SLUTRAPPORT/FINAL REPORT
NR. 2021-166

Marianne Nissen Lund:

IMAGE: Improvement of the quality and stability of UHT treated dairy products by enzymatic tailoring of the milk carbohydrate profile

IMAGE: Forbedring af kvalitet og stabilitet af UHT behandlede mejeriprodukter ved enzymatisk design af mælke-sukkerprofil

Mejeribrugets
ForskningsFond

March 2021

Final report

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

1. Title of the project

In Danish: IMAGE: Forbedring af kvalitet og stabilitet af UHT behandlede mejeriprodukter ved enzymatisk design af mælkesukkerprofil

In English: IMAGE: Improvement of the quality and stability of UHT treated dairy products by enzymatic tailoring of the milk carbohydrate profile

2. Project manager

Professor Marianne Nissen Lund, Department of Food Science (FOOD), University of Copenhagen (UCPH), Rolighedsvej 26, DK-1958 Frederiksberg C, Phone: 3533 3547, Email: mnl@food.ku.dk

3. Other project staff

PhD student Wei Zhang, FOOD-UCPH, employed at Uppsala University now

Assis prof. Cristian de Gobba, FOOD-UCPH, gobba@food.ku.dk

Assoc prof. Karsten Olsen, FOOD-UCPH, ko@food.ku.dk

Assis prof. Marie Sofie Møller, Department of Systems Biology, DTU, msmo@dtu.dk

Prof. Birte Svensson, Department of Systems Biology, DTU, bsn@bio.dtu.dk

Senior scientist Valentin Rauh, Arla Foods amba, varau@arlafoods.com

Senior scientist John Sørensen, Arla Foods amba, now retired

4. Sources of funding

DDRF, FOOD-UCPH, and China Scholarship Council (CSC)

5. Project period

Project period with DDRF funding: 1st July 2016 – 31st October 2018

Total project period, if sub-project within a larger project: 1st October 2015 – 30th March 2019

6. Project summary

In Danish:

Formålet med IMAGE var at undersøge, hvordan Maillard-reaktioner kan hæmmes i laktose-fri UHT-behandlet mælk ved at benytte et enzym, som ikke kun kløver laktose til galaktose og glukose, men også sætter galaktose-enheder sammen til galakto-oligosaccharider (GOS). Dermed vil der ende med at være færre reducerende sukre, som kan indgå i Maillard-reaktioner, og i sidste ende forventes det at UHT-mælken vil få en forlænget holdbarhed og udvikle brunfarvning senere end for en normal laktose-fri, UHT-behandlet mælk.

Modelforsøg viste, at GOS gav anledning til færre Maillard-reaktionsprodukter end monosaccharider under varmebehandling (75-130 °C) af kaseinopløsninger ved pH 6,8. GOS blev dog fundet at danne mere 3-deoxyglucosone, en dicarbonyl, som er et reaktivt Maillard-intermediat. Dette var et overraskende resultat, og kunne give anledning til flere advanced glycation endproducts (AGEs), men af de AGEs, det er muligt at analysere for, var der ikke nogen effekt. Et opfølgende studie viste, at dannelse af 3-deoxyglucosone kun krævede varmebehandling ved 40 °C i 24 timer af GOS opløst i fosfat-buffer ved pH 6,8.

En GOS-mælk blev fremstillet enzymatisk og UHT-behandlet. Den enzymatiske behandling blev optimeret, så der blev dannet ca. 35% GOS, hvilket er højere GOS-niveauer, end hvad der tidligere er rapporteret. I den friske, UHT-behandlede GOS-mælk blev der dannet tilsvarende høje niveauer af 3-deoxyglucosone, som fundet i kasein-modelsystemerne, men dette gav kun anledning til øgede niveauer af 5-hydroxymethylfurfural (HMF) (Maillard-markør der dannes fra 3-deoxyglucosone) og ikke af AGEs. Til sammenligning blev der målt høje niveauer af både HMF og AGEs i frisk, UHT-behandlet laktose-fri mælk sammenlignet med konventionel UHT-mælk.

