Changes in the viscosity, textural properties, and water status in yogurt gel upon supplementation with green and Pu-erh teas

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ABSTRACT

The present research was established to study the effect of green tea and Pu-erh tea (PT) additives on the mechanical and hydration properties of yogurt gels using a combination of nuclear magnetic resonance, rheological, and textural studies. Tea infusions (0–15 mL/100 mL) were added to batch milk before fermentation with yogurt culture. Obtained dairy products were analyzed for the water mobility and organization, viscosity, and texture profile. Results of the rheological and nuclear magnetic resonance studies suggested that stabilization of the yogurt gel structure was achieved upon supplementation with tea infusions. Generally, green tea fortification produced yogurts with more consolidated gel structure, tighter interacting water, and less susceptibility to shearing and temperature changes than PT treatments. In contrast, PT yogurts were more viscous and characterized by 8 to 17% lower hardness values. This knowledge can be useful for developing novel dairy products with desired structure and consistency.

Key words: dairy product, tea infusion, viscosity, textural analysis, nuclear magnetic resonance relaxation

INTRODUCTION

Yogurt is the most popular cultured milk product that originates from countries around the Balkans and Eastern Mediterranean Sea. Physically, it is an acid milk gel formed during fermentation performed by starter lactic acid bacteria such as *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* (Jaros and Rohm, 2003). Different types of yogurt are produced in regard to the structure and composition, but fruit or flavored varieties are the most popular among consum-

ers. Addition of fruit preparations and other bioactive supplements (e.g., tea or coffee extracts) may enhance the nutritional and health-promoting value of fermented milk. However, textural properties, lack of whey separation, aroma, and acidity are key characteristics for consumer quality acceptance (Tamime and Robinson, 1999; O'Rell and Chandan, 2006).

Yogurt constitutes a mixture of biopolymers (proteins, polysaccharides, and fats). The microstructure of milk protein gels and their rheological properties affect texture, sensory properties, and storage stability (Benezech and Maingonnat, 1994; Cayot et al., 2008). The knowledge of the rheological properties in food systems is especially important from a technological point of view (Jaros and Rohm, 2003; Purwandari et al., 2007). Because of their complex microstructure, yogurt gels are susceptible to irreversible damages as a result of the action of time, temperature, and shear forces. Yogurt is a product that may be described as a non-Newtonian fluid with shear-thinning characteristics, exhibiting time-dependent changes of viscosity (Afonso and Maia, 1999; Loveday et al., 2013). It is characterized by unstable structure prone to shear damage, with only slight recovery on postshear resting or under the action of low shear forces (De Lorenzi et al., 1995). Thus far, the rheological properties of yogurts have been subjected to many studies. Their characteristics may differ depending on the milk base formulation, studied subject, type of conducted experiment, and data analysis. Some of the reports have focused on the effect of the microstructure on the rheological properties (Kalab and Emmons 1974; Tegatz and Morris, 1990; Lee and Lucey, 2010; Krzeminski et al., 2014), whereas other authors have studied the effects of the technological processes (Schmidt et al., 1980; Labropoulos et al., 1984). Milk base composition, types of bacterial cultures (with special emphasis on the exopolysaccharides-producing strains; Sodini et al., 2004; Folkenberg et al., 2006), or additives were considered as factors influencing texture, sensory features, or composition of yogurts (Ramas-

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wamy and Basak, 1992; Najgebauer-Lejko et al., 2014). The obtained results have been subjected to analyses using the logarithmic time Weltman model as well as the Herschel-Bulkley model (Ramaswamy and Basak, 1992; Mullineux and Simmons, 2008; Najgebauer-Lejko et al., 2014). The flow curves (De Lorenzi et al., 1995) and oscillation tests (Najgebauer-Lejko et al., 2014) have also been reported. Correlations between textural sensory attributes and corresponding instrumental parameters were also studied (Skriver et al., 1999; Salvador and Fiszman, 2004).

