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High Hydrostatic Pressure Effects on Color and Milk Fat Globule of Ewe's Milk.

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ABSTRACT

The influence of high hydrostatic pressure treatments on lipolysis, color, and size distribution of milk fat globule (MFG) on ewe's milk was studied. Treatments consisted of combinations of pressure (100 to 500 MPa) and temperature (4, 25, and 50 ºC) for 10 min. Pressurized samples showed FFA levels lower than control. Pressurizations at 25 and 50 ºC showed a tendency to increase MFG in the range 1-2 μm and decrease MFG between the range 2 to 10 μm, whereas at 4 ºC was the reverse. A decrease of lightness (L*) value, and an increase of (-a*) and (+b*) values occurred in color with pressure increase.

Key Words: hydrostatic pressure, ewe's milk, lipolysis, fat globule.

INTRODUCTION

Over the last few years the demand for fresh foods with a high nutritional, safety and sensory quality by food industries and consumers has been growing so new technologies such as electric or magnetic fields, ionizing radiation, light pulses, and high hydrostatic pressure (**HHP**) (Mertens and Knorr 1992) are being developed to satisfy the demand and replace conventional treatments (preservatives and thermal processes).

HHP treatment is a physical process, which is based on two principles; the first, its application is uniform and instantaneous throughout a food and the second, pressure accelerates the processes that have a volume decrease, whereas it inhibits processes involving a volume increase.

The new food process HHP (100 to 600 MPa) can inactivate microorganisms (Hoover and others 1989; Styles and others 1991) without damaging food constituents or altering the taste and flavor (for example milk, meat, vegetable juices, and so on). This process permits non-inactivation of the enzymes that are important for cheese maturation (Cheftel 1991; Earnshaw 1992).

The first experiments on foods (milk, vegetables, and fruits) were by Hite (1899) and Hite and others (1914). Subsequently, a great number of studies on foods and beverages for the potential use of HHP have been published (Carlez and others 1993; Ponce and others 1998), but few studies were carried out on microbial inactivation of ewe's milk (Gervilla and others 1997a, 1999a). There have been no studies on the effect of HHP on physicochemical properties of ewe's milk.

Ewe's milk production is increasing in Mediterranean countries where it is mostly used for cheese making. The popularity of raw milk cheeses has led to

increasing interest in the study of physicochemical behavior of ewe's milk under HHP, especially since microbial inactivation was possible (Gervilla and others 1997b, 1999b).

In fresh whole milk, the lipids are found as dispersed milk fat globules (**MFG**). These MFG (98% triglycerides) are surrounded by a native membrane which acts to prevent flocculation and coalescence of MFG and also protects against enzymatic action of native lipase (EC 3.1.1.34). During milk processing (cooling, agitation, homogenization, and heat treatments) the membrane of MFG is altered making the action of lipase to triglycerides possible and increasing the levels of the free fatty acids (**FFA**) in milk. This process is called lipolysis. If the concentration of FFA rises above a critical level, it results in an off-flavor defect known as hydrolytic rancidity (rancid milk), for that, lipolysis is a good index on the damage of the MFG membrane (Anderson and Needs 1983).

On the other hand, information about the size distribution and structure of MFG is of interest from a biophysical, nutritional and technological point of view. The latter aspect is of increasing importance in milk separation and butter and cheese making.

The objective of this work was to evaluate the effect on some physicochemical properties (lipolysis, size and distribution of MFG, and color) of ewe's milk under HHP treatments.

MATERIALS AND METHODS

Collection of milk samples

Fresh raw Manchega ewe's milk was obtained from the first milking in the morning of a rural dairy farm (Can Gelats Nou, Riudarenes, Spain) (collaborator station). The time between milk collection in sterile glass bottles and preparation of control and HHP treatments of samples did not exceed 1 h (approximately 25 ºC).

Determination of free fatty acids (FFA)

The FFA content of the samples was measured by the Boureau of Dairy Industries method (FIL-IDF 1991a), except that automatic titration (Liquid Handling Station LHS 100, BRAND GmbH, Wertheim, Germany) was used. The results are expressed in mequiv FFA / 100 g fat. Immediately after the milk arrived at the laboratory, in each experiment, one sample was heated at 60 ºC for 30 min to destroy lipase activity, and frozen (-30 ºC) until tested for initial FFA (**FFA-A**). A second sample was stored at 4 ºC for 24 h, then heated (60 ºC / 30 min) and frozen (-30 ºC) until tested for FFA content (**FFA-B**). The others were subjected to HHP treatments before storage and then heated (60 ºC / 30 min) and frozen (-30 ºC) until tested for FFA content (**FFA-C**). Spontaneous lipolysis (**SL**) was defined as the difference between FFA-B and FFA-A. Induced lipolysis (**IL**) was defined as the difference between FFA-C and FFA-A.

