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A gas chromatography-mass spectrometry-based metabolomic approach for the characterization of goat milk compared with cow milk

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ABSTRACT

In this work, the polar metabolite pool of commercial caprine milk was studied by gas chromatography-mass spectrometry and multivariate statistical data analysis. Experimental data were compared with those of cow milk and the discriminant analysis correctly classified milk. By the same means, differences due to heat treatments (UHT or pasteurization) on milk samples were also investigated. Results of the 2 discriminant analyses were combined, with the aim of finding the discriminant metabolites unique for each class and shared by 2 classes. Valine and glycine were specific to goat milk, talose and malic acid to cow milk, and hydroxyglutaric acid to pasteurized samples. Glucose and fructose were shared by cow milk and UHT-treated samples, whereas ribose was shared by pasteurized and goat milk. Other discriminant variables were not attributed to specific metabolites. Furthermore, with the aim to reduce food fraud, the issue of adulteration of caprine milk by addition of cheaper bovine milk has been also addressed. To this goal, mixtures of goat and cow milk were prepared by adding the latter in a range from 0 to 100% (vol/vol) and studied by multivariate regression analysis. The error in the level of cow milk detectable was approximately 5%. These overall results demonstrated that, through the combined approach of gas chromatography-mass spectrometry and multivariate statistical data analysis, we were able to discriminate between milk typologies on the basis of their polar metabolite profiles and to propose a new analytical method to easily discover food fraud and to protect goat milk uniqueness. The use of appropriate visualization tools improved the interpretation of multivariate model results.

Key words: goat milk, gas chromatography-mass spectrometry, metabolomics, heat treatment, food fraud

INTRODUCTION

Goat (*Capra hircus*) milk and related dairy products have nowadays gained a valuable industry niche (Dubeuf et al., 2004). Many commercial parameters indicate that the diffusion of goat dairy products is increasing as whole milk, fermented milk derivatives, dried or evaporated milk, and for the production of cheese. The nutritional and health benefits of goat milk are of the utmost relevance for people affected by food allergies, with bovine milk proteins the dominant food cause. Although controversial, superior digestibility of goat milk compared with cow milk, attributed to the higher content of the α_{s2}-CN variant rather than α_{s1}-CN, lower naturally homogenized fat globule size, and the higher proportion of medium-chain triacylglycerols has been commonly accepted. Despite the huge number of reports on its nutraceutical properties (Silanikove et al., 2010; Ceballos et al., 2009), the use of goat milk for nutraceutical needs still deserves in-depth discussion and documentation (Haenlein, 2004). Milk is a very complex mixture of several components in different physical states. Milk composition is influenced by a range of different factors (e.g., diet, genetics, number and stage of lactation, seasonal variation, SCC, and milk processing; Goetsch et al., 2011). These factors may have remarkable quantitative effects on milk nutrients as well as on the physical and technological properties of milk (e.g., coagulation properties, heat stability, and fermentation quality of the milk; Dubeuf et al., 2004). Whereas lipids and lactose are the 2 major caloric nutrients, milk also contains a wide variety of bioactive compounds, including immunoglobulins and other immune proteins, peptides, nucleotides, oligosaccharides, and metabolites (Raynal-Ljutovac et al., 2008; Sundekilde et al., 2013). Sugars, free amino acids, organic acids, and other low-molecular-weight compounds compose the metabolite pool of milk. The different origin and sources of these compounds contribute to the variability of milk metabolite profiles. Milk metabolites often reflect metabolic activity in the mammary gland or metabolism in the whole organism, or both; they may also originate from enzymatic reactions or from microorganisms present in

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raw milk, or both (Sundekilde et al., 2013). Moreover, before entering the market, milks undergo different heat treatments that determine their commercial value and their quality; these treatments can modify the overall metabolite composition of milk. It was found that levels of monosaccharides in milk change because of thermal processing (Mendoza et al., 2005) and during storage (Troyano et al., 1996). The characterization of the metabolite profile in a biological matrix is well performed by metabolomics; this science is based on the use of analytical methods, such as GC-MS (Marincola et al., 2012), nuclear magnetic resonance (Locci et al., 2011), and direct analysis in real time-mass spectrometry (Hrbek et al., 2014), coupled with multivariate statistical data analysis (**MVA**). Metabolomic studies have been extensively applied in the areas of nutrition sciences and food matrices, such as milk (Chen et al., 2004; Boudonck et al., 2009; Klein et al., 2010; Marincola et al., 2012; Harzia et al., 2013; Sundekilde et al., 2013; Hrbek et al., 2014).

