and mozzarella for buffalo), the spectra showed unambiguously only species-specific markers. Analysis of homemade multispecies cheeses, designed on the basis of manufacturing practices and fraudulent purposes, highlights the ability of this method to detect real fingerprints of milk used; in all cheeses tested, only peptide markers of milk employed

Table 4. Multianalyte multiple reaction monitoring experiment for goat, sheep, buffalo, and cow[1]

	Marker					Mascot matching		
Item	Q1, *m/z*	Z, Q1	Q3, *m/z*	Time, min	CE	Peptide match	Peptide fragmentation	Protein match
Goat	620.3	2		15.1	34	YLGYLEQLLK		gi|311943 αS1-casein [*Capra hircus*]
			963.6				y	
			743.6				y	
			445.6				y*++	
			249.2				b++	
			277.3				b	
			373.4				y	
			501.7				y	
			334.2				b	
Sheep	622.8	2		1.9	34	TPEVDNEALEK		gi|165839 β-lactoglobulin [*Ovis* spp.]
			917.4				y	
			818.4				y	
			703.6				y	
			460.4				y	
			328.2				b	
			389.3				y	
	974.3	2		12.6	50	(NDLLTENVPYCDAPAQK)		Unassigned
			696.6				y++	
			315.1				y++	
			450.5				b++	
			580.6				b++	
	878.4	2		13.4	46	(ALVVGGAGFVGSHLCRR)		Unassigned
			772.6				b	
			464.5				b++	
			284.2				b	
			249.2				b++	
	761.6	U		15.3	41	(NLTSIVSLHQVNIAREFGER)		Unassigned
			547.3				b++	
			319.2				y++	
			398.2				b^0	
Buffalo	716.7	2		6.6	38	(AFKPTELGEVITK)		Unassigned
			460.7				y	
			888.5				y	
			361.2				y	
			759.6				y	
			589.7				y	
	706.3	3		9.3	39	(QWQNARAAMGHENVVIHR)		unassigned
			428.4				b++	
			279.2				b++	
Cow	549.8	2		4.1	31	AMKPWIQPK		gi|27806963 Alfa-S2-casein precursor [*Bos taurus*]
			768.5				y	
			671.5				y	
	830.9	2		11.0	44	(MSHLVLSNVGISFTR)		Unassigned
			681.5				b	
			568.6				b	
			882.6				b	
			356.5				b	
			779.7				y	
			680.6				y	
	976.4	2		11.4	50	(STDSLQGNGRWPAKPPIK)		Unassigned
			785.3				b^0	
			882.5				y++	
			391.2				b	
			304.2				b	
			504.3				b	
	1,112.0	2		14.8	57	(EVVSSTALTDAIHALRTPEGR)		Unassigned
			594.6				b++	
			337.6				b++	
			444.7				b++	
			251.6				b++	

[1]Q1, *m/z* = ratio mass/charge of ion (precursor) generated in Q1, first quadrupole; Z, Q1 = charge of precursor ion; Q3, *m/z* = ratio mass/charge of ion (product) generated in Q3, third quadrupole, by fragmentation of precursor ion; CE = collision energy; Peptide match = amino acidic composition of peptide well matching marker characteristics; Peptide fragmentation = product ions, with the same (Q3) *m/z* of marker, obtained by cleavage of amido-bond, with the charge retained on N-terminus (b) or on C-terminus (y); Protein match = when indicated, the protein able to produce matched peptides under set conditions.

for their manufacturing were detected, as shown in Figure 2A for a goat-sheep-cow mix and in Figure 2B for a buffalo-cow mix. In our study, detection limits were not investigated; however, the protocol applied to experimentally designed cheeses was able to detect a single species milk at the lower concentrations commonly used in a mixture for technological and organoleptic improvement or often required to justify a fraudulent purpose (Tables 1 and 2).