More data about structural and compositional features of yogurts can be obtained from nuclear magnetic resonance (NMR) analysis in time domain. The NMR spin-spin (T_2) and spin-lattice relaxation (T_1) times provide information about the mobility of water, other food ingredients, and their interactions. They can be indicators of structural and compositional changes occurring in food, which has been shown in some applications on dairy products (Karoui and De Baeremaeker, 2007). In yogurt production, the strong effect of formation of casein protein gel on water mobility was revealed by NMR relaxometry (Mok et al., 2008). The recipe and processing of yogurts influenced water-holding capacity as determined by distribution of water phases observed from the T_2 measurement (Hinrichs et al., 2003). It was also shown that addition of nonfat milk solids to yogurts significantly modified T_1 and T_2 relaxation times (Yu et al., 2016). To date, there are no reports using NMR relaxation studies on the effects of nonmilk additives on the structural properties of yogurt, while several studies have demonstrated that milk proteins have the ability to interact with phenolic compounds (Yüksel et al., 2010; Ozdal et al., 2013). Therefore, it is expected that such modifications in the protein matrix should be reflected in altered rheological, textural, and water-holding properties of milk gels, including yogurt, which are very sensitive to any rearrangements in the protein network. There are already reports about the characteristics of yogurt with the addition of tea extracts (Jaziri et al., 2009; Najgebauer-Lejko et al., 2011; Amirdivani and Baba, 2013; Marhamatizadeh et al., 2013; Najgebauer-Lejko, 2014), but they only provide limited information on the effect of tea supplementation on the rheological and textural parameters of yogurt, as well as on the interaction of water with proteins. In the previous publication plain, green tea (GT) and Pu-erh tea (PT) yogurts were subjected to the analyses of acidity, viscoelastic properties, back extrusion parameters, susceptibility to syneresis, and instrumental color (Najgebauer-Lejko et al., 2014). Therefore, the aim of the present research was to broaden the knowledge of the effect of GT and PT additives on water mobility, textural properties,

and rheological characteristics under the shear conditions of yogurts. We hypothesized that the combination of the NMR relaxation, rheological, and instrumental texture measurements would be an effective approach to determine changes in the structure of the yogurt gel network caused by the addition of 2 types of tea infusions.

MATERIALS AND METHODS

Materials

Chinese leaf GT (Yunnan Tea Garden Group Shareholding Co., Ltd., Kunming, China), PT (individually packed tea blocks, 3.0 ± 0.2 g; Yunnan Haichao Group, Haichao Tea Blocks Co., Ltd., China), and pasteurized cow milk (2% fat, Mlekpól, Grajewo, Poland) were purchased from the local store. Instant nonfat milk powder was obtained from Dairy Company in Gostyn (Poland) and direct-vat-set lyophilized yogurt culture (YC-180; composed of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*) from Chr. Hansen (Hoersholm, Denmark).

Preparation of Tea Water Extracts

Freshly boiled water (800 mL) was added to weighed tea leaves (40 g), mixed thoroughly, and held for 15 min in a beaker covered with a watch glass. Then the tea leaves were removed, and infusions cooled to ambient temperature. Freshly prepared GT or PT water extracts were added to milk just after inoculation with lactic acid bacteria. Boiled and cooled water was added to yogurt milk for the treatments with 0 to 10% (vol/vol) concentration of tea extracts to obtain yogurts with the same content of milk solids.

Preparation of Yogurts with Tea

Nonfat milk powder was added to liquid milk (6 g/100 mL) preheated to 50°C, mixed for 10 min, and repasteurized at 85°C for 10 min. After cooling to 44°C, the milk was inoculated with yogurt culture (0.05 g/L) and divided into 4 portions. Tea water extracts (GT or PT) and water were added to inoculated milk to produce the following treatments: natural yogurt without supplementation (no tea extract; NY), yogurts with 5 mL/100 mL of GT (GT_{5%}) or PT (PT_{5%}), yogurts with 10 mL/100 mL of GT (GT_{10%}) or PT (PT_{10%}), yogurts with 15 mL/100 mL of GT (GT_{15%}) or PT (PT_{15%}). Prepared yogurt milk was poured into 200-mL sterile glass jars. The incubation was carried out at 43°C until the pH of ~4.7 was achieved. Yogurts were cooled and stored at 4°C before analyses. The final pH values of yogurts at the time of the analyses were in the

range of 4.30 to 4.35 without any significant differences between treatments (Najgebauer-Lejko et al., 2014).