Determination of milk fat globules (MFG) size and distribution

Size distribution of MFG was determined using a Coulter Counter® system model ZM (Coulter Electronics Ltd., Luton, U.K.). Control (unpressurized whole milk) and samples (pressurized whole milk) were kept at 23 ºC for 1 h until analysis. Isoton®II (Coulter Euro Diagnostics GmbH, Krefeld, Germany) was used for appropriate dilution.

The MFG diameters were classified into 12 size classes (1-2, 2-3, 3-4, 4-5, 5-6, 6-7, 7-8, 8-9, 9-10, 10-15, 15-20, and 20-50 μm). The various moments (S_n) of the distribution function were calculated using the number of particles of each size class per unit volume (N_i) and the mean diameter of the corresponding size class (d_i), according

$$S_n = \sum_{i=1} d_i^n N_i$$

to the following equation:

(n-th moment of the distribution functions).

From these moments various parameters characterizing the mean size of MFG were obtained (Rüegg and Blanc 1982):

Total number of MFG / mL: N / mL = S_0

Number mean diameter: $d_n = S_1 / S_0$

Volume mean diameter: $d_v = (S_3 / S_0)^{1/3}$

Volume / surface mean diameter: $d_{vs} = S_3 / S_2$

Volume moment mean diameter: $d_{vm} = S_4 / S_3$

Distribution width: $c_s = (S_2 S_4 / S_3^2 - 1)^{1/2}$

Creaming parameter: $H = S_5 / S_3$

Determination of color parameters

Color measurements were made using a portable HunterLab spectrocolorimeter (MiniScan XE^{TM}, Hunter Associates Laboratory, Inc., Reston, Vir, USA). Control (unpressurized whole milk) and samples (pressurized whole milk) were kept at 23 ºC for 1 h and measured for values of color coordinates: L* (lightness), a* (redness to greenness), and b* (yellowness to blueness). ΔE (total color differences) values were computed using $\Delta E^* = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$ (MiniScan XE^{TM}, manual version 1.2, user's guide 1995, Hunter Associates Laboratory, Inc., Reston, Vir, USA). WI (whiteness) was obtained directly from software of the HunterLab spectrocolorimeter (using ASTM method E313; MiniScan XE^{TM}, Hunter Associates Laboratory, Inc., Reston, Vir, USA). The instrument was calibrated with a white standard reference prior to the measurements and operated with a wide aperture at a 10 ºangle of illuminant D65.

Confocal light scanning microscopy observations

Microscopy observations of control (unpressurized whole milk) and samples (pressurized whole milk) were taken using confocal light scanning microscopy (**CLSM**) (Leica TCS4D, Heidelberg, Germany). Control and samples were stained with Nile Blue (Brooker 1991) 0.01% (w/v) in water, at a proportion 1 : 1 (milk : Nile Blue). When milk was exposed to 568 nm with a Kr/Ar laser (Leica TCS4D, Heidelberg, Germany), the images obtained corresponded to both protein and fat phase, whereas with 488 nm only the fat phase was observed.

High hydrostatic pressure processing

Samples were pressurized by using discontinuous high hydrostatic pressure equipment (ACB, Nantes, France) with a 2 L capacity pressure chamber. The time needed to achieve maximum pressure (500 MPa) was 2 min. The chamber and water (hydrostatic fluid medium) inside were cooled or heated to treatment temperature with a constant flow of ethylene glycol-water (1 : 1) solution within the walls of the vessel. Samples were kept for 5 to 10 min at atmospheric pressure in the chamber until temperature equilibrium was established. The temperature of the samples was monitored by a thermocouple to evaluate the most extreme temperature conditions suffered by the samples.

Time, temperature, and pressure parameters were selected on the basis of previous studies for inactivating microorganisms (Gervilla and others 2000). The responses of different physicochemical parameters to treatments at different conditions of pressure (100, 200, 300, 400, and 500 MPa), temperature (4, 25, and 50 ºC), and time (10 and 30 min, only for color testing) were studied.