Despite the few cheese samples suitable for each species, we tried to find a linear fit between quantitative composition and analytical response of any peptide transitions. For cheeses made with a buffalo-cow milk mix, the MRM transition 830.864/779.7 (Q1/Q3 m/z) of a cow peptide showed a good correlation (r = 0.9913), as shown in Figure 3A. For cheeses made with a mix of cow, sheep, and goat milk, the better correlation results are summarized in Table 5 and shown in Figures 3B–D. These preliminary results show the promising potential of this method from a quantitative point of view, making future studies worthwhile.

CONCLUSIONS

In this work we applied a bottom-up proteomic approach to assess the milk species involved in cheese

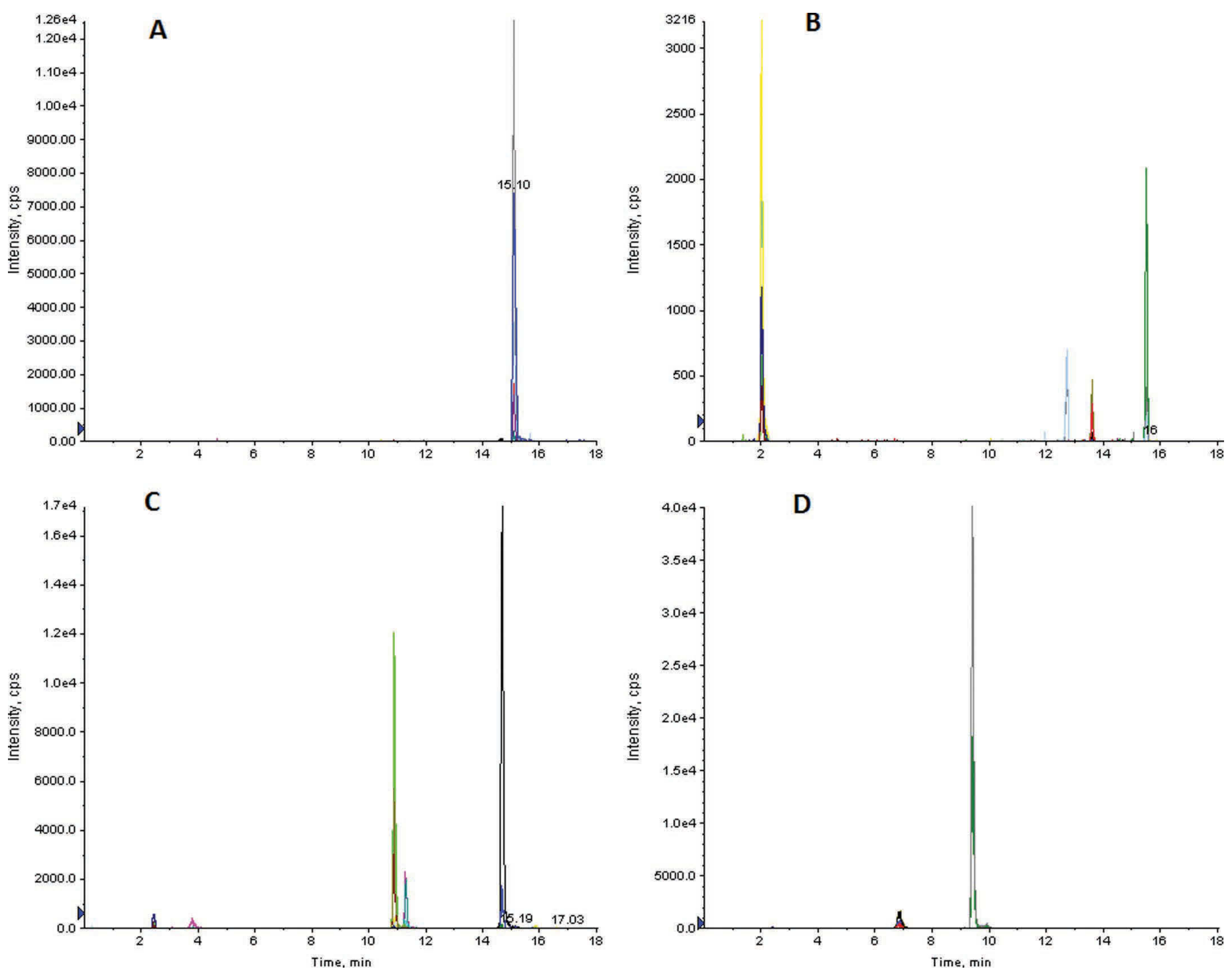

Figure 1. Extracted ion chromatograms of the multianalyte multiple reaction monitoring (MRM) experiment for goat (A), sheep (B), cow (C), and buffalo milk (D), resulting from the tryptic digestion protocol we developed. The peak at 2.4 min is not a marker but a peptide common to cow and buffalo with a positive response to first sheep marker transitions not screened in our multianalyte MRM experiment. Color version available online.

