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## Effects of pasteurization and freeze-drying on human milk oligosaccharides

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**Background and Objectives:** Human milk oligosaccharides (HMOs) are known as important factors in neurologic and immunologic development of neonates. Moreover, freeze-drying seems to be promising storage method for better functions of human milk banks. However, the effects of pasteurization and freeze-drying on HMOs were not evaluated yet. The purpose of this study is to analyze and compare HMOs profiles before and after the pasteurization and freeze-drying.

**Methods and Study Design:** Totally nine fresh human milk samples were collected from three healthy mothers at first, second, and third week after delivery from each mother. The samples were treated with Holder pasteurization and freeze-drying. HMOs profiles were analyzed by MALDI-TOF/TOF mass spectrometry and compared between samples before and after the treatments by bioinformatics analysis.

**Results:** Human milk samples showed significantly different HMO patterns between mothers. However, HMOs were not affected by lactation periods within 3 weeks after delivery ( $r^2=0.972 \sim 0.999$ ,  $P < 0.001$ ). Moreover, both of pasteurization and freeze-drying were found not to affect HMO patterns in a correlation analysis ( $r^2=0.989 \sim 0.999$ ,  $P < 0.001$ ).

**Conclusion:** HMOs were found not to be affected by pasteurization and freeze-drying. In addition, there were large differences between mothers. As significant changes of HMOs were not found after these treatments, we hope that introducing freeze-drying in human milk banks would be supported by the present study. However, the storage length without composition changes of HMOs after freeze-drying needs to be evaluated in the further study.

**Running Title:** Effects of pasteurization and freeze-drying on HMOs

**Keywords:** Freeze-drying, Human milk, Mass spectrometry, Oligosaccharides, Pasteurization

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