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Factors affecting variations in the detailed fatty acid profile of Mediterranean buffalo milk determined by 2-dimensional gas chromatography

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ABSTRACT

Buffalo milk is the world's second most widely produced milk, and increasing attention is being paid to its composition, particularly the fatty acid profile. The objectives of the present study were (1) to characterize the fatty acid composition of Mediterranean buffalo milk, and (2) to investigate potential sources of variation in the buffalo milk fatty acid profile. We determined the profile of 69 fatty acid traits in 272 individual samples of Mediterranean buffalo milk using gas chromatography. In total, 51 individual fatty acids were identified: 24 saturated fatty acids, 13 monounsaturated fatty acids, and 14 polyunsaturated fatty acids. The major individual fatty acids in buffalo milk were in the order 16:0, 18:1 *cis-9*, 14:0, and 18:0. Saturated fatty acids were the predominant fraction in buffalo milk fat (70.49%); monounsaturated and polyunsaturated fatty acids were at 25.95 and 3.54%, respectively. Adopting a classification based on carbon-chain length, we found that medium-chain fatty acids (11–16 carbons) represented the greater part (53.7%) of the fatty acid fraction of buffalo milk, whereas long-chain fatty acids (17–24 carbons) and short-chain fatty acids (4–10 carbons) accounted for 32.73 and 9.72%, respectively. The n-3 and n-6 fatty acids were 0.46 and 1.77%, respectively. The main conjugated linoleic acid, rumenic acid, represented 0.45% of total milk fatty acids. Herd/test date and stage of lactation were confirmed as important sources of variation in the fatty acid profile of buffalo milk. The percentages of short-chain and medium-chain fatty acids in buffalo milk increased in early lactation (+0.6 and +3.5%, respectively), whereas long-chain fatty acids decreased (−4.2%). The only exception to this pattern was butyric acid, which linearly decreased from the beginning of lactation, confirmation that its synthesis is independent of malonyl-CoA. These results seem to suggest that in early lactation the mobilization of energy reserves may have less influence on the fatty acid profile of buffalo milk than that of cow milk, probably due to a shorter and less severe period of negative energy balance. Parity affected the profiles of a few traits and had the most significant effects on branched-chain fatty acids. This work provided a detailed overview of the fatty acid profile in buffalo milk including also those fatty acids present in small concentrations, which may have beneficial effects for human health. Our results contributed also to increase the knowledge about the effects of some of the major factors affecting buffalo production traits and fatty acid concentrations in milk, and consequently its technological and nutritional properties.
Key words: Mediterranean buffalo, milk fatty acid, 2-dimensional gas chromatography, sources of variation

INTRODUCTION

Buffaloes (*Bubalus bubalis*) are the world's the second largest source of milk, producing 102 billion liters each year (13.3% of all milk produced vs. 636 billion liters of cow milk, 82.7% of the total) (FAOSTAT, 2013). Buffalo milk is a rich source of nutrients and therefore plays an important role in human nutrition, particularly in developing countries (e.g., ∼70% of buffalo milk is produced in India; FAOSTAT 2013). Indeed, buffalo milk has higher contents of protein and fat (Ahmad et al., 2013) and minerals (Stocco et al., 2016) than cow milk, whereas some specific classes of gangliosides seem to be only present in buffalo milk (Colarow et al., 2003). The high fat content of buffalo milk makes it also highly suitable for processing (Menard et al., 2010), and in developed countries it is mainly used for the production of a variety of foodstuffs, such as butter, butter oil (ghee), soft and hard cheeses, condensed and evaporated milk, ice cream, yogurt, buttermilk, and in Italy, the highly popular buffalo mozzarella obtained under the European Union's protected designation of origin scheme.

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Buffalo dairy farms in Italy are traditionally concentrated in the Campania region where the protected designation of origin cheese "Mozzarella di Bufala Campana" is produced. Recently, however, the demand for buffalo mozzarella has greatly increased, resulting in increasing numbers of buffaloes (Hanaa et al., 2015) and considerable expansion in buffalo farming even into the northern regions of Italy (Tiezzi et al., 2009).

Over the last decade, many studies have been done on the fatty acid composition of bovine milk fat (**MF**) due to its potential beneficial or negative effects on human health. For instance, CLA has been shown to have anticarcinogenic, antiobesity, antidiabetic, and antihypertensive properties in humans (Koba and Yanagita, 2014), as well as benefic effects for reproductive performance and carcass traits in ruminants (Mir et al., 2000). Furthermore, the effects of the large groups of fatty acids (saturated vs. unsaturated, n-3 vs. n-6, and so on) on the cardiovascular system have been challenged by recent meta-analyses (Siri-Tarino et al., 2010; de Souza et al., 2015). Indeed, more attention has being paid to the effect of individual fatty acids on human health and disease instead of macro-categories, and novel roles for specific fatty acids have been (and probably will be) proposed. Thereby, it would be desirable to obtain a comprehensive profile of fatty acids in the various food matrices (e.g., milk), including those less known, present in small concentrations, or both.

To our knowledge, the available studies aimed at evaluating the fatty acid profile of buffalo milk have been carried out on a relatively small number of animals (e.g., Menard et al., 2010; Zotos and Bampidis, 2014) or considered only the most representative and well-known fatty acids groups and individual fatty acids (e.g., Tonhati et al., 2011).

Therefore, the aims of this work were (1) to determine by GC × GC (2-dimensional GC) the detailed milk fatty acid profile in a large number of Mediterranean buffaloes; and (2) to evaluate potential sources of variation affecting the profiles of the identified milk fatty acid traits.

MATERIALS AND METHODS

Animals and Sampling

Milk samples were collected once during the evening milking from 272 buffaloes reared under intensive farming conditions in 6 herds located in northern Italy from January to May 2013. Farms and facilities were comparable to those characterizing the dairy herds in northern Italy with open barns, loose animals, and milking parlors. Buffaloes were fed year-round TMR based on corn silage, cereals, grass hay, wheat straw, and protein meals, supplemented with vitamins and minerals [i.e., the same ingredients of the TMR diets of dairy cows reared in a the same geographical area, the Po valley (Dal Maso et al., 2009)]. The number of animals sampled from each herd were 81, 31, 30, 60, 30, and 40. Animals were selected from each herd to represent all lactation stages and a range of parities. Each buffalo was given a BCS by a trained operator, according to Edmonson et al. (1989). The milk samples (no preservative was added) were immediately refrigerated at 4°C and transferred to the Cheese-Making Laboratory of the Department of Agronomy, Food, Natural Resources, Animals and Environment of the University of Padua (Legnaro, Padua, Italy).

Data on the buffaloes, herds, and single test/day milk yields (**MY**) were provided by the breeders' associations of the Veneto and Friuli Venezia-Giulia regions.

Lipid Extraction and Gas Chromatography Analysis

Milk fat was extracted from milk subsamples (5 mL) by accelerated solvent extraction (ASE 200, Dionex Corp., Sunnyvale, CA). After thawing, samples were homogenized using a Hydromatrix (Agilent Technologies, Santa Clara, CA) and transferred to 22-mL stainless steel extraction cells for ASE extraction using petroleum ether:isopropanol (2:1, vol/vol). The extraction conditions were as follows: temperature, 120°C; pressure, 10.34 MPa; static time, 1 min; number of static cycles, 3. Solvent evaporation was carried out under vacuum (37°C) using a Rotavapor (Rotavapor1 R-205, Buchi Italia s.r.l., Cornaredo, Italy). The extracted fat was weighed and subsamples of about 40 mg were transferred to culture tubes for immediate transesterification and methylation, according to Christie (2001), using 1 *M* sodium methoxide in methanol at room temperature. Methyl 12-tridecenoate was used as an internal standard (#U-35 M, Nu-Chek Prep Inc., Elysian, MN). The resulting FAME solution was stored at −20°C before GC analysis.

The fatty acid profiles of the samples obtained were analyzed using a GC × GC instrument (Agilent 7890A, Agilent Technologies) equipped with a modulator (Agilent G3486ACFT), an automatic sampler (Agilent 7693), and a flame-ionization detector connected to chromatography data system software (Agilent Chem Station). Two columns in series were used to obtain better separation and identification of fatty acids: first column 75 m × 180 μm (internal diameter) × 0.14 μm film thickness (SP-2560, 23348U, Supelco, Bellefonte, PA); second column 3.8 m × 250 μm (internal diameter) × 0.25 μm film thickness (HP-5MS, J&W 19091S-