

# EFFECT OF HIGH HYDROSTATIC PRESSURE PROCESSING ON RHEOLOGICAL AND TEXTURAL PROPERTIES OF PROBIOTIC LOW-FAT YOGURT FERMENTED BY DIFFERENT STARTER CULTURES

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## ABSTRACT

*The effect of milk processing on rheological and textural properties of probiotic low-fat yogurt (fermented by two different starter cultures) was studied. Skim milk fortified with skim milk powder was subjected to three treatments: (1) thermal treatment at 85C for 30 min; (2) high hydrostatic pressure (HHP) at 676 MPa for 5 min; and (3) combined treatments of HHP (676 MPa for 5 min) and heat (85C for 30 min). The processed milk was fermented using two different starter cultures containing Streptococcus thermophilus, Lactobacillus delbrueckii ssp. bulgaricus, Lactobacillus acidophilus and Bifidobacterium longum at inoculation rates of 0.1 and 0.2%. Rheological parameters were determined and a texture profile analysis was carried out. Yogurts presented different rheological behaviors according to the treatment used, which could be attributed to structural phenomena. The combined HHP and heat treatment of milks resulted in yogurt gels with higher consistency index values than gels obtained from thermally treated milk. The type of starter culture and inoculation rate, providing different fermentation pathways, also affected the consistency index and textural properties significantly. The combined HHP and heat treatment of milks before fermentation, and an inoculation rate of 0.1% (for both cultures), led to desirable rheological and textural properties in yogurt, which presented a creamy and thick consistency that does not require the addition of stabilizers.*

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## INTRODUCTION

In recent years, low-calorie and low-fat foods have won popularity among consumers. Yogurt, a fermented dairy product, has gained special prominence and economic importance because of its high nutritional value and health benefits. The consumption of yogurt has steadily increased over the last 30 years in the U.S.A. (Economic Research Service 2002) and in other parts of the world.

Fermented dairy products have been consumed for nutritional reasons and maintenance of good health for a long time (Vinderola and Reinheimer 1999). The food industry has noticed this shift, and during the last few years, there has been a fast growth in the market of diet and functional foods, including fermented dairy products. The quality of fermented dairy products depends on the food's texture and body, because the amount of solids is very low. Therefore, the physical properties of cultured milk are the major criteria for quality assessment. For instance, the most important textural characteristics of yogurt are firmness and the ability to retain water (Hassan *et al.* 1996b). The physical properties of cultured milk are also affected by many other factors, including composition and heat treatment, mechanical handling of coagulum and the type of culture (Hassan *et al.* 1996a).

Probiotics are beneficial live microorganisms that, when given to human beings through food (functional foods), affect the host beneficially. Probiotics are beneficial because they produce enzymes that help the body digest food. They also produce B-complex vitamins and, in cases of diarrhea, help in the neutralization of pathogenic microorganisms responsible for infections. Probiotic yogurt occupies a very satisfactory position in the dairy products market, and there is a clear trend to increase its consumption in the next few years. Additional healthy aspects, like an additive-free product, will make this increase much more favorable. Therefore, the type of culture is one of the most critical factors influencing the textural and rheological properties of yogurt, making selection of the appropriate culture of great importance (Vlahopoulou and Bell 1993).

For example, the total solid content can affect the type of yogurt. During fermentation of milk into yogurt, the pH falls to around 4.4 and the destabilized micelles aggregate into a three-dimensional matrix in which whey is trapped (Rawson and Marshall 1997). The use of stabilizers to improve texture and reduce whey separation is common. Other strategies to increase the total solid content include the addition of milk solids and/or whey protein concentrate (Mistry and Hassan 1992).

High hydrostatic pressure (HHP) processing has been a promising non-thermal food processing method in many countries. The small-scale produc-

tion of pressurized foods has become a reality in Japan (fruit-based products and other foods), France (orange juice) and the U.S.A. (avocado spread). Large-volume pressure vessels (500 L) are currently available for such products from manufacturers. For example, high-pressure-treated milk has been successfully used to manufacture a low-fat set-type yogurt (12% total solids) with a creamy, thick consistency that does not require the addition of polysaccharides (Moorman *et al.* 1996).

