Rheological Characterization of Omega-3 Enriched Skimmed Milk during Production and Storage

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ABSTRACT

The aim of this study was to investigate and compare rheological effects when replacing the original fat face in milk with blends of polyunsaturated fish-, camelina- and oat oil. This was obtained using traditional dairy technology without any addition of emulsifying agents. The intention with the study was to make enriched products as stable as possible during storage. Besides, these products should behave more or less similar to the physical behaviour of original stored marketed milk

Fat free milk was enriched with unsaturated omega-3 fat, resulting in two concentrations; 1.0 and 2.0 wt %. Rheological characterization of the enriched milk was studied after blending, homogenization and further during 8 days storage at 4^{o}C. The results were compared with the same rheological measurements on traditionally low fat milk consisting of 1% milkfat.

INTRODUCTION

Consumer`s demand for food products presenting nutritional benefits, has increased over the years. As a result of this trend, food products have been enriched with ingredients claiming nutritional benefits. It may be additives added such as fibres, pigments, vitamins, microorganisms, minerals, proteins, polyunsaturated fatty acids [1,2,3,4,5,6].

In this study skimmed milk was used as medium for an enriched oil-in-water emulsion for investigation of rheological phenomena. The enrichment was added and composed of different polyunsaturated oil blendings. This treatment was based on the fact that monounsaturated fatty acids (MUFA) like C18:1 and polyunsaturated fatty acids (PUFA) such as C18:2 and C18:3 contribute to lowering serum cholesterol levels in blood. This is in contrast to saturated fatty acids (SFA) such as C: 12, C: 14 and C:16, which have been proved to increase the serum cholesterol levels in blood [7,8,9]. Regarding milk fat, a rough estimate is that the SFA fraction normally account for about 65% of the total fatty acids (g/100g total fatty acids)[10].

Milk is an emulsion or colloid of fat globules within a water-based fluid that contains dissolved carbohydrates[11]. Homogenization is applied to improve stability of the fat phase in milk during storage. The smaller milk fat globule formed during homogenization, dramatically decreases the cream separation rate caused by the density difference between milk fat and the aqueous phase (Stoke`s law). To some extent, it also prevents coalescence. Homogenization results in an emulsion more stable than milk directly from the udder. This phenomenon contributes also to increased shelf-life of the product, mainly caused by adsorbed milk proteins (basically casein micelles) at the interface of the

smaller fat globules formed[12]. Homogenization is therefore a technical treatment making stable emulsions without adding foreign additives. This technological treatment is used for years within the dairy industry making marketed milk.

Regarding homogenization different equipment exists depending on the pressure utilized. Pressure between 15-20 MPa is normally used in commercial milk treatment in combination with pasteurization at 72°C for 15 s. This treatment results in a dramatically reduction of the fat globule size mainly due to shear stress and cavitation[12]. High pressure homogenization (HPH) is based on the regular homogenization technique at pressure > 50-100 MPa. Micro fluidization is another homogenization technique by which the fluid is forced under high pressure up to 150 MPa[13]. At a given pressure, a micro fluidizer produces significantly narrower fat globule size variation compared to the effect of a regular homogenizer.

The native milk fat globule size variation spans from < 1 to about 20µm. Average size is initially around 3-5 µm. Upon homogenization the average size is reduced to around 1 µm, resulting in a 4 to 10 fold increase of the interface between the fat droplets and the aqueous medium[14].

The context within this study was to investigate emulsion stability of oil enriched milk after a mild homogenization treatment and following storage. Commercial homogenization treatment between 15-20 MPa was therefore the natural choice in this study.

Viscosity data from fluid food samples are often derived from single point measurements. Such measurements give normally little or no information about the flow behaviour of the fluid[4]. A continuing rotational viscosity measurement of the emulsions was therefore used in this study.

The objectives with this study were as follows:

- Investigate rheological parameters of homogenized fat enriched milk, when milk fat is replaced by other sources of fats and oils without addition of emulsifying agents.
- Investigate the feasibility of a rotational rheometer instrument equipped with a cone-plate system to study texture characteristics in oil-in-water emulsions.
- Investigate stability and possible rheological changes in the emulsions during storage at +4 ^{o}C including temperature sweeps from +4 ^{o}C to +20 ^{o}C and back to +4 ^{o}C.

