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► **To cite this version:**

François Chevalier, Emanuele Armaforte, A Ryan, Al Kelly. Proteins and proteolysis in pre-term and term human milk samples. The 38th UCC Annual Research Conference hosts on Food, Nutrition and Consumer Sciences, Sep 2008, Cork, Ireland. hal-04244470

HAL Id: hal-04244470

<https://hal.science/hal-04244470>

Submitted on 18 Oct 2023

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Proteins and proteolysis in pre-term and term human milk samples



UCC

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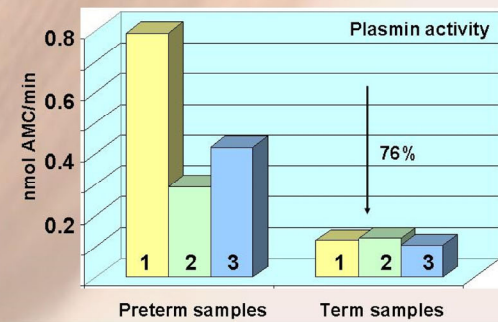
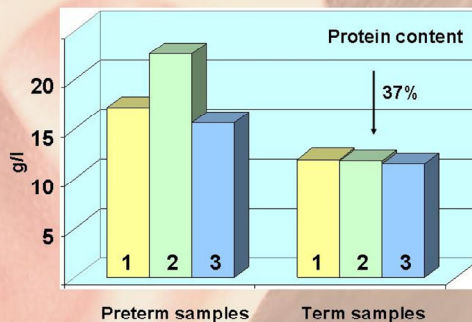
The composition of milk obtained from mothers giving birth prematurely is of interest because of the key role of this milk as a nutrient source for the premature infant. Preterm milk of early lactation is generally found to have higher levels of protein, non-protein nitrogen, fat, energy, sodium, and chloride, but lower levels of lactose and greater inter-individual variation. However, by the end of the first month of lactation the preterm mother's milk is very similar in nutrient composition to term milk. The objective of this work was to investigate the protein composition of breast milk for pre-term babies and to compare it with the "normal" breast milk in term of protein concentration, plasmin activity and protein variety and abundance.

Compositions of breast milk between samples from mothers of pre-term and term infants

Six breast milk samples (3 samples from mothers giving birth prematurely and 3 control normal milk) were selected and compared for their protein content and their plasmin activity.

According to the kjeldahl method, the mean **protein content** is 18.4 g/l for preterm milk samples and 11.7 g/l for term milk samples (37% decrease).

According to the plasmin activity assay (Richardson and Pearce, 1981), the mean **plasmin activity** is 0.252 nmol AMC/min for preterm milk samples and 0.059 nmol AMC/min for term milk samples (76% decrease).



Two-dimensional electrophoresis: building a reference map with the identification of the 50 most abundant spots of human milk

Experimental conditions

Protein solubilisation: 9M urea, 4% CHAPS, 0.05% Triton X100, 65 mM DTT

Analytical gels (7.5 µl of milk / gels)

1st dimension : Precast 7 cm strips pH 3-10 (Bio-Rad)

with the Protean IEF Cell (Bio-Rad)

2nd dimension : 12.5% SDS-polyacrylamide gel, using the Criterion® Dodeca

Cell electrophoresis unit (Bio-Rad)

Preparative gels (20 µl of milk / gels)

1st dimension : Precast 17 cm strips pH 3-10 (Bio-Rad)

with the Protean IEF Cell (Bio-Rad)

2nd dimension : 12.5% SDS-polyacrylamide gel, using the Protean II XL Cell

electrophoresis unit (Bio-Rad)

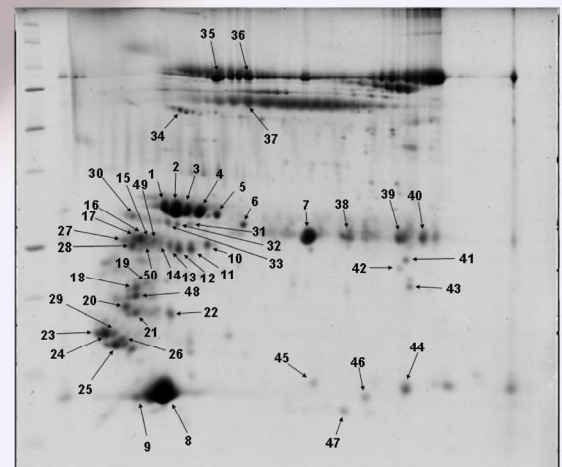
Staining conditions : Colloidal Coomassie blue

Gel images are digitalized with a GS 800 densitometer (Bio-Rad)

Gel are analyzed using the PDQuest software v.7.3.1.

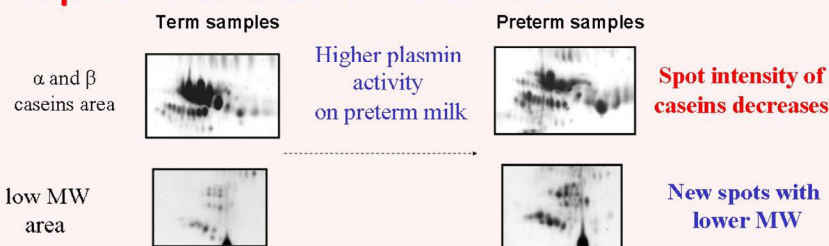
Mass spectrometry analysis: MALDI-TOF peptide mass fingerprint with a

Axima CFR plus mass spectrometer (Shimadzu Biotech)



1-5: β -casein; 6: β -casein / immunoglobulins; 7: β -casein; 8-9: α -lactalbumin; 10-17: α -casein; 18-19: α 1-casein / β -casein; 20-22: α 1-casein; 23: protease serine 3; 24-26: α 1-casein; 27-28: immunoglobulin J chain; 29-30: α 1-casein; 31: α 1-casein / β -casein / anti-pneumococcal antibody; 32: α 1-casein / β -casein; 33: α 1-casein / β -casein / α -lactalbumin; 34: α 1-antitrypsin / κ -casein; 35-36: lactoferrin; 37: lacto-transferrin / immunoglobulin; 38: immunoglobulin K; 39-40: immunoglobulin G kappa chain; 41-44: β -casein; 45: fatty acid binding protein; 46: β -casein / α -lactalbumin; 47: β 2-microglobulin; 48: lactoferrin / α 1-casein / β -casein; 49-50: α 1-casein.

Proposed mechanism and conclusions



With a higher protein content and a higher plasmin activity, preterm milk samples display a higher number of spots with a decreased intensity of the major native casein spots.

In addition, new specific low molecular weight spots are observed on preterm milk sample, resulting from the hydrolysis of caseins. The increased variety of "pre-digested" proteins on preterm sample can lead to a easier digestion of breast milk for premature babies.

Quantification data from the image analysis highlighted that pre-term milk samples are richer in immunoglobulins and antibodies, leading to hypothesis of a milk "naturally enriched" in proteins but also in bio-active compounds able to protect the pre-term babies during their early life.



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ACKNOWLEDGEMENT

This project is funded through the Department of Agriculture and Food under the National Development Plan 2000-2006.