Harte *et al.* (2003) reported that yogurt made from milk subjected to HHP (400–500 MPa) and thermal treatment (85C for 30 min) showed increased yield stress, elastic modulus and resistance to normal penetration, while having reduced syneresis, compared with yogurts made from thermally treated milk and from raw milk. Thus, the use of HHP offers microbiologically safe and additive-free low-fat yogurt with improved characteristics, such as reduced syneresis, better texture, increased shelf life and high nutritional and sensory qualities (Trujillo *et al.* 2002; Harte *et al.* 2003). For instance, it was reported that HHP improves acid coagulation of milk without detrimental effects on important quality characteristics such as taste, flavor, vitamins and nutrients (Trujillo *et al.* 2002).

Although a certain amount of attention has been directed toward the sensory properties of probiotic yogurt, most publications have focused on the health aspects. Little information is available concerning the growth of probiotic bacteria in HHP-treated milk. Moreover, there is limited published information concerning the technological production of fermented probiotic dairy products and the rheological and textural properties of these microorganisms in HHP-treated milk.

This study will allow researchers to improve the textural properties of traditional yogurt and to develop novel varieties with improved functional properties. The specific objectives of this study were to determine and compare the effects on the textural and rheological properties of superior quality stirred probiotic yogurt prepared with different probiotic cultures.

## MATERIALS AND METHODS

### Heat Treatment

Skim milk (0–0.2% fat and 9.17–9.20% total solids) was purchased from the Washington State University (WSU) Dairy Creamery and was fortified with skim milk powder to increase the total solids to 14%. The fortified milk was then subjected to thermal treatments at 85C for 30 min using a plate heater with magnetic stirrer. The milk was cooled to 42C in a water bath for the yogurt preparation.

## Pressure Treatment

Samples of fortified milk (700 mL) were placed in polyethylene plastic bags and heat-sealed. Pressure treatments were carried out using an isostatic pressure system (Engineered Pressure Systems, Inc., Haverhill, MA) with a chamber size of 0.10 m in diameter and 0.25 m in height. The medium for hydrostatic pressurization was 5% Mobil Hydrasol 78 water solution. The samples were subjected to HHP at 676 MPa for 5 min at room temperature, according to previous research of Harte *et al.* (2002). Targeted pressure was achieved in 4–5 min and depressurization took less than 1 min.

## Yogurt Preparation

Processed milk (thermal, HHP or submitted to both treatments) was inoculated (0.1 or 0.2% v/v) with two different freeze-dried probiotic yogurt starter cultures (YO MIX 236 or DPL ABY 611) supplied by Rhodia, Inc. (Madison, WI) and Danisco USA, Inc. (Milwaukee, WI), respectively. These starter cultures consisted of a mixture of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Lactobacillus acidophilus* and *Bifidobacterium longum*. The fermentation was carried out at 43C until the pH value reached  $4.6 \pm 0.1$ . The yogurt was cooled to 20C in an ice bath and then stirred with a mechanical mixer for 30 s using a standardized procedure in all experiments. The cooled yogurt was then poured into 100-mL cups and stored at 4C for 15–16 h. Stirred yogurt samples were withdrawn from storage for rheology and texture evaluation.

## Rheological and Textural Properties

The effect of combined HHP and thermal treatments was studied and compared with the other two methods individually, by determining the rheological properties (yield stress, consistency index and flow behavior index) and textural properties (texture profile analysis, TPA) in order to obtain a high-quality probiotic yogurt with less syneresis and longer shelf life. All determinations were carried out in triplicate.

Total solids content was measured by drying the sample in a vacuum oven at 70C for 24 h (Case *et al.* 1985). The pH was measured using a digital 420-A pH meter (Orion Research, Inc., Boston, MA).

