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Rheological Properties of Yoghurt Made with Milk Submitted to Manothermosonication

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Manothermosonication (MTS) treatments, the simultaneous application of heat and ultrasound under moderate pressure, of milk during 12 s at 20 kHz ultrasound amplitude, 2 kg pressure, and 40 °C allowed elaboration of yoghurts with rheological properties superior to those of control yoghurts elaborated with untreated milk. Measurements performed on intact samples (compression tests, relaxation tests, and texture profile analysis) and on slowly stirred samples (flow curves, apparent viscosity, yield stress, and viscoelastic properties) showed that MTS yoghurts had stronger structures, which resulted in higher values of almost all of the many relevant rheological parameters. Homogenization of fat globules brought about by MTS treatments is not responsible for the superior properties of MTS yoghurts, because the control yoghurt was also elaborated with homogenized milk. These results show that MTS could be a useful tool to improve the texture of yoghurts.

KEYWORDS: Manothermosonication; milk; yoghurt; texture; homogenization

INTRODUCTION

Manothermosonication (MTS), the simultaneous application of heat and high-energy ultrasonic waves under moderate pressure, is a recently developed tool that is able to inactivate food-related enzymes and microorganisms at much higher rates than thermal treatments of identical temperature (*1*). MTS effects on enzymes and microorganisms have been mainly studied on model systems. For example, MTS is able to inactivate proteases and lipases of psychrotrophic microorganisms, the limiting factor of UHT milk shelf life (*2*). Ultrasound is also able to inactivate several microbial species, ranging from heat-labile bacteria (*3*) to heat-resistant bacterial spores (*4*). Therefore, it allows reduction of the intensity (time or temperature or both) of thermal treatments necessary to inactivate deletereous enzymatic activities and microbial populations.

Ultrasound effects are mainly related to the cavitation phenomenon. Cavitation is the formation, growth, and, in some cases, implosion of bubbles inside liquids. Implosion of cavitation bubbles leads to energy acumulations in hot spots where temperatures of 5000 °C and pressures of 1000 atm have been measured (*5*). As a result of these conditions water molecules can be broken, generating highly reactive free radicals that can react with and modify several molecules (*6*). Mechanical stress, generated by shock waves derived from bubble implosion or from microstreaming derived from bubble's size oscillations, is also able to break big macromolecules or particles (*7*). Because of the nature of these ultrasound effects on molecules or particles dissolved or suspended in liquids it is quite reasonable to expect that the big multimolecular structures contained in milk, mainly casein micelles and fat globules, can be affected by ultrasound irradiation.

High energy ultrasound has several uses in the food industry, ranging from homogenization and emulsification (*8*), to mass transfer enhancement (*9*) to the improvement of proteolytic (*10*) reactions for ingredient elaboration. Ultrasound uses in the dairy industry have been recently reviewed by Villamiel et al. (*11*). The milk homogenization ability of ultrasound was described as early as 1969 by Walstra (*12*). Fat globules are routinely homogenized (prior to inoculation of milk with yoghurt starters) to improve yoghurt consistency and to prevent serum separation in the final product (*13*). These effects are not only due to fat globule size reduction but also to the effects of pressure on other milk constituents, mainly proteins. Ultrasound homogenization of milk has been studied more recently by Villamiel and de Jong (*14*) and Wu et al. (*15*). Both groups of authors reported the ability of high-energy ultrasound to homogenize fat globules. Villamiel and de Jong (*14*) found that ultrasound-assisted homogenization was very dependent on simultaneous thermal treatment, whereas Wu et al. (*15*) studied the relationship between ultrasound intensity and its homogenization ability. The latter authors also found by using simple methods that ultrasound treatment prior to starter inoculation improved, in some experiments, yoghurt viscosity, something they attributed to the milk homogenization effects of ultrasound. Wu et al. (*15*) also found that fermentation time could be reduced by ultrasound treatments

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prior to starter inoculation. Reduction of fermentation time by the use of ultrasound has been also cited by Mason and Paniwynk (*8*).

Yoghurt is one of the most consumed dairy products. Flavor and consistency are its main quality parameters. Consistency of yoghurt is dependent on its structure, a protein network formed by casein micelles strings and/or clusters entrapping serum and fat globules (*16*). Complex interactions can be established between these three components (see Lucey and Singh (*17*) for a review). This network is relatively weak and it is formed by acidification of milk by a mixed culture of *Streptococcus thermophilus* and *Lactobacillus delbruekii* ssp. *bulgaricus*, although proteolytic processes could also be involved. As ultrasound has been shown to affect fat globule size (*14*, *15*) and dairy protein denaturation (*14*), it is expected that yoghurt structure, which is highly dependent on fat globule size and denaturation and aggregation state of proteins, is also affected. The aim of this work is to investigate the ability of milk submitted to manothermosonication to be used for yoghurt elaboration. To do this we have conducted a thorough study of yoghurt rheology using intact yoghurt samples and samples that were slowly stirred, as yoghurts usually are by consumers prior to consumption.

MATERIALS AND METHODS

Milk Supply. Thermized (60 °C/15 s) cow milk (1.6% fat content) was kindly supplied by a local dairy factory either homogenized (for control yoghurt elaboration) or not homogenized (for MTS yoghurt elaboration).

MTS Treatments. MTS treatments were carried out in a continuous system equipped with a Branson 450 sonicator (*18*). Milk was circulated at a flow rate of 32 mL/min through a sonication chamber of 6 mL. Residence time was also 12 s. MTS treatments were performed at 40 °C. Sonication parameters were 117 μm amplitude, 20 kHz frequency, and 2 kg/cm^2 pressure. Treatments were performed in triplicate, each one from a different milk batch.