Den UHT-behandlede GOS-mælk blev ikke lagret, da GOS var ustabile ved lagring. Derimod blev konventionel og laktose-fri UHT-mælk lagret ved 20, 30 og 40 °C i et år og dannelse af dicarbonyler, 15 forskellige AGEs og HMF blev kvantificeret over lagringstiden. Den konventionelle UHT-mælk var meget stabil ved lagring ved 20 °C (ingen signifikant udvikling af Maillard-reaktionsprodukter) over et år, mens den laktose-fri UHT-mælk var mere udsat for dannelse af Maillard-produkter, især ved højere lagringstemperaturer.

Kasein (alfa, beta og kappa)-kasein interaktioner blev bestemt ved Surface Plasmon Resonance Spectroscopy, og tilstedeværelse af forskellige sukkerarter påvirkede ikke protein-protein interaktionerne.

In English:

The aim of the IMAGE project was to study how Maillard reactions can be inhibited in lactose-free UHT-treated milk by using an enzyme that not only cleaves lactose into galactose and glucose but also puts galactose units together into galacto-oligosaccharides (GOS). In this way, there will be fewer reducing sugars that can be included in Maillard reactions, and ultimately, it is expected that the UHT milk will have an extended shelf life and develop browning later than a normal lactose-free, UHT-treated milk.

Model experiments showed that GOS gave rise to fewer Maillard reaction products than monosaccharides under heat treatment (75-130 °C) of casein solutions at pH 6.8. However, GOS was found to produce more 3-deoxyglucosone, a dicarbonyl, which is a reactive Maillard-intermediate. This was a surprising result and could give rise to more advanced glycation end products (AGEs), but of the AGEs it is possible to analyse for, there was no effect. A follow-up study showed that the formation of 3-deoxyglucosone only required heat treatment at 40 °C for 24 hours of GOS dissolved in phosphate buffer at pH 6.8.

One GOS milk was produced enzymatically and UHT-treated. The enzymatic treatment was optimised so that approx. 35% of GOS were formed, which is higher GOS levels than previously reported. In the fresh, UHT-treated GOS milk, similarly high levels of 3-deoxyglucosone were formed as those found in the casein model systems, but this only gave rise to increased levels of 5-hydroxymethylfurfural (HMF) (Maillard marker formed from 3-deoxyglucosone) and not of

AGEs. By comparison, high levels of both HMF and AGEs were measured in fresh, UHT-treated lactose-free milk compared to conventional UHT milk.

The UHT-treated GOS milk was not stored as GOS was unstable during storage. On the contrary, conventional and lactose-free UHT milk was stored at 20, 30 and 40 °C for one year, and the formation of dicarbonyls, 15 different AGEs and HMF were quantified during the storage time. The conventional UHT milk was very stable in terms of storage at 20 °C (no significant development of Maillard reaction products) over a year, while the lactose-free UHT milk was more prone to the formation of Maillard products, especially at higher storage temperatures.

Casein (alpha, beta and kappa)-casein interactions were determined using Surface Plasmon Resonance Spectroscopy, and the presence of various sugars did not affect the protein-protein interactions.

7. Project aim

In Danish:

At vise at ændring af sukkerprofilen i mælk ved omdannelse af laktose til galakto-oligosakkarider vil forbedre lagerstabilitet og kvalitet af laktose-reducerede UHT-mejeriprodukter.

At bestemme i hvilken grad ændring af sukkerprofilen i mælk mod et oligosakkarid-rigt produkt vil påvirke sukker-protein og protein-protein interaktioner og proteindenaturering og efterfølgende, hvad dette betyder for de fysiske karakteristika af mælken og stabilitet under transport og lagring.

Samlet set er formålet at give den danske mejeriindustri ny basal viden, som kan bidrage til strategier til produktion af langtidsholdbar, laktose-fri mælk af høj kvalitet til eksportmarkeder.

In English:

To show that altering the carbohydrate profile of milk by converting lactose into galacto-oligosaccharides will improve shelf stability and quality of lactose-reduced UHT dairy products.

To determine to what extent changing the carbohydrate profile of milk towards an oligosaccharide-rich product will affect carbohydrate-protein (glycation and subsequent age gelation), protein-protein interactions (aggregation, age gelation) and protein denaturation and subsequently what this will mean for physical, chemical and sensory properties of UHT milk and stability during transport and storage.

Taken together, the objective is to provide the Danish dairy industry with new, basic knowledge that can contribute to strategies for producing high-quality, long-life liquid lactose-reduced milk products for domestic and export markets.