Rheological properties were measured using a Physica rheometer (model 320, Paar Physica USA, Inc., Glen Allen, VA). The measurements were made at 10C using concentric cylinders (CC27). The temperature control was maintained by water circulation from an external water bath through the jacket surrounding the rotor and cup assembly. Shear rates ranging from 0.1 to 300/s (with logarithmic scale increased at every 10 s) under programmed upward

and downward curves were used, and corresponding shear stress data were obtained. The rheological parameters were obtained at shear rates ranging from 0.1 to 103/s using Origin Software 5.0 version (Northampton, MA) and adjusted by the Herschel–Bulkley model.

A texture analyzer with a 2-kg compression load cell (TA-XT2 Texture, Stable Micro Systems, Texture Technologies, Scarsdale, NY) was used to evaluate the texture profiles. The analysis was carried out through a double compression test using an aluminum cylinder (P/50, 50 mm in diameter). The cylinder penetrated 35% of strain on the surface of the coagulum, and the crosshead speed was 1 mm/s for 12 s. Three replicate samples (70 g of yogurt) were prepared at 5C for each type of yogurt. Szczesniak *et al.* (1963) showed that the textural attributes or parameters that resulted from the TPA force–time curve are well correlated with sensory evaluation.

Szczesniak *et al.* (1963) defined chewiness as “the energy required to masticate a solid food” and gumminess as “the energy to disintegrate a semi-solid food.” Typical parameters quantified were cohesiveness (the extent to which a material can be deformed before it ruptures), hardness (the force necessary to attain a given deformation), springiness or elasticity (the rate at which the deformed material returns to its undeformed state after removal of deforming force) and adhesiveness (the work necessary to overcome the attractive forces between the surface of the yogurt and the surface of other material with which it comes in contact) (Rawson and Marshall 1997).

### Statistical Analyses

All experiments (Table 1) were repeated in triplicate on individual yogurt samples. Statistical analyses were performed using a randomized block design, using SAS Statistical Software (SAS Institute, Inc., Cary, NC) by Tukey’s pairwise comparisons at the 99% confidence level.

## RESULTS AND DISCUSSION

The shear stress/rate relationship (upward and downward curves) of the yogurt determined using the Herschel–Bulkley model is shown in Tables 2 and 3. These products could be characterized as non-Newtonian fluids with thixotropic flow behavior resulting from the structural breakdown during the shearing cycle. This is observed by the difference between the upward and downward curves of the shear rate/stress relationship of the yogurts when applying the Herschel–Bulkley model. These results were consistent with those reported in the literature for yogurt.

The yogurts presented different rheological behaviors according to the treatment used ( $P < 0.01$ ), which can be attributed to structural phenomena.

TABLE 1.  
EXPERIMENTAL DESIGN OF PROBIOTIC LOW-FAT  
YOGURT PREPARATION

Run	Culture type	Inoculation (%)	Treatment
1	DPL ABY 611	0.1	Heat
2	DPL ABY 611	0.1	HHP
3	DPL ABY 611	0.1	HHP + heat
4	DPL ABY 611	0.2	Heat
5	DPL ABY 611	0.2	HHP
6	DPL ABY 611	0.2	HPP + heat
7	YO MIX 236	0.1	Heat
8	YO MIX 236	0.1	HHP
9	YO MIX 236	0.1	HPP + heat
10	YO MIX 236	0.2	Heat
11	YO MIX 236	0.2	HHP
12	YO MIX 236	0.2	HPP + heat

Heat: 85C for 30 min.

High hydrostatic pressure (HHP): 676 MPa for 5 min.

