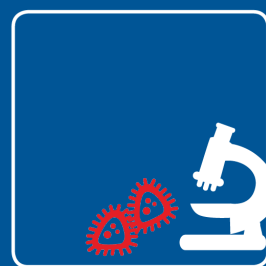


Marianne Nissen Lund:
Effekt af UHT og opbevaring på den
biologiske kvalitet af flydende
modermælkserstatninger

UHT treatment and storage effects on the biological
quality of liquid infant formulas



Final report

for collaborative projects funded via the Danish Dairy Research Foundation (DDRF)

1. Title of the project

Danish: Effekt af UHT og opbevaring på den biologiske kvalitet af flydende modernælkserstatninger

English: Effect of UHT and storage on the biological quality of liquid infant formula

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4. Sources of funding

Milk Levy Fund, GUDP (Green Development and Demonstration Program)

The project was integrated with the GUDP-project: INFANT-I: Tailored processing of bioactive ingredients for high-end formula.

5. Project period

Project period with DDRF funding: November 2018/December 2021

Total project period, if sub-project within a larger project: July 2018/June 2023

6. Project summary

Danish:

UHT (ultrahøj temperatur) behandling og lagring af modermælkserstatninger benyttes verden over. Vi har vist, at en kombination af indirekte UHT og langvarig lagring af modermælkserstatninger kan igangsætte inflammatoriske effekter, og at mindre hård UHT-behandling, som for eksempel direkte UHT, mindsker skader på proteiner og tilpasser de biologiske effekter af flydende modermælkserstatning og derved reducerer inflammatoriske reaktioner. Desuden påvirker milde lagringsforhold holdbarheden af produktet. Vi har udviklet nye metoder til kvantificering af Maillard-reaktionsprodukter, iværksat cellestudier for at bestemme de anti-inflammatoriske og inflammatoriske effekter af peptider fra disse produkter og testet produkterne i dyreforsøg. Den kombinerede analytiske platform, cellestudierne og dyreforsøgene viser, at indirekte UHT-behandling af flydende modermælkserstatninger kombineret med lagring for at fremtvinge Maillard-reaktionen, fremtvinger inflammation i makrofager og i for tidligt fødte smågrise, der anvendes som model for for tidligt fødte spædbørn. Udvælgelse af mildere UHT-behandlinger og valg af en optimal lagringstid kan forbedre helbredstilstanden og forhindre sygdom.

English:

UHT (ultra high temperature) treatment and storage of infant formulas is utilized worldwide. We showed that combined indirect UHT and prolonged storage of infant formulas may initiate inflammatory effects and that less severe UHT treatment, such as direct UHT, lowers protein damage and modulate the biological effects of the liquid infant formulas, reducing inflammatory reactions. Moreover, more gentle storage conditions affect the shelf-life of the product. We have developed new methods for quantifying Maillard reaction products, set up cell studies to determine the anti-inflammatory and inflammatory effects of peptides derived from these products and tested these products in animal studies. The combined analytical platform, together with the cell studies and animal studies indicate that indirect-UHT treatment of ready to feed liquid infant formulas, combined with storage to induce the Maillard reaction, induces inflammation in macrophages and in preterm piglets, used as a model for preterm infants. Selection of more gentle UHT treatment conditions and selection of an optimal storage time and temperature may improve disease prevention/health care.

7. Project aim

Danish*:

De specifikke mål for dette projekt er:

WP1: Maillardreaktionen (oxidering, AGEs, proteinaggregering, fordøjelighed) karakteriseres efter

pasteurisering, varierende opbevaringstid af produkter (råmælk, modermælkserstatning), som er underkastet skånsom eller hård UHT-behandling.

WP2: Tarm-inflammatoriske effekter af alle WP1 produkter vurderes i en tarmcelle model baseret på grise.

WP3: Inflammatoriske effekter af udvalgte produkter fra WP1 og WP2 testes i en model af tidligt fødte grise.

English*:

The specific objectives of this project are:

WP1: The Maillard reaction (oxidation, AGEs, protein aggregation, digestibility) is characterized after pasteurisation, variable storage time for products (colostrum, milk formula) subjected to gentle or harsh UHT treatment.

WP2: Inflammatory effects of all products from WP1 are assessed in a pig intestinal cell model.

WP3: Gut inflammatory effects of selected products from WP1 and WP2 are tested in a preterm pig model.

