
© 2008 AVA Agrar-Verlag Allgäu

Reprinted with permission.
Soybean oil with the highest density, surface tension and yield stress formed the most stable emulsions; among other oils similar tendency was not found. The influence of the oil type on emulsion properties is not well documented. Few publications demonstrated superiority of sunflower to soybean oil, in relation to the emulsion stability, and pointed at a significance of the nature of the oil phase in shaping emulsion properties (8,2). However, it seems that method and conditions of the oil manufacture and raw material variety do not allow to make any comparisons.

### 4. Conclusions

The continuous phase properties significantly influenced physicochemical properties of the emulsions. Increasing protein concentrations of the continuous phase followed by higher surface tension, density, conductivity and viscosity caused a decrease in emulsion stability and an increase in the emulsion surface tension and conductivity. The oil type had a significant impact on the emulsion properties; however, besides soybean oil with the highest density, surface tension and yield stress, the properties of the other oil-based emulsions were not attributed to measured oil properties. An increase in surface tension and electrical conductivity may be an indication of flocculation.

### Acknowledgment

This research has been supported by the Polish Ministry of Scientific Research and Information Technology under Grant 2P06T00829 (2005-2008).

### 5. References

(11) Statistica 7.1 Statsoft Inc., USA (2005)
1. Introduction

Heat treatment of milk can lower the quality of milk proteins by the Maillard reaction between reducing sugars and free amino groups of proteins. Lysine is the essential amino acid most affected as its free ε-amino group is very reactive (1,2).

The first stable intermediate of the Maillard reaction in normal milk is lactuloselysine (ε-N-deoxyalactosyl-L-lysine), which is biologically unavailable (2,3). Lysine damage can be estimated on the basis of furosine (ε-N-2-furoylmethyl-L-lysine), which can be released from the lysine derivatives with acid hydrolysis (2,4). Several studies on formation of furosine during UHT-treatment or storage of unhydrolysed UHT-milk have been published (e.g. 5, 6, 7, 8). Direct UHT treatment is usually found to be more gentle than indirect UHT-treatment (5, 6, 7).

The effect of heat treatments and storage on lactose hydrolysed milk has been studied in only few studies (4, 9, 10, 11, 12). In lactose hydrolysed milks lysine damage caused by the Maillard reaction is often more significant due to higher molar level of reducing sugars. MENDOZA et al. (9) studied the formation of furosine in lactose hydrolysed milk in UHT-process at different heating temperatures and times. Significant formation of furosine took place (17-184 mg/100 g protein) and hydrolysis of lactose after the heat treatment was suggested in order to avoid lysine damage. MARCONI et al. (10) found furosine levels of 942 and 1070 mg/100 g protein in commercial lactose hydrolysed UHT-milks. EVANGELISTI et al. (11) studied furosine formation in lactose hydrolysed UHT-milks during storage at 4°C and 20°C and found 134 and 206 µg furosine per 100 g protein within 5 d after production. After 3 months' storage at 20°C the levels rose to 401 and 555 mg/100 g protein respectively. According to the authors storage <4°C should be compulsory to limit protein damage. The range of published furosine levels of lactose hydrolysed UHT-milks is very wide (9, 10, 11). During recent years lactose hydrolysed and lactose free milks have gained significant market share in several market areas (13). Nutritional value of these milks should stay as good as possible.

In this study the effect of lactose hydrolysis on formation of furosine in skim milk during storage at 5°C, 22°C, 30°C and 45°C for 3 months was followed after direct UHT treatment. Lactose was hydrolysed either before or after the UHT-treatment. The amount of available lysine was estimated on the basis of furosine and also measured as OPA-reactive lysine. Concentrations of glucose, galactose, fructose and lactulose and colour of the milks were measured.

2. Materials and methods

Milk was normal skim milk received at Valio's UHT-plant, Turenki, Finland. Production of the three different UHT milk samples (unhydrolysed, prehydrolysed and posthydrolysed) and the direct UHT process (141°C, 2-4s) is described in earlier article (14). The same milk batch was used both for the unhydrolysed and posthydrolysed milk tests. Very similar milk was used for the posthydrolysed milk test (Table 1).