8. Background for the project

To a large extent, long-life milk is exported to Asia and Africa in particular. The long shelf-life of the milk is achieved by UHT treatment that kills bacteria. However, the heat treatment also initiates chemical reactions between sugars and proteins. These reactions are called Maillard reactions and give rise to browning and formation of unwanted flavours, especially during uncontrolled storage conditions. In lactose-free milk, lactose is hydrolysed into galactose and glucose, where in particular, galactose is significantly more reactive in Maillard reactions. This means that the problems with browning and undesirable off-flavours are even greater in lactose-free UHT milk. The shelf-life of lactose-free UHT milk is therefore also significantly lower than the shelf-life of conventional UHT milk.

Lactose-free milk is usually produced by the use of an enzyme, lactase, which cleaves lactose into glucose and galactose. Some lactases can also incorporate galactose into galacto-oligosaccharide (GOS), where several galactose units are bound together. This means that there are a total of fewer galactose units to be included in the Maillard reactions. The hypothesis in the IMAGE project was, therefore, to investigate whether the conversion of galactose to GOS inhibits Maillard reactions in UHT milk and the effect of this on taste and colour.

9. Sub-activities in the entire project period

WP 1 Identification and quantification of Maillard reactions in heat treated GOS-enriched dairy models.

WP 2.1 Storage dependent chemical and physical changes during storage of UHT milk.

WP 2.2 Elucidating non-Maillard interactions of oligosaccharides and milk proteins and implications for storage stability of UHT milk.

Gantt chart

WP	Project activity		2016				2017				2018			
MFF funding														
PhD: Wei Zhang		x	x	x	x	x	x	x	x	x	x	x	x	
Postdoc: Mahesha Poojary														
WP1 Identification and quantification of Maillard reactions in heat treated GOS-enriched dairy models														
1	Establish quantitative methods: Dicarbonyls, GOS, HMF, furfural, milk proteins	x	x			x								
1	Characterisation of Maillard reactions heat-treated GOS-protein model systems		x	x	x	x								
1	Characterisation of sugar degradation in heat-treated GOS model systems				x	x	x							
1	Writing up 1-2 paper(s)						x	x						
WP2 Elucidating the effect of carbohydrate profile on stability, sensory, and nutritional properties of UHT milk during storage														
WP 2.1: Storage dependent chemical and sensory changes associated with GOS-enriched milk														
2.1	Test of enzyme efficiency and optimal conditions							x						
2.1	Production of UHT milk and storage (20, 30, 40C)							x	x	x	x			
2.1	Sensory analysis (Arla)							x	x	x	x			
2.1	Characterisation of stored UHT milk							x	x	x	x	x		
2.1	Writing up 1-2 paper(s)								x			x		
WP 2.2: Elucidating non-Maillard interactions of GOS and milk proteins and implications for storage stability of UHT milk														
2.2	Effect of GOS on physical parameters of milk													
2.2	GOS-protein binding/protein-protein binding													
2.2	Writing of 1-2 paper(s)									x		x		
	Writing up PhD thesis												x	
	MFF steering committee meetings							x		x		x		x

10. Deviations

The aim of WP 2.1 was to carry out a storage experiment with enzymatically processed milk, where lactose was converted into GOS instead of monosaccharides, but GOS was not stable, so the GOS milk was removed from the storage experiment, as it did not make sense to continue the experiment. A paper was published on the fresh GOS-UHT milk. Instead, the storage experiment was conducted with conventional and lactose-free milk, which was characterised in great detail in relation to the development of Maillard reaction products at 20 ° C, 30 ° C, and 40 ° C for 12 months. The trial was conducted without delay in relation to the time schedule.

Colin Ray was initially the project manager and grant holder of the project, but when he left UCPH, Marianne Nissen Lund took over the project management from Colin Ray. At the same time, Ali Osman also stopped working at Arla, so Valentin Rauh and John Sørensen took over from Ali Osman. The collaboration with Arla Foods was very good and without delay in the project schedule, despite the fact that the project originators left their respective positions.