*: WPs blev ændret til fire i det endelige projekt (INFANT-I)/WPs were changed into 4 WPs in the final project (INFANT-I):

WP1: Exploration and demonstration of gentle processing techniques

WP2: Chemical and structural characterization of proteins

WP3: In vitro digestibility and bioactivity of protein ingredients and IF

WP4: Physiological effects in a piglet model of preterm infants

8. Background for the project

Most infant formulas (IFs) are formulated using bovine milk protein ingredients, particularly whey protein, because of its high nutritional value. Whey protein ingredients normally undergo harsh technological processes, which involve thermal treatments that lead to structural and chemical protein modifications and lower protein bioactivity. Organic whey is derived as the main product from cheese production where the cheese is the side stream. The demand for organic whey-based ingredients for IFs is increasing even though the demand for organic cheese is limited. Organic whey proteins can be isolated directly from milk as serum protein concentrate (SPC) by milk fractionation to accommodate market needs. Milk fractionation has the potential to provide organic ingredients for a more efficient IF process with less waste and a reduced CO₂ footprint. Interestingly, proteins in SPC may be more preserved due to more gentle processing conditions. However, the bioactivity and health-derived effects of SPC protein for infants is not documented. There is an urgent need for development of organic IF with retained protein structure, and even more for preterm infants that have an immature gut and immune system and whose only source of nutrition is often IF because of lack of mother's own milk. Thus, the project aimed to create the understanding of how protein modifications in IF ingredients influence protein bioactivity and digestibility and derived effects on gut function and health. This knowledge is used to improve protein bioactivity and digestibility in protein ingredients for IF by tailored gentle processing.

9. Sub-activities in the entire project period

WP No.	Milestones	Status			Explanation If a milestone is only partially achieved or abandoned, provide a brief explanation for it.
		Achieved	Partially achieved	Abandoned	
WP1	UHT treated IF produced by M9.	x			The milestone has been completed.
WP2, WP3	UHT treated IF stored and protein quality characterized by in vitro assays (modifications, bioactivity, digestibility) by M27.	x			Delayed due to equipment breakdown and COVID-19 shutdown until month 27. The milestone has been completed.
WP3	UHT treated milk stored and characterized by in vitro assays (bioactivity, digestibility) by M30	x			The milestone has been completed.
WP4	Physiological effects of IF investigated in piglets by M30.	x			The milestone has been completed.
WP2, WP3, WP4	Relevant in vitro markers for protein modification and bioactivity have been identified by correlation to in vivo endpoints by M33.	x			Delayed due to COVID-19 shutdown until month 33. The milestone has been completed.
WP1	SPC-based IF with improved bioactivity has been developed by gentle processing by M48.	x			Delayed due to stress of PhD students and COVID-19 shutdown until month 36. Finally it was delayed until M48. The milestone has been completed.

WP2, WP3, WP4	Physiological effects of SPC-based IF with improved bioactivity investigated in piglets by M45.	x			Rewritten to deal with ingredients only: SPC-based ingredients with varying processing degree/protein damage has been produced and linked to physiological gut health effects in piglets by 48. The milestone has been completed.
WP4	Amino acid uptake of SPC-based IF with improved bioactivity investigated in pigs by month 48			x	The milestone was abandoned, as initial studies showed that the premature pigs, which are used in this project as a model for babies, become too stressed during the intervention that studies on amino acid uptake require (DIAAS). A request to change this milestone was prepared in a separate document for GUDP to address the SPC-based infant formulas referred to in milestone 6.
WP4	New: Physiological effects of gentle processed SPC formula with improved bioactivity investigated in pigs by month 60	x			Based on the results in milestone 6 (pig study 2), testing of the SPC formula is changed to prioritize further characterization of the effects of the SPC ingredient for increased documentation.

10. Deviations

Most project activities proceeded according to plan, but the COVID-19 shutdown resulted in delays in some activities and therefore in the whole project plan. The project group has worked constructively together to find solutions to the major challenges we faced. In addition, both PhD students (employed in WP1 and WP4) were on sick leave with stress for different periods of time; this did not cause significant changes in the WP1 activities but caused a delay in preparation of one of the manuscripts from WP4. Moreover, the WP3 leader and original leader of the MFF project was on sick leave for a longer period of time and ended the employment at KU before the project ended. The other senior researchers in the project group have taken over the responsibility of finalizing WP3, but this has caused a delay in publication of the WP3 manuscripts. This has been a collective effort across departments and institutions. All experimental analysis and data analysis was performed, one manuscript has been submitted and the rest of the manuscripts will soon be submitted.