2.1 Analyses

Samples were analysed every 4 weeks for furosine, glucose and galactose and reactive lysine. Furosine was analysed according to (15). Glucose, galactose, fructose and lactulose were measured using high-performance anion-exchange chromatography with pulsed amperometric detection with a modified method of DE SLEGTE (16). Lysine blockage was estimated on the basis of furosine according to FINOT et al. (4) and EVANGELISTI et al. (11). Reactive lysine values were corrected by multiplying with 0.8 before applying the FINOT principle as suggested by EVANGELISTI et al. (11). Reactive lysine was analysed according to VIGO et al. (17) dissolving liquid milk samples in 10% SDS for at least 1 hour. Possible interference of the free amino groups of amino acids, amines and small peptides was checked according to (18). Milk samples were analysed for fat (19), protein and total nitrogen (20), lactose (21). Advanced Maillard reaction was followed by measuring colour with a colorimeter Minolta CR-210 (Japan). The instrument detects the light reflected by the sample and presents the result in the CIELab colour system where the colour is described by three dimensions L (lightness), a and b (chromaticity coordinates). The colour change (ΔE) was calculated by the equation

\[ \Delta E = \sqrt{(L_t - L_0)^2 + (a_t - a_0)^2 + (b_t - b_0)^2} \]  

where t denotes the storage time and 0 the beginning of the storage (22). A white standard plate was used for the calibration of the device. A 1 cm layer of the sample was poured on a petri dish for the measurement and the analysis was carried out at room temperature. Measurement was performed in six repetitions.

<table>
<thead>
<tr>
<th>Table 1: Composition of the UHT-treated milks directly after packaging</th>
<th>Unhydrolysed</th>
<th>Prehydrolysed</th>
<th>Posthydrolysed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (%)</td>
<td>3.42</td>
<td>3.54</td>
<td>3.49</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>0.08</td>
<td>0.08</td>
<td>0.07</td>
</tr>
<tr>
<td>Lactose (%)</td>
<td>4.48</td>
<td>0.10</td>
<td>2.57</td>
</tr>
<tr>
<td>Glucose (%)</td>
<td>-</td>
<td>2.36</td>
<td>1.20</td>
</tr>
<tr>
<td>Galactose (%)</td>
<td>-</td>
<td>2.26</td>
<td>1.02</td>
</tr>
</tbody>
</table>

3. Results

Table 1 shows the composition of the freshly packed UHT-milks. The compositions of the raw milks used for this study are presented elsewhere (14). After the storage of two weeks lactose hydrolysed milks had roughly the same contents of monosaccharides (Fig. 3 a-b).

3.1 Changes in furosine during storage

Development of furosine in unhydrolysed and hydrolysed milk is shown in Figures 1 (a-b). A clear difference between prehydrolysed and posthydrolysed milk can be seen when stored at 5°C. At this temperature development of furosine in posthydrolysed milk is close to that of unhydrolysed milk. However, at the temperature of 22°C the difference between prehydrolysed and posthydrolysed milk is already very small. In 30 and 45°C no difference can be seen between prehydrolysed and posthydrolysed milks. The development of furosine in lactose hydrolysed milks is clearly faster than in unhydrolysed milk at all storage temperatures.
3.2 Changes in blocked lysine and reactive lysine during storage

The content of reactive lysine measured with OPA-reagent describes the level of unreacted lysine in milk samples. However, reactive lysine was not possible to be measured reliably by the OPA-method if significant proteolysis had occurred (17). In our previous study (14) it was shown that proteolysis proceeded in lactose hydrolysed milks stored at 22°C and above this temperature. Therefore only 5°C samples were included in the analyses. According to the OPA-method no significant changes took place during the storage at this temperature (data not shown).

Table 2 shows the lysine blockage estimated on basis of the furosine content. This shows that the difference between the different milks at 5°C is small but clear. In prehydrolysed milk the lysine blockage was highest followed by the posthydrolysed milk. In unhydrolysed milk the blockage was lowest. At 22°C lysine blockage was also rather small but lactose hydrolysed milks differed more clearly from the unhydrolysed one. At 30 and 45°C the differences became larger and at 45°C the extent of the lysine damage was so large that it had a clearly detrimental effect on the nutritional value of the lactose hydrolysed milks. The higher the storage temperature, the closer the lysine blockage values in pre- and posthydrolysed milks grew during the storage.