Composition and physicochemical analysis of milk

The total solids content was determined by drying at 102 ± 2 ºC in an oven to constant weight (FIL-IDF 1987). Ash content was determined by gravimetric analysis after

the sample had been calcinated in an oven at 550 ºC (FIL-IDF 1964). Fat content was determined by the Gerber method (FIL-IDF 1991b). Total nitrogen was calculated using the digestion block method, a modification of the Kjeldahl method (FIL-IDF 1993). The pH was measured using a pH meter (micro-pH 2001, Crison Instruments S.A., Alella, Spain) (Richardson 1985).

Statistical analysis

Each experiment was run three times with duplicate analysis in each replicate. An analysis of variance (ANOVA) was performed using the general linear models (GLM) procedure of Statistical Analysis System software (SAS®, Version 6.03, SAS Institute, Inc., Cary, NC, USA). Duncan's new multiple range test and Student-Newman-Keuls test were used to obtain pairwise comparisons among sample means. Evaluations were based on a 5% significance level ($P < 0.05$).

RESULTS AND DISCUSSION

Ewe's milk composition was: total solids = 18.71 (± 1.63); fat = 7.55 (± 1.49); total protein = 5.81 (± 0.19); ash = 1.19 (± 0.11); expressed in percentages on wet weight basis. The pH was 6.68 (± 0.11).

Color

The statistical analysis of the independent variables of treatment (pressure, temperature, and time) was studied in regard to the dependent variable total color differences (**DE**). Taken as a whole, the experiments showed that ΔE rates increased with pressure (500 > 400 > 300 > 200 > 100 MPa; $P < 0.05$), temperature (4 > 25 > 50 ºC; $P < 0.05$), and time (30 > 10 min; $P < 0.05$). From the analysis of the F-value ($r^2 = 0.99958$), pressure was the main factor and then temperature, explaining 78 and 19%, respectively, of the variability of the statistical model.

Lightness values (L*) ranged from 90.32 to 85.09. A decrease of L* ($P < 0.05$), and an increase ($P < 0.05$) of greenness (-a*) and yellowness (+b*) was observed when pressure was increased (Table 1). Also at 4 ºC pressure produced the greatest differences in ΔE with minimal L* values and maximal –a* and +b* values. In general, low pressures (100 and 200 MPa) tend to decrease the effect of temperature per se in the ΔE values.

Some authors reported similar results in the behavior of milk color under HHP treatments (Adapa and others 1997; Johnston and others 1992) and the differences between absolute L*, a*, and b* values of these authors and our own could be due to the percentage of fat (skim cow's milk and whole ewe's milk). On the other hand, the decrease in L* value could have been mainly due to disintegration of casein micelles by pressure into small fragments that increase the translucence of the milk (Johnston 1995). Schmidt and

Buchheim (1970) observed and demonstrated by electron microscopy that casein micelle disintegration was induced by HHP treatments.

FFA lipolysis

The results of statistical analysis of HHP treatment variables showed significant differences between temperatures (25 > 4 > 50 ºC; $P < 0.05$), pressure (100 = 200 > 300 = 400 > 500 MPa; $P < 0.05$), and non-significant differences ($P > 0.05$) were found between 100 and 200 MPa and between 300 and 400 MPa. Differences between treatments are shown in Table 2. For the analysis of F-value ($r^2 = 0.98875$), temperature was the main factor, explaining 95% of the variability of statistical model. The response was the result of SL = (FFA-B) – (FFA-A) or IL = (FFA-C) – (FFA-A).

Mean FFA-A levels ranged from 0.48 to 0.80 mequiv FFA / 100 g fat. Non-significant differences were shown between the different experiments in SL, with an increase of about 1 mequiv FFA / 100 g fat. Pressurization at 4 and 50 ºC produced lower lipolysis ($P < 0.05$) than at 25 ºC, and we can also see that an increase of pressure reduced the content of FFA in induced lipolysis. In most results IL produced lower FFA content than SL, only pressurizations at 25 ºC showed similar results to those obtained in SL. Jandal (1996) showed that lipolysis in ewe's milk can be reduced by heating and cooling at 50 and 5 ºC for 1 h, while the lipolysis can be enhanced by agitation and the addition of certain chemicals.

The low increase in FFA when we applied HHP treatments might be due to the total or partial inactivation of the native lipoprotein lipase (**LPL**) of milk by pressure, or compositional and structural changes of the MFG membrane by adsorption of disintegrated casein micelles on its surfaces (Law and others 1998), or adsorption of the denatured whey proteins (Felipe and others 1997) which would considerably increase the strength of the membrane, prevent melting and leakage of the fat (Dalgleish and Banks 1991) and hinder

the accessibility of LPL to the fat; also the induction of fat crystallization by pressure (Buchheim and Abou El-Nour 1992) could reduce the action of LPL to the fat of MFG.