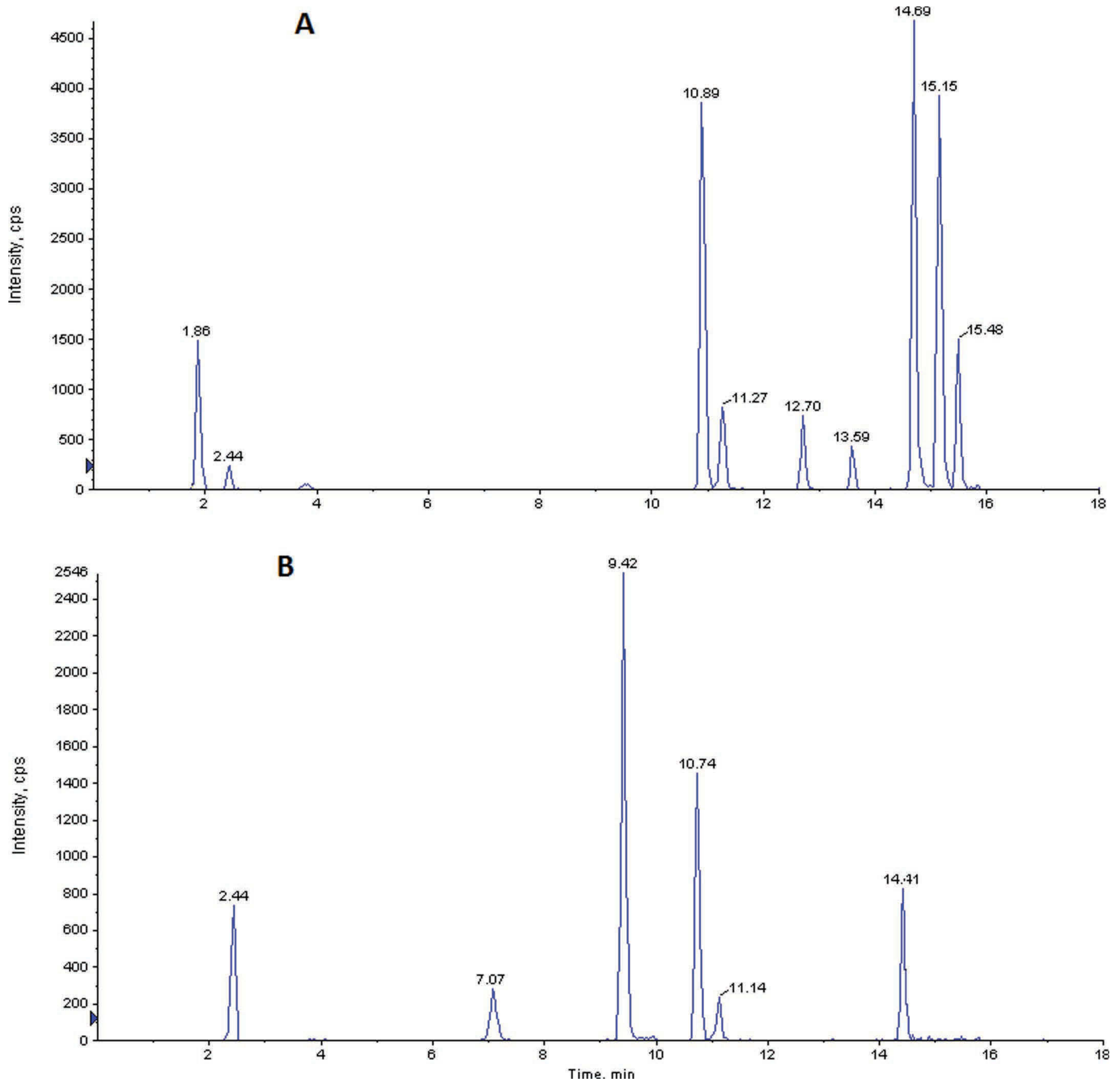

Figure 2. Liquid chromatography-MS/MS chromatograms obtained from the analysis of tryptic-digested cheese manufacturing with different milk composition: 33% sheep, 33% cow, and 33% goat (chromatogram A, corresponding to cheese 4); as well as 80% buffalo and 20% cow (chromatogram B, corresponding to cheese 5). Refer to Figure 1 for information on the peak at 2.4 min. Color version available online.

production. For this purpose, we developed an easy digestion protocol where whole milk and cheese were subject to trypsin digestion, after DTT reduction and iodoacetamide alkylation, without drying and defatting pretreatments, thus saving time and solvents. Trypsin-digested samples of cow, sheep, goat, and buffalo milk, analyzed by HPLC-MS/MS, provided species-specific peptides, some of them recognized by Mascot as derived from well-known species-specific proteins. A multianalyte MRM method, built with these specific peptides, was successfully applied to cheeses with different composition, showing high specificity in detec-