TABLE 2.  
FLOW PARAMETERS OF YOGURT PREPARED WITH CULTURE DPL ABY 611 USING  
0.1 AND 0.2% OF STARTER CULTURE, USING THE HERSCHEL-BULKLEY MODEL

	$\tau_0$ (Pa)	$K$ (Pa·s <sup><i>n</i></sup> )	<i>n</i>	$R^2$	$\tau_0$ (Pa)	$K$ (Pa·s <sup><i>n</i></sup> )	<i>n</i>	$R^2$
0.1%	Upward curves				Downward curves			
Heat	2.297	1.539	0.724	0.996	0.855	0.222	0.969	0.990
HHP	0.851	1.995	0.611	0.986	0.060	0.100	0.980	0.994
HHP + heat	3.428	4.569	0.507	0.980	1.581	0.271	0.949	0.986
0.2%	Upward curves				Downward curves			
Heat	2.073	2.072	0.624	0.9937	1.310	0.283	0.881	0.9859
HHP	0.646	0.371	0.886	0.9971	0.064	0.135	0.978	0.9912
HHP + heat	3.083	2.132	0.651	0.9861	0.862	0.206	0.953	0.9932

Shear rates ranged from 0.1 to 300/s and measurements were made at 10C.

$\tau_0$ , yield stress (Pa);  $K$ , consistency index (Pa·s<sup>*n*</sup>); *n*, flow behavior index (dimensionless);  $R^2$ , determination coefficient; HHP, high hydrostatic pressure.

The differences could also be explained by a different capacity of the protein to interact with casein micelles. Denatured whey proteins, obtained by heating process, are an important cross-linking agent. Samples prepared with milk treated by HHP combined with heat using 0.1% of DPL ABY 611 culture presented the higher consistency index; however, there is no significant difference between heat and HHP treatments alone. The type of culture and

TABLE 3.  
FLOW PARAMETERS OF YOGURT PREPARED WITH CULTURE YO MIX 236 USING  
0.1 AND 0.2% OF STARTER CULTURE, USING THE HERSCHEL–BULKLEY MODEL

	$\tau_0$ (Pa)	$K$ (Pa·s <sup><i>n</i></sup> )	<i>n</i>	$R^2$	$\tau_0$ (Pa)	$K$ (Pa·s <sup><i>n</i></sup> )	<i>n</i>	$R^2$
0.1%	Upward curves				Downward curves			
Heat	1.158	1.269	0.840	0.9946	0.115	0.019	1.412	0.9937
HHP	1.646	4.276	0.537	0.9814	0.617	0.145	0.944	0.9880
HHP + heat	3.355	4.320	0.564	0.9929	1.380	0.713	0.689	0.9728
0.2%	Upward curves				Downward curves			
Heat	3.227	3.106	0.651	0.9931	1.627	1.022	0.664	0.9719
HHP	1.743	0.368	0.984	0.9938	0.849	0.144	0.967	0.9899
HHP + heat	2.054	1.928	0.653	0.9934	1.152	0.349	0.788	0.9792

Shear rates ranged from 0.1 to 300/s and measurements were made at 10C.

$\tau_0$ , yield stress (Pa);  $K$ , consistency index (Pa·s<sup>*n*</sup>); *n*, flow behavior index (dimensionless);  $R^2$ , determination coefficient; HHP, high hydrostatic pressure.

inoculation rate, which provided different fermentation pathways, also affected the consistency index significantly ( $P < 0.01$ ).

Yield stress (upward curves) showed no significant difference ( $P < 0.01$ ) between culture types (DPL ABY 611 or YO MIX 236), although treatment and inoculation rate differed significantly ( $P < 0.01$ ) from the others. Yogurt prepared with milk treated by HHP combined with heat using 0.1% of DPL ABY 611 showed the highest yield stress.

For both cultures of yogurts prepared, the consistency index decreased when increasing the concentration of culture. These results agree with those obtained by Saxelin *et al.* (1999). They reported that probiotic strains combined with *S. thermophilus* and *L. bulgaricus* reduced viscosity compared with the yogurt culture alone.

During heat treatment of milk, the main change that occurs is denaturation and aggregation of whey proteins with caseins, through  $\kappa$ -casein binding, and fat globules (Corredig and Dalgleish 1999). Complexation of  $\beta$ -lactoglobulin with  $\kappa$ -casein gives the casein micelles a hairy or spiky appearance. During gelation, the casein micelles thus altered form to branched chains rather than clusters, the latter being common in curd made from unheated milk (Barrantes *et al.* 1996). Cross-linking or bridging of denaturated whey protein associated with the casein micelles results in an increase in numbers and strength of bonds between protein particles (Lukey *et al.* 1997).

HHP can alter both structures of casein and whey proteins. The denaturation of whey protein by HHP was reported by Gaucheron *et al.* (1997), Datta and Deeth (1999) and Trujillo *et al.* (2002). An increase in the viscosity of

$\beta$ -lactoglobulin stabilized emulsions following HHP, including the generation of gel-like characteristics, was reported by Dickinson and James (1998), while  $\alpha$ -lactoalbumin showed more resistance to pressure denaturation (Hinrichs and Kessler 1997). The application of HHP to skim milk at room temperatures leads to a decrease in the mean hydrodynamic diameter of casein particles, with a decrease in milk turbidity and lightness, and an increase in viscosity of the milk (Johnston *et al.* 1992). The presence of small particles would explain the decrease in apparent lightness (Gaucheron *et al.* 1997). Needs *et al.* (2000), in a microstructure study, also observed in pressure-treated milk held at 4°C that the micelles were fragmented, forming small irregularly shaped particles, which are often formed into clumps and chains. During yogurt preparation, the irregular micelle fragments in milk changes to round, separate and homogeneous compact micelles (Harte *et al.* 2003), but they also observed that HHP treatment alone (676 MPa for 5 min) is not suitable for promoting whey protein denaturation and further aggregation of  $\beta$ -lactoglobulin with casein in order to obtain a creamy, thick consistency with no addition of stabilizers.

The combined HHP and heat treatment of milks and fermentation with 0.1 and 0.2% of DPL ABY 611 and 0.1% of YO MIX 236 resulted in yogurt gels with higher consistency index than gels obtained from thermally treated milk. In another study, yogurt gels prepared from HHP at 676 MPa for 30 min showed equivalent rheological curves compared with yogurt gels obtained from heated milk. Yogurt gels prepared from HHP for shorter times (676 MPa for 5 min) exhibited weak structured gels (Harte *et al.* 2002). In this study, the results showed the synergistic effect of combined treatment. Furthermore, some differences could be related to the fermentation process. The gel firmness of the yogurt depended on the starter culture (DPL ABY 611 or YO MIX 236), which modified the gel properties. The viscous characteristics of the acid gel are increased when texturing starters are used because of the interaction of exopolysaccharides (EPS) with the casein network (Sodini *et al.* 2004). However, Hassan *et al.* (1996b), Hess *et al.* (1997) and Rohm and Kovac (1994) observed a decrease in firmness when using a texturing starter. Furthermore, Beal *et al.* (1999) found that strain association, temperature and final pH had significant effects on yogurt viscosity. The texturing character of *S. thermophilus*, for instance, increased with decreasing temperature and final pH. Dannenberg and Kessler (1988) also found that yield stress of skimmed milk yogurt was related to the extent of whey protein denaturation; the higher the level of denaturation, the higher the number of labile bounds in the gel structure.

Tables 4 and 5 show the results obtained using the TA-XT2 texture analyzer in measuring the textures of different yogurt samples prepared under the same protocol. The texture of stirred yogurt is the result of both acid aggregation of casein micelles and production of EPS by ropy strains during incubation (Cerning 1995).

TABLE 4.  
PROBIOTIC YOGURT DPL ABY 611 TEXTURE PROFILE EVALUATED USING THE TA-XT2  
TEXTURE ANALYZER, TOTAL SOLIDS AND pH VALUE

	0.1%			0.2%		
	Heat	HHP	HHP + heat	Heat	HHP	HHP + heat
Hardness	28.52	24.15	46.14	28.14	23.68	32.15
Fracturability (g)	5.42	5.16	5.49	4.96	5.28	5.24
Adhesiveness (g-s)	-41.24	-13.76	-129.37	-25.32	-7.93	-51.10
Springiness	0.96	0.98	0.90	0.98	3.08	0.94
Cohesiveness	0.76	0.81	0.66	0.76	0.93	0.72
Gumminess	21.59	19.49	30.67	21.31	21.94	23.12
Resilience	0.27	0.34	0.15	0.31	0.34	0.25
Total solids	14.12	14.11	14.44	13.11	14.90	14.26
pH	4.68	4.59	4.48	4.70	4.65	4.60

HHP, high hydrostatic pressure.