11. Project results

Initially a review paper on strategies to control Maillard reactions in foods was written by Colin Ray and Marianne N. Lund (paper 1); a simplified overview of Maillard reactions from this review is given in Figure 1. Formation of HMF is not included in the Figure, but is formed by further degradation of 3-deoxyglucosone. Formation of Strecker aldehydes is important for off-flavor of UHT milk, but was not evaluated in the IMAGE project, since this had just been thoroughly investigated in the PhD study of Therese Jansson (e.g. Jansson et al. 2014. *J. Agric. Food Chem.* 62, 7886-7896).

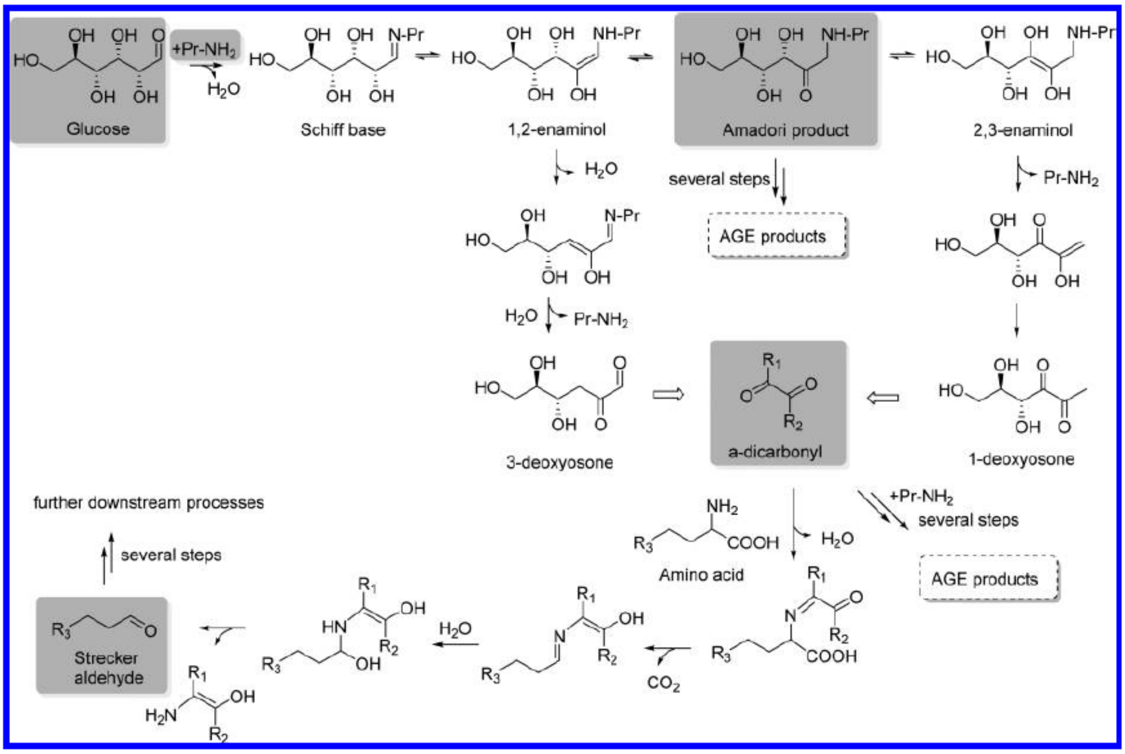

Figure 1. Simplified scheme of the Maillard reaction of a reducing sugar (glucose; an aldose) and a protein amine group to form a Schiff base, which can rearrange to an Amadori product (or Heyns product in the case of ketoses) via an 1,2-enaminol. The Amadori product can then undergo enolization to 1,2-enaminol or 2,3-enaminol and form deoxyosones (α-dicarbonyl compounds), which react rapidly with additional nucleophiles to form Strecker aldehydes. A number of the Maillard reaction intermediates can generate AGEs as marked in the scheme. Reactants and intermediates that have been modified to control Maillard reactions are highlighted in gray.

WP1 Identification and quantification of Maillard reactions in heat treated GOS-enriched dairy models by Wei Zhang

<u>Paper 2:</u> Four caseinate-carbohydrate models, LAC, MON, GOSP and HMON, were prepared to represent four types of milk as also indicated in Table 1. The composition of the LAC was prepared according to the molar ratio of sugar to lysine of 10:1, as in milk. The MON was prepared according to the composition of a lactose hydrolyzed milk, while the GOSP was prepared to represent a GOS-enriched lactose hydrolyzed milk using a β-galactosidase. In some lactose hydrolyzed milks the initial lactose is reduced by filtration in order to avoid increase in sweetness after hydrolysis, and

the HMON represents this type of milk. The two remaining models, GAL and GOS, were included in order to have models with comparative concentrations of GAL and GOS to that of LAC.