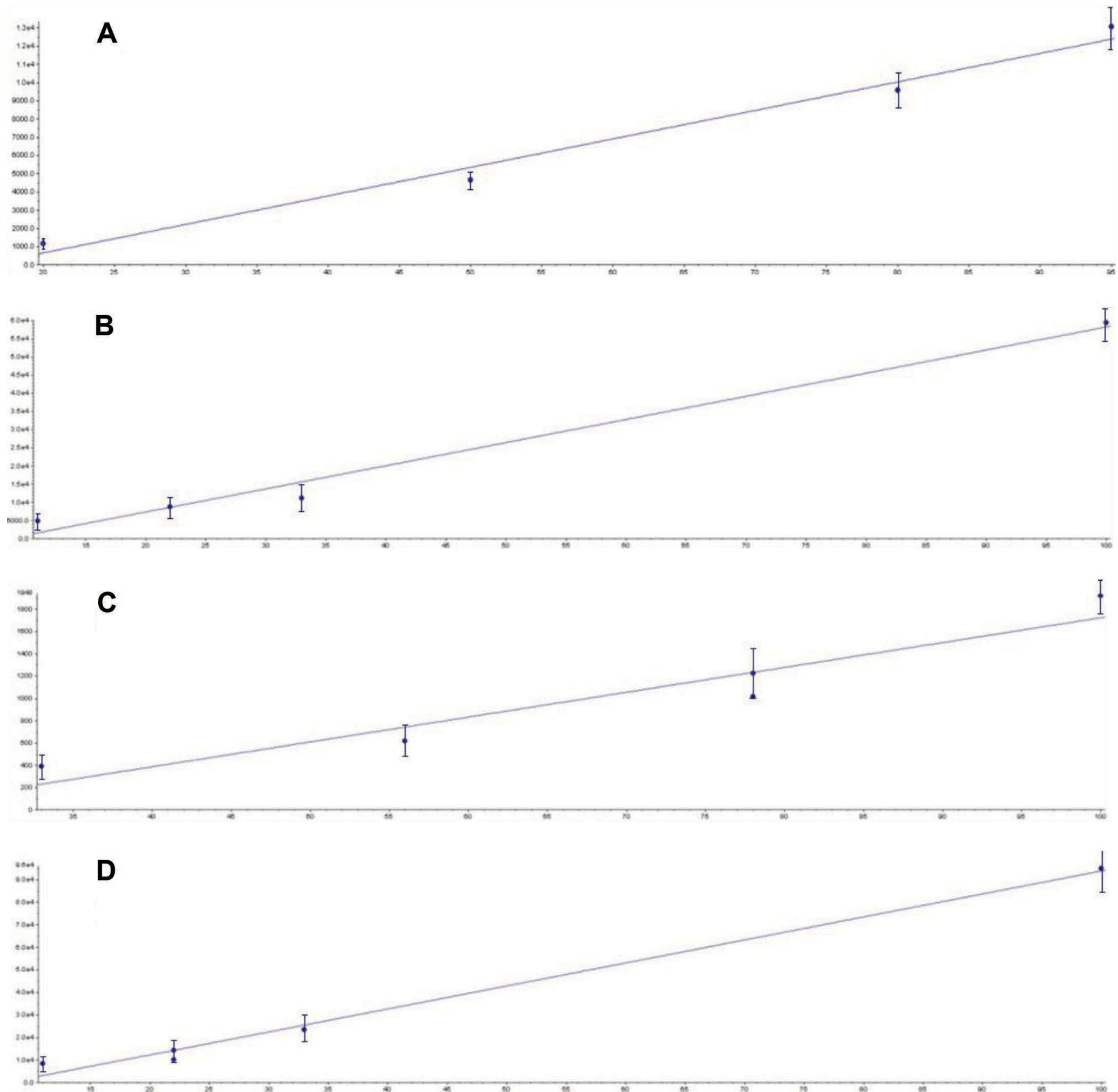

Figure 3. Correlation study between cheese composition and response for the selected multiple reaction monitoring transitions: (A) cow and buffalo milk (830.864/779.7); (B) sheep, goat, and cow milk (622.807/389.3); (C) sheep, goat, and cow milk (620.301/249.21); and (D) sheep, goat, and cow (1112.017/594.6). Color version available online.

tion of species involved. Neither aging nor production method seems to affect the response, demonstrating that chosen peptides act as species markers for dairy products. A preliminary quantitative test was run with promising results, as some specific MRM peptide transitions showed good correlation between percentage of species milk used in cheese manufacturing and chromatographic response. Beyond that aspect, which will be investigated further, the MRM multianalyte method developed can be used as a powerful and easy tool to assess the true species milk composition of cheese products.

Table 5. Cheese from sheep, goat, and cow milk: correlation between multiple reaction monitoring transitions response and composition

Item	Species percentage,[1] %				Q1/Q3	r
Sheep	100	78[2]	56	33	620.301/249.2	0.9920
Goat	100	33	22	11	622.807/389.3	0.9544
Cow	100	33	22[2]	11	1,112.017/594.6	0.9955