The Content of Polyphenols in Tea Infusions

The contents of catechins (flavan-3-ols) in tea infusion were analyzed by means of HPLC with a reversed-phase column (Thermo Scientific, Sunnyvale, CA; 25 mm × 0.4 cm × 5 μm) and L-7420 LaChrom UV/Vis detector (Merck-Hitachi, Tokyo, Japan), and tea pigments (i.e., theaflavin, thearubigin, and theabrownin) were determined by the spectrophotometric method using an UV/Vis Helios Gamma spectrophotometer (Thermo Electron Corp., Cambridge, UK) for the absorbance measurement. Procedures and results of polyphenolic analyses were discussed widely in the previous work (Najgebauer-Lejko et al., 2014). Polyphenolic profiles of GT and PT infusions are shown in Supplemental Figure S1 (<https://doi.org/10.3168/jds.2020-19032>).

Rheological Studies

Measurements were made using a RS6000 rheometer (Haake, Karlsruhe, Germany). A cone-plate geometry was used. Cone parameters were as follows: diameter 35 mm, angle 2°. The measuring gap size was equal to 0.105 mm.

Flow Curves. The rheological measurements included a qualitative hysteresis loop test, which demonstrated that the system exhibited thixotropy when sheared. Flow curves were obtained at 8°C and 25°C, respectively, in the range of shear rate of 1 to 300 s⁻¹. The linear increase (up flow curve) and decrease (down flow curve) of shear rate was carried out for over 5 min in the same shear-rate range.

Estimation of Discrete Distributions of Time Constant $\eta_p(t_p)$. The method of reduced variables was used in interpretation of experimental data. Values of shift coefficients a_C and a_T were determined based on an algorithm presented in Honerkamp and Weese (1993). Obtained results of rheological tests were used to calculate the parameters of rheological equations of state for shear-thinning systems (De Kee et al., 1980):

$$\eta(\dot{\gamma}) = \tau_0 \times \dot{\gamma}^{-1} + \sum_{p=1}^{\infty} \eta_p \times \exp(-t_p \times \dot{\gamma}), \quad [1]$$

where τ_0 (Pa) and $\dot{\gamma}^{-1}$ are yield stress and shear rate, respectively. In both cases, t_p (s) and η_p (Pa × s) values can be interpreted as time constants and their intensity. Finally, as a result of estimation process, the discrete distributions $\eta_p(t_p)$ of time constants t_p could be obtained (Ptaszek, 2012). Estimation of the parameters of

the rheological equations of the state was determined by means of the Tikhonov regularization method (Weese, 1992).

For comparison, the parameters of the most common power-law equation Herschel-Bulkley model and Cross model (Ahmed et al., 2016) were estimated. The Herschel-Bulkley model is as follows:

$$\eta(\dot{\gamma}_j) = \tau_0 \times \dot{\gamma}_j^{-1} + k \times \dot{\gamma}_j^{n-1}. \quad [2]$$

The Cross model is as follows:

$$\eta = \eta_{\infty} + \frac{\eta_0 - \eta_{\infty}}{1 + (\tau \times \dot{\gamma})^m}. \quad [3]$$

The following objective function was minimized using the Marquardt-Levenberg method (Press et al., 2007):

$$\chi_{M-L}^2 = \sum_{j=1}^N \left(\eta_j^{\delta} - \hat{\eta}_j \right)^2 \rightarrow \min_{\tau_0, k, n \geq 0}. \quad [4]$$

The goodness of fit for Eq. 2 was assessed by computing the values of the following function:

$$\chi^2 = \sum_{j=1}^N \left(\frac{\eta_j^{\delta} - \hat{\eta}_j}{\eta_j^{\delta}} \right)^2. \quad [5]$$

The value of dissipated energy ΔE was calculated numerically with the help of the trapezoidal method (Press et al., 2007).

Textural Studies

The penetrometric texture profile analysis (TPA) test was carried out using TA-XTPlus texture analyzer (Stable Micro Systems–Haslemere, Surrey, UK) with a cylindrical probe of 20 mm in diameter and 40 mm in height, which penetrated the undisturbed samples of yogurt. Two cycles were applied to a depth of 25 mm at the rate of 1 mm s⁻¹. As a result, a plot of force versus time was obtained for each sample using the Texture Expert for Windows v. 1.05 software (Stable Micro Systems, Haslemere, UK). The following factors were determined using an algorithm fracture TPA: hardness (N), adhesiveness ($|N \times m| \times 10^{-3}$), cohesiveness (dimensionless), and gumminess (N).