3.3 Changes in colour

The colour change in milk during the storage was negligible at 5°C. At 22°C colour change was also very small but slightly higher in prehydrolysed milk than in posthydrolysed unhydrolysed milk. At 30 and 45°C the colour change was significant and there was not much difference between prehydrolysed and posthydrolysed milk. Both lactose hydrolysed milks showed significantly larger colour change than unhydrolysed milk. After 12 weeks of storage at 45°C the colour of lactose hydrolysed milks resembled café au lait. The colour change at 5, 22, 30 and 45°C is shown in Table 3. When the colour change is approximately 1 or larger, it can be noticed by a human eye.

The change in colour correlated well with the change in furosine (Fig. 2).

3.4 Changes in carbohydrate contents

Glucose and galactose contents in lactose hydrolysed milks were stable during the storage at different temperatures (Fig. 3 a-b). A small decrease in glucose and galactose content was noticed at the highest tem-
Table 3: Colour change during the storage at different temperatures

<table>
<thead>
<tr>
<th>Time (wks)</th>
<th>5°C Unhydrolysed</th>
<th>5°C Pre-hydrolysed</th>
<th>5°C Post-hydrolysed</th>
<th>22°C Unhydrolysed</th>
<th>22°C Pre-hydrolysed</th>
<th>22°C Post-hydrolysed</th>
<th>30°C Unhydrolysed</th>
<th>30°C Pre-hydrolysed</th>
<th>30°C Post-hydrolysed</th>
<th>45°C Unhydrolysed</th>
<th>45°C Pre-hydrolysed</th>
<th>45°C Post-hydrolysed</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>1.7±1.1</td>
<td>2.2±1.0</td>
<td>0.8±0.6</td>
<td>2.4±1.5</td>
<td>3.0±0.7</td>
<td>1.9±0.5</td>
<td>1.5±0.6</td>
<td>2.7±0.3</td>
<td>2.2±0.9</td>
<td>1.7±0.4</td>
<td>0.8±0.6</td>
<td>1.9±0.5</td>
</tr>
<tr>
<td>8</td>
<td>0.8±0.5</td>
<td>1.0±0.2</td>
<td>0.9±0.6</td>
<td>1.9±0.6</td>
<td>2.9±0.8</td>
<td>1.5±0.6</td>
<td>3.7±0.6</td>
<td>3.6±0.3</td>
<td>2.3±0.9</td>
<td>2.4±0.9</td>
<td>2.2±0.9</td>
<td>2.2±0.9</td>
</tr>
<tr>
<td>12</td>
<td>0.5±0.3</td>
<td>2.4±1.0</td>
<td>1.7±0.5</td>
<td>1.9±0.6</td>
<td>3.3±1.2</td>
<td>2.0±0.5</td>
<td>3.6±0.6</td>
<td>4.2±0.3</td>
<td>2.9±0.9</td>
<td>3.4±0.9</td>
<td>2.9±0.9</td>
<td>2.9±0.9</td>
</tr>
</tbody>
</table>

Fig. 2: The correlation between the colour change and the change in furosine in milk on the basis of lactose unhydrolysed and hydrolysed UHT-milks.

22°C after 3 months furosine levels were lower for hydrolysed milk than those found by EVANGELIsti et al. (11) at 20°C (284-311 vs. 401-555 mg/100 g protein). This was probably due to the differences in heat treatments of the UHT-processes.

Hydrolysis of lactose after the UHT-treatment caused a lower formation of furosine than hydrolysis before the UHT-treatment (Fig. 1). The difference was clear when stored at 5°C. However, when stored at room temperature or above the order of hydrolysis stage and UHT-treatment had only a small effect on furosine content and on the colour of the milk (Fig. 1 and Table 3). Earlier it was suggested that heating prior to hydrolysis would help to avoid the Maillard reaction (9). However, storage temperatures had a significantly larger effect on the quality of milk than the order of hydrolysis and UHT-treatment. Lysine blockage became significant in lactose hydrolysed milks stored at 30°C or above.