Fat Globule Size Distribution. Fat globule size was measured by optical microscopy with a phase contrast microscope from Nikon (model L-Ke). A 20-μL sample of a 1:20 milk dilution was put on a microscope slide and visually analyzed with an immersion objective. Fat globule size was estimated with an ocular micrometer. This allows estimation of fat globule sizes in the 0.8−8 μm range. Fat globules of at least four randomly chosen microscope fields were counted. The assay was done in duplicate for each milk sample.

Yoghurt Elaboration. The milk used for elaboration of control yoghurts was thermized and homogenized milk (fat content 1.6%) containing 2% (w/v) added nonfat milk powder (supplied by NESTEC). The same milk containing also 2% (w/v) added nonfat milk powder, but not homogenized, was MTS treated and used for production of MTS yoghurts. Control and MTS-treated milk were heated at 92 °C for 6 min and cooled to 44 °C. Then, they were inoculated with a starter (YBCN 143 starter from CHR Hansen); the content of each starter flask, intended for 150 L of yoghurt, was diluted up to a final volume of 140 mL with 96 mL of skimmed UHT milk and 1.166 mL was inoculated to 1.25 L of milk, distributed in aliquots (50-mL aliquots in jars (5 cm diameter) for texture analysis, 10-mL aliquots in plastic tubes for syneresis determination, and 0.5-mL aliquots in Eppendorf tubes for pH control), and they were incubated at 44 °C until the pH value reached 4.60 (pH was measured for each timepoint in triplicate). Then, fermentation was stopped by putting all the samples at 2 °C. They were left at this temperature overnight. All the rheological measurements were done the day after the yoghurts were elaborated. All the jars, tubes, pipets, and glass material used were previously sterilized.

Textural Analysis on Intact Yoghurt Samples. Penetration tests, compression−relaxation tests, and texture profile analysis (TPA) were performed on intact yoghurt samples using a TA-XT2i texture analyzer (Stable Microsystems, Goaldming, England) equipped with an aluminum cylinder probe (1 in. diameter). Samples were assayed in quadruplicate with each test immediately after they were taken out of the cold room (temperature set at 2 °C). Jar diameter was about 2-fold larger than the probe diameter, which was enough to minimize side-wall effects.

Compression Tests. Compression tests were performed at 1 mm/s speed and 15 mm length to measure the force necessary to break down the sample structure.

Stress−Relaxation Tests. Tests to measure stress and relaxation were performed by maintaining a 2.5 mm deformation (achieved at 1 mm/s) for 2 min. Force-relaxation was monitored as a function of time. From this test, the following data were obtained: (1) initial maximum force, the force in the contact area at time 0, where it is a maximum; (2) minimum residual force, the force that is not alleviated by the sample at the end of the test (2 min), i.e., the nonrelaxable force; and (3) relaxable force, the force that the sample is able to recuperate at the end of the test. The sum of the two last forces is the initial maximum force. The quotient of minimum residual force to initial maximum force expresses the percentage of structure that is broken down and does not recover at the end of the test.

Texture Profile Analysis. The texture analyses were performed by two sequential compression events (2 mm penetration at 1 mm/s) separated by a rest phase of 30 s. Hardness, fracturability, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience values were calculated from the obtained profiles using the software provided by Stable Microsystems.

Textural Analysis on Carefully Stirred Samples. Yoghurt gels were stirred by manually rotating them very slowly (ca. 2−3 s each rotation) 3 times with a tablespoon inside the jar. The yoghurts appeared visually homogeneous after this procedure. The following tests were performed with a Bohlin CS controlled stress rheometer using a plate and plate geometry (PP40) with 1 mm gap setting at 8 °C constant temperature. All the assays were performed in triplicate.

Flow. Flow curves were performed upward/downward at shear rates between 10 s^{-1} and 290 s^{-1}. Both delay time and integration time were set at 5 s. The upward and downward parts of the plots data obtained were separately adjusted to the power law equation: shear stress $= K \times$ shear rate^n, where K is the consistency index, and n, the power law index, expresses the flow behavior as Newtonian (n is close to 1) or non-Newtonian (n is far from 1).

Viscosity. Apparent viscosity was measured at 112 s^{-1} for about two minutes. This shear rate value lies in the linear portion of the flow curve. Both delay time and integration time were set at 5 s. The difference in initial and final apparent viscosity was calculated using the following formula: % broken structure = ((initial viscosity − final viscosity)/initial viscosity) × 100.

Yield. Yield test measures the stress, or yield point, that has to be applied to a sample until it begins to flow. Yield stress was calculated by applying a stress gradient between 0.5 and 20 Pascal.

Oscillation. Oscillation tests allow the calculation of storage modulus (G') and loss modulus (G''), which are related to the energy stored and released or to the energy dissipated into heat, respectively, during a periodic application of a strain. They were done at 0.02 strain and 5 Hz frequency. These strain and frequency values are inside the linear viscoelastic range, which was previously calculated (data not shown).

Water Holding Capacity. Water holding capacity was measured in quadruplicate after 15-mL plastic tubes containing 10 mL of yoghurt were centrifuged at 3000*g* for 10 min. It is expressed in % (weight of serum released/total sample weight).

Statistical Analysis. Groups of data were compared by a Student's *t* test using the SPSS statistical package.

RESULTS

Homogenization. The fat-globule size distribution of MTS-treated and control milks are shown in **Table 1**. Almost 100% of the fat globules are smaller than 0.8 μm in MTS-treated milk, whereas raw milk contains only about one-third of the fat globules of this size, with the rest being larger.

Fermentation. The course of fermentation was followed by pH measurement. It varied over time in each batch of control

Table 1. Fat Globule Size Distribution (%) in Control and MTS-Treated Milk

size (μm)	control milk	MTS-treated milk
0–1.6	31.5	99.9
1.6–3.2	51.3	
3.3–4.8	12.7	
4.8–6.4	3.5	
6.4–8.0	0.8	
>8	0.2	

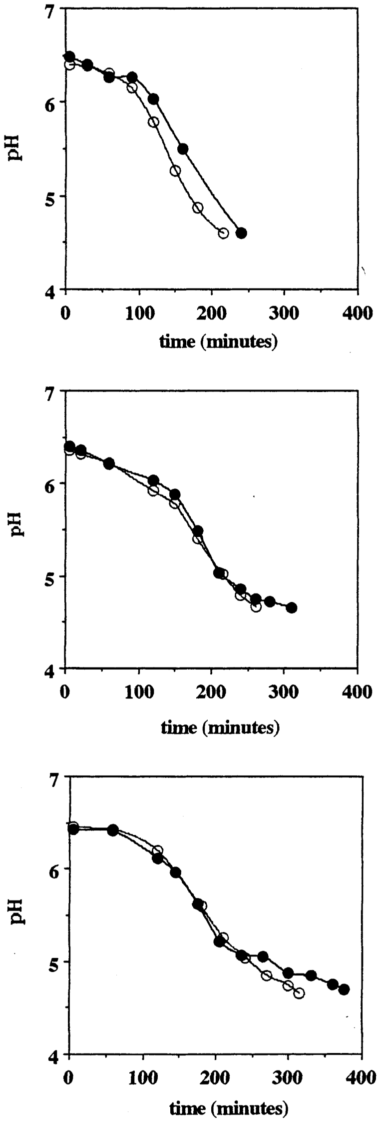

Figure 1. Decline of pH values in each of the three fermentation experiments. MTS-treated yoghurts (filled circles, ●); control yoghurts (open circles, ○).

Table 2. Rheological Parameters of Intact Yoghurt Samples[a]

	control yoghurts	MTS-treated yoghurts
compression tests		
penetration force (N)	0.641 ± 0.052	0.904 ± 0.009 (+++)
	0.770 ± 0.046	1.157 ± 0.033 (+++)
	0.939 ± 0.048	1.407 ± 0.078 (+++)
stress relaxation tests		
maximum force (N)	0.421 ± 0.133	0.681 ± 0.021 (++)
	0.689 ± 0.018	0.968 ± 0.065 (+++)
	0.886 ± 0.075	1.212 ± 0.043 (+++)
minimum residual force (N)	0.097 ± 0.030	0.146 ± 0.006 (+)
	0.119 ± 0.012	0.173 ± 0.009 (+++)
	0.154 ± 0.010	0.187 ± 0.014 (+)
relaxation force (N)	0.324 ± 0.103	0.535 ± 0.018 (++)
	0.570 ± 0.011	0.795 ± 0.057 (+++)
	0.704 ± 0.056	1.025 ± 0.059 (+++)
% lost structure	23.0 ± 0.4	21.4 ± 0.7 (+)
	17.3 ± 1.4	17.9 ± 0.5 (NS)
	17.9 ± 0.8	15.4 ± 3.7 (NS)
texture profile analysis (TPA)		
hardness (g)	47.6 ± 2.4	68.0 ± 2.6 (+++)
	66.4 ± 2.2	88.3 ± 2.6 (+++)
	81.7 ± 5.0	122.3 ± 3.8 (+++)
fracturability	18.9 ± 0.026	17.8 ± 0.013 (NS)
	18.5 ± 0.035	15.5 ± 0.019 (NS)
	20.6 ± 0.018	18.6 ± 0.032 (NS)
adhesiveness (g.s)	3.3 ± 5.00	29.2 ± 20.5(+)
	32.8 ± 24.11	89.9 ± 39.7 (NS)
	46.9 ± 31.81	52.8 ± 44.5 (NS)
springiness	1.02 ± 0.02	1.02 ± 0.02 (NS)
	1.00 ± 0.00	0.99 ± 0.02 (NS)
	0.99 ± 0.01	1.00 ± 0.02 (NS)
cohesiveness	0.679 ± 0.010	0.672 ± 0.007 (NS)
	0.650 ± 0.016	0.690 ± 0.002 (NS)
	0.655 ± 0.036	0.644 ± 0.003 (NS)
gumminess	32.3 ± 1.9	45.7 ± 1.4 (+++)
	43.1 ± 1.0	60.9 ± 1.4 (+++)
	53.4 ± 2.9	78.7 ± 3.5 (+++)
chewiness	32.8 ± 2.4	46.6 ± 1.9 (+++)
	43.1 ± 0.9	60.3 ± 1.4 (+++)
	53.1 ± 3.0	78.3 ± 3.1 (+++)

[a] Abbreviations and symbols: NS, not significant; (+) $p < 0.05$; (++) $p < 0.01$; (+++) $p < 0.001$.

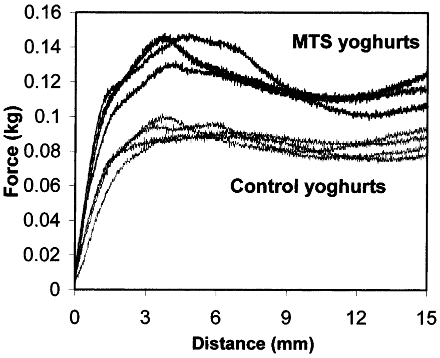

Figure 2. Compression tests of four MTS-treated and four control yoghurts from one single fermentation experiment.

yoghurts in the different experiments (**Figure 1**), which also occurs frequently in the industry even when no variation in the fermentation process is introduced. Fermentation of MTS-treated milk took longer than fermentation of control milk in all cases. The difference (10−20%) was statistically significant ($p < 0.05$) and was mostly due to a retardation of the final part of the process. However, three experiments do not allow us to draw definitive conclusions about the significance of this phenomenon. Furthermore, results reported by Wu et al. (*15*) show exactly the opposite: they find that fermentation is accelerated in the last portion of the fermentation process when milk is sonicated (without external pressure application which results in much lower ultrasonic intensity), before starter addition.

Texture of Intact Yoghurt Samples. Results of textural analysis of intact yoghurt samples in the three experiments are summarized in **Table 2**. Typical penetration tests are shown in **Figure 2**. Penetration force was consistently higher, 1.156 vs 0.783 N (48% higher in average), for MTS than for control yoghurts. Stress-relaxation tests (**Figure 3**) monitor force-relaxation as a function of time. Several parameters can be

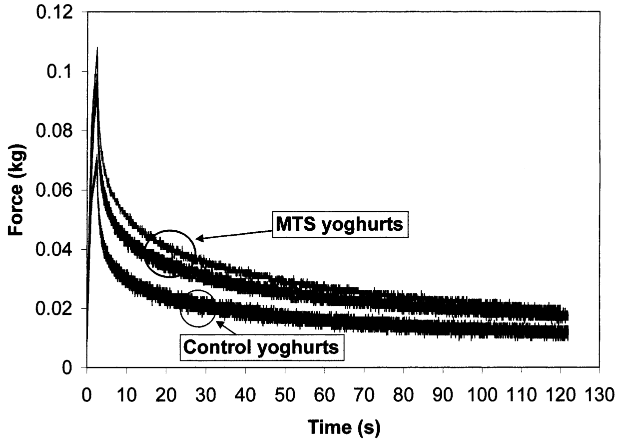

Figure 3. Stress relaxation tests of four MTS-treated and four control yoghurts of one single fermentation experiment.

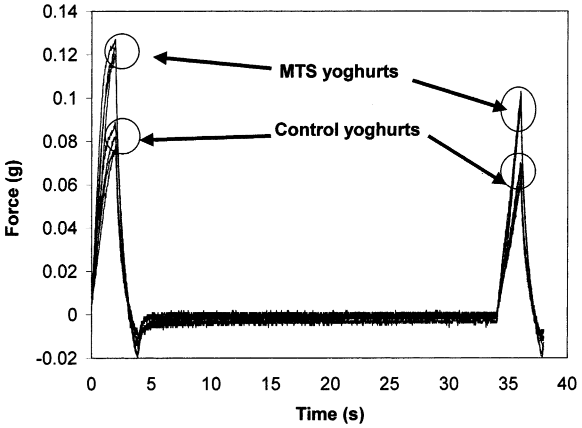

Figure 4. Texture profile analysis (TPA) of four MTS-treated and four control yoghurts of one single experiment.

Table 3. Rheological Parameters of Stirred Yoghurt Samples

	control yoghurts	MTS-treated yoghurts
flow curves (upward)		
consistency (Pa.s^n)	19.2 ± 4.8	42.5 ± 3.4 (++)
	27.0 ± 4.0	49.3 ± 7.9 (+)
	37.6 ± 4.3	66.2 ± 10.2 (+)
flow behavior	0.31 ± 0.04	0.15 ± 0.01 (+)
	0.22 ± 0.02	0.12 ± 0.02 (++)
	0.17 ± 0.03	0.11 ± 0.03 (+++)
flow curves (downward)		
consistency (Pa.s^n)	0.55 ± 0.01	0.96 ± 0.19 (++)
	0.87 ± 0.24	1.68 ± 0.21 (++)
	1.28 ± 0.13	2.61 ± 0.70 (+)
flow behavior	0.88 ± 0.04	0.76 ± 0.04 (++)
	0.77 ± 0.01	0.65 ± 0.01 (+++)
	0.71 ± 0.01	0.61 ± 0.003 (+++)
yield stress (Pa)	2.78 ± 1.13	4.68 ± 1.20 (NS)
	4.07 ± 1.12	7.33 ± 1.30 (++)
	6.7 ± 1.13	10.6 ± 1.10 (+)
apparent viscosity (mPA.s)		
initial	893 ± 75	1150 ± 109 (+)
	768 ± 16	1095 ± 48 (+++)
	943 ± 34	1271 ± 124 (+)
final	412 ± 39.0	409 ± 75.2 (NS)
	349 ± 8.9	394 ± 4.5 (++)
	385 ± 13.0	491 ± 39.8 (+)
% lost structure	54 ± 2.7	65 ± 2.7 (++)
	54 ± 1.2	64 ± 1.0 (+++)
	59 ± 0.6	61 ± 0.99 (+)
oscillation tests		
G' (Pa)	72 ± 11	106 ± 17 (+)
	97 ± 9	141 ± 18 (+)
	133 ± 12	225 ± 33 (+)
G'' (Pa)	30 ± 6	35 ± 11 (NS)
	37 ± 3	55 ± 9 (+)
	50 ± 3	80 ± 11 (++)

[a] Abbreviations and symbols used: NS, not significant; (+) $p < 0.05$; (++) $p < 0.01$; (+++) $p < 0.001$.

obtained from such tests: MTS samples showed higher initial maximum force (0.954 vs 0.656 N), higher minimum residual force (0.169 vs 0.123 N), higher relaxable force (0.785 vs 0.533N), and a slightly lower relative loss of structure (18.2 vs 19.4%). Plots of texture profile analysis (TPA) tests are shown in **Figure 4**. Results show MTS yoghurts to be harder (92.84 vs 65.22 g) and more adhesive (16.2 vs 12.7); they showed also higher guminess and chewiness (61.7 vs 43.0 and 61.7 vs 43.0, respectively), whereas their springiness and cohesiveness where almost the same as those of control yoghurts. In addition, MTS yoghurts lost less structure after the first compression event than control yoghurts (18.6% vs 20.6%).

Water holding capacity measurements also showed significant differences between MTS and control samples. MTS samples liberated less serum (14.8%) than control samples (18.8%). This difference is statistically significant ($p < 0.05$) and represents about 40% more serum liberated by control yoghurts.

Texture of Stirred Yoghurt Samples. Results of textural analysis of stirred yoghurt samples in the three experiments are summarized in **Table 3**. Yoghurts were carefully stirred before sample application as consumers usually do before yoghurts are eaten. This is the most used method to perform viscometry or oscillation tests on yoghurts (*19*−*23*) probably because it is not easy to find and standardize a mechanical and reproducible method to stir yoghurts without breaking substantial amounts of their structure. Instead, manual slow stirring appears to be the most effective way to preserve yoghurt structure and at the same time allow obtention of yoghurt samples that can be measured in a rheometer equipped with a parallel plate geometry. Fermenting milk in the rheometer measuring system could be an alternative, but it has its own technical difficulties (evaporation, separation of the sample from the measuring plates). Furthermore, it is very time-consuming: only one yoghurt sample can be produced every 4−6 h which means that milk has to be stored for some days after thermal or MTS treatments before a set of rheological test can be performed. As milk is not sterile after processing, it would change during the storage period making the results very different at the beginning and at the end of the experiment.

Figure 5 shows typical flow curves of control and MTS samples. They are qualitatively similar. Both show time-dependent flow: there is a considerable hysteresis loop between the downward and the upward portion of the plot. But quantitatively they are different: consistency indexes (obtained from the upward curve and the downward curve) of MTS yoghurts are about twice as high as that of control yoghurts (52.7 vs 27.9 Pa•s^n and 1.75 vs 0.90 Pa•s^n for the upward and downward curves, respectively). Both MTS and control samples are non-Newtonian, but more so the MTS yoghurts, whose n value is 0.13 vs 0.23 of the control yoghurts. The non-Newtonian behavior tends to disappear when the yoghurt structure is broken by shearing during the test: n values of the downward curves are much closer to one than n values of the upward curve, but n values of MTS downward curves are still lower than that of control ones (0.61 vs 0.71).

Apparent viscosity was measured at 112 s^{-1} for about two minutes. Both samples showed rheodiluyent behavior but more

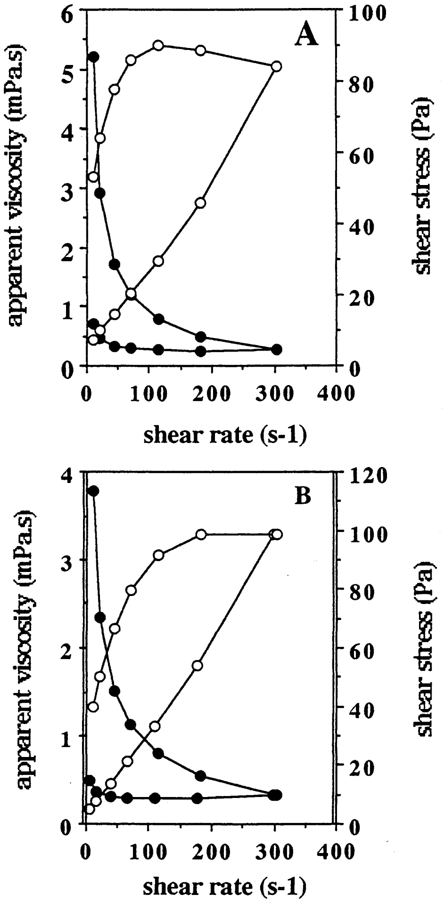

Figure 5. Typical flow curves of MTS-treated (A) and control yoghurts (B). Apparent viscosity (filled circles, ●); shear stress (empty circles, ○).

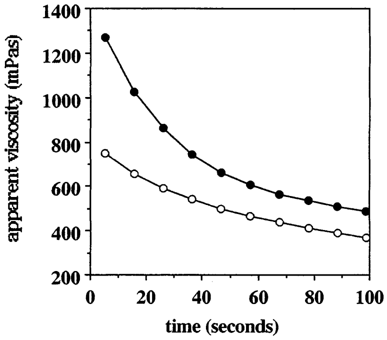

Figure 6. Apparent viscosity at constant shear rate (112 s^{-1}) of one MTS-treated yoghurt (filled circles, ●) and one control yoghurt (open circles, ○).

so for the MTS samples (**Figure 6**). MTS yoghurts showed higher initial apparent viscosity values, 1172 vs 868 mPa·s, but this difference was smaller, 431 vs 382 mPa·s, at the end of the test. This represents a reduction of 63.3% and 55.7% of initial viscosity in MTS and control samples, respectively. The difference in initial apparent viscosity values could have been higher if we had selected shorter delay and integration times for the measurements. As can be seen in the plots, the decrease in viscosity is much higher in MTS samples at the beginning of the test. As the first measurement is taken with a delay of at least 5 s we are losing an important portion of the test. However, delay and integration times that are too short result in lower accuracy of measurements, so a compromise between measurement accuracy and test amplitude had to be found.

