

Textural design of cheese for melting applications by exchanging the type of fat

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ABSTRACT

A model system made from concentrated skim milk and various vegetable fats was used to investigate the influence of different fats on textural and melting properties of cheese. Solid fats were present as stiff and non-spherical fat globules in the casein matrix and acted as structural interference during milk coagulation. Consequently, the solid fats resulted in weaker casein networks that were less elastic during heating and showed better melting properties.

INTRODUCTION

The amount of cheese used in cooking or in processed foods is increasing. For such applications the rheological properties of the cheese at high temperatures (50 to 100 °C) become important¹. Furthermore, increasing amounts of vegetable oils and fats are used in cheese production to replace milk fat, mainly due to reduce production costs.

The two phases (i.e. the fat and the protein/water phase) in cheese have disparate rheological properties before and during heating. Milk fat melts in the temperature interval of 0-40 °C while the softening of the protein network occurs mainly at 30-70°C². Together the rheological properties of the two phases provide the melting characteristics of the cheese. By changing the type of fat the textural and melting properties of the cheese can be designed, however it is not known in detail how this technology can be used.

In this study the rheological properties of casein gels made from concentrated milk and different types of vegetable fats were investigated at cooking temperatures.

MATERIALS AND METHODS

Preparation of concentrated milk

Low-pasteurized skim milk was concentrated by means of microfiltration (MF) to remove a substantial part of whey proteins and water. The MF process was carried out using an APV MF-CM-C1 unit (APV, Silkeborg, Denmark) equipped with 0.1- μm nominal pore diameter ceramic Membralox[®] membranes (Pall Norden AB, Lund, Sweden).

After the concentration processes, the MF concentrate was filled into 150-mL plastic bags and quickly cooled and frozen (-23 °C). Compared to fresh MF concentrate, the coagulation properties of frozen stored concentrate were not significantly different³.

Chemical analysis of MF concentrate, unconcentrated skim milk (i.e. the feed in the MF process) was performed as previously described by Karlsson et al³.

Vegetable and milk fats

Three vegetable oils and fats classified as liquid oil (i.e. canola oil), semi-solid fat (i.e. a partly hydrogenated vegetable fat) and hard stock (i.e. palm stearin) with different melting profiles were supplied by

AarhusKalshamn AB (Karlshamn, Sweden). Anhydrous milk fat (AMF) was supplied from Arla Foods A/S (Viby, Denmark). The melting profiles of all fats were analysed with differential scanning calorimetry (DSC, Mettler Toledo, Greifensee, Switzerland).

Preparation of emulsions and gels

MF concentrate was thawed for 1 h at 30°C. Sodium azide [0.02 % (w/w); Merck, Darmstadt, Germany] and glucono- δ -lactone (GLD; Acros Organics, Geel, Belgium) was added to the MF concentrate to prevent microbial spoilage and to achieve a pH of 5.8, respectively. Fats were melted at 55 °C and then homogenised (20 bars) with the MF concentrate (55 °C) to obtain emulsions 45 % (w/w) fat in dry matter. The emulsions were stored at 55 °C (24 h) and sizes of particles in emulsions were measured with a Malvern Mastersizer Micro Plus (Malvern Instruments, Malvern, UK).

Emulsion was poured in beakers and rennet [0.010 International Milk Clotting Units (IMCU) g⁻¹, CHY-MAX Extra Liquid, Chr. Hansen A/S, Hørsholm, Denmark] was added at 55 °C to induce coagulation. The beakers were placed at 30 °C for 24 h and then at 4 °C for another 24 h before analysis.

Uniaxial compression and stress relaxation

Uniaxial compression and uniaxial stress relaxation was performed with an Instron 5564 Universal Testing Machine (Instron Ltd, High Wycombe, UK) equipped with lubricated plates.

For compression at 20 °C, cylindrical samples of gels were prepared (height 18.3 mm, diameter 15.0 mm) and conditioned for 30 min at room temperature (20 °C). The compression was performed with a constant velocity of 500 mm/min. From the force and displacement data, with the assumption that the sample maintained its volume and cylindrical shape during compression, the stress (σ) and the Hencky strain (ϵ_H) was calculated and plotted for each measurement⁴. Stress (σ_f) and strain at

fracture (ϵ_f) was determined as σ and ϵ_H at the local maximum of the σ - ϵ_H curve. An elastic modulus (E) was calculated as the slope of the σ - ϵ_H curve at $0.05 < \epsilon_H < 0.1$. Five cylindrical samples from each gel were formed and analysed.