The Maillard reaction influences adversely the digestibility of the protein in milk. MöLLER et al. (23) reported that the modification of the protein structures induced by lactulosyl-lysine formation makes hydrolysis of the peptide bond more difficult during digestion, probably through steric hindrance. This can make some amino acids less bioavailable. In our earlier study (14) it was noticed that in lactose hydrolysed UHT-milks the protein fraction can be more hydrolysed than in unhydrolysed UHT-milk, which means that the release of the new free α-amino groups may enhance the Maillard reaction further.

4. Discussion

MARCONI et al. (10) studied the contents of furosine in lactose hydrolysed and unhydrolysed commercial UHT-milks. Furosine levels of 162-1071 mg/100 g protein were found for hydrolysed milks and 80-235 mg/100 g protein for unhydrolysed milks. In this study furosine levels of 26-1486 mg/100 g protein were found for hydrolysed milks and 23-514 mg/100 g protein for unhydrolysed milk depending on the storage temperature and time. The wider range in furosine values in this study than in other studies (10, 11, 8) was due to the wider range of storage temperatures. In this study at
stored at 45°C and lowest at 5°C, where the colour changes were almost nonexistent. This can give a rough tool to estimate the furosine content of the lactose hydrolysed UHT milk during storage by measuring the colour change. From furosine content it is possible to estimate the level of blocked lysine in milk. RUIFÍAN-HENARES et al. (24) studied furosine and colour change in enteral formulas during storage at elevated temperatures. A similar correlation between their furosine and colour results can be calculated at temperatures 4-30°C as in this study. The main carbohydrate in enteral formulas was dextrinomaltose and colour was measured with a different method (Δελ20pol). PAGLIARINI et al. (26) found that the colour change (ΔE) during heating of skim milk at constant temperature of 90-130°C followed zero order kinetics with the activation energy of 101.8 KJ/mol.

5. Conclusions

If lactose hydrolysed UHT milk is stored at high ambient temperature, the Maillard reaction cannot be avoided by performing the lactose hydrolysis step after the heat treatment. The storage temperature is a more significant factor for the furosine formation and lysine blockage than the order of the hydrolysis and UHT treatment stages. Storage of the lactose hydrolysed UHT milks at 30 or 45°C caused a significant blockage of lysine during 12 weeks storage. Change in the colour of UHT milk during storage can be used to roughly estimate the formation of furosine and the loss of available lysine in milk.

Acknowledgements

The authors want to thank Leena Tykkyläinen, Outi Kerojoki and Riitta Puttonen for their skilful analyses. We thank Seppo Hamina and his team for the careful test runs for preparing the samples, Dr. Matti Harju for comments and criticism and Mona Söderström for language consultancy.

6. References

(14) TOSSAVAINEN, O., KALLIOINEN, H.: Proteolytic changes in lactose hydrolysed UHT milks during storage, Milchwissenschaft (submitted)
(19) IDF 10:1996 Modified. Valio R&D/Chemistry method 40

Tossavainen, Effect of hydrolysis on UHT milk

Erratum


On page 411 the below table and text should read as follows:

<table>
<thead>
<tr>
<th>Table 1: Analysis results of raw material milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test run</td>
</tr>
<tr>
<td>Hydrolysed</td>
</tr>
<tr>
<td>pH</td>
</tr>
<tr>
<td>Fat</td>
</tr>
<tr>
<td>Dry matter (%)</td>
</tr>
<tr>
<td>Sensory evaluation (max.5)</td>
</tr>
<tr>
<td>Standard plate count cfu/ml</td>
</tr>
<tr>
<td>Coliforms (cfu/ml)</td>
</tr>
<tr>
<td>B. cereus (cfu/ml)</td>
</tr>
<tr>
<td>Temperature (°C)</td>
</tr>
</tbody>
</table>

2.1 The description of the UHT-process

The UHT-treatment of milks was carried out by a direct UHT process based on infusion technology (APV, Denmark). Milk was first preheated to 75°C for about 20 s by plate heat exchangers and then heated very rapidly to 141°C by steam infusion. After a holding time of 2-4 s the milk was first cooled in a flash vessel to 70°C, homogenised with 180/50 bar and cooled by plate heat exchanger to 20°C.