There was also some delay in some of the analyses in WP2 due to unforeseen technical challenges. The quantification method previously developed by our group for AGEs and amino-acid cross-links were found to have some drawbacks in terms of reproducibility, and a new LC-MS method was developed. During the method development, it was found out that commercial standards used for quantification were of lower purity than stated by the provider (Iris-Biotech). Through extensive communication with the company, we managed to solve the problem. Moreover, experiments performed during method development allowed very important observations, which helped to explain the inconsistencies reported by different research groups (worldwide) on the concentration of hydroimidazolone structures in foods. The results were published in Food Chemistry and the paper was selected as editor's choice in IMARS (International Maillard Reaction Society) highlights. After the development of the method, all other activities were accelerated.

We had expected to run a study on UHT-treated milk, for comparison with UHT-treated infant formula, in the animal studies but due to logistical reasons and for economic considerations we decided, in close collaboration with industrial experts at AFI, to focus on liquid infant formulas and their ingredients, as this was considered the major priority. Caco-2 cells, used as a model for intestinal epithelial cells, were not very sensitive to peptides released from the products, and therefore we utilized THP-1 cells, a model of macrophages to study the anti-inflammatory/inflammatory effects of the peptides. However, for co-culture experiments, Caco-2 cells were used to simulate the intestinal epithelium and apical/basolaterally transported peptides were then allowed to stimulate THP-1 macrophages.

11. Project results

INFANT-I aimed to document that tailored gentle processing of organic whey fractionated directly from milk and used for IF formulation improves gut function and health of infants by improving protein bioactivity and minimising processing-induced protein damage. This was achieved through four WPs with well-defined objectives for each:

WP1. To establish optimal processing solutions by minimizing heat load, while securing microbiological safety, by tailoring the use of gentle processing technologies instead of using conventional processing for protein ingredients.

WP2. To quantify chemical modifications and characterise structural changes of protein ingredients produced in WP1 to understand how gentle processing can be tailored to protect proteins from damage.

WP3. To understand how chemical and structural modifications affect digestibility and bioactivity of protein ingredients produced in WP1 in *in vitro* assays and intestinal cells.

WP4. To characterise gut function and health in piglets fed IF with protein ingredients produced by tailored gentle processing.

To achieve these purposes, different activities were performed in each WP as described below:

WP1: Liquid IFs with different heat treatments (low pasteurized (LP), direct (D) and indirect (ID) UHT) was manufactured at Arla Foods ingredients (AFI) and a following storage trial at 40 °C for 6 months to promote formation of Maillard reaction products (MRPs) was performed at KU FOOD. After the storage trial was completed, structural changes in UHT treated liquid WPC-based IF were characterized. Colour and pH were monitored and structural changes were characterized by dynamic light scattering, SDS-PAGE and centrifugal field-flow fractionation (FFF) coupled with multi-angle light scattering (MALS) and UV detectors. Both D and ID UHT treatment of IF led to structural changes, where the higher heat load of the ID-UHT treatment caused more pronounced effects. FFF-MALS-UV analysis allowed characterisation of complex aggregates, where structural changes continued to be most pronounced in indirect UHT-treated IF samples, and different storage-induced aggregation behaviour was observed. Both UHT treatments caused formation of reducible disulphide-linked aggregates, while non-reducible aggregates were formed during indirect UHT treatment and during storage of D-UHT and ID-UHT samples (Paper 1-1). A gentle milk fractionation process was used to produce serum protein concentrate (SPC)-based protein ingredients (liquid in this part of the project) and the traditional whey protein concentrate (WPC) ingredient (powder) by AFI, and the powdered IFs made from these ingredients were produced. These ingredients were characterized first and then the IFs were analyzed to demonstrate the differences between the samples. Structural protein modifications were evaluated, and MRPs (furosine, α -dicarbonyls, furans, and advanced glycation end products) were quantified in the protein ingredients and IFs. It was found that IF processing (spray drying) was responsible for higher levels of protein modifications compared to the levels observed in the SPC and WPC ingredients. The increased processing of WPC led to increased levels of disulfide-linked aggregates, amino acid cross-links (lanthionine; LAN) and lysinoalanine; LAL), and large soluble aggregates (Paper 1-2). In continuation of this, various process steps were investigated to identify where in the process modifications of proteins are introduced. It was found that the lactose content has a major effect on the protein modifications during IF processing and adjusting the lactose to protein ratio during processing was efficient for regulation of protein modifications. The presence of lactose had a limiting factor on process-induced aggregation of whey proteins whereas absence of lactose resulted in decreased formation of MRPs. The results suggested that partial dry-blending of lactose (30:70, lactose:whey

protein) with remaining spray dried ingredients could be a feasible approach for manufacturing of powdered IF with improved protein quality (Paper 1-3).