The risk linked to food fraud is increasing due to the global and composite nature of food supply chains. With the aim to reduce this risk and in view of detecting economically driven adulterations, in this study, the issue of adulteration of caprine milk by the addition of cheaper bovine milk was addressed. The commercial value of goat milk is much higher than that of cow milk due to lower productivity and little market demand; therefore, the addition of cow milk to goat milk can allow economic advantages and becomes a fraud when the mixture is sold with label. Taking into account this option, in this paper, an attempt to assess whether the metabolomic approach could be suitable tool for discovering such fraud was carried out. The literature reports several attempts to find suitable methods to detect milk adulteration. Quantification of cow milk adulteration of goat milk, based on solvent separation of whey proteins, followed by HPLC with electrospray ionization mass spectrometry, was performed by Chen et al. (2004); levels as low as 5% of cow milk were detected. Levieux and Venien (1994) proposed an ELISA to detect cow β-LG at 5 ng/mL. Antonilli et al. (2005), by inspection of the ratios of some FAME, offered some parameters suitable for discovering such fraud. Proton nuclear magnetic resonance low-molecular-weight metabolite fingerprinting was applied for the quantification of the relative amount of cow and sheep milk in mixtures (Lamanna et al., 2011).

In this work, for the first time, the goat milk metabolite profile, composed by polar and hydrophilic low-molecular-weight compounds, was characterized by the means of GC-MS and compared, through the application of discriminant multivariate analysis, with cow milk. Differences in milk metabolite profiles correlated with heat treatments: UHT and pasteurization were also investigated. Moreover, milk mixtures of goat milk with increasing quantities of cow milk were prepared and their GC-MS metabolic profiles used to construct a suitable model, based on orthogonal projections to latent structures (**OPLS**) regression, to detect adulteration of goat milk with cow milk.

MATERIALS AND METHODS

Chemicals and Reagents

Methanol, chloroform, hexane, pyridine, methoxamine hydrochloride, potassium chloride, *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide, lactic acid, valine, butyric acid, urea, glycine, succinic acid, fumaric acid, serine, malic acid, proline, alanine, creatinine, glutamine, phosphoric acid, fructose, glucose, galactose, gluconic acid, palmitic acid, inositol and stearic acid were purchased from Sigma-Aldrich (Milan, Italy). Bidistilled water was obtained from a Milli-Q purification system (Millipore S.p.A., Milan, Italy) before use.

Samples

Seventeen commercial samples of goat whole milk (**G1**–**G17**) and 14 samples of cow whole milk (**C1**–**C14**) were acquired in local markets; all samples were within the expiration date. Seventeen milk samples were subjected to UHT and 14 samples were subjected to pasteurization processes. Furthermore, 9 mixtures were prepared by adding different aliquots (%, vol/vol) of cow milk to goat milk as follows: 0, 5, 10, 20, 40, 50, 60, 80, and 100%.

Extraction and Derivatization

To obtain rupture of the milk micelles, 15 mL of sample was sonicated for 15 min; 100 μL of milk was transferred to an Eppendorf tube and then 250 μL of methanol and 125 μL of chloroform were added. Samples were vortexed every 15 min 4 times and then 380 μL of chloroform and 90 μL of aqueous 0.2 *M* potassium chloride were added. The suspension was centrifuged at 13,572 × *g* for 10 min at 4°C. After centrifugation, the aqueous layer was transferred to a glass vial and dried by a gentle nitrogen stream and derivatized with 50 μL of pyridine containing methoxamine hydrochloride at 10 mg/mL. After 17 h, 100 μL of *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide was added and after 1 h, samples were resuspended with 600 μL of hexane.