Hardness was defined by the force peak of the first compression cycle (force needed to attain a given deformation). Adhesiveness referred to the negative area under the curve between compression cycles (work needed

to overcome attractive force between yogurt and the surface of the probe). Cohesiveness was computed as the ratio of the positive force area under the curve during the second cycle to the positive area during the first cycle (the strength of the yogurt's internal bonds). Gumminess (force needed to disintegrate a semisolid yogurt sample to a state ready to swallow) was achieved by multiplication of hardness and cohesiveness (Tunick, 2000).

NMR Relaxation Studies

The spin-spin and spin-lattice relaxation times (T_2 and T_1) were acquired using a Bruker Minispec NMR spectrometer (Bruker BioSpin, Poznań, Poland) operating at 60 MHz for ^1H at stabilized temperatures: 8°C, 10°C, 15°C, 20°C and 25°C. The Carr-Purcell-Meiboom-Gill pulse sequence $[90^\circ - (\tau - 180^\circ - \tau - \text{echo})_n]$ was used for T_2 measurements. The value of τ was 0.1 ms, and the number of echoes (n) was 3,600. Data were averaged over 16 scans with a recycle delay time of 6 s. The echo envelope was fitted by a single exponential function, and also deconvoluted as a continuous distribution of transversal relaxation times. For the T_1 measurement, an inversion recovery pulse sequence was used with an interpulse spacing that ranged from 50 ms to 10 s. Carr-Purcell-Meiboom-Gill decays and T_1 curves were analyzed with application of OriginPro 9.6 software (OriginLab Corporation, Northampton, MA).

Values of T_1 for yogurts supplemented with both teas were multiplied by scaling factor b_C :

$$b_C = \frac{T_{1i}^{5\%}}{T_{1i}^j}, \quad [6]$$

where i means GT- or PT-supplemented yogurts, j stands for the level of supplementation (10 mL/100 mL and 15 mL/100 mL).

Statistical Analysis

All analyses were run on the second day after production in 3 independent tests and in duplicates. Results are expressed as means $\pm$ standard deviations. For textural studies, 1-way ANOVA was performed at the significance level of $P < 0.05$, and the significance of differences between the means was estimated according to the Duncan test using Statistica 12.0 software (StatSoft, Inc., Tulsa, OK).

RESULTS AND DISCUSSION

Flow Properties

The rheological characteristic of NY is presented in Figure 1 in the form of apparent viscosity changes. The values of apparent viscosity have been calculated based on classical flow curves carried out in the condition of increasing shear rate (up flow curve) and decreasing shear rate (down flow curve) and plotted together to create a hysteresis loop. The values of apparent viscosity calculated for increasing shear rate have been shown as experimental points with lines obtained according to the De Kee et al. (1980) model described by Eq. 1.

The changes of apparent viscosity (up flow curve) exhibited shear-thinning properties and a complex dependence on shear rate. The shape of $\eta(\dot{\gamma})$ dependence presented in log-log scale was not linear as expected for typical shear-thinning systems. Moreover, the rheological nature of NY was temperature-dependent. The apparent viscosity measured in the middle range of shear rates increased as the temperature rose from 8°C to 10°C, whereas a further increase of the temperature to 15°C or 20°C did not lead to any significant viscosity changes. This unexpected behavior is reflected in Table 1, where we have presented the goodness of fitting to experimental data for popular rheological models. As shown by the values of χ^2 , the H-B model didn't meet the experimental data because the complexity of rheological behavior cannot be described by a simple model. Only the values of consistency coefficient (k) indicated untypical effect of temperature on apparent viscosity:

Figure 1. Apparent viscosity (η) dependence on shear rate ($\dot{\gamma}$) for plain natural yogurt and discrete time distributions (inside) calculated from Eq. 1 for selected temperatures. The apparent viscosity values have been shown in log-log scale. The values of viscosity calculated from flow curve for increasing shear rate (up flow curve) were plotted with lines demonstrating the De Kee et al. (1980) model (Eq. 1). Experimental points located below represented apparent viscosity based on down flow curve—flow curve obtained by decreasing shear rate.