[1]The amount of single species milk in experimental cheeses manufacturing as shown in Table 1.

[2]The same species percentage occurs in 2 experimental cheeses.

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REFERENCES

Ansari, P., N. Stoppacher, J. Rudolf, R. Schuhmacher, and S. Baumg. 2011. Selection of possible marker peptides for the detection of major ruminant milk proteins in food by liquid chromatography-tandem mass spectrometry. Anal. Bioanal. Chem. 399:1105–1115.

Bai, W. L., R. H. Yin, S. J. Zhao, Q. L. Dou, J. C. Yang, and W. Q. Jiang. 2009. Rapid detection of bovine milk in yak milk using a polymerase chain reaction technique. J. Dairy Sci. 92:1354–1360.

Blasi, F., G. Lombardi, P. Damiani, M. S. Simonetti, L. Giua, and L. Cossignani. 2013. Triacylglycerol stereospecific analysis and linear discriminant analysis for milk speciation. J. Dairy Res. 80:144–151.

Calvano, C. D., C. De Ceglie, A. Monopoli, and C. G. Zambonin. 2012. Detection of sheep and goat milk adulterations by direct MALDI-TOF MS analysis of milk tryptic digests. J. Mass Spectrom. 47:1141–1149.

Cartoni, G. P., F. Coccioli, R. Jasionowska, and M. Masci. 1998. Determination of cow milk in buffalo milk and buffalo mozzarella cheese by electrophoresis of the whey protein fractions. Ital J Food Sci. 10:127–135.