Elastic properties of heated gels (50, 60 or 70 °C) were also measured after heating in an oven by compressing (500 mm/min) discs (diameter 25 mm, height 10 mm) of gels between two lubricated plates (diameter 25 mm). Compression curves (i.e. σ - ϵ_H) were constructed and E was calculated as described above. After compression to a height of 2 mm, the samples were let to relax for two minutes while the force was registered. The registered data was used to obtain stress relaxation curves (stress vs. time) and the relaxation time (time to relax to 15 % of initial stress) was determined. All experiments were performed in triplicates.

Rheological properties by small amplitude oscillatory shear (SAOS)

Rheological properties during small amplitude oscillatory shear (SAOS) were investigated with a Bohlin C-VOR (Malvern Instruments, Malvern, UK) equipped with serrated parallel plates (diameter 25 mm). A disc (thickness 2 mm, diameter 25 mm) of gel was formed and mounted between the plates in the rheometer. Areas of the gel exposed to air were covered with a layer of lithium grease and the gel was then let to relax for 15 min (10 °C). Before measurement start, the gap was adjusted so the normal force on the upper plate was 0.5 N. During measurement, the elastic modulus (G') and the phase angle ($\tan \delta$) were measured at strain 0.002, frequency 1 Hz and temperatures from 10 to 80 °C.

Microstructure by confocal laser scanning microscopy (CLSM)

Microstructure in gels were investigated before and after heating (10 minutes at 70 °C) with a Leica TCS 4D confocal laser scanner with a Leitz DM RB/E* microscope and an argon/krypton laser (Leica

Microsystems, Heidelberg, Germany). Protein and fat in a thin gel slice were simultaneously labelled with 0,05 µg/ml of Rhodamine B (Sigma-Aldrich Inc., St.Luis, MO) and 0,5 µg/ml of Bodipy D-3992 (Invitrogen Molecular Probes, Eugene, OR) in 0.01 M phosphate-buffered saline at pH 6,5, respectively. After incubation (30 min), the gel sample was placed onto a cover slip and samples were viewed using a 63 x plan apo n.a. 1.20 water immersion objective. The following filter setting was applied: for Bodipy, excitation wavelength 488 nm, beam splitter DD 488/568 (double dichroic, reflects at 488 and 568 nm), emission detection band 500-613 nm; and for Rhodamine B isothiocyanate, excitation wave length 568 nm, beam splitter DD 488/568, emission detection band 580-720 nm. To avoid cross-talk between the two lines, samples were scanned sequentially. Some samples of emulsions were also investigated by CLSM according to the same procedure as for gels.

RESULTS

Melting profiles of oils and fats

Melting of fat crystals in canola oil occurs at temperatures below 0 °C and the oil is fluid at room temperature (i.e. 20 °C). Also a large proportion of triacylglycerides in milk fat melts below 0 °C and the milk fat forms a viscous fluid at room temperature although a large proportion of triacylglycerides is still in the crystallised form. The partly hydrogenated vegetable fat is a fat that has been developed to replace cocoa butter. Thus, the main proportion of the triacylglycerides melts between 30 and 40 °C and the bulk fat is solid at 20 °C. Palm stearin is the high-temperature melting fraction from palm oil and was shown to have an endset of melting at 55.6 °C.

Chemical composition of MF concentrate

The ceramic membranes of the MF module retained casein micelles while the whey proteins could permeate the

membranes. Thus, the MF concentrate had a high total solids content with a reduced amount of whey proteins (Table 1).

Fat was added to obtain a concentration of 45 % (w/w) in dry matter in the emulsions. No water was expelled from the gels, thus the chemical composition of emulsions and gels were identical.

Uniaxial compression

The type of fat very much influenced the fracture properties of gels at 20 °C. Gels with canola oil showed a much lower σ_f and ϵ_f than gels with milk fat, palm stearin and partly hydrogenated vegetable fat (Fig. 1). Gels with milk fat and palm stearin had a slightly lower σ_f and ϵ_f than gels with partly hydrogenated vegetable fat.