Table 1. Carbohydrate-caseinate model systems investigated in the present study. The protein source was sodium caseinate (3% w/v) and pH was adjusted by phosphate buffer (0.1 M, pH 6.8).

Models	Carbohydrate composition	Representative for milk type
LAC*	0.15 M lactose	Conventional
MON	0.30 M monosaccharides: 0.15 M galactose and 0.15 M glucose	Lactose hydrolyzed
GOSP	0.17 M new vivinal GOS added 0.05 M glucose and 0.019 M galactose	Milk added GOS-producing enzyme
GAL*	0.15 M galactose	-
HMON*	0.15M half monosaccharides: 0.075 M galactose and 0.075 M glucose	Filtered and lactose hydrolyzed
GOS*	0.15M vivinal GOS	-

*Contains same concentration of reducing carbohydrates

The models were incubated at 75 °C for 32 hours and 130 °C for 60 min to facilitate Maillard reactions and investigate the effect of the different carbohydrates.

Browning is often evaluated by measurement of absorbance at 420 nm and is correlated to the formation of melanoidins, which is the final stage of the Maillard reaction. At 130 °C, the GOSP and GOS developed less browning during heating compared to the models containing monosaccharides (MON, GAL and HMON). At 75 °C, the MON showed the highest browning intensity, followed by GOSP, GAL, HMON and LAC. The monosaccharide-containing models (MON, GAL, HMON) had a significantly faster amine loss compared to the other models at 130 °C, which was in agreement with development of browning. GOSs showed a lower blocking of free amines and browning development associated with Maillard reaction compared with galactose at 75 °C. Furosine is formed from acid hydrolysis of Amadori products and is therefore often used as a marker for the ketoamines that are formed in the early stage of Maillard reactions. GOS-containing models was low compared to the other models at 130 °C. MON system accumulated approximately three-fold as much furosine as compared to the rest of the model systems at 75 °C. α-Dicarbonyls are reactive intermediates formed during both Maillard reactions and sugar condensations. Rapid accumulation of 3-deoxyglucosone (3-DG) and 3-deoxygalactosone (3-DGal) were detected in GOSP in the beginning of the incubation at 130 °C and at 75 °C (after 15 min and 8 hour, respectively). Next, the accumulated C6 α-dicarbonyls tended to decrease in concentration to similar levels as those observed for GOS- and galactose-containing models. In LAC, more 3-DGal was detected and no 3-DG was detected, which may be described by the lactose isomerization to lactulose, followed by fragmentation to galactose which subsequently induced 3-DGal. 5-Hydroxymethylfurfural (HMF) is a product formed from 3-deoxysones during both sugar condensation and Maillard reactions. The GOS-containing models had the highest formation of HMF at both 130 °C and 75 °C followed by the MON, then GAL and HMONs and finally the LAC. The HMF results did not follow the same trend as observed for development of color, loss of amines and formation of Amadori product where GOS-containing models were found to have lower reactivity than most of the other models. In conclusion, GOS-containing caseinate model systems had less consumption of free amines, less formation of Amadori product and less development of brown color during incubation at 75 °C for 32 h and 130 °C for 60 min compared to monosaccharide-containing caseinate model systems. These results indicate that conversion of lactose in milk into GOS by addition of suitable β-galactosidases rather than hydrolysis could potentially inhibit Maillard reactions during storage of UHT treated milk. However, GOS-containing model systems produced more HMF and more α-dicarbonyls compared to monosaccharide-containing models, which suggests that GOS is less heat stable and more susceptible to sugar condensation reactions during incubation at both 75 °C and 130 °C. Therefore, a stability the study of the carbohydrates at lower temperatures (40 and 50 °C) was conducted in order to get further insight into the degradation of GOS (Paper 3).

