Commercially Processed Human Milk and Human Milk-Derived Fortifier Retain Bioactive Human Milk Oligosaccharides

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Background and Aims:

Human milk (HM) contains many bioactive substances including human milk oligosaccharides (HMO). HMO are a family of structurally diverse sugars that are highly abundant in and unique to HM. HMO constitute the third most abundant component in HM, following lactose and fat, and are more abundant than protein. The best-characterized function of HMO is their prebiotic activity support of gut colonization by commensal microbial flora. In breastfed infants, HMO promote gut colonization by *Bifidobacterium spp.* in the period following birth. These, and other, commensal bacteria serve diverse roles in health in infancy and throughout the lifespan. HMO may reduce NEC and sepsis by supporting gut colonization by commensal bacteria over pathobionts that underlie many diseases in premature infants. We sought to explore the impact of standard pasteurization of HM on the concentration, spectrum, and activity of HMO present in donor HM and a human milk-derived HM protein fortifier.

Methods:

We used HPAEC-PAD using commercial HMO standards to annotate the spectrum and extrapolate the concentration of HMO in donor HM and in a HM-derived human milk protein fortifier. A simplified HMO extraction method was developed to avoid chemical reagents and solid phase extraction (Fig. 1). TNO i-screen assays (TNO, Zeist, Netherlands) were performed in triplicate using standardized pools of adult fecal matter. Samples were incubated at 37C for 24 hours under anaerobic conditions. HMO in a HM extract were added at the concentrations noted in Figure 3. Bacterial DNA was isolated and 16S V4 rRNA amplicons were prepared and sequencing performed using Illumina MiSeq. Downstream sequence analysis was performed using a standardized pipeline. Reads were assembled and taxonomic classification performed using Ribosomal Database Project (University of Illinois, Urbana) with composition presented as OTU.

Figure 1. Pooled donor HM and HM-derived human milk fortifier were centrifuged and the fat layer aspirated. The aqueous phase was filtered using a 10kDa Amicon filter and the HMO filtrate collected and diluted in deionized water prior to analysis using a Carpac analytical column (ThermoFisher, Carlsbad, CA).

Results:

We identified a similar spectrum and concentration of neutral and sialylated HMO in pasteurized donor HM and human milk-derived protein fortifier similar to HM before processing (Fig. 2 and Table I).

Results:

Figure 2. Left column chromatograms show neutral (top) and sialylated (bottom) HMO detected in pooled donor HM before (black tracing) and after pasteurization (green tracing). Right column chromatograms show neutral (top) and sialylated (bottom) HMO detected in HM-derived HM fortifier.

HMO Source	Neutral	Sialylated	Total
Donor HM (g/L) n=3	4.12 ± 0.40	0.26 ± 0.03	4.38 ± 0.38
HM-derived HM fortifier (g/L) n=5	3.71 ± 0.28	0.35 ± 0.06	4.07 ± 0.34

Table I. Concentration of neutral, sialylated HMO subsets and total HMO in processed donor HM and HM-derived-human milk fortifier as determined by HPAEC-PAD. Results expressed as the mean ± SD. n=number of independent determinations.

To determine the activity of HMO following pasteurization, we examined the response of fecal microbiota to stimulate growth of *Bifidobacterium spp.* and other commensal bacteria using a TNO-i-screen assay (Fig. 3).

Figure 3. The percentage of OTU by genus in adult fecal samples in response to the addition of HMO filtrate or control vehicle. Results presented as % of total OTU.

Conclusions:

Standard pasteurization of HM preserves the spectrum, content and activity of naturally occurring HMO. Our data support that supplementation and fortification of mother's own milk with exclusive human milk-derived products further complements the clinical benefits of exclusive human milk feeding in preterm and other high-risk newborns.