Table 1. Composition of skim milk, MF concentrate and MF permeate. All values (% w/w) are means of three replicates.

	Skim milk	MF concentrate	MF permeate
Casein	2.8	16.7	0.1
Whey protein	0.5	1.2	0.2
Total solids	9.3	26.1	5.7

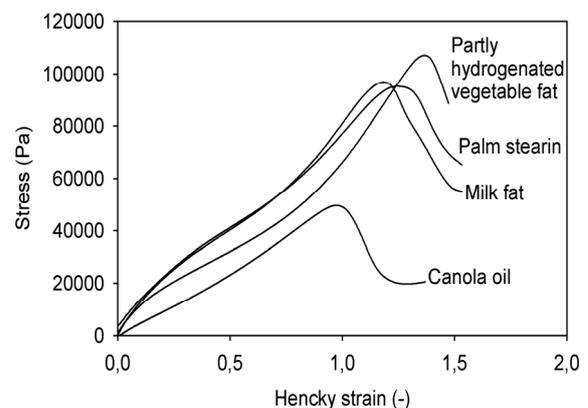


Figure 1. Representative Stress-Hencky strain curves from uniaxial compression of casein gels with different fats at 30°C. The local maximum of the curves represents the fracture of the gels.

Elastic modulus (E) is an expression for the density of cross-links in the gel matrix. Gels containing fats with a high level of

crystallisation at 20 °C (i.e. palm stearin and partly hydrogenated vegetable fat) were more elastic than gels with fluid canola oil. However, gels with milk fat were found to be most elastic although the level of crystallisation at 20 °C is not high.

Uniaxial compressions of gels at 50, 60 and 70 °C showed, as expected, that all gels were plastic, thus no fracture could be detected. However, from the compression curves E could be calculated (Fig. 2). After heating at 50 °C, palm stearin was not melted and gels containing this fat had the largest value for Young's modulus among all gels while gels with canola oil had a higher elasticity than gels with milk fat and partly hydrogenated vegetable fat. After heating at 60 °C the elasticity of all gels were at approximately the same level while gels with partly hydrogenated vegetable fat and palm stearin was lower than for other gels after heating at 70 °C.

Uniaxial stress-relaxation test

The relaxation time after an 80 % uniaxial deformation of heated gels provided a measure for the flow properties of gels at various temperatures. The relaxation time (i.e. time to relax to 15 % of initial stress) decreased and gels became more fluid when the temperature increased from 50 to 70 °C (Fig 3). Gels with canola oil had the longer relaxation times and gels containing palm stearin the shortest relaxation times at all testing temperatures. Gels with milk fat and partly hydrogenated vegetable fat had medium long relaxation times.

Small amplitude oscillatory shear (SAOS)

The elastic properties, described by G' , in gels decreased during heating from 10 to 70 °C for all gels (Fig. 4). At 10 °C, G' of gels with canola oil was much lower than for gels with the other fats. However, when the temperature increased and reached the approximate end-set temperature of fat melting for the various fats the G' of all the gels decreased below G' of the gel with

canola oil. Thus, gels with partly hydrogenated vegetable fat and palm stearin became less elastic than gels with canola oil at approximately 35 and 45 °C, respectively. At heating from 70 to 80 °C, G' increased or was constant for all gels.

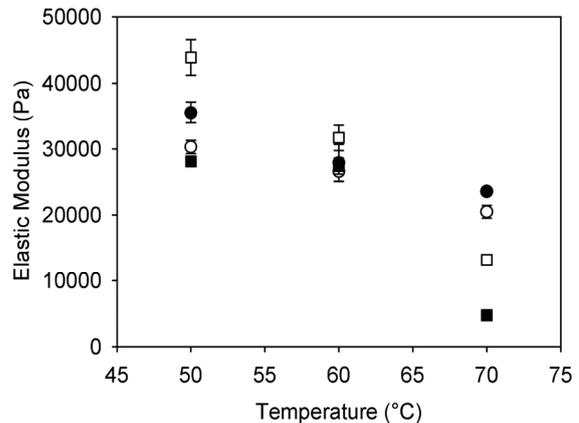


Figure 2. Elastic modulus for casein gels with canola oil (●), milk fat (○), partly hydrogenated vegetable fat (■) and palm stearin (□) during uniaxial compression at different temperatures (n=3).