Table 1. Rheological parameters of yogurts (sample volume of 0.5 mL)¹

Yogurt type ²	Herschel-Bulkley				Cross, $\eta_{\infty} = 0.1\text{Pas}$				Scaling coefficients $a_C \times a_T$ values ³		
	τ_0 (Pa)	k (Pa·s ⁿ)	n	χ^2	η_0 (Pas)	τ (s)	m	ΔE (mJ)	Tea concentration in yogurt (mL/100 mL)	8°C	25°C
NY8	75	2.25	0.6	114	500	1.5	1.3	3.24	—	—	—
NY10	70	3.00		16	200	0.5	1.3	4.36			
NY15				17				3.48			
NY20				12				2.19			
NY25	70	2.00		10	40	0.4	1.0	1.32			
GTY	120	3.0	0.2	50	1,000	2.5	1.3	3.17	5	1	0.50
									10	0.90	1.20
									15	0.80	1.30
PTY	70	3.4	0.6	80	100	5.0	1.7	2.50	5	1	1.35
									10	0.99	1.80
									15	1.04	2.25

¹ τ_0 (Pa) = yield stress; k (Pa·sⁿ) = consistency coefficient; n = rheological index; χ^2 = goodness of fit for Eq. [2]; η_0 (Pas) = initial viscosity of Cross model; τ (s) = time constant from Cross model; m = exponent from Cross model; ΔE = dissipated energy; a_C and a_T = scaling factors.

²NY8–25 = natural yogurt (without tea infusion) in different temperatures; GTY = yogurt with green tea infusion; PTY = yogurt with Pu-erh tea infusion. GTY and PTY estimation is based on the temperature and concentration scaling flow curve.

³Reference temperature 8°C and tea concentration 5 mL/100 mL.

at 8°C, k took the value of $2.25 \text{ Pas}^{0.6}$, whereas k took a higher value of $3.00 \text{ Pas}^{0.6}$ at 10°C, 15°C, and 20°C. The results were also fitted to a Cross model (Table 1). The parameter τ , an indicator of relaxation phenomenon, decreased with rising temperature as well as yield stress. Due to the complex nature of temperature effect on rheological behavior, it was impossible to scale apparent viscosity experimental points with shift coefficient a_T . The main reason was an extremely different course of $\eta(\gamma)$ curves obtained at 10, 15, and 20°C, when compared with results at 8 and 25°C. The values of apparent viscosity measured at these 3 temperatures were similar within the whole range of shear rates.

The selected apparent viscosity curves (up flow curves at 8°C and 25°C) were fitted by the De Kee et al. (1980) model (Eq. 1). As a solution of regularization process, the discrete distributions of the time constants $\eta_p(t_p)$ were determined and shown in Figure 1. In the case of rheological data obtained at the temperature of 8°C, the discrete distribution consisted of 6 elements with prevailing time constant in the range of about 1 s. Increasing the temperature to 25°C resulted in the more complex distribution of the time constants, which included main times constant of 0.1 s. Temperature increase caused certain changes in the structure of the distribution of the time constants, whereas the range of the time constants t_p was not subjected to any variations.

The course of η , calculated on the base of the up flow curve, differed from the down flow curve, indicating the hysteresis phenomenon, which is characteristic for rheounstable systems. The quantity of energy that dissipated during shearing (Table 1) decreased with

a temperature increase above 10°C. This loss of dissipated energy ΔE did not result from the lowering of the hysteresis phenomenon (closing of the course of flow up and down curves), but from the gradual decrease of the apparent viscosity affected by the temperature increase.

The addition of GT water extract to yogurt base had a great effect on the yogurt rheological features (Figure 2a). Even though the apparent viscosity values were subjected to changes in the range similar to that observed for the plain yogurt and the course of the flow curves was characteristic for shear thinning, the temperature and concentration scaling of the rheological properties was possible (Table 1). This means that the friction mechanism during shearing was not affected by the tea extract concentration in yogurts. Moreover, this indicated that the temperature increase did not change the yogurt structure. The addition of GT extract had, in this case, a stabilizing effect. However, the up curve obtained for the yogurt with 5% GT supplementation at 25°C, which despite the scaling did not coincide with the master curve determined for other data, was an exception (square symbol on the figure). The reflection of the behavior of these products could be found in the distributions of the time constants determined from Eq. (1). Even though the flow curves formed the hysteresis loops, the $\eta_p(t_p)$ distributions were of similar shape and lay in the 0.1 to 10 s range of time constants. They differed regarding intensity, which resulted from the differences in the values of apparent viscosity of the up and down curves. Despite different experimental conditions (i.e., flow range in this work vs. small deformations in the previous study; Najgebauer-Lejko et

al., 2014) the obtained yogurts supplemented with GT extracts maintained scaling ability of the rheological properties in relation to the concentration.