TDP coulter

The statistical analysis of pressure, temperature and diameter was studied as a function of frequency number of distribution. The experiments, taken as a whole showed that with respect to the effect of pressure (200 = 300 > 100 = 400 = 500 MPa; $P < 0.05$) non-significant differences ($P > 0.05$) were found between 200 and 300 MPa and between 100, 400, and 500 MPa. Temperature effect (25 = 50 > 4 ºC; $P < 0.05$) showed non-significant differences ($P > 0.05$) between 25 and 50 ºC. Diameter (1-2 > 2-3 = 3-4 = 4-5 = 5-6 > 6-7 = 7-8 = 8-9 = 9-10 μm; $P < 0.05$) showed non-significant differences ($P > 0.05$) from 2 to 6 μm and from 6 to 10 μm. For the analysis of the F-value ($r^2 = 0.96889$) pressure and temperature were the main factors, both explaining 84% of the variability of the statistical model. The differences in size distribution between initial and treated samples are shown in Fig. 1.

Pressurizations at room (25 ºC) and moderately high (50 ºC) temperatures showed a tendency to increase MFG in the range 1-2 μm and decrease MFG between the range 2 to 10 μm, whereas at low (4 ºC) temperature the tendency was the reverse. The effect of temperature per se at 4 and 50 ºC (Fig. 1) in size distribution was a decrease of about 3-4% in MFG of 1-2 μm and a slight increase of the larger MFG. Pressure contribution showed greater differences in size distribution at 200 and 300 MPa with an increase ($P < 0.05$) of about 6-10% (at 25 and 50 ºC) and a decrease ($P < 0.05$) of about 6-8% (at 4 ºC) in MFG of 1-2 μm. Taken as a whole, the fewest differences in size distribution were observed when pressures under 200 MPa or above 300 MPa were applied.

It seemed that the highest pressures such as 400 and 500 MPa hardened the MFG membrane and did not allow their coalesce or fission. Low temperatures (4 ºC) produced an adsorption of the small MFG, coalescing with the medium MFG. Maybe the formation of crystals of triglycerides by pressure and low temperatures could influence this fact. On the contrary, temperatures at 25 and 50 ºC facilitated the fission of medium MFG to small MFG. These temperatures (25 and 50 ºC) could affect the fluency and dilation of the membrane lipids and that together with the pressure could deform the MFG until producing small MFG.

The effect of HHP treatments on the most important parameters of the size distribution of the MFG are shown in Table 3. The number of MFG ranged between 2 to 3×10^9 MFG / mL of milk. The different parameters to characterize an average MFG size (d_n, d_v, d_{vs}, and d_{vm}) were mainly calculated for comparison with other data. d_{vs}, which corresponds to the arithmetic mean of the surface-weighted distribution, is less dependent on uncertainties in the number of small globules and is, therefore, a more useful average. For the same reason the distribution width is better described on the basis of the coefficient of variation of the surface-weighted distribution (c_s) (Rüegg and Blanc 1982). Average diameter (d_{vs}) ranged from 4.3 to 5.1 μm with 400-500 MPa at 50 and 4 ºC, respectively. There are not many data on ewe's milk and other species which permit calculation and comparison of average MFG size. In comparison with the average size of unpressurized MFG, the average diameter of pressurized MFG hardly changed between 100 and 500 MPa. These results showed that the average diameter of MFG was apparently not affected by pressures up to 500 MPa. Similar behavior showed a cream (30% fat w/v) from cow's milk when it was pressurized from 100 to 400 MPa at 37 ºC for 10 min by Kanno and others (1998). Pressurizations at low temperatures (4 ºC) showed the highest average creaming factor (H), as H characterizes the stability of a fat emulsion. Apparently,

pressurizations at 4 ºC would produce ewe's milk that would tend to cream off faster than pressurizations at 25 and 50 ºC.

CLSM observations

The observations by CLSM agree with the results obtained from analysis of MFG distribution. Figure 2 shows MFG size and distribution images of control and milk samples at different HHP conditions. Image A (unpressurized) represents a typical ewe's MFG distribution with a uniform emulsion and spherical form of MFG. Image B (500 MPa at 50 ºC) shows a MFG distribution similar to unpressurized milk. In samples submitted to pressures between 200 and 300 MPa at 25 or 50 ºC, as in image C (200 MPa at 25 ºC), an increase of small MFG (1-2 μm) can be observed. On the other hand, samples treated at low temperature (4 ºC), as in image D (200 MPa at 4 ºC), showed an increase of the medium MFG, with a more uniform dispersion than untreated milk.