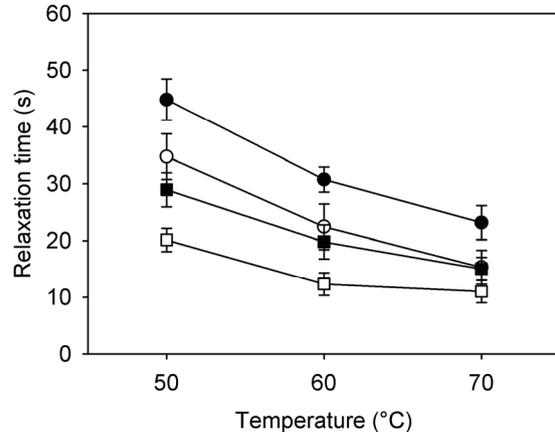


Figure 3. Relaxation times (i.e. time to relax to 15 % of initial stress) of gels with canola oil (●), milk fat (○), partly hydrogenated vegetable fat (■) and palm stearin (□) after an 80-% uniaxial compression at different temperatures (n=3).

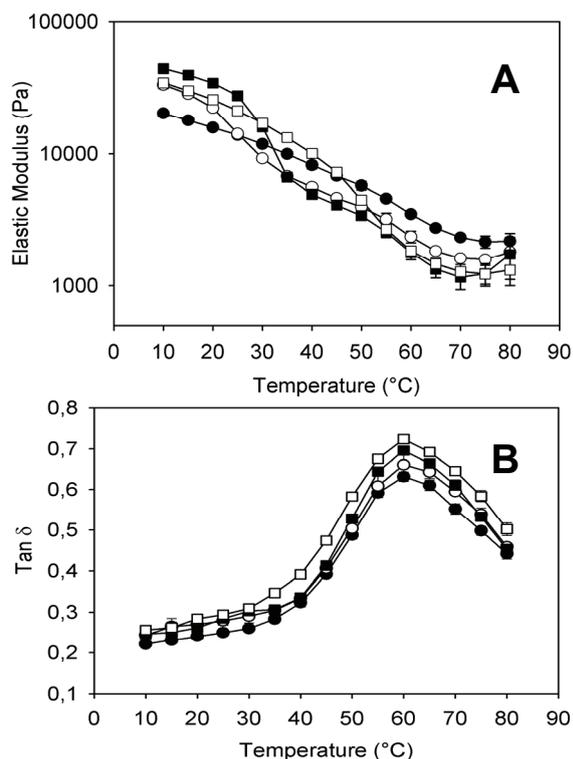


Figure 4. Rheological properties, described by the elastic modulus (A) and phase angle (B), of casein gels with canola oil (●), milk fat (○), partly hydrogenated vegetable fat (■) and palm stearin (□) during heating (n=2).

The phase angle is a measure for the relationship between viscous and elastic properties at small deformations and increased when the temperature increased from 10 to approximately 60 °C. Above 60 °C, the phase angle decreased again. The differences in the phase angle were small between the gels.

Microstructure in emulsions and gels

The particle size distribution, measured by static light scattering, was shown to be bimodal with the first peak in the range 0.5 to 10 μm and a smaller peak in the particle size interval 10-500 μm for emulsions with all fats. The latter peak was smaller and its size varied more between the different samples, thus this peak was probably due to that not the entire sample of milk

concentrate was properly dissolved in the sample unit of the Master Sizer.

