

Effects of pasteurization and refrigerated storage on human milk neurobiomarkers concentrations

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Valorisation

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concentrations

The aim of this thesis was to investigate the effects of storage on specific HM constituents. In detail, the issues addressed are:

- i) the state of art of HoP effects on HM composition;
- ii) the changes in HM qualitative protein profile following HoP procedure;
- iii) the potential side-effects due to HP procedure on biomarkers such as Activin A, S100B, HO-1;
- iv) the potential side-effects due to prolonged refrigerated storage on AM.

Relevance: Fresh own mother's breast milk is considered the first choice in preterm infant feeding, and is able to provide health benefits that are of vital importance for preterm infants in NICUs. HM feeding in preterm infants decreases infection, NEC and mortality rates, while improves neurocognitive and cardiovascular outcomes. HM can be considered a species-specific biological "dynamic" system, containing bioactive and immunomodulatory factors which ensure adequate host defense against infections, actively modulate the immune response, modify the intestinal bacterial flora and, regulate optimal newborn growth including CNS. Selected biomarkers have a special role in human milk, such as: i) Neurotrophic factors — activin A; ii) calcium binding proteins — S100B; iii) oxidative stress biomarkers —HO-1, iv) vasoactive agents – AM. For this reason, international neonatal networks are committed to increase breastfeeding rates at discharge from the NICU whenever possible.

When mother's own milk is not available, the use of DM is highly recommended since it retains most of these benefits. DM must be sourced from established HMB under specific safety guidelines for storage and processing, which foresee a HoP requiring HM extraction and refrigerated storage. However, storage and processing of DM may reduce some of its biological components and therefore result in a partial loss of its health benefits.

Target group: The target population addressed in this thesis is the preterm newborn (i.e., newborns whose birth occurs <37 weeks gestational age). This group comprises 5-10% of all birth in Europe, whereas in USA the rate is about 12–13%.

Innovation: Currently, it remains a matter of debate whether the studies available in Literature adequately describe the impact of HoP and refrigerated storage on milk properties, and if the nutritional qualities of milk are preserved after these treatments. In fact, substantial discrepancies exist not only between the protocols applied in different studies, but also between the standard operating procedures adopted by HMBs and the experimental methods reported in research protocols.

The studies conducted in the present thesis were performed according to protocols designed to:

- i) match the actual procedures of storage, handling and processing of DM practiced by HMBs;

- ii) provide results relevant for the actual clinical management of DM. Moreover, our studies determined the effects of HoP and refrigeration on specific biomarkers, which were not previously investigated.

Activities: The results reported in the present thesis confirm that DM treatments can affect, to some extent, the milk components. In detail, differences in the protein profile after HoP were found, although only in 30% of the tested samples. The main detectable protein profile changes were observed in colostrum, thus further supporting the use of DM (since no evident changes were shown in mature milk). HoP did not cause any significant change in the concentration of activin A and HO-1 in DM, also after correction for GA and maturation degree of the human milk. Conversely, HoP significantly decreased the concentration of the S100B protein. The finding is of relevance bearing in mind that role of the protein in CNS fetal and neonatal development.

Concerning refrigeration procedures, our studies show that AM levels are affected by storage procedures (at 96-h from storage, up to 98% of the total peptide amount is degraded).

Notably, the present thesis showed first the presence of activin A, HO-1 and AM in milk collected from preterm deliveries. Overall, results further support the clinical evidence that DM retains the nutritional properties of HM, and provide further insight on which bioactive components these can be attributed to.

Schedule and implementation: The present thesis contributes to a better understanding of the potential effects of storage on HM and primarily of HoP on DM. Our findings may also represent an opening view to:

- i) develop best practices balancing the treatments required to ensure microbiological safety of DM;
- ii) maintain DM benefits avoiding refrigerated storage for more than 24 hours; and
- iii) accept only mature milk (less susceptible to protein degradation) from donors.

Future research should focus on improvements of milk processing in HMB (particularly heat treatment) and on further evaluation of the potential clinical benefits of processed and fortified DM. In particular, future studies must be aimed at improving the biological quality and safety of DM and should be:

- i) designed to investigate the pre-analytical stability of these components according to the storage procedures;
- ii) intended to evaluate innovative test technologies, such as metabolomics;
- iii) focused on new pasteurization techniques (high-temperature short-term pasteurization, thermos-ultrasonic treatment, high-pressure processing, and Ohmic heat treatment);
- iv) aimed to evaluate analytical techniques able to assess the protein changes due to thermic treatments, as well as their interaction with sugars and lipids;
- v) designed to evaluate the effects of HoP on other biomarkers involved in growth and developing of newborns.

Moreover our findings open up to further investigations aimed at elucidating the protein stability during industrial processes for the preparation of artificial milk such as pasteurization and spray-drying, which have already been shown to affect milk composition and properties.

Further data concerning the metabolic fate of the most important milk biomarkers in the gastro-enteric tract is needed to corroborate the hypothesis that these participate in the nutritional effects of milk and in its immuno-regulatory and trophic role for intestine and brain development.