The addition of the PT extract also affected the rheological properties of yogurts (Figure 2b). In particular, complete temperature and concentration scaling ability of the apparent viscosity could be observed. This effect was probably caused by the presence of polyphenolic compounds, which are able to interact with milk proteins (Ozdal et al., 2013). On the other hand, the rheological studies performed within the linear viscoelastic region revealed that PTY exhibited viscous fluid-like properties (Najgebauer-Lejko et al., 2014). The course of the scaled up flow curves visibly diverged from the power model (Table 1). The distributions of the time constants for the cumulative up and down curves were different from one another. The first one included a bimodal peak with a maximum for 0.1 s and 2 s, whereas for the down curve had 1 peak with a maximum at 1 s and the second lower peak at 0.1 s were observed.

The rheological behavior depicted as the function of apparent viscosity against shear rate revealed that the viscosity values of all yogurts decreased with increasing shear rates in the same range (i.e., from 100 to 0.1 Pa s). The analysis of the rheological properties defined by the comparison of the values obtained from the popular rheological models (Table 1) indicated that there were some differences in the behavior of the analyzed yogurts. Estimated yield stress values (Table 1) obtained for the NY and PTY were equal to approximately 70 Pa, whereas considerably higher values (120 Pa) of this parameter were determined for GTY. Yield stress is defined as the value of shear stress above which the material starts to flow. Thus, it is a measure of the interactions between the components in the product. The obtained results for this parameter indicated that GTY were less susceptible to shearing than other treatments. This suggests that a stronger network was formed upon the modification with GT polyphenolic compounds. The results obtained from the rheological studies performed in the flow range were consistent with the previous observations made within the linear range, which indicated the lack of scaling of the G' (loss modulus) curves (Najgebauer-Lejko et al., 2014).

The consistency index obtained from high-deformation rheological studies is another important factor from a technological point of view (Eq. 2). The values of this parameter for the NY fluctuated in the range of 2.0 to 3.0 $\text{Pa} \times \text{s}^n$, whereas for the GTY they took the average value of 3.0 and for PTY 3.4 (Table 1). This suggests a more viscous character of tea yogurts, especially those supplemented with PT. Yogurts with GT extract exhibited more shear-thinning properties, as reflected in the lower flow index (n , Table 1) when

compared with the NY and PTY. The obtained results suggest that GTY were less susceptible to deformation during shearing (at lower shear stress values applied).

Textural Properties

Results of the texture studies are given in Table 2. Texture profile analysis revealed that textural properties of the obtained yogurts depended on the type of tea used as a supplement, and in some cases on its concentration. Fermented milks with addition of GT water extract were characterized by the higher hardness in TPA than those supplemented with PT, but did not differ significantly from the NY. Avci et al. (2010) observed increased hardness values of yogurts enriched with GT extracts and explained this phenomenon with the milk protein cross linking with tea flavanols. Pu-erh tea fortification at the concentration of 15% resulted in yogurts with considerably lower adhesiveness and higher cohesiveness value than the nonsupplemented yogurt. The increase in cohesiveness, which is related to the forces involved in the internal bonds of the product, may indicate that greater number of linkages in the gel network broken during stress application were reformed after stress was ceased (Sandoval-Castilla, Lobato-Callero, Aguirre-Mandujano and Vernon-Carter, 2004). On the other hand, 5% addition of PT contributed to the decrease in gumminess when compared with non-supplemented and GT-supplemented yogurts. Different textural properties of yogurts with GT and PT addition performed in TPA may result from the differences in the compositions of both types of tea, and consequently varied the protein-binding affinity of polyphenolic compounds depending on their mass (Supplemental Figure S1, <https://doi.org/10.3168/jds.2020-19032>).

Generally, results of TPA (Table 2) revealed that PTY produced weaker, less adhesive structures than the GT-fermented milks. However, the differences were significant only for certain tea concentrations, and no significant changes were observed for GT yogurts in relation to NY. This may indicate that the changes that occurred in the acidified milk gel structures upon the addition of tea extracts were too small to be detected by the instrumental texture analysis.