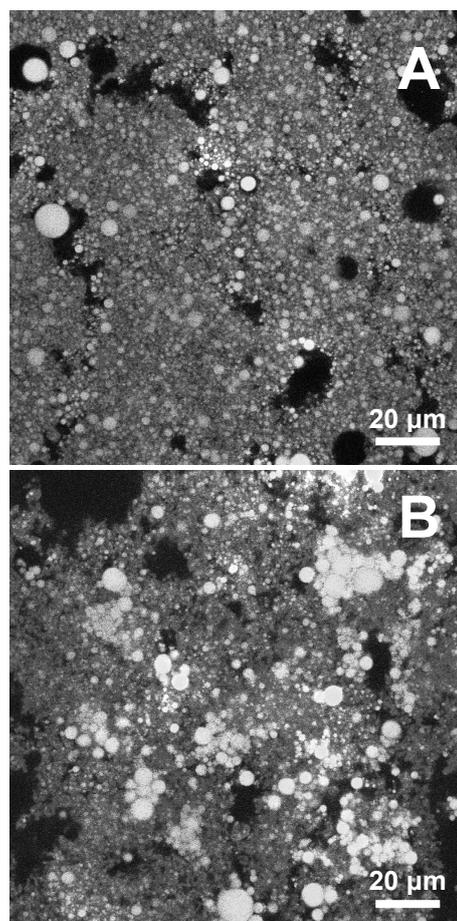


Figure 5. Microstructure of gels with canola oil at 20 °C (A) and at 70 °C (B). Fat globules appear as light grey, proteins as dark grey and the serum phase as black.

Micrographs of gels with different fats showed that the diameters of fat globules were less than 15 μm and that fat globules of canola oil (Fig. 5A) was spherical and fat globules of palm stearin was non-spherical (Fig. 6A) at 20 °C. Fat globules of milk fat and partly hydrogenated vegetable fat resembled fat globules of canola oil and palm stearin, respectively. The casein network was dense in all gels and entrapped the fat as well as pools of serum.

During melting, fat globules of all fats agglomerated or coalesced (Figs. 5B and 6B). The protein network contracted somewhat and cavities of the serum phase

became in general more defined and larger. The fat pools of the partly hydrogenated vegetable fat and the palm stearin remained unspherical also during and after heating to 70 °C.

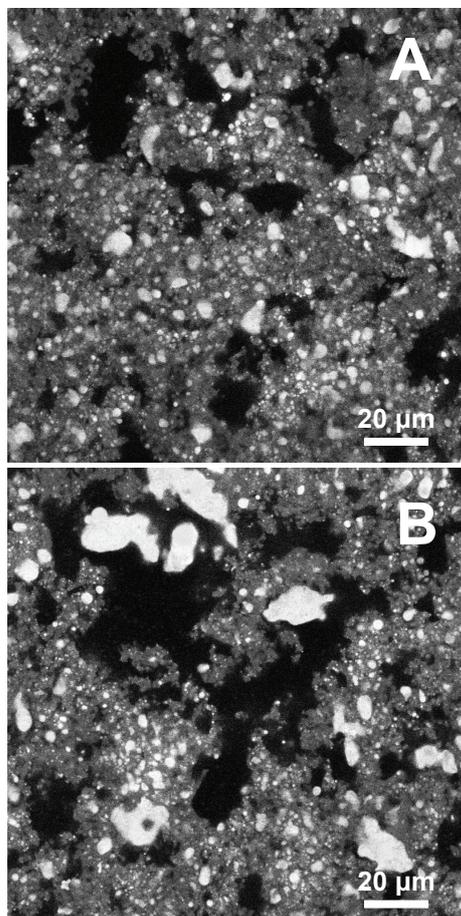


Figure 6. Microstructure of gels with palm stearin at 20 °C (A) and at 70 °C (B). Fat globules/pools appear as light grey, proteins as dark grey and the serum phase as black.

DISCUSSION

Textural properties of gels

The rheological and microstructural properties of rennet-induced casein gels are affected by various parameters (e.g. temperature, pH, fat concentration)^{5,6}, however in this study all those parameters were constant and only the type of fat was investigated.

The fat globules in the casein gels of this study were connected to the casein network

and contributed to the textural properties of the gels. During homogenisation of the MF concentrate, the fat globules and their oil/water-interfaces are stabilised by proteins, mainly casein, otherwise only present in the serum phase in unhomogenised milk⁵.

During rennet-induced milk coagulation, the casein micelles in milk will form a three-dimensional gel network⁴. Also the caseins at the oil/water-interface of the fat globules, and therefore the fat globules, will become a part of the gel network and contribute to the textural properties of the gel⁷. Thus, it was not surprising that presence of fat globules with palm stearin or partly hydrogenated vegetable oil (i.e. high degree of crystallisation) in gels was measured to result in harder gels than when the fat globules contained canola oil (Fig. 1).