TABLE 5.  
PROBIOTIC YOGURT YO MIX 236 TEXTURE PROFILE EVALUATED USING THE TA-XT2  
TEXTURE ANALYZER, TOTAL SOLIDS AND pH VALUE

	0.1%			0.2%		
	Heat	HHP	HHP + heat	Heat	HHP	HHP + heat
Hardness	28.11	35.26	44.28	40.53	22.66	46.84
Fracturability (g)	5.42	6.16	5.86	5.33	5.82	6.69
Adhesiveness (g-s)	-17.54	-90.72	-113.06	-74.43	-24.92	-112.63
Springiness	1.00	0.92	0.92	0.94	4.18	0.94
Cohesiveness	0.80	0.70	0.67	0.71	1.08	0.68
Gumminess	22.56	24.81	29.56	28.96	24.57	31.80
Resilience	0.34	0.20	0.15	0.20	0.51	0.17
Total solids	15.10	13.88	14.26	14.16	14.34	14.95
pH	4.54	4.56	4.50	4.60	4.56	4.57

HHP, high hydrostatic pressure.

The texture profile was different according to the treatment, culture type and inoculation rate used. This observation was confirmed by a statistical analysis, comprising analysis of variance and a multiple comparison of means (data not shown). Yogurts prepared with milk treated by HHP combined with heat presented more hardness ( $P < 0.01$ ). Combined effects of HHP and heat resulted in a high level of protein denaturation. Dannenberg and Kessler (1988) reported that yogurt gel firmness was strongly related to the level of  $\beta$ -lactoglobulin denaturation for up to 60% denaturation. Between 60 and 90%  $\beta$ -lactoglobulin denaturation, the effect of heating intensity became less

evident, and therefore significant differences were observed above 90%. Additionally, severe heating intensities involving more than 90% denaturation of  $\beta$ -lactoglobulin led to a slight reduction in the firmness of yogurt gel.

It was also observed that all results of consistency index ( $K$ ) showed high correlation with hardness ( $r^2 > 0.83\%$  for yogurts fermented by 0.1% starter culture and  $r^2 > 0.76\%$  for yogurts fermented by 0.2% starter culture). Yogurt made from combined treatments using 0.1% starter culture showed higher yield stress and consistency index values, which can be clearly correlated with its high hardness and gumminess.

The milk treatment and starter culture also had a significant effect on the gumminess of yogurt ( $P < 0.01$ ); however, the inoculation rate showed no major differences. Yogurt prepared with combined HHP and heat fermented by YO MIX 236 culture showed the higher values for gumminess. However, the gumminess was correlated only with yield stress of yogurts fermented by 0.1% starter culture,  $r^2 > 0.92\%$ , independently of milk treatment.

After fermentation, the pH value of yogurts varied from 4.48 to 4.70. Oliveira *et al.* (2002) reported similar values during the manufacture of lactic beverage containing probiotic starter cultures. However, the pH value of fermented milk products tended to decrease during storage because of postacidification, a result of starter culture activity. In the case of yogurt, if the pH reaches below 4.0, syneresis becomes evident because of curd contraction owing to the reduction of hydration of water (Brandão 1995).

Furthermore, the pH value has an influence on the viability of probiotic cultures in fermented milk. The survival of *L. acidophilus* and *Bifidobacterium bifidum* in Argentinean yogurt was studied during refrigerated storage by Vinderola *et al.* (2000). The authors found that a decrease in pH reduced the viable cell count of these microorganisms. Thamer and Penna (2004) reported similar results. The highest probiotic microorganism populations were observed in dairy beverages with lower acidity.