WP2: Initially it was planned that the existing in-house quantification method for MRPs and amino acid cross-links would be used in the project. However, preliminary experiments with the UHT-treated liquid WPC-based IF samples showed it was not possible to obtain reproducible results with the method. We therefore developed a new method for this analysis. During the method development, it was also found that different AGEs have different stabilities towards the acidic conditions used for protein hydrolysis (a necessary step during sample preparation for quantitative analysis), which have created inconsistent results among different research groups. A systematic investigation of the acid hydrolysis provided very important results and revealed that methylglyoxal derived hydroimidazolone (and glyoxal derived hydroimidazolone) isomers isomerizes to each other during acid hydrolysis, which affects the overall quantification of these MRPs. These findings were reported in Paper 2-1 and have gained recognition over short time and were selected as the editor's choice in IMARS (International Maillard Reaction Society) highlights. The findings were presented as a poster in IMARS-14 conference in September 2021 and led to a collaboration with University of Maastricht, where kinetics of isomerization of hydroimidazolone structures have been investigated (Paper 2-3, in preparation). After establishing the quantification method, all activities in the project were accelerated. Protein modifications in liquid WPC-based IFs were quantified for WP3 and WP4 and the results obtained from the storage trial with UHT-treated IF are published in Paper 2-2. ID-UHT treatment induced significantly higher concentrations of α -dicarbonyl compounds (glyoxal, methylglyoxal, 3-deoxyglucosone and 3-deoxygalactosone) compared to D-UHT, which facilitated increased formation of AGEs (N- ϵ -(carboxymethyl)lysine, methylglyoxal- and glyoxal-derived hydroimidazolones) in unstored IFs. During storage for 6 months, concentrations of furosine and AGEs increased while α -dicarbonyl compounds decreased. This is the first study reporting the effects of UHT treatment and subsequent storage on the formation of MRPs and amino acid cross-links in liquid IF. Gut tissues, kidney and serum samples from piglets (Pig study 1) were analyzed with the aforementioned method and quantification of AGEs in the tissues of piglets was completed together with CML quantification in the tissues. In general, more AGEs were found to be accumulated in tissues of piglets fed with stored and UHT-treated IF (ID-60 days) compared to the ones fed with a control low pasteurized formula. The results were published in Paper 4-1. The findings of this study were also presented as a poster in IMARS-14 conference in September 2021 and the poster won the best poster award. SPC-based and WPC-based protein ingredients from D1.3 (used in Pig study 2) were characterized in terms of MRPs with the aforementioned quantification method. Quantification of AGEs (including CML) in animal tissues from pig study 2 was completed and were shared with WP4. Additional analysis, such as amino acid analysis in samples used in pig study 2 and lactoferrin analysis by LC-MS, was also completed. The results are published in Paper 4-3.

WP3: Digestibility of proteins in liquid WPC-based IFs and the release of bioactive peptides upon simulated infant digestion was determined *in vitro* and the results are published in Paper 3-1. It was found that protein aggregates were formed in correlation to the extent of heat treatment and the aggregates remained undigested after gastric digestion. After intestinal digestion (sequential pepsin and pancreatin digestion), large amounts of whey proteins remained undigested in LP but increased in UHT-treated IF, indicating heat treatment and storage facilitated the digestion of unaggregated proteins. On the other hand, aggregated proteins remained undigested especially in the long-stored ID-UHT. The peptides were characterized by LC-MS analysis after *in vitro* digestion and the extracted peptides were analyzed in cell models (RAW 264 and THP-1 cells). It was found that more bioactive peptides were released from LP and D-UHT IF and those showed lower inflammatory responses whereas storage of IF caused release of less bioactive peptides and increased inflammation (Paper 3-3, in preparation). The peptides were also tested in a co-cultured Caco-2 cell and THP-1 cell model to investigate the absorption of peptides and their respective immunomodulatory effects. It was found that peptides from LP, unstored D-UHT and in some cases ID-UHT lowered the gene expression of inflammatory markers whereas peptides from stored ID-UHT (120 days) increased the inflammation. Digestibility and bioactivity of SPC-based ingredients and WPC-based ingredients, as well as SPC-based powder IF and WPC-based powder IF were determined. It was found that reducible protein aggregates were present in the WPC ingredient and extra heat-treated SPC ingredients, but not in the SPC ingredient. The ingredients all showed good digestibility after intestinal digestion and the WPC sample was more digestible compared to SPC samples but released a low level of bioactive peptides (Paper 3-2, submitted). UHT treated bovine whole milk produced by direct and indirect UHT treatment and stored at 40 °C for up to 120 days, and low-pasteurized milk was included as control. Protein aggregate formation was found to increase with increasing