MATERIALS AND METHODS

Milk and oil ingredients in enriched milk emulsions

Traditionally low fat milk consisting of 1% milk fat was purchased from a local retailer in 1l carton containers produced by Q-Dairies, Norway; Table 1. Skimmed milk powder was obtained from TINE BA, Oslo, Norway; Table 2.

Refined cod liver oil with added antioxidants (< 2%) was provided by Borregaard Industries Ltd, division Denomega Pure Health, Norway. Oat oil was obtained from CreaNutrition, Swedish Oat Fiber AB, Sweden. Crude cold pressed oil from Camelina seeds was provided by Bioforsk, Norwegian Ministry of Agriculture and Food; Table 3.

Table 1. Main constituents of homogenized marketed milk containing 1% milk fat (from Q-Dairies).

Constituent	(w%)
Lactose	4.8
Protein	3.3
Fat	1.0*

* 65% SFA, 30% MUFA, 4.5% PUFA[10]

Table 2. Main content of spray dried skimmed milk powder used in the study, pH

6.65. Adapted from Tine`s product specification P1499, version 2.

Constituent	Content (w%)	
Water	< 3.7	
Dry matter	96.5	
Dry matter consists of;		
- Protein	35.5	
Casein		28.4
Whey protein		7.1
- Lactose	52.5	
- Fat	0.8	
SFA		0.5
MUFA		0.2
-Asch	7.8	

Table 3. Percentages of main fatty acids present in fish-, camelina- and oat oil used in this study analysed according to AOCS official methods [15, 16] (provided by NOFIMA and Denomega).

Fatty Acids %	Fish oil	Camelina oil	Oat oil
SFA			
C14:0	3,8	0,1	0,2
C16:0	13,5	5,4	15,8
C18:0	4	2,4	1,3
C20:0	–	1,3	0,1
MUFA			
C16:1(*n*-7)	5,5	–	0,2
C18:1(*n*-7)	4	0,7	0,7
C18:1(*n*-9)	19	12,5	37,7
C20:1(*n*-9)	–	14,7	0,7
C20:1(*n*-11)	1,3	–	–
C22:1(*n*-9)	–	3,0	0,1
C22:1(*n*-11)	4,6	–	–
PUFA			
C18:2(*n*-6)	4,4	15,6	41,5
C18:3(*n*-3)	1,2	37,9	1,4
C18:4(*n*-3)	1,9	–	–
C20:2(*n*-6)	–	2,2	–
C20:4(*n*-3)	0,9	1,9	–
C20:5(*n*-3)	10	–	–
C21:5(*n*-3)	0,6	–	–
C22:5(*n*-3)	2,2		
C22:6(*n*-3)	11,9	–	–
Other	11,2	2,4	0,3

Milk fat consists normally about 64 % SFA, 30% MUFA and 4.5% PUFA. SFA includes fatty acids in the range from C4:0 – C18:0 with about 10% C14:0, 25% C16:0 and 12% C18:0[17].

Preparations of enriched milk emulsions

The enriched skim milk samples were produced according to the flow diagram in Fig.1. Skimmed milk powder (100g/l) was mixed with water (20°C) using an Ultra Turrax Super Dispax SD 45/2 (IKA-Werke GmbH & Co. KG, Staufen, Germany). The resulting milk with a fat content less than 0.01% was then pasteurized (72°C for 15 s) and cooled to room temperature. Pure fish oil or blends of fish- and vegetable oils were then added to the milk (1 wt% or 2 wt%). Fish- and vegetable oil were mixed together in ratio 90:10. The blended milk sample were subsequently homogenized (18 MPa) in a (two-valve) homogenizer (Rannie, No. 4580/71, Denmark) at room temperature.

Instrumental analysis and experimental set-up

The Physica MCR301 rheometer (Paar Physica, Anton Paar, Stuttgart, Germany, 2010) fitted with a CP-50-1-SN 20814 cone probe was used (diameter of probe 50 mm, angle 1°). The viscosity of the emulsions was measured by rotational viscometry.