The influence of degree of fat crystallisation on the rheological properties of the gels was also seen during the SAOS measurements. Fats with a high level of crystallisation at 10 to 20 °C and high melting temperatures resulted in gels with higher elasticities (i.e. G') at room temperature (Fig. 4). Furthermore, when the melting of the different fats occurred, a relative drop in G' was detected.

Microstructure of casein gels

The size of fat globules has been observed to not change during rennet-coagulation or cheese manufacture⁷. This was also estimated to be the case when sizes of fat globules in emulsions were compared to the sizes of fat globules in gels.

The rennet was added at 55 °C but the temperature soon dropped and the coagulation mainly occurred at 30 °C. Thus, the gel network developed at a temperature when some fats (i.e. partly hydrogenated vegetable fat and palm stearin) had crystallised. The degree of fat crystallisation affected the shape of the fat globules. Fat globules of palm stearin were non-spherical due to the crystallisation of the fat (Fig. 6A) while fat globules of non-crystallised canola

oil were spherical (Fig. 5A). The difference in fat globule shape could not be seen to have affected the structure of the casein network. However, the interaction between fat globules and the casein network seemed to be more extensive in gels with canola oil than in gels with palm stearin. Probably fat globules with crystallised fat were less flexible and could to the same extent not be embedded in the casein network.

During heating the fat globules underwent agglomeration or coalescence (Figs 5B and 6B), however the spherical and non-spherical shape of fat globules from canola oil and palm stearin, respectively, was maintained. The melting of the fat and the rearrangements in the casein network during heating is the main reasons for the coalescence and development of larger fat globules during heating⁸.

Melting properties

The “meltability” of cheese is characterised by the property of the cheese to melt and form a viscous fluid with certain flow properties upon heating⁹. Increased meltability accomplished by melting at low temperatures and/or formation of a low-viscous fluid. Low elasticity (i.e. E and G'), a large phase angle (i.e. $\tan \delta$) and short relaxation times are rheological parameters, which have been found to correlate to increased cheese meltability^{1,9}.

Chemical composition of the casein network influences the melting properties of cheese, however also the physical structural of the casein network can be speculated to be important⁴. The fat phase (i.e. fat globules) will also affect the melting properties and low melting temperatures of the fat could be of advantage^{10,11}. However, in the present study, gels with canola oil (i.e. low melting temperature) had higher E , longer relaxation times and higher G' at cooking temperatures (i.e. 50 to 80 °C) than gels with palm stearin and partly hydrogenated vegetable oil (Figs. 2, 3 and 4). In general, the rheological parameters showed that casein gels were less elastic and

were more prone to flow at cooking temperatures when the fat had a high melting temperature. This was opposite to what was expected.

The rheological results of gels above 60 °C when all fats was melted, indicated that the casein network was weaker and less elastic in gels with fats with high melting temperatures. The solid state (i.e. crystallisation) of the fat probably affected the development of the casein network and/or the interaction between the casein network and the fat globules during rennet-induced coagulation. Non-spherical and stiff fat globules (e.g. from palm stearin) acted as structural interference during the development of the casein network and caused a weaker casein network (Fig. 6A). The difference in the strength of the casein networks of different gels was, however, first apparent at high temperatures when all the different fats were melted and solid fat could not contribute to the elasticity of gels.

CONCLUSIONS

We have found substantial differences in the textural properties and microstructure of casein gels with fats with different melting profiles. Fats with a high melting temperature were present as non-spherical fat globules, which to a lower degree interacted with the casein network and acted as structural interference during gel coagulation. The application of fats with high melting temperatures increased the elasticity of gels at room temperature although they also indirectly caused weaker casein networks. The difference in elasticity of casein networks of gels became apparent at temperatures above 60 °C when all fats were melted and the rheological properties of the gels was mainly determined by the casein matrix. Gels with fat with high melting temperatures were determined to have rheological parameters of more extensive flow and lower elasticity at cooking temperatures.

The results show that vegetable fats with higher melting temperatures than milk fat

can be used to design cheeses, for which a hard texture is desired for shredding at room temperatures but low viscosity and elasticity is desired for the cheese after melting.

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