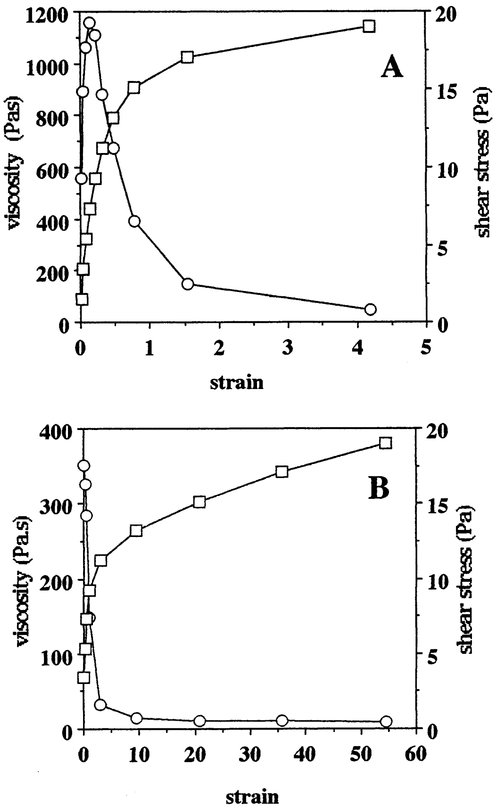

Figure 7. Yield stress determination of one MTS-treated (A) and one ontrol yoghurt (B). Note that the scales of both *y* and *x* axes are very different.

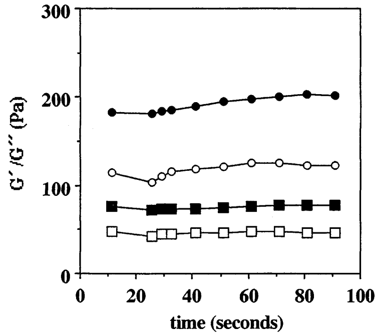

Figure 8. Oscillation tests of MT-treated (filled symbols) and control yoghurts (empty symbols). Storage modulus, *G*′, (circles). Loss modulus, *G*″, (squares).

Yield stress was calculated by applying a stress gradient between 0.5 and 20 Pa. Typical determinations are shown in **Figure 7**. MTS samples showed higher yield points (7.54 vs 4.52 Pa) than control samples.

Oscillation tests for determination of storage modulus (*G*′) and loss modulus (*G*″) of MTS and control samples are shown in **Figure 8.** *G*′ values of MTS samples were about 50% higher on average than *G*′ values of the control sample (157 vs 101 Pa).

DISCUSSION

One of the main driving forces for research in nonthermal technologies for food preservation has been to avoid, or reduce, the deletereous effects (in some instances) of heat on both organoleptic and nutritional properties of foods (*24*). The original goal of nonthermal technologies was to find physical principles able to stop or eliminate bacterial and enzymatic activities at time/temperatures conditions below the usual thermal treatments of foods (*25*). This goal had to be achieved at a reasonable cost, in terms of energy and equipment, and without damaging other desirable properties of foods. But some of the physical principles used in nonthermal technologies for food processing have added properties besides their bactericidal or enzyme -inactivation abilities. For example, high-pressure treatment of milk has been shown to increase the yield of cheese manufacture and even its texture (*26*), and manothermosonication (*27*) or high pressure (Crelier and Juillerat, unpublished observation) treatments of tomato pastes increased the apparent viscosity of this product. It is quite probable that the use of nonthermal technologies shall find a marketplace more because of their ability to improve or modify textural or other organoleptical properties, than because of its increased bactericidal or enzyme-inactivation capacities; because both bacterial and enzymatic activities can be relatively well controlled by conventional thermal means, except in some really sensitive foodstuffs. In this work, we describe extensively another example of texture improvement by the use of ultrasound in combination with moderate pressures, which we call manothermosonication.

Homogenization Effects. We have demonstrated that the combination of low pressure with ultrasonic irradiation improves substantially the homogenizing effects of the latter alone. Results reported by Villamiel and de Jong (*14*) showed that irradiation of milk at low temperature (<30 °C) for over 70 s yielded a biphasic distribution of fat globule sizes with two peaks at about 3.5 μm and 0.7 μm. They could achieve a more extensive homogenization degree only by elevating the temperature to 70 °C, although treatment conditions used in this work are different from those used by Villamiel and de Jong. This is another manifestation of the enormous increase of ultrasonic irradiation intensity when simultaneous moderate pressures are applied (provided that ultrasound equipment is powerful enough to maintain ultrasound amplitude and frequency). However, manothermosonication-assisted homogenization, at least under the conditions we have used here, is in no way better than standard homogenization. Manothermosonicated milk still has around 3% of the total fat content inside fat globules of 1.6 μm diameter or larger, whereas this is not the case for milk used for control yoghurt (*28*). This means that the fat globule homogenizing effect of manothermosonication treatments is not the cause of the textural differences between control and MTS yoghurts.

Fermentation Time. We have observed with the three batches of milk that fermentation of the three MTS yoghurts took longer than fermentation of the control yoghurts. This difference is statistically significant. Ultrasound has been found to reduce fermentation time if applied during the fermentation process, something that has been ascribed to the liberation of fermentative enzymes (*29*). Wu et al. (*15*) stated that ultrasonic irradiation before starter inoculation does not cause an appreciable reduction in fermentation time, although they saw statistically significant differences between sonicated and control samples.

Heat treatment of milk can have complex effects on fermentation time. These effects can be stimulatory or inhibitory on fermentation (*13*) depending on time and temperature conditions. They are due to several factors but mainly to the apparition of degradation products of milk proteins, which can have stimulatory or inhibitory effects on fermentation kinetics. The expulsion of oxygen has stimulatory effects on yoghurt starter cultures, but this probably does not play a role in the fermentation kinetics in our experiments although ultrasound has proven to be an efficient means to degas milk (*30*). Manothermosonication treatments were performed in a closed circuit under pressure (by definition) and strong agitation which does not allowed liberation of any dissolved gas.