Paper 3: The formation of seven α-dicarbonyls was characterized in solutions containing dairy related carbohydrates (galactose, glucose, lactose, and galacto-oligosaccharides (GOS)) during incubations at 40 and 50 °C with and without Nα-acetyl-L-lysine at pH 6.8 for up to 2 months. The concentrations of α-dicarbonyls in samples of monosaccharides with Nα-acetyl-L-lysine were found to be 3-deoxyglucosone (3-DG) > 3-deoxygalactosone (3-DGal) > glyoxal > glucosone, galactosone > methylglyoxal > diacetyl. The presence of Nα-acetyl-L-lysine resulted in up to 100-fold higher concentrations of C6 α-dicarbonyls but lesser formation of glyoxal in the monosaccharide-containing models compared to what was observed in the absence of Nα-acetyl-L-lysine. Galactose incubated with Nα-acetyl-L-lysine generated the highest concentrations of 3-DGal (up to 130 μM), glyoxal (up to 100 μM), and methylglyoxal (up to 9 μM) compared to the other carbohydrates during incubation. Surprisingly, 3-DG (1500 μM) and 3-DGal (80 μM) were formed at levels of 2 orders of magnitude higher in solutions of GOS in the absence of Nα-acetyl-L-lysine as compared to the other carbohydrates at 40 °C, while GOS generated the lowest levels of glyoxal. GOS are widely used as an ingredient in various types of foods products, and it is therefore of importance to consider the risk of generating high levels of the reactive C6 α-dicarbonyl, 3-DG, in these types of products. This study contributes to the understanding of major α- dicarbonyl formation as affected by the presence of primary amines in GOS-, lactose-, and galactose-containing solutions under moderate heating in liquid foods.

Paper 4: An advanced LC-MS/MS based method was developed and validated for the simultaneous identification and quantification of 15 different AGEs, furosine (an indicator of Amadori products), 2 protein-derived cross-links (lanthionine and lysinoalanine) and 2 amino acids (Lys and Arg). The analytes were separated on a reversed phase C-18 column and quantified accurately based on the isotope dilution method, where 9 stable isotope-labelled internal standards were used to quantify 20 different analytes using an Orbitrap mass analyzer. The method showed acceptable linearity, accuracy and precision. The LOD and LOQ values in plasma were in the range of 0.30–19.02 and 0.87–57.06 ng/mL, respectively. The recovery values at the three spiked levels were in the range of 71–110%, with some exceptions. The intraday and interday precision were in the range of 1.5–13.2%, however, quantification of N-ε-(carboxymethyl)lysine accompanied slightly higher interday precision (30.7%). The applicability of the method was successfully assessed by analyzing AGEs and protein cross-links in six different complex matrices including Ultra-High Temperature (UHT) processed milk, roasted chicken breast meat, roasted chicken skin, roasted pork liver, bovine plasma and perfusion liquid.

WP2.1 Storage dependent chemical and physical changes during storage of UHT milk

Paper 5: The formation of 3-deoxyglucosone (3-DG), 3-deoxygalactosone (3-DGal), 5-hydroxymethyl furfural (HMF), pyrraline, as well as fifteen advanced glycation end products (AGEs), including lysine-derived (CML, CEL, GOLD, MOLD, pentosidine), and arginine-derived (MG-H1, G-H1, G-H2, argpyrimidine) AGEs, were thoroughly investigated in both lactose-free UHT milk (LF) and conventional UHT milk (CON) during storage at 20°C, 30 °C and 40°C for up to one year. The accumulation of 3-DG and 3-DGal in LF-UHT milk ranged from 20-fold to 44-fold higher than what was observed in CON-UHT milk after one year of storage depending on temperature. The formation of HMF and pyrraline was only 2- to 5-fold higher in LF-UHT milk compared to CON-UHT milk during storage. Three routes for the disappearance of 3-DG and 3-DGal were proposed, including formation of HMF, pyrraline and other Maillard products (MRP), where formation of HMF was found to be a key step in milk.

The level of AGEs in milk is following: CML > MG-H1 > CEL > G-H1 > G-H2 > GOLD > GALA, argpyrimidine, GOLA, MOLD, pentosidine. The total amount AGEs derived from three nucleophile amino acid is following: lysine > arginine. Significant higher level of CEL and MG-H1 was observed in LF than in CON. This study significantly expands the available AGE database (15 AGEs), validates the AGE quantification methodology and proposes the AGEs formation mechanism in milk. These studies contribute to a better understanding of the differences in Maillard reaction pathways between CON and LF-UHT milk under extended storage conditions at realistic temperatures.

