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A profile of sphingolipids and related compounds tentatively identified in yak milk

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ABSTRACT

This work characterized a fraction of constituents in yak milk within the realm of approximately 1,000 to 3,000 Da using matrix-assisted laser desorption/ionization (MALDI) time-of-flight mass spectrometry. Eleven samples of yak milk powder from the Sichuan province of China were received by the Department of Food Science, University of Wisconsin–Madison, and stored at room temperature until analysis. Sample preparation involved delipidation and deproteinization of yak milk samples and cold ethanol precipitation. Subsequently, MALDI time-of-flight mass spectrometry was performed in positive ion, reflector mode (AB Sciex TOF/TOF 4800 MALDI; ABSCIEX, Foster City, CA). The instrument was first calibrated with the manufacturer's 6-peptide mixture, and each spectrum was internally calibrated using the accurate mass of ACTH Fragment 18–39 standard peptide (protonated mass at *m/z* 2464.199) present in each sample. Laser power was adjusted for the calibration standards and for each sample so that the signal obtained for the most-abundant ion in each spectrum could be maximized, or kept below $\sim 2 \times 10^4$ to preserve spectral quality. Structure and name based on mass were matched using the Metlin metabolite database (https://metlin.scripps.edu/index.php). Results of the current work for yak milk powder showed a large variety of sphingolipid structures with clusters around 1,200, 1,600, and 2,000 Da. The profiling matched several glycosphingolipids, such as gangliosides GA1, GD1a, GD1b, GD3, GM1, GM2, GM3, and GT2 and several other unique moieties, including deaminated neuraminic acid (KDN) oligosaccharides, and fucose containing gangliosides. Matrix preparation and MALDI time-of-flight parameters were important factors established in this work to allow high resolution profiling of complex sphingolipids in yak powder milk.
Key words: yak milk, sphingolipid, ganglioside

INTRODUCTION

Milk contains a wide array of complex constituents important for the health of the mammalian offspring. Such constituents include the major components such as protein, milkfat, and lactose as well as some relatively low-molecular-weight, biologically important compounds including oligosaccharides, phospholipids, and sphingolipids (**SL**). Although the functions of these latter, minor compounds are not fully understood, they are thought to serve various important metabolic and functional roles to the imbiber beyond merely providing calories. The various roles of these compounds include functioning as prebiotics, prevention adhesion of pathogens to the human epithelial cells (Niñonuevo and Lebrilla, 2009), and functioning in cell membrane and neuron development as well as in various roles in the development of the immune system (O'Hara and Shanahan, 2006). For example, some of these compounds benefit human health as prebiotics through such pathways as the conversion of exogenously acquired L-fucose to guanosine diphosphate-L-fucose, which is then incorporated into multiple surface capsular polysaccharides and glycoproteins important for human symbionts (Coyne et al., 2005).

Structurally, these compounds routinely contain carbohydrate oligomers composed of various monosaccharide subunits bonded through covalent glycosidic linkages with sugar moieties or conjugated to other biomolecules, such as proteins and lipids. The glycan moieties of glycoproteins are a major class of oligosaccharides attached to proteins via amide or acyl linkages. Sphingolipids are considered a type of lipid material composed of sphingoid bases and aliphatic amino alcohols including sphingosine (2-amino-4-octadecene-1,3-

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diol). As a result of these complexities, complete identification and nomenclature of these compounds is notably complex and often may defy complete, positive molecular characterization.

In general, SL are thought to be concentrated in the milkfat globule bilayer membrane, primarily associated with the outer leaflet of the membrane. Although not thought to serve a direct role in human nutrition, the digestive products of SL are regarded as demonstrating substantial effects on various systems involving cell regulation including the development and prevention of disease (see Vesper et al., 1999). Although additional studies are in order, beneficial effects appear to be significant in the realm of gastrointestinal heath (Rueda et al., 1998), cancer prevention (Parodi, 1997), and atherosclerosis and have prompted interests in the commercial preparation of milkfat globule membrane as a food ingredient (Spitsberg, 2005). We propose that an improved understanding of the molecular structure of SL in diverse foods will aid the use of such a food fraction for human health.