Very sporadically we could observe formation of MFG aggregates and MFG that lose their circularity overall when pressurization was at low temperature (4 ºC), but we believe that these observations were not representative.

CONCLUSIONS

The more extremely high hydrostatic pressure (HHP) treatments did not produce sufficiently significant changes that would cause consumers to reject the milk color. The HHP treatments did not increase spontaneous lipolysis and reduced de FFA content in induced lipolysis when the treatments were carried out at 4 and 50 ºC. The HHP treatments at 25 and 50 ºC measured the number of small MFG (1-2 μm) by affecting the size and distribution of MFG and increased the stability of milk to creaming off, but the opposite effect resulted at 4 ºC.

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Table 1: Effect of HHP treatments on color parameters in ewe's milk

Treatments	L*		a*		b*		ΔE		WI	
MPa / °C /min	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
White Standard	89.15	0.021	-2.49	0.071	9.88	0.290	0.00	0.000	25.56	1.662
0.1 / 25 / 30	89.34	0.014	-2.34	0.009	9.70	0.013	0.20	0.014	26.79	0.062
100 / 25 / 15	89.25	0.005	-2.52	0.024	9,72	0.016	0.13	0.019	26.58	0.075
200 / 25 / 15	88.94	0.005	-2.51	0.005	10.04	0.013	0.44	0.013	24.51	0.070
300 / 25 / 15	89.17	0.000	-2.31	0.006	10.41	0.014	0.75	0.013	22.74	0.063
400 / 25 / 15	87.65	0.009	-2.56	0.013	11.02	0.008	2.03	0.009	17.76	0.041
500 / 25 / 15	86.25	0.014	-3.16	0.013	11.26	0.008	3.40	0.013	15.04	0.045
500 / 25 / 30	85.97	0.013	-3.22	0.009	11.39	0.008	3.72	0.013	14.05	0.043
0.1 / 50 / 30	89.20	0.005	-2.24	0.005	9.63	0.022	0.20	0.009	26.97	0.113
100 / 50 / 15	89.22	0.010	-2.47	0.013	9.70	0.006	0.07	0.013	26.69	0.035
200 / 50 / 15	89.05	0.009	-2.42	0.000	9.80	0.006	0.17	0.005	25.97	0.029
300 / 50 / 15	90.32	0.008	-1.95	0.000	10.38	0.000	1.44	0.005	24.24	0.018
400 / 50 / 15	89.23	0.012	-2.24	0.009	11.01	0.021	1.35	0.017	19.60	0.129
500 / 50 / 15	87.92	0.005	-3.00	0.009	11.21	0.009	2.06	0.009	17.10	0.066
500 / 50 / 30	88.35	0.005	-2.90	0.006	10.72	0.009	1.40	0.013	20.23	0.053
0.1 / 4 / 30	89.15	0.006	-2.43	0.013	10.28	0.015	0.23	0.013	23.33	0.066
100 / 4 / 15	89.07	0.013	-2.48	0.009	10.07	0.021	0.08	0.019	24.39	0.109
200 / 4 / 15	88.58	0.010	-2.67	0.005	10.28	0.014	0.60	0.013	22.73	0.065
300 / 4 / 15	87.35	0.005	-2.74	0.006	11.42	0.008	2.24	0.009	15.25	0.056
400 / 4 / 15	85.81	0.009	-2.81	0.010	12.05	0.012	3.87	0.008	10.31	0.070
500 / 4 / 15	85.41	0.009	-3.03	0.013	12.16	0.005	4.29	0.009	9.37	0.022
500 / 4 / 30	85.09	0.013	-3.13	0.014	12.42	0.006	4.70	0.014	7.77	0.031

Data are the means (n = 6) and standard deviation (SD) of three independent experiments.
L*: lightness; a*: redness to greenness; b*: yellowness to blueness.
ΔE: total color difference; WI: whiteness index.