Paper 6: Commercially available β-D-galactosidases with transgalactosylating activity was used to incorporate galatose into galactooligosaccharides (GOS). The aim of this study was to examine the extent of Maillard reactions in a lactose-reduced GOS milk compared to LH and CON milk after UHT processing. The GOS milk had significant lower levels of lysine- and arginine-derived AGEs compared to LH milk, while their concentrations were similar to those found in CON milk. The total concentration of measured Arg-derived AGEs was similar to the total concentration of Lys-derived AGEs in the three types of milk, indicating that Arg is an important source of AGEs in milks. Interestingly, the GOS milk generated threefold higher concentrations (up to 330 ± 6 µM) of 3-deoxyglucosone (3-DG, a C6 α-dicarbonyl). These results demonstrate that GOS milk could be a potential alternative for LH milk for lactose-intolerant individuals, although further studies are needed to understand the increased formation of 3-DG in GOS-containing milk.

WP 2.2 Elucidating non-Maillard interactions of oligosaccharides and milk proteins and implications for storage stability of UHT milk

Paper 7: The method for the investigation of GOS-protein and protein-protein interactions by using Surface Plasmon Resonance (SPR) technology was optimized to make it possible to immobilize α-, β- and κ-casein onto the chip surface, and to measure the interaction of different caseins in presence or absence of GOS and lactose. Based on these results it was possible to calculate the steady state affinity constant (K_D) between the different proteins. Soluble α_s-casein had roughly 10 times lower affinity against immobilized α_s-casein and β-casein than soluble β-casein, and 40 to 60 times lower affinity than κ-casein. An opposite behavior, instead, was seen when κ-casein was immobilized. In this case α_s-casein showed the highest affinity, followed by β-casein and κ-casein. Interesting to notice was that no binding reversibility was observed: the K_D of soluble β-casein against immobilized α_s-casein was 21 times higher than the opposite interaction (soluble α_s-casein against immobilized β-casein). After calculating the K_D of the different caseins, lactose or GOS were added to the solubilized casein in order to test if the presence of sugars affected the interaction between proteins. The results show that the presence of lactose or GOS had a minor effect on interaction of caseins. It is possible to see differences between the samples, but the values were all in the same range. Finally, we tried to calculate the constants of association (K_a) and dissociation (K_d) of the various interactions using a 1:1 binding model. Although it was possible to obtain numerical values, it is our opinion that the 1:1 fitting model cannot be used with caseins, since the curves formed in the sensorgrams are not in accordance to the fitting and to a 1:1 binding sensorgram model.

These studies showed that neither GOS nor lactose were able to interact with caseins at room temperature and short incubation times.

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

The very detailed characterisation of Maillard reaction products in conventional lactose-free UHT milk over one year of storage at 20-40 °C is extremely useful for developing strategies for inhibiting Maillard reactions in UHT milk. Some of the reaction products that are formed may be harmful to health (a highly debated area in the current literature), so the large quantitative data material produced in this project enables a much more targeted effort to inhibit Maillard reaction products in long-lasting dairy products in the future. This clear and quantitative data can also help to assess whether the levels in milk are an issue from potential future legislation on which levels are acceptable.

In addition, the formation of dicarbonyls in GOS is an important discovery, as this risks affecting the formation of additional Maillard reaction products in other dairy products, for example infant formula, and this must be investigated further.

13. Communication and knowledge sharing about the project

Papers in international journals: 7

Easily read papers: 2

Student theses: 1

Oral presentations at scientific conferences, symposiums etc.: 0

Oral presentations at meetings: 0

Scientific publications in peer-reviewed international journals:

Papers 1 and 4 were prepared in collaboration with the PELUM and ICOM projects [PELUM: Second generation lactose free ultra-high-temperature processed milk drinks with functional polyphenols for export markets; funded by Innovation Fund Denmark] and [ICOM: Inhibition and control of Maillard reactions in dairy foods by plant polyphenols; funded by Independent Research Fund Denmark].