Yak is a bovid species found throughout the Himalayan region of south central Asia, the Tibetan Plateau, and as far as Mongolia and Russia. Yaks are adapted to the extreme conditions of high altitudes and climate of these regions (Medhammar et al., 2012) and provide an almost singular, sustaining food source for the people who live in these harsh conditions. Yak milk and its derivatives, such as butter and fermented products, are consumed in these regions, providing a sustaining dietary source of health-imparting nutrients, calories, and compositional factors, such as protein and minerals. Given the unique environmental conditions wherein the yak has evolved and the ability of yak milk to act as a significant source of nutrition for many of the Tibetan peoples in this area, we suggest that yak milk may have some unique components or component profiles that influence human health, namely SL pro-

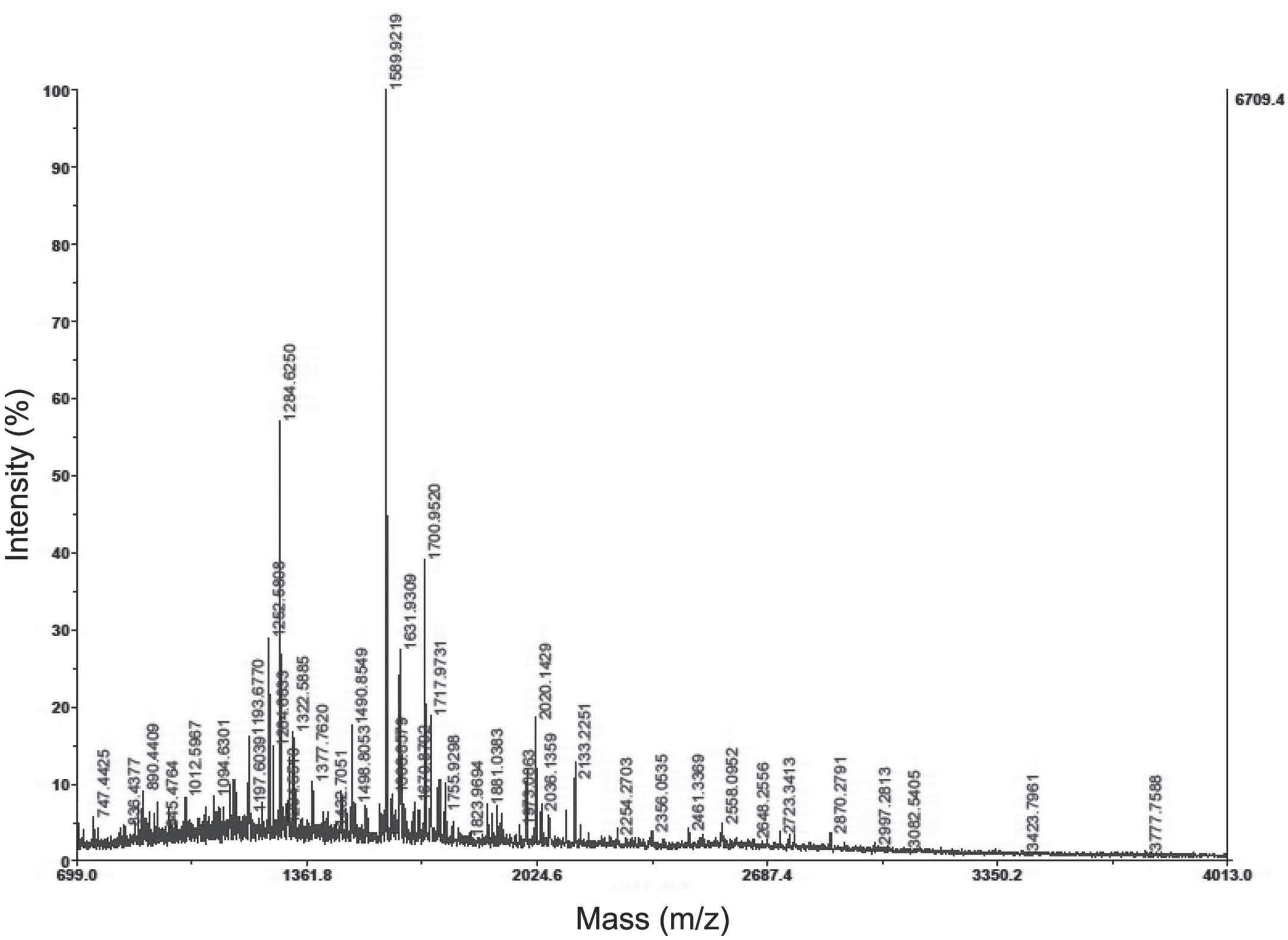

Figure 1. Representative mass spectrum of matrix-assisted laser desorption/ionization time-of-flight analysis of yak milk. m/z = mass-to-charge ratio. Color version available online.