Table 2: Effect of HHP treatments on lipolysis (free fatty acids content)[a] in ewe's milk

	Pressure	25°C		Pressure	50°C		Pressure	4°C	
	MPa	Mean	SD	MPa	Mean	SD	MPa	Mean	SD
[b]SL	0.1	0.87	0.03	0.1	1.04	0.04	0.1	1.00	0.04
[c]IL	100	0.82	0.03	100	0.27	0.04	100	0.33	0.03
IL	200	0.91	0.03	200	0.12	0.03	200	0.33	0.04
IL	300	0.98	0.03	300	-0.31	0.06	300	0.30	0.05
IL	400	0.89	0.03	400	-0.10	0.03	400	0.21	0.02
IL	500	0.69	0.03	500	-0.11	0.06	500	0.25	0.04

Data are the means ($n = 6$) and standard deviation (SD) of three independent experiments.
[a] Results are expressed as: mequivFFA / 100g fat.
[b] Spontaneous lipolysis (stored at 4°C for 24 h).
[c] Induced lipolysis by HHP treatments.

Table 3: Effect of HHP treatment on some parameters used to characterize the particle size distribution on ewe's milk fat globules

Treatments	N / mL		dn (μm)		dv (μm)		dvs (μm)		dvm (μm)		Cs (%)		H (μm^2)	
MPa / °C	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	2.00×10^9	0.05×10^9	3.06	0.07	3.95	0.07	5.00	0.09	6.39	0.11	52.86	1.88	63.32	4.41
0.1 / 25	2.01×10^9	0.04×10^9	3.04	0.02	3.96	0.03	4.99	0.07	6.33	0.10	54.12	1.21	66.19	3.96
100 / 25	2.02×10^9	0.05×10^9	2.97	0.05	3.83	0.05	4.82	0.08	5.87	0.11	46.60	2.08	45.64	8.36
200 / 25	2.04×10^9	0.06×10^9	2.73	0.05	3.59	0.05	4.64	0.09	5.99	0.10	53.77	1.19	55.70	5.29
300 / 25	2.03×10^9	0.06×10^9	2.78	0.07	3.66	0.06	4.73	0.11	6.11	0.14	54.01	1.22	57.43	6.21
400 / 25	2.05×10^9	0.05×10^9	2.91	0.08	3.77	0.08	4.78	0.11	6.04	0.13	51.17	2.48	54.34	7.41
500 / 25	2.03×10^9	0.05×10^9	2.99	0.06	3.89	0.06	4.95	0.09	6.29	0.11	51.99	2.36	59.57	6.93
Control	1.88×10^9	0.07×10^9	2.73	0.09	3.51	0.09	4.46	0.12	5.54	0.14	49.32	3.88	40.81	10.13
0.1 / 50	2.03×10^9	0.05×10^9	2.83	0.05	3.63	0.05	4.59	0.09	6.09	0.12	57.29	3.44	64.14	9.24
100 / 50	2.04×10^9	0.06×10^9	2.68	0.03	3.45	0.03	4.36	0.11	5.44	0.19	49.53	4.87	42.40	14.38
200 / 50	1.70×10^9	0.07×10^9	2.60	0.05	3.43	0.05	4.46	0.12	5.89	0.18	56.53	4.62	55.74	15.16
300 / 50	1.71×10^9	0.07×10^9	2.81	0.11	3.66	0.09	4.74	0.14	6.56	0.21	61.97	4.88	75.37	17.12
400 / 50	1.94×10^9	0.06×10^9	2.65	0.12	3.38	0.11	4.29	0.12	5.79	0.20	59.25	5.02	59.21	12.11
500 / 50	1.95×10^9	0.05×10^9	2.67	0.09	3.41	0.09	4.33	0.11	5.83	0.21	60.18	4.97	60.37	12.08
Control	3.29×10^9	0.06×10^9	2.50	0.10	3.46	0.11	4.76	0.12	6.64	0.14	62.79	3.31	74.05	9.02
0.1 / 4	3.27×10^9	0.06×10^9	2.58	0.07	3.54	0.07	4.82	0.08	6.65	0.11	61.72	3.07	73.32	7.94
100 / 4	3.20×10^9	0.05×10^9	2.64	0.07	3.63	0.08	4.93	0.11	6.63	0.14	58.65	3.12	68.31	10.02
200 / 4	3.20×10^9	0.04×10^9	2.69	0.04	3.70	0.06	5.02	0.10	6.69	0.14	57.63	4.08	68.15	12.21
300 / 4	3.18×10^9	0.05×10^9	2.72	0.04	3.76	0.05	5.11	0.09	7.05	0.12	61.57	3.67	81.84	13.12
400 / 4	3.25×10^9	0.05×10^9	2.59	0.08	3.65	0.09	5.09	0.12	7.24	0.16	65.04	4.15	85.46	12.61
500 / 4	3.28×10^9	0.06×10^9	2.56	0.11	3.62	0.11	5.09	0.12	7.70	0.18	71.68	4.31	97.92	11.72

Data are the means ($n = 6$) and standard deviation (SD) of three independent experiments.

Parameters calculated and units in bracket. N/mL: total number of milk fat globules per milliliter; dn: number mean diameter (μm); dv: volume mean diameter (μm); dvs: volume per surface mean diameter (μm); dvm: volume moment mean diameter (μm); Cs: distribution width (% of dvs); H: creaming parameter (μm^2).

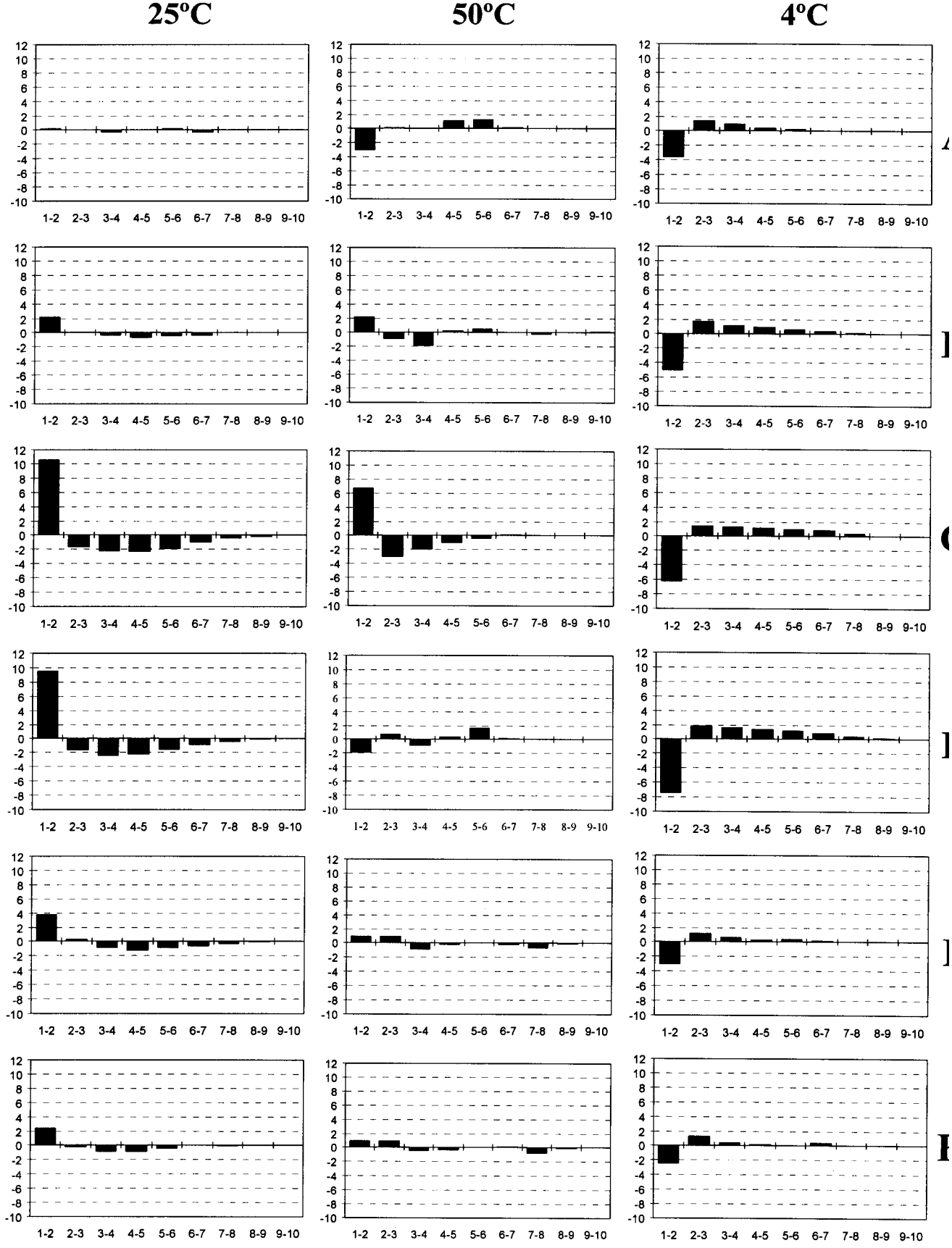

FIGURE 1. Effect of HHP treatments on size and distribution of ewe's milk fat globules. Results are expressed as percentage differences (%) between controls and treated samples. Vertical column graphics were treated at the same temperature, and horizontal column graphics at the same pressure. Y-axes are expressed as % differences (in number) between treated samples and control, and X-axes are expressed as diameter (μm) of milk fat globules. Files A: 0.1 MPa (control), B: 100 MPa, C: 200 MPa, D: 300 MPa, E: 400 MPa, F: 500 MPa.