1. Marianne N. Lund & Colin A. Ray (2017) Control of Maillard reactions in foods: Strategies and chemical mechanisms: J. Agric. Food Chem, 65, 4537-4552. DOI: 10.1021/acs.jafc.7b00882. Review paper, status as highly cited on Web of Knowledge

2. Wei Zhang, Colin Ray, Mahesha M. Poojary, Therese Jansson, Karsten Olsen & Marianne N. Lund (2019) Inhibition of Maillard reactions by replacing galactose with galacto-oligosaccharides in casein model systems. *J. Agric. Food Chem.* 67, 875-886. DOI: 10.1021/acs.jafc.8b05565

3. Wei Zhang, Mahesha M. Poojary, Karsten Olsen, Colin A. Ray & Marianne N. Lund (2019) Formation of α-dicarbonyls from dairy related carbohydrates with and without Nα-acetyl-L-lysine during incubation at 40 °C and 50 °C. *J. Agric. Food Chem.*, 67, 6350-6358. DOI: 10.1021/acs.jafc.9b01532

4. Mahesha M. Poojary, Wei Zhang, Ines Greco, Cristian de Gobba, Karsten Olsen & Marianne N. Lund (2020) Liquid chromatography quadrupole-Orbitrap mass spectrometry for the simultaneous analysis of advanced glycation end products and protein-derived cross-links in food and biological matrices. *J. Chrom. A*, 1615, 460767. https://doi.org/10.1016/j.chroma.2019.460767

5. Wei Zhang, Mahesha M. Poojary, Valentin Rauh, Colin A. Ray, Karsten Olsen & Marianne N. Lund (2019) Quantitation of α-dicarbonyls and advanced glycation endproducts in conventional and lactose-hydrolyzed UHT milk during 1 year of storage. *J. Agric. Food Chem.,* 67, 12863-12874. https://doi.org/10.1021/acs.jafc.9b05037

6. Wei Zhang, Mahesha M. Poojary, Valentin Rauh, Colin A. Ray, Karsten Olsen, K. & Marianne N. Lund (2020) Limitation of Maillard reactions in lactose-reduced UHT milk via enzymatic conversion of lactose into galactooligosaccharides during production. *J. Agric. Food Chem.*, 68, 3568-3575. DOI: 10.1021/acs.jafc.9b07824

7. Cristian De Gobba, Marie S. Møller, Valentin Rauh, Birte Svensson & Marianne N. Lund (2020) Casein-Casein Interactions in the Presence of Dairy Associated Carbohydrates Analyzed Using Surface Plasmon Resonance. *Int. Dairy J.*, 105, 104686. https://doi.org/10.1016/j.idairyj.2020.104686

Easily read papers:

Lund, M.N., Rauh, V. (2020) Forbedret UHT-mælk med enzymer? Mælkeritidende, 2, 16-17

Lund, M.N., Rauh, V. (2017). Bedre UHT mælk med enzymer. [Mælkeritidende, 23, 12-13]

14. Contribution to master and PhD education

Education of PhD student Wei Zhang. PhD thesis: Mechanism of Maillard reactions in lactose-hydrolyzed UHT milk and strategy of inhibition by galacto-oligosaccharides, March 2019, University of Copenhagen. [Link]

15. New contacts/projects

The proposed enzymatic strategy of IMAGE turned out not to work due to poor performance of the GOS-producing enzyme, but the methodology developed in IMAGE is now being used extensively in other dairy-related research projects:

In the ICOM project (Inhibition and control of Maillard reactions in dairy products by plant polyphenols) funded by Independent Research Fund Denmark (collaboration with Arla Foods and Arla Foods Ingredients), the effect of plant polyphenols on Maillard reactions in dairy foods is investigated.

In the INFANT-I project (Tailored processing of bioactive ingredients for high-end infant formulas) funded by GUDP (collaboration with Arla Foods Ingredients) the effect of gentle processing protein ingredients on Maillard reactions in infant formula is investigated.

In the DOABLE project (Effect of Maillard reactions and lipid peroxidation on AGEs formation in dairy-plant oil blends) funded by Arla Foods, the effect of dicarbonyl formation during lipid oxidation and subsequent AGEs formation is investigated.

16. Signature and date

The project is formally finalised when the project manager and DDRF-representative (e.g. steering committee leader) have signed this final report.

Date: 9/3-2021 Signature, Project manager: MNLund

Date: 15 March 2021 Signature, DDRF-representative: