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E Armaforte, François Chevalier, M. F. Caboni, M. A. E. Auty, A. L. Kelly. Study of protein complex formation during storage and gelation of UHT milk. The 38th UCC Annual Research Conference hosts on Food, Nutrition and Consumer Sciences, Sep 2008, Cork, Ireland. hal-04244482

**HAL Id: hal-04244482**

**<https://hal.science/hal-04244482>**

Submitted on 18 Oct 2023

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# Study of protein complex formation during storage and gelation of UHT milk



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The protein fraction of ultra-high-temperature (UHT)-treated skimmed milk was investigated to study the physical instability of this product, which can give rise to sedimentation and gelation during storage. Skimmed milk was divided into 12 batches (Table 1) to study the effect of pre-heat treatment, addition of phosphates, plasmin and proteases inhibitor on the formation of pellets or gels during storage at 30°C after UHT treatment at 135°C for 3 sec. Protein complex formation during storage and gelation of UHT milk were studied using proteomic tools.

## Protein complex formation

The main target of this project was to give a scientific interpretation to different behaviours that UHT milk samples showed during storage (Fig 1). Starting from the observation of the samples, this work carried out the understanding of the protein complex formation and their structure through proteomic and microscopical studies.

Samples	+ Phosphate	+ Pre-heat T	+ AP	+ Plasmin
A1a				
A1b			•	
A1c				•
A2a		•		
A2b		•	•	
A2c		•		•
B1a	•			
B1b	•		•	
B1c	•			•
B2a	•	•		
B2b	•	•	•	
B2c	•	•		•

Table 1: description of samples according to treatments or added compounds; AP: anti-proteases

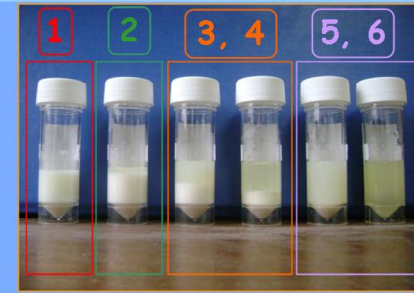


Figure 1: different degrees of protein complexes after 8 weeks: 1: Control; 2: UHT milk + pre-heat T + anti-protease; 3: UHT milk + anti-protease; 4: UHT milk + phosphates + anti-protease; 5: UHT milk + phosphates + pre-heat T + plasmin; 6: UHT milk + plasmin.

## Mono and two-dimensional electrophoresis and confocal microscopy

Experimental conditions

Protein solubilisation: 9M urea, 4% CHAPS, 0.05% Triton X100.

### Mono-dimensional gels

12.5% SDS-polyacrylamide gel, using the Protean II XL Cell electrophoresis unit (Bio-Rad)

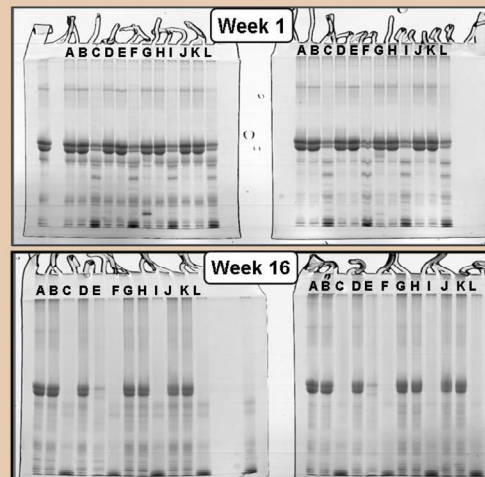
### Analytical gels (3.0 µl of milk / gels)

1<sup>st</sup> dimension : Precast 7 cm strips pH 3-10 (Bio-Rad) with the Protean IEF Cell (Bio-Rad)

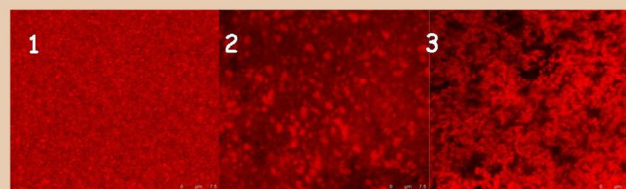
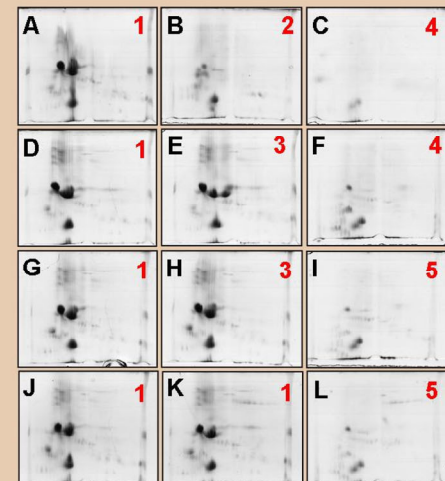
2<sup>nd</sup> dimension : 12.5% SDS-polyacrylamide gel, using the Criterion® Dodeca Cell electrophoresis unit (Bio-Rad)

Staining conditions : 1D: Standard Coomassie blue, 2D: Colloidal Coomassie blue

Gel images were digitalized with a GS 800 densitometer (Bio-Rad) and gels are analyzed using the Quantity one and PDQuest softwares v.7.3.1.



1D gels (1 and 16 weeks of storage) and 2D gels (8 weeks of storage) of UHT milk samples : A: UHT milk control, B: UHT milk + anti-protease, C: UHT milk + plasmin, D: UHT milk + pre-heat treatment, E: UHT milk + pre-heat treatment + anti-protease, F: UHT milk + pre-heat treatment + plasmin, G: UHT milk + phosphates, H: UHT milk + phosphates + anti-protease, I: UHT milk + phosphates + plasmin, J: UHT milk + phosphates + pre-heat treatment, K: UHT milk + phosphates + pre-heat treatment + anti-protease, L: UHT milk + phosphates + pre-heat treatment + plasmin. Red numbers: 1: milk appearance; 2: complete gelation; 3: partial gelation; 4: strong sedimentation with clear supernatant; 5: light sedimentation with cloudy supernatant



### Confocal Microscopy

sample 1: UHT milk control  
sample 2: UHT milk with plasmin  
sample 3: UHT milk with anti-protease

## Conclusions

- Plasmin affects the protein pattern as clearly visible from 1D and 2D gels, in which main bands or spots decrease their concentration or disappear completely, while new spots, coming from hydrolysis of main proteins, appear;
- Gelation occurred most quickly samples with anti-proteases after 4-5 weeks of storage or samples without any added compounds after long time storage;
- Gels originating from different samples have different structure, as highlighted from confocal microscopy. While samples with plasmin showed high particle heterogeneity due to proteolytic processes, samples with anti-proteases showed a structure similar to yoghurt gels.
- Pre-heat treatment and phosphates seems to have a protective effect on milk in term of stability of samples and protein patterns;
- Further studies, such as Scanning Electron Microscopy (SEM) and identification of spots of interest by Peptide Mass Fingerprinting and MALDI-TOF are being carried out



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## ACKNOWLEDGEMENT

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