The Peltier temperature was set to 4 °C. The skimmed milk samples were placed directly on the Peltier plate; Fig 2. The CP-50-1-SN 20814 cone probe MP31, was then lowered slowly squeezing the samples gently to obtain the desired cone – plate clearance.

Rotational shear rate sweeps from 200 1/s to 400 1/s were recorded at +4 °C. Temperature-sweeps from +4 °C to +20 °C and back to +4 °C were recorded at a shear rate of 300 1/s.

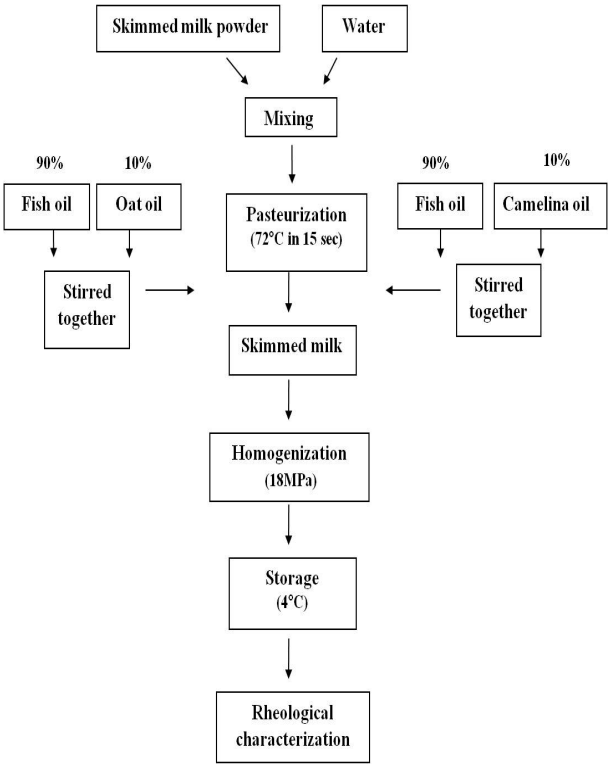

Figure 1. Production of enriched skim milk samples using traditional dairy technology. without addition of emulsifying agents. The samples contained 1.0 or 2.0% (wt%) blends of polyunsaturated oils from fish and vegetable. In addition refined fish oil was used by oneself as a third alternative of enriched skim milk.

Statistical analysis

All rheological measurements were carried out in triplicate. Unless indicated otherwise, results are expressed as average ±standard deviation (SD) using all available data. One-way analysis of variance unstacked ANOVA, Minitab 16, Fisher 99% individual confidence interval, was used as statistical test method in order to confirm statistical significance of differences among samples tested ($p<0.05$).

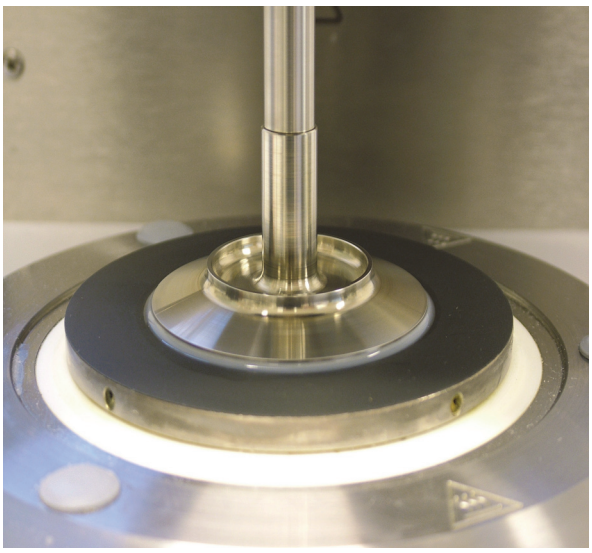

Figure 2. Milk sample measured by rotational viscometry using a CP-50-1-SN 20814 cone probe (diameter of 50 mm, angle 1^{o}).

RESULTS

Typical results of the viscosity determination with the Paar Physica rheometer for aqueous oil-in-water emulsions investigated are shown in Fig. 3, 4 and 5. All figures are focusing on viscosity as a function of shear rate.