Covalent modification of proteins by ultrasound is a well-described fact (*31*). We do not know if, under the conditions of our experiments, proteins are degraded and small molecules are liberated, but this is quite possible taking into account the free radical production ability of MTS (*32*). The detailed study of this question would require a much simpler system than milk (with added skim milk powder), which could be very interesting but was outside of the scope of this work.

It is very interesting to mention that longer fermentation times have been shown to correlate well with increased firmness of yoghurts (*33*). We observed very different fermentation times in each of the control yoghurt batches, and we observed also increased consistency and viscosity in yoghurts fermented for longer times. If this rule holds true, it is quite reasonable to find a firmer structure in yoghurts produced with manothermosonicated milk compared to yoghurt made from control milk simply because fermentation takes longer in MTS yoghurts.

Rheological Properties of MTS Yoghurts. In the present work we have found that the textural characteristics of control and MTS yoghurts are quantitatively very different. Assays performed on both intact and slowly stirred yoghurts showed that MTS yoghurts have a firmer structure. This can be attributed to the effect of fermentation time, but other possibilities should be taken into account. Texture (high viscosity and consistency) is a very important characteristic of yoghurt. It is directly related to yoghurt structure. which is based on strings or clusters of casein micelles interacting physically which each other and with denatured serum proteins (mostly β-lactoglobulin) entrapping serum and fat globules. To obtain high consistency and viscosity yoghurts, the industry usually follows one or a combination of the following approaches: (1) milk supplementation with nonfat dairy solids; (2) milk homogenization; and (3) heat treatments to denature serum proteins to allow a better interaction of these with caseins. Clearly, in the conditions used in this work, point 1 cannot be considered as responsible for the better textural properties of MTS yoghurts because both types of yoghurts were supplemented with the same amounts of nonfat dairy solids. About point 2, the reduction of fat globule size caused by homogenization does not seem to be the fact that could explain the superior properties of MTS yoghurts. Indeed, standard homogenization is slightly more efficient than MTS homogenization (see above). However, it could be possible that MTS could cause some qualitative changes in the fat globule membrane which would modify the ability of fat globules to interact with themselves and/or casein micelles, improving the gelling properties. Anyway, this is still a matter of controversy even in standard homogenization procedures (*34*). About point 3, it is quite reasonable to suspect that MTS effects on milk proteins contribute to the results we obtained. We support this affirmation on several facts. First, ultrasound is able to denature, or even split, proteins. Denatured serum proteins enhance firmness of yoghurt. Indeed this is the reason milk is heat treated before starter addition. Villamiel and de Jong (*14*) found increased denaturation of both β-lactoglobulin and α-lactalbumin (measured as their adsorption to the casein fraction) in sonicated milks compared to control milks although they used much lower intensity irradiation conditions than we have used. Denaturation of these proteins results in exposure of free sulfydryl groups. These are considered responsible for the “cooked” character of milks. MTS milk had a strong “cooked” flavor which was not detectable in the yoghurts (Vercet and Lopez-Buesa, unpublished observation). Cooked flavor has been also described in sonicated milk by Sauter ((*35*). Therefore, we think that it is quite possible than serum protein denaturation by ultrasound can play a role in the increased consistency and viscosity of MTS yoghurts. However, direct effects of manothermosonication on casein micelles or individual caseins cannot be ruled out, although Villamiel and de Jong (*14*) did not found any effect on the individual caseins. This is because big multimeric protein complexes are more sensitive to the shearing forces created by microstreaming and bubble implosion than are the single dissolved monomeric proteins. We have also observed by using a formagraph, which follows milk coagulation after chymosin addition, that manothemosonicated milk takes much longer to coagulate and yields a weaker coagulum (Lopez-Buesa and Vercet, unpublished observation) than nonsonicated milks, a phenomenon that was observed also by Munckacsi and Elhami (*36*), which points to destabilization of micellar structure in manothermosonicated/sonicated milk, although these authors also treated milk with UV irradiation.

CONCLUSIONS

Application of pressure dramatically enhances the homogenization power of ultrasound. This is not enough to state that manothermosonication is more effective than standard procedures to homogenize milk. However, MTS treatments could be a suitable option for the yoghurt industry because they improve, by still unknown mechanisms, the texture of yoghurts.