severity of heat treatment and storage time. Most aggregates and whey proteins were digested during *in vitro* digestion, releasing small peptides (<3.5 kDa). Digested low-pasteurized and direct UHT-treated milk displayed anti-inflammatory activity by inhibiting the NFκB pathway in THP-1-derived macrophages. This activity was reduced in digested indirect UHT-treated and stored samples (Paper 3-4, in preparation).

WP4: Pig study 1 was performed with liquid WPC-based IFs, and gut and immunity analyses were completed at KU IVH. Data from WP2 and WP3 related to IF samples were used to make the conclusion that feeding UHT-treated IFs, particularly after prolonged storage, adversely affects gut maturation and function in preterm pigs used as a model of preterm infants (Paper 4-1). In particular, relative to LP, ID-UHT contained lower levels of lactoferrin and IgG. Additional storage (40 °C, 60 days) reduced antimicrobial capacity and increased non-reducible protein aggregates and MRPs (up to 13-fold). Pigs fed stored UHT IF had more diarrhea and showed signs of intestinal inflammation (necrotizing enterocolitis) compared with pigs fed LP and UHT IF (not stored). These clinical effects were accompanied by accumulation of MRPs, protein cross-links, and inflammatory responses in the gut. The accumulation of MRPs were also determined in kidney and serum samples from pigs. It was found that feeding stored UHT-treated IF might impair systemic immunity and to a minor extent kidney development in preterm newborns. These systemic effects were likely derived from the impact of MRPs on gut inflammation (Paper 4-2).

Pig study 2 was performed with SPC- and WPC-based ingredients. The physiological effects of SPC were investigated in premature pigs as a model for sensitive newborns and compared with the effect of extra heat-treated and long-term stored SPC and reference WPC-based IF. The study showed that the SPC ingredient contained no protein aggregates and more native lactoferrin, and despite higher Maillard reaction product (MRP) formation, the clinical response and most gut and immune parameters were similar to WPC-fed pigs. SPC feeding negatively impacted intestinal MRP accumulation, mucosa and bacterial diversity. In contrast, circulating T-cells were decreased and oxidative stress- and inflammation-related genes were upregulated in WPC pigs. Protein aggregation and MRP formation increased in HTS-SPC, leading to reduced antibacterial activity, lactase/maltase ratio, circulating neutrophils and cytotoxic T-cells besides increased gut MRP accumulation and expression of *TNFAIP3*.

In conclusion, the gently-processed SPC had more native protein, but higher MRP levels than WPC, resulting in similar tolerability but subclinical adverse gut effects in preterm pigs. Additional heat treatment and storage further induced MRP formation, gut inflammation and intestinal mucosal damage (Paper 4-3).

Overall, the study follows up on the first pig study in WP4 with the effect of heat treatment and storage on protein ingredients for breast milk substitutes which apparently have a negative effect on intestinal maturation in sensitive newborns. The SPC ingredient, which is formulated into IF by a more efficient IF process with less waste and a reduced CO₂ footprint, has an overall similar tolerability compared to standard WPC-containing IF, when fed to preterm piglets. However, no additional positive effects of SPC as an ingredient in the IF are seen, potentially due to higher MRP content as a result of its higher lactose content.

Conclusion

Overall, the project has provided documentation for the effects of heat treatments and formulation on nutritional quality during production of dairy ingredients and IF. Prolonged, warm storage of ingredients and IF is the major driver for adverse gastrointestinal effects in piglets fed ingredients and IF. Hence, it is strongly encouraged to store products cool and dry according to manufacturers' instructions to preserve nutritional quality.