Using one-way analysis of variance ANOVA, Minitab, Fig. 3 indicates no significant differences between the samples of either pure milk or the oil enriched products. The results are the same both regarding 1% and 2 % enrichment and during 8 days storage at +4 °C. The only rheological difference observed, was between the pure polyunsaturated oils themselves. The viscosity of oat oil was higher and significantly different from the viscosity of fish and camelina oil.

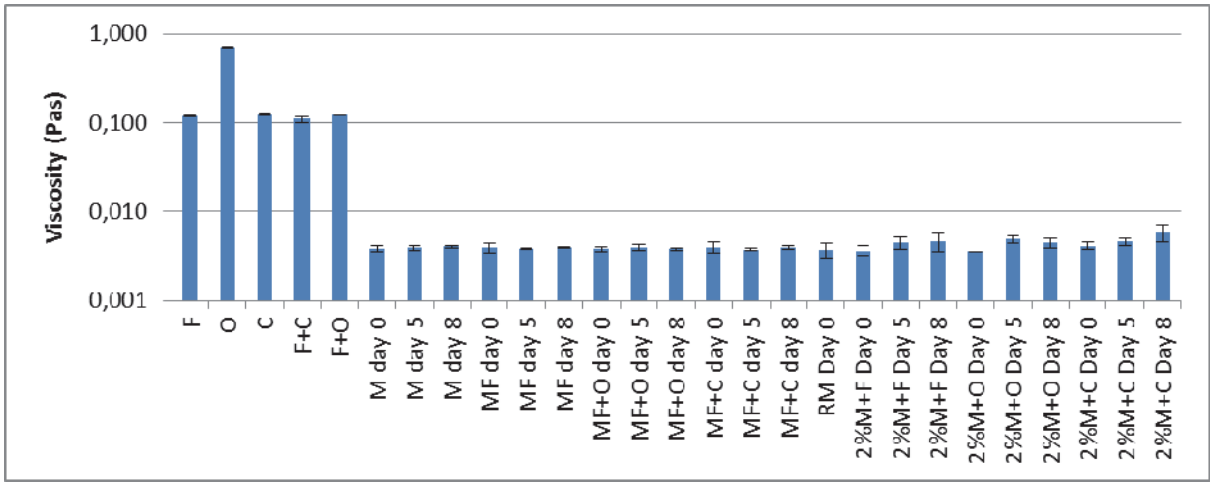

Figure 3. Average viscosity in pure oils (fish F, camelina C and oat O) and blends of the same oils. Viscosity in homogenized milk M (1% fat) and in enriched homogenized skim milk M (added 1% or 2% oils) at time 0 and after 5 and 8 days storage at +4 °C. (Error bars indicate standard deviation).

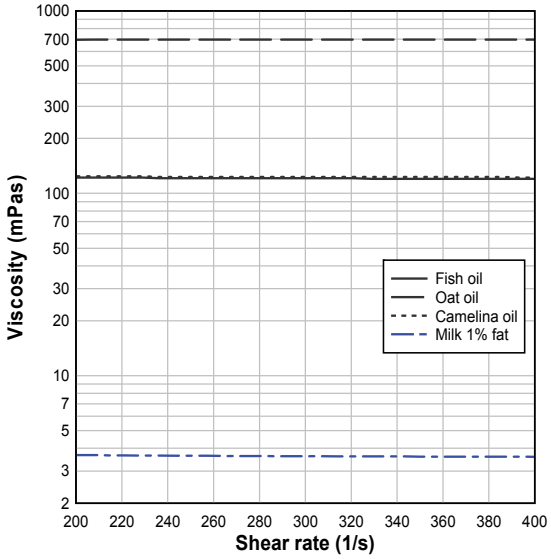

Figure 4. Rotational shear rate sweeps from 200 1/s to 400 1/s at +4 °C for homogenized milk, pure fish oil, pure camelina oil and pure oat oil.

Fig 4 gives information about the viscosity of pure oils and of homogenized milk with 1% fat during a rotational shear rate sweep at +4°C. As can be observed from the fig. 4, there are no change in viscosity during the sweeps. But there are different level of viscosity between the milk and the pure oils. Among the oils – the oat oil seem more viscose than the camelina and the fish oil. The two last mentioned oils acted more or less in the same way.