Although *L. acidophilus* tolerates acidity, a rapid decrease in their number has been observed under acidic conditions (Shah and Jelen 1990; Lankaputhra and Shah 1995). *Bifidobacteria* are not as acid-tolerant as *L. acidophilus*. The growth of the latter microorganism ceases below 4.0, while the growth of *Bifidobacteria* ssp. is retarded below pH 5.0 (Shah 1997). Thus, in order to obtain a higher population of *Bifidobacteria*, Almeida *et al.* (2001) standardized the pH value of probiotic fermented dairy beverages above 5.0.

The aggregation strength in yogurt is also related to the yogurt's total solids and pH value (Tables 4 and 5). The increase in the hardness of yogurt observed at low pH could be explained by the effect of pH on the electric charge of casein, as suggested by Harwalkar and Kalab (1986). These researchers reported an increase of 20% in gel firmness when the final pH was decreased from 4.50 to 3.85. They assumed that it was caused by the higher

intramolecular repulsion as a result of the increase in the positive charge of casein at lower pH, below the isoelectric point of caseins. This would tend to swell the casein particles, resulting in an increased rigidity of the milk gel. However, they observed larger pores in the protein network at low pH. It reduced intermolecular interactions, which resulted in the formation of an open structure more susceptible to forming grains and a lumpy texture when gel is stirred. Such a porous structure also makes the whey separation easier (Harwalkar and Kalab 1986).

The effects of treatments on milk and yogurt are also reflected in changes in the color values. HHP-treated milk had lower  $L^*$ ,  $a^*$  and  $b^*$  values than either heat and combined heat and HHP-treated milk (data not shown). Yogurt and heat-treated milk had higher values of  $L^*$ ,  $a^*$  and  $b^*$  because of changes in the light-scattering properties of milk. The disruption of micelles under high pressure caused a significant change in the appearance of the milk, which was quantified by measuring the color. Heat treatment also affected these characteristics. The decrease in  $L^*$  (lightness) and increase of greenness ( $-a^*$ ) and yellowness ( $+b^*$ ) were also observed by Gervilla *et al.* (2001) when ewe's milk was treated by HHP. Harte *et al.* (2003) observed high  $L^*$  values (increased whiteness) in milk subjected to HHP followed by thermal treatment and related to reaggregation of disrupted micelles. The HHP treatment reduced the lightness of raw or thermally treated milks and a small decrease in color was observed when milk was subjected to HHP. Complementary studies regarding the effect of milk treatment on acidification, physicochemical characteristics, probiotic cell counts and microstructure of probiotic low fat yogurt were conducted by Penna *et al.* (2006a,b).

An interesting relationship between acidification and texture was observed for culture DPLABY 611; the lower the amount of starter culture, the higher the hardness and adhesiveness. Starter culture YO MIX 236 showed the opposite behavior. The duration of the fermentation had a positive effect on texture development irrespective of final pH; the slower the acidification, the longer the fermentation time and the higher the viscosity. This emphasizes that the textural properties of yogurt may be governed by the duration of fermentation. Results of Beal *et al.* (1999) and Garcia-Garibay and Marshall (1991) support this proposition. It could be explained by the firmer structure of the gel resulting from acid coagulation at low pH. Therefore, the different fermentation times related to the various experimental conditions may affect product viscosity.

## CONCLUSIONS

The milk treatment before yogurt fermentation significantly affected the rheological and textural properties of probiotic yogurts. Starter culture and the

inoculation rate that governs the fermentation also modified the gel properties. Combined HHP (676 MPa for 5 min) and heat (85°C for 30 min) treatment of milk before fermentation and a 0.1% inoculation rate (for both cultures) led to attractive rheological and textural properties. The combined HHP and heat-treated yogurt presented a creamy and thick consistency that does not require the addition of stabilizers.

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