The extent of protein modifications induced by direct UHT treatment of liquid IF is similar to the extent induced by pasteurization. On the other hand, indirect UHT treatment causes increased protein modification levels. These findings may encourage producers of liquid IF and other liquid formulations to reduce the heat load applied during processing with reduced energy consumption and CO₂ footprint as a result.

The findings on adjusting the lactose to protein ratio during IF processing for regulation of protein modifications represent important information for ingredient- and IF producers that aim to enhance the nutritional value of products.

The SPC ingredient, which is formulated into IF by a more efficient IF process with less waste and a reduced CO₂ footprint, has an overall similar tolerability compared to standard WPC-containing IF, when fed to preterm piglets.

12. The relevance of the results, including relevance for the dairy industry

Overall, the aim of INFANT-I was to document that tailored gently processed SPC-based IF provides positive physiological effects through improved protein bioactivity and less protein damage compared to conventionally processed whey protein ingredients.

To achieve this output, it was necessary to involve all project participants for generation of the knowledge and understanding of how gentle processing affects protein structure and modifications on a molecular level and to correlate this to protein digestibility and bioactivity. This output involved activities from all project WPs.

Protein modification data and the obtained molecular understanding of the influence of processing on the quality of milk protein ingredients are actively used in the commercial part of the AFI organization. Specifically, the obtained knowledge is used by AFI towards costumers to demonstrate that gentle processing and sustainability is of key importance to AFI. Furthermore, the documented quality of proteins is communicated to customers to promote value-added sales.

The finding that even though the SPC ingredient is more native than powdered WPC, the advantage is evened out by the spray drying of the final IF has led to an increased focus in AFI on minimising heat load in the drying step in all AFI's infant formula production. Specifically, the temperature of the pre-heating step before the drying towers at an AFI production site has been lowered significantly. This may have a positive effect on the nativeness and level of modifications in the final product. In line with this, AFI is currently communicating published results from the project to customers that produce infant formula to facilitate discussions on the impact of processing and storage on the quality of ingredients and infant formula to promote sales of AFI products. Furthermore, knowledge from the INFANT-I project has been transferred to and is actively used in new product development projects that focus on innovating new milk- and whey-based ingredients with optimal nutrient and functional quality based on production processes with as minimal environmental impact as possible. As an example, the findings of WP1 and WP2 regarding the effects of heat treatments and storage conditions on liquid infant formula was a cornerstone of a strategic project in 2024 in AFI. The project investigated how minimising overall heat load in production of ready-to-drink products (RTDs) can preserve nutritional and functional parameters of AFI's protein ingredients and provide uniqueness in final consumer products.

13. Communication and knowledge sharing about the project

Papers in international journals:

Paper 1-1

Lund, P., Nielsen, S.B., Nielsen, C.F., Ray, C.A., Lund, M.N. (2021) Impact of UHT treatment and storage on liquid infant formula: Complex structural changes uncovered by centrifugal field-flow fractionation with multi-angle light scattering, *Food Chem.*, 348, 129145. <https://doi.org/10.1016/j.foodchem.2021.129145>

Paper 1-2

Lund, P., Bechshøft, M.R., Ray, C.A., Lund, M.N. (2022) Effect of processing of whey protein ingredient on Maillard reactions and protein structural changes in powdered infant formula, *J. Agric. Food Chem.*, 70, 1, 319–332. <https://doi.org/10.1021/acs.jafc.1c05612>

Paper 1-3

Lund, P., Mardal, F., Ray, C.A., Lund, M.N. (2022) Probing the cumulative effects of unit operations in powdered infant formula: Dry blending of lactose as a strategy to improve protein quality? *Int. Dairy J.*, 132, 105397. <https://doi.org/10.1016/j.idairyj.2022.105397>

Paper 2-1

Akillioğlu, H.G., Lund, M.N. (2022) Quantification of advanced glycation end products and amino acid cross-links in foods by high-resolution mass spectrometry: Applicability of acid hydrolysis, *Food Chem.*, 366, 130601. <https://doi.org/10.1016/j.foodchem.2021.130601>

Paper 2-2

Akillioğlu, H.G., Chatterton, D.E.W., Lund, M.N. (2022) Maillard reaction products and amino acid cross-links in liquid infant formula: Effects of UHT treatment and storage, *Food Chem.*, 396, 133687. <https://doi.org/10.1016/j.foodchem.2022.133687>