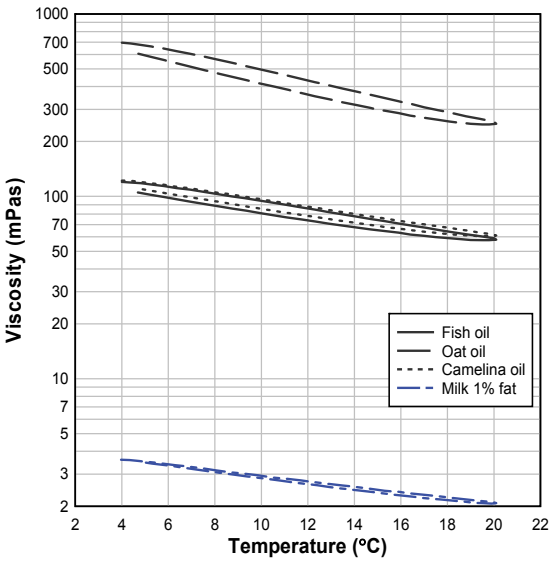

Figure 5. Temperature-sweeps from +4 °C to +20 °C and back to +4 °C at a shear rate of 300 1/s. Homogenized milk, pure fish oil, pure camelina oil and pure oat oil.

Fig. 5 illustrates results of temperature sweeps at a shear rate of 300 1/s for the same samples as shown in fig. 4. A relatively small decrease in viscosity is observed for all the samples when increasing temperature from +4 ^{o}C to +20 ^{o}C. Lowering the temperature to +4 ^{o}C again, the samples almost retained to the same viscosity level as before. These sweeps indicate an almost reversible course of events cased by this treatment.

DISCUSSION

In emulsions, several types of instability can occur; like creaming, flocculation, coalescence and disruption. Often these different processes take place simultaneously. To prevent the phases from separating, milk is usually homogenized[18]. As mentioned, homogenization of milk causes disruption of milk fat globules into smaller ones. The milk fat-plasma interface is thereby considerably enlarged, usually by a factor of 4 to 10. The new interface is then covered with milk protein, predominantly micellar casein[19]. This treatment creates stable milk during storage whether homogenization is performed for milk treated with low pasteurization (72°C in 15 seconds) or Ultra High Temperature Treatment (UHT - 145°C for a few seconds).

In this study a mild form of homogenization treatment was used in order to stabilize skim milk enriched with polyunsaturated oils. The idea was to utilize the milk protein itself as a natural emulsifier for the polyunsaturated oil droplets produced during homogenization in the enriched milk (1% and 2%). The enriched milk samples without added foreign emulsifier, were all subsequently homogenized at 18 MPa (180 bar). The same pressure is normally used for processing of marketed milk by the dairy industry.

The results presented in this study, demonstrated stability and no detectable rheological changes of the homogenized enriched skimmed milk samples during storage of the emulsions at +4 °C. Traditional dairy homogenization at 18 MPa seemed to be a suitable technological treatment for production of stable fat enriched milk. This treatment prevented addition of any emulsifying agents, when milk fat was replaced by polyunsaturated sources of fats and oils.

CONCLUSIONS

Using continuing rotational viscosity measurement on milk and enriched homogenized milk emulsions, the conclusions from this study can be summarized as follows:

- No significant viscosity differences were observed between samples of either pure milk of 1% fat, or homogenized polyunsaturated oil enriched milk during preparation and storage. The results are the same both regarding 1% and 2 % oil enrichment, and during 8 days storage at +4 °C.
- A rotational rheometer instrument equipped with a cone-plate system seemed feasible to study texture characteristics in oil-in-water emulsions.
- Only reversible changes were observed when temperature-sweeps from +4 ^{o}C to +20 ^{o}C and back to +4 ^{o}C were carried out in the tested emulsions in this study.
- The only difference observed, was between the pure polyunsaturated oils. The viscosity of oat oil was higher and significant different from the viscosity of fish and camelina oil.

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