LITERATURE CITED

(1) Burgos, J. Manothermosonication. In *Encyclopedia of Food Microbiology*; Robinson, R K., Batt, C. A., Patel, P. D., Eds.; Academic Press: New York, 1999; pp 1462−1469.
(2) Vercet A.; Lopez, P.; Burgos, J. Inactivation of heat-resistant lipase and protease from *Pseudomonas fluorescens* by manothermosonication. *J. Dairy Sci*. **1997**, *80*, 29−36.
(3) Wrigley, D. M.; Llorca, N. G. Decrease of *salmonella typhimurium* in skim milk and egg by heat and ultrasonic wave treatment. *J. Food Prot*. **1992**, *55*, 678−680.
(4) Garcia, M. L.; Burgos, J.; Sanz, B.; Ordoñez, J. A. Effect of heat and ultrasonic waves on the survival of two strains of *Bacillus subtilis*. *J. Appl. Bacteriol*. **1989**, *67*, 619−628.
(5) Suslick, K. *Ultrasound: Its Chemical, Physical and Biological Effects*; VCH: New York, 1988.
(6) Riesz, P.; Kondo, T. Free radical formation induced by ultrasound and its biological implications. *Free Radical Biol. Med*. **1992**, *13*, 247−270.
(7) Basedow, A. M.; Ebert, K. H. Ultrasonic degradation of polymers in solution. *Adv. Polym. Sci.* **1977**, *22*, 83−148.
(8) Mason, T. J.; Paniwnyk, L. The uses of ultrasound in food processing. *Adv. Sonochem*. **1996**, *4*, 177−203.
(9) Sánchez, E. S.; Simal, S.; Femenia, A.; Roselló, C. Effect of acoustic brining on the transport of sodium chloride and water in Mahón cheese. *Eur. Food Res. Technol*. **1996**, *212*, 39−43.
(10) Lopez, P.; Oria, R.; Vercet, A. Estimulación de la actividad de proteasas mediante manotermosonicación. 2001. Spanish patent application P200101662.
(11) Villamiel, M.; van Hamerveld, E. H.; de Jong, P. Review: Effect of ultrasound processing on the quality of dairy products. *Milchwissenschaft* **1999**, *54*, 69−73.
(12) Walstra, P. Preliminary note on the mechanism of homogenisation. *Neth. Milk Dairy J.* **1969**, *23*, 290−292.
(13) Tamime, A. Y.; Robinson, R. K. *Yoghurt Science and Technology*; CRC Press: Boca Raton, FL, 1999; pp 55−60, 64−65.
(14) Villamiel, M.; de Jong, P. Influence of high-intensity ultrasound and heat treatment in continuous flow on fat, proteins, and native enzymes of milk. *J. Agric. Food Chem*. **2000**, *48*, 472−478.
(15) Wu, H.; Hulbert, G. J.; Mount, J. R. Effects of ultrasound on milk homogenisation and fermentation with yoghurt starter. *Innov. Food Sci. Emerg. Technol*. **2001**, 211−218.
(16) Kalab, M.; Allan-Wojtas, P.; Phipps-Todd, B. E. Development of microstructure in set-style nonfat yoghurt. A review. *Food Microstr*. **1983**, *2*, 51−66.
(17) Lucey, J. A.; Singh, H. Formation and physical properties of acid milk gels: a review. *Food Res. Int.* **1998**, *30*, 529−542.
(18) Lopez, P.; Vercet, A.; Sanchez, C.; Burgos, J. Inactivation of tomato pectic enzymes by manothermosonication. *Z. Lebensm.-Unters. Forsch.* **1998**, *207*, 249−252.
(19) Bottazi, V. Factors influencing gel formation and thixotropy of yoghurt. *Sci. Tec. Lat-Cas*. **1986**, *27*, 402−414.
(20) Parnell-Clunies, E. M.; Kakuda, Y.; Deman, J. M. Influence of heat treatment of milk on the flow properties of yoghurt. *J. Food Sci*. **1986**, *51*, 1459−1462.
(21) Guirguis, N.; Hickey, M. W.; Freeman, R. Some factor affecting nodulation in yoghurt. *Aust. J. Dairy Technol*. **1987**, *42*, 45−47.
(22) Dannenberg, F.; Kessler, H. G. Effcet of denaturation of β-lactoglobulin on texture properties of set-style nonfat yoghurts. 2. Firmness and flow properties. *Milchwissenschaft* **1988**, *43*, 700−704.
(23) Mottar, J.; Bassier, A.; Joniau, M. Baert, J. Effect of heat-induced association of whey proteins and casein micelles on yoghurt texture. *J. Dairy Sci.* **1989**, *72*, 2247−2256.
(24) Mertens, B.; Knorr, D. Developments of nonthermal processes for food preservation. *Food Technol.* **1992**, *46*, 124−133.
(25) Gould, G. W. *New Methods of Food Preservation*; Blackie: London, 1995.
(26) Lopez-Fandiño R.; Carrascosa, A. V.; Olano, A. The effects of high pressure on whey protein denaturation and cheese making properties of raw milk. *J. Dairy Sci*. **1996**, *79*, 929−936.
(27) Vercet, A.; Sánchez, C.; Burgos. J.; Montañés, L.; Lopez-Buesa, P. The effects of manothermosonication on tomato pectic enzymes and tomato paste rheological properties. *J. Food Eng*. **2002**, *53*, 273−278.
(28) Phipps, L. W. The high-pressure dairy homogenizer; National Institute of Rural Development: Reading, U.K., 1985.
(29) Sakakibara, M.; Wang, D.; Ikeda, K.; Suzuki, K. Effect of ultrasonic irradiation on production of fermented milk with *Lactobacillus delbruekii*. *Ultrason. Sonochem*. **1994**, *1*, 107−110.
(30) Villamiel, M.; Verdurmen, R.; de Jong, P. Degassing of milk by high-intensity ultrasound. *Milchwissenschaft* **2000**, *55*, 123−125.
(31) El'Piner, E. A. In *Ultrasounds: Physical, Chemical and Biological Effects*; Consultants Bureau: New York, 1964; pp 149−229.
(32) Vercet, A.; Lopez, P.; Burgos, J. Free radical production by manothermosonication. *Ultrasonics* **1998**, *36*, 615−618.
(33) Yuguchi, H.; Tanai, S.; Iwatsuki, K.; Okonogi, S. The influence of fermentation velocity on the properties of yoghurt. *Jpn. J. Zootech. Sci*. **1989**, *60*, 742−746.
(34) Paquin, P. Technological properties of high-pressure homogenizers: the effect of fat globules, milk proteins and polysaccharides. *Int. Dairy J.* **1999**, *9*, 329−335.
(35) Sauter, E. FRG Patent 892.109, 1953 (Cited in Villamiel et al., 1999, ref *11*).
(36) Munckacsi, F.; Elhami, M. Effect of ultrasonic and ultraviolet irradiation on the chemical and bacteriological quality of milk. *Egypt. J. Dairy Sci*. **1976**, *4*, 1−6.

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