Physico-chemical changes during acidification by a yoghurt starter of heat-treated milk

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Understanding the built up of acid gels by changing the fermentation temperature, as a low gelation rate can result in a higher firmness.
Gelation was followed on heat-treated milk
✓ by static measurements (at a final equilibrium) ✓ by dynamic measurements (in a continuous on-line mode)

Methods and materials

Milk

Reconstituted skim milk at 11% solid content (Ingredia, Arras, France)
Heat treatment at 90°C for 10 min
Acidification with *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* at 42 and 30°C or, when specified, with glucono-delta-lactone (GDL) at 30°C for 17 h

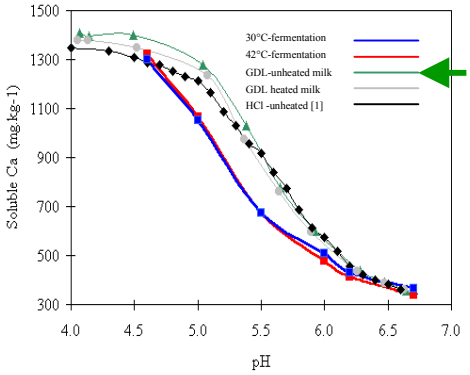
Static methods

pH drop stopped by addition of 2.05 g.kg^{-1} sodium azide and stirring
Fractionation of milk into a permeate and a supernatant at 42 or 30°C
Permeate: on Centriflo membrane (CF25)
Supernatant: at 150000 g for 70 min
Soluble calcium (Ca) on permeate by atomic absorption spectroscopy [1]
Soluble casein by RP-HPLC [2]

Dynamic methods

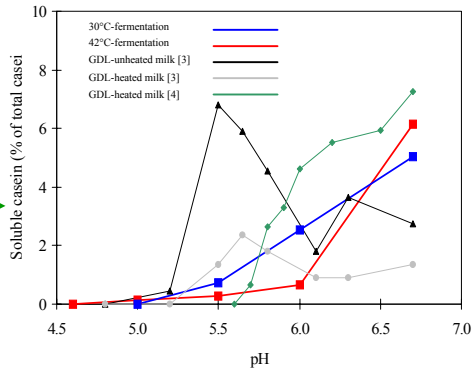
Sinusoidal oscillation with coaxial cylinder at 2 % strain at 1 Hz (4 experiments/ temperature)
H-NMR relaxation in a low field spectrometer (Bruker, 20MHz, 0.47 T) fitted in a 1-exponential (42°C) or a 2-exponential decay (30°C) (2 experiments/ temperature)
Front-face fluorescence (FFF) of intrinsic tryptophane (TRP) at l_{exc}=290 nm and principal component analysis (PCA) (4 experiments/ temperature)

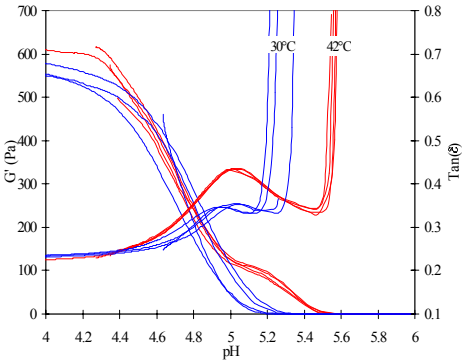
Results and discussion

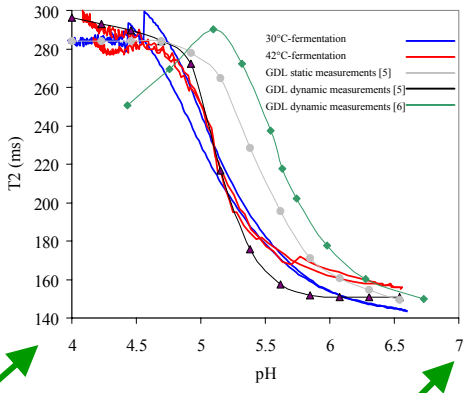


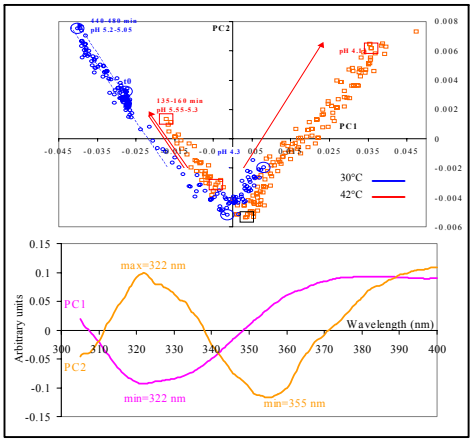
- Fermentation ⇒ shift of soluble Ca toward low pH values
- Ca solubilisation: 30°C = 42°C

- Heat treatment ⇒ shift of soluble casein toward higher pH values
- Casein solubilisation: 30°C > 42°C









^{1}H-RMN

- Fermentation ⇒ shift toward acid pH related to the shift of soluble Ca
- Sensitive to the mode of acidification
- Heterogeneity of protons : 42°C, pH 5, mono-exponential ⇒ a short + a long T2; 30°C, pH 4.5 ⇒ a break in the T2 curve
- Higher magnitude of T2 at 30°C
- Higher rate of T2 increase at 42°C

FFF + PCA : information about the environment of TRP residues during acidification

- PC1 was sensitive to fermentation time and PC2 to fermentation temperature
- 3 steps:

✒ from initial pH values to the gel point : TRP environment became more hydrophobic
✒ from the gel point to pH 4.5 (30°C) or 4.7-4.9 (42°C): TRP environment became less hydrophobic
✒ final firmness increase: TRP environment more hydrophobic

Conclusion

- Shift in Ca and T2 with dynamic measurements due to the limited diffusion of protons
- Proteins were not in the same state during fermentation than after long time equilibrium as for acidification with GDL

[1] Le Graet et al., 1993 [2] Laligant et al., 2002
[3] Singh et al., 1996 [4] Law, 1996
[5] Mariette et al., 1998 [6] Famelart et al., 1997

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