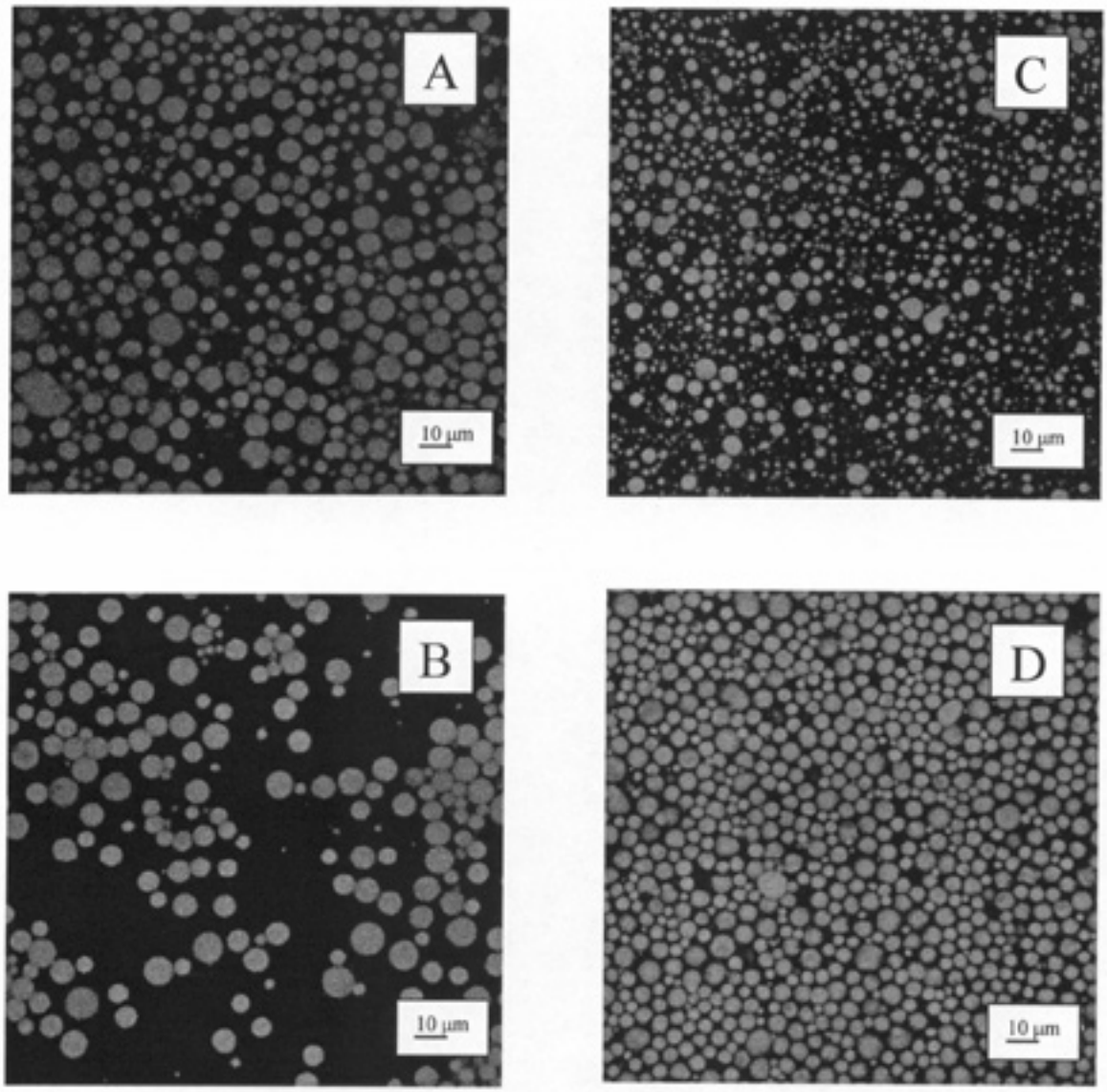

FIGURE 2. Confocal light scanning microscopy observations of ewe's milk after HHP treatments. Image A: control; Image B: 500 MPa at 50°C; Image C: 200 MPa at 25°C; and Image D: 200 MPa at 4°C. Bars 10 µm.