Cerquaglia, O., M. Sottocorno, L. Pellegrino, and M. Ingi. 2011. Detection of cow's milk, fat or whey in ewe and buffalo ricotta by HPLC determination of β-carotene. Ital J Food Sci. 23:367–372.

Chen, R. K., L. W. Chang, Y. Y. Chung, M. H. Lee, and Y. C. Ling. 2004. Quantification of cow milk adulteration in goat milk using high performance liquid chromatography with electrospray ionization spectrometry. Rapid Commun. Mass Spectrom. 18:1167–1171.

Chmilenko, F. A., N. P. Minaeva, and L. P. Sidorova. 2011. Complex chromatographic determination of the adulteration of dairy products: A new approach. J. Anal. Chem. 66:572–581.

Cozzolino, R., S. Passalacqua, S. Salemi, P. Malvagna, E. Spina, and D. Garozzo. 2001. Identification of adulteration in milk by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. J. Mass Spectrom. 36:1031–1037.

Cuollo, M., S. Caira, O. Fierro, G. Pinto, G. Picariello, and F. Addeo. 2010. Toward milk speciation through the monitoring of casein proteotypic peptides. Rapid Commun. Mass Spectrom. 24:1687–1696.

Czerwenka, C., L. Müller, and W. Lindner. 2010. Detection of the adulteration of water buffalo milk and mozzarella with cow's milks by liquid chromatography-mass spectrometry analysis of β-lactoglobulin variants. Food Chem. 122:901–908.

Drummond, M., B. S. A. F. Brasil, L. S. Dalsecco, R. S. A. F. Brasil, L. V. Teixeira, and D. A. A. Oliveira. 2013. A versatile real-time PCR method to quantify bovine contamination in buffalo products. Food Contr. 29:131–137.

Guarino, C., F. Fuselli, A. La Mantia, L. Longo, A. Faberi, and R. M. Marianella. 2010. Peptidomic approach, based on liquid chromatography electrospray ionization tandem mass spectrometry, for detecting sheep's milk in goat's and cow's cheeses. Rapid Commun. Mass Spectrom. 24:705–713.

Harvey, D. J. 2003. Methods in Molecular Biology. Vol. 211. Protein Sequencing Protocols, 2nd ed. Humana Press Inc., Totowa, NJ.

Herreo-Martínez, J. M., E. Simo-Alfonso, G. Ramis-Ramos, C. Gelfi, and P. G. Righetti. 2000. Determination of cow's milk and ripening time in nonbovine cheese by capillary electrophoresis of the ethanolewater protein fraction. Electrophoresis 21:633–640.

Hurley, I. P., R. C. Coleman, H. E. Irelan, and J. H. Williams. 2004. Measurement of bovine IgG by indirect competitive ELISA as a means of detecting milk adulteration. J. Dairy Sci. 87:543–549.

Monaci, L., and A. Visconti. 2009. Mass spectrometry-based proteomics methods for analysis of food allergens. TrAC. Trends Analyt. Chem. 28:581–591.

Müller, L., P. Barták, P. Bednář, I. Fryšová, J. Ševčík, and K. Lemr. 2008. Capillary electrophoreis-mass spectrometry—A fast and reliable tool for the monitoring of milk adulteration. Electrophoresis 29:2088–2093.

Wal, J. M. 2004. Bovine milk allergenicity. Ann. Allergy Asthma Immunol. 93(Suppl. 3):S2–S11.

Yang, Y., D. Bu, X. Zhao, P. Sun, J. Wang, and L. Zhou. 2013. Proteomic analysis of cow, yak, buffalo, goat and camel milk whey proteins: Quantitative differential expression patterns. J. Proteome Res. 12:1660–1667.

Yang, Y., N. Zheng, J. Yang, D. Bu, J. Wang, L. Ma, and P. Peng Sun. 2014. Animal species milk identification by comparison of two-dimensional gel map profile and mass spectrometry approach. Int. Dairy J. 35:15–20.