Paper 3-1

Ye, Y., Engholm-Keller, K., Fang, Y., Nielsen, C.F., Jorda, A., Lund, M.N., Chatterton, D.E.W. (2022) UHT treatment and storage of liquid infant formula affects protein digestion and release of bioactive peptides, *Food Funct.*, 13, 344-355. <https://doi.org/10.1039/D1FO02619D>

Paper 4-1

Sun, J., Akillioğlu, H.G., Aasmul-Olsen, K., Ye, Y., Rasmussen, P.L., Zhao, X., Brunse, A., Nielsen, C.F., Chatterton, D.E.W., Sangild, P.T., Lund, M.N., Bering, S. (2022) Ultra-High Temperature Treatment and Storage of Infant Formula Induces Dietary Protein Modifications, Gut Dysfunction, and Inflammation in Preterm Pigs, *Mol. Nutr. Food Res.*, 66, 20, 2200132. <https://doi.org/10.1002/mnfr.202200132>

Paper 4-2

Sun, J., Akillioğlu, H.G., Bojesen, A.M., Zhong, J., Muk, T., Pan, X., Lund, M.N., Sangild, P.T., Nguyen, D.N., Bering, S.B. (2023) Ultra-high temperature treatment of liquid infant formula, systemic immunity and kidney development in preterm neonates, *Mol. Nutr. Food Res.*, 67, 2300318. <https://doi.org/10.1002/mnfr.202300318>

Paper 4-3

Aasmul-Olsen, K., Akillioğlu, H.G., Christiansen, L.I., Engholm-Keller, K., Brunse, A., Stefanova, D.V., Bjørnshave, A., Bechshøft, M.R., Skovgaard, K., Thymann, T., Sangild, P.T., Lund, M.N., Bering, S.B. (2024). A gently processed skim milk-derived whey protein concentrate for infant formula: effects on gut development and immunity in preterm pigs, *Mol. Nutr. Food Res.*, 68, 2300458. <https://doi.org/10.1002/mnfr.202300458>

Submitted manuscripts:

Paper 3-2

Ye, Y., Fang, Y., Engholm-Keller, K., Bechshøft, M.R., Chatterton, D.E.W., Nguyen, D.N., Sangild, P.T., Bering, S.B., Lund, M.N. Effect of processing of whey protein ingredients for infant formula on protein digestibility, release of bioactive peptides, and inflammation.

Publications in preparation:

Paper 2-3

Akillioğlu, H.G., Scheijen, J., Schalkwijk, C., Lund, M.N. Multiresponse kinetic modelling of hydroimidazolone isomerization during different protein hydrolysis conditions.

Paper 3-3

Fang, Y., Akillioğlu, H.G., Ye, Y., Zhao, X., Engholm-Keller, K., Chatterton, D.E.W., Lund, M.N., Bering, S.B. Thermal processing and storage of liquid infant formula affects the release of immunomodulatory peptides after *in vitro* infant digestion.

Paper 3-4

Ye, Y., Fang, Y., Engholm-Keller, K., Bechshøft, M.R., Chatterton, D.E.W., Nguyen, D.N., Sangild, P.T., Bering, S.B., Lund, M.N. Effect of UHT treatments and storage on protein digestibility and bioactivity of whole milk.

Oral and poster presentations at scientific conferences, symposiums etc.:

- Sun, J., Akillioğlu, H.G., Aasmul-Olsen, K., Ye, Y., Lund, P., Zhao, X., Brunse, A., Christiansen, L.L., Chatterton, D.E.W., Engholm-Keller, K., Nielsen, C.F., Bechshøft, M.R., Bjørnshave, A., Thymann, T., Sangild, P.T., Lund, M.N., Bering, S.B. (2022) High temperature processing and subsequent storage of infant formulas induces protein modifications, gut dysfunction and inflammation in preterm pigs. 19th IMGC (International Milk Genomics Consortium) symposium, 18-20/10-22, Davis, California, USA (*conference abstract and oral presentation*).
- Aasmul-Olsen, K., Akillioğlu, H.A., Lund, M.N., Bjørnshave, A., Bechshøft, M.R., Thymann, T., Sangild, P.T., Bering, S.B. (2022) Improved whey protein processing for gut and immunity development in formula-fed sensitive neonates. 53rd ESPGHAN (European Society for Pediatric Gastroenterology, Hepatology and Nutrition) Annual Meeting, 22-25/6-22, Copenhagen, Denmark (*conference abstract and poster presentation*).
- Akillioğlu, H.G., Sun, J., Aasmul-Olsen, K., Lund, P., Yuhui, Y., Zhao, X., Chatterton, D.E.W., Sangild, P.T., Lund, M.N., Bering, S.B. (2021) Ultra-high temperature (UHT) treatment and prolonged storage of liquid infant formula induces protein modifications, gut dysfunction and inflammation in preterm pigs. IMARS-14: Protein glycation in food, health and disease, Doha, 20-24/9-21 (*conference abstract and poster presentation*). **Best Poster Award**
- Akillioğlu, H.A., Lund, M.N. (2021) Quantification of methylglyoxal derived hydroimidazolone in food products: Re-evaluation of acid hydrolysis. IMARS-14: Protein glycation in food, health and disease, Doha, 20-24/9-21 (*conference abstract and poster presentation*).
- Sun, J., Akillioğlu, H.G., Aasmul-Olsen, K., Lund, P., Yuhui, Y., Zhao, X., Chatterton, D.E.W., Sangild, P.T., Lund, M.N., Bering, S.B. (2021) Ultra-high temperature (UHT) treatment and prolonged storage of liquid infant formula induces protein modifications, gut dysfunction and inflammation in preterm pigs. Congress of joint European Neonatal Societies (jENS), 14-18/9-21, international online event (*conference abstract and poster*).
- Lund, P., Bechshøft, M.R., Ray, C.A., Lund, M.N. (2021) New type of whey protein ingredient for improved quality of infant formula? Dairy Science and Technology Symposium - Delivering with Dairy: from Primary Production to Primary Purpose, 21-25/6-2021, international online event (*abstract for PhD flash presentation*).
- Lund, P., Bechshøft, M.R., Mardal, F., Lund, M.N. (2020) Gentle processing of protein ingredient – characterising effects on protein modifications in infant formula. 9th International Whey Conference, 6-9/9-2020, Dublin, Ireland (*conference abstract*).
- Sun, J., Aasmul-Olsen, K., Nguyen, D.N., Akillioğlu, H.G., Lund, M.N., Nielsen, C.F., Christensen, M., Sangild, P.T., Bering, S.B. (2020) An experimental liquid formula subjected to ultra-high temperature (UHT) treatment and long-term storage adversely affects gut, kidney and systemic immunity in preterm pigs. 6th World Congress of Pediatric Gastroenterology, Hepatology and Nutrition (WCPGHAN), Copenhagen, Denmark, 3-6/6-2020 (*conference abstract and poster*).
- Sun, J., Aasmul-Olsen, K., Nguyen, D.N., Akillioğlu, H.G., Lund, M.N., Nielsen, C.F., Christensen, M., Sangild, P.T., Bering, S.B. (2020) A model infant formula subjected to indirect ultra-high temperature (UHT) treatment and long-term storage adversely affects gut, kidney and immune functions in preterm pigs. International Milk Genomics Consortium (IMGC) conference, Aarhus, DK (*conference abstract and poster*).

Oral presentations at meetings:

- Akillioğlu, H.G. (2023) Analysis of arginine-derived advanced glycation end-products (AGEs) in foods: application of acid hydrolysis. Arla Foods Ingredients Science Meeting, Nr Vium, Denmark, 30/01/2023 (*invited talk*).
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14. Contribution to master and PhD education

Pernille Lund Rasmussen, PhD, University of Copenhagen, Department of Food Science.

Yuhui Ye, PhD, University of Copenhagen, Department of Food Science.

Karoline Aasmul-Olsen, PhD, University of Copenhagen, Department of Veterinary and Animal Sciences.

15. New contacts/projects

The INFANT-I project has allowed KU to build up further competences and generate knowledge within the fields of gentle processing techniques, chemical and structural characterization of proteins, protein bioactivity, and how these processing-induced modifications affect gut function and health. These competences have improved our position in the international research arena, and have made us more attractive as collaboration partners in future research projects with industry and international academic partners. We have already been invited to join other scientific projects through our collaborations.

Generation of this unique knowledge that covers a broad range of scientific disciplines have been published in high-impact factor scientific journals and the knowledge are used for teaching MSc and PhD students in food/dairy science and nutrition.