In the case of textural studies, including TPA or back extrusion test (**BET**), the interactions between food constituents can be described by the parameters such as hardness or firmness (Table 2). These and other textural parameters concern normal stresses generated by the probe movement during the compression and decompression cycles. Results of the rheological properties measured in the flow range and textural parameters of the NY are related to one another and provide valuable information regarding functional properties (Lee and

Figure 2. Apparent viscosity (η ; after temperature and concentration scaling) dependence on shear rate (γ) for (a) yogurt with green tea infusion and (b) yogurt with Pu-erh tea infusion and discrete time distributions (inside) calculated from Eq. 1 for up and down flow curve. The apparent viscosity values have been shown in log-log scale. The values of viscosity calculated from flow curve for increasing shear rate (up flow curve) were plotted with lines demonstrating the De Kee et al. (1980) model (Eq. 1). Experimental points located below represented apparent viscosity based on down flow curve—flow curve obtained by decreasing shear rate.

Lucey, 2010). Thus, greater hardness values obtained from the TPA may support the conclusion drawn above for the yield-stress results: the GT additive produced a more consolidated yogurt gel matrix when compared with PTY.

The comparison of the results obtained for hardness and firmness presented in our previous work (Najgebauer-Lejko et al., 2014) revealed that GTY, which produced greater hardness during TPA ($P \leq 0.05$ for GTY_{5%} vs. PTY_{5%} and GTY_{15%} vs. PTY_{15%}), were simultaneously slightly less firm in BET (however, not significantly, $P > 0.05$) when compared with the plain yogurt and PTY (Table 2). Both hardness and firmness are defined as the force needed to attain given deformation during compression of a probe into the product, but unlike TPA, BET involves extrusion of the yogurt sample through the narrow annular gap between the probe and the container wall. As a result, greater forces are attained in BET (~8 N vs. 1 N in TPA presented herein). Thus, it could be hypothesized that BET results could be well correlated with the viscosity of yogurt measured under shearing conditions. However, such correlation was not confirmed between results obtained from the study performed on the yogurts prepared from microfluidized or conventionally homogenized milk by Ciron et al. (2011). Results obtained from the BET presented in our previous work (which indicated the tendency to produce gels with increased consistency, cohesiveness, and index of viscosity parameters affected by the tea, especially PT addition; Najgebauer-Lejko et al., 2014) correspond well with the results of the rheological studies that revealed a more viscous character of tea yogurts (especially PTY). These results are in contradiction to the data reported by Amirdivani and Baba (2013), who found that yogurts with 2% GT extract addition were less viscous and runnier than the nonsupplemented yogurt. However, the authors high-

lighted that the apparent viscosity of the GT yogurts tended to increase during refrigerated storage, unlike the plain one.

Results from the texture experiment confirmed observations made during the rheological studies. Yogurts with GT extract exhibited more shear-thinning properties when compared with the NY and PTY. It suggested that GTY were less susceptible to deformation during shearing and compression with a small-diameter probe (during TPA), but when higher shear stress values or higher compression and extrusion forces were applied, the structure of the acid gel underwent more visible changes. This may be connected with a more consolidated gel network, but with a large number of weak interactions (namely characteristic for protein-polyphenol associates hydrogen bonds), as stated for the acidified milk gels fortified with gallic or tannic acid by Harbourne et al. (2011). On the other hand, PTY were more prone to deformation (lower yield stress in the rheological experiment and hardness in the texture study), more viscous (higher consistency index and index of viscosity), and characterized by the greater ability to rebuild the structure (lower flow behavior index, higher cohesiveness).

NMR Relaxation Versus Mechanical Properties

The effect of GT and PT supplementation on the water mobility and organization in yogurt was studied by spin-spin and spin-lattice relaxation times. The T_1 and T_2 relaxation times of water molecules were observed for all studied samples. The relaxation times were reduced significantly from values of bulk water, indicating that the water molecules were in fast exchange between water associated with proteins (hydrated water) and free water. Figure 3a shows the T_2 values of natural and supplemented yogurts measured for 5 different temper-

Table 2. The results of texture profile analysis (TPA) and firmness from the back extrusion test (BET) of yogurts with and without tea additive (mean values $\pm$ SD, $n = 6$ or 24 for sensory analysis)

Yogurt type ¹	TPA parameters				BET firmness ² (N)
	Hardness (N)	Adhesiveness (N $\times$ m $\times 10^{-3}$)	Cohesiveness (-)	Gumminess (N)	
NY	1.20 $\pm$ 0.09 ^{abc}	6.35 $\pm$ 0.38 ^a	0.50 $\pm$ 0.02 ^a	0.59 $\pm$ 0.03 ^a	8.48 $\pm$ 0.09 ^{ab}
GTY ₅	1.28 $\pm$ 0.16 ^{abc}	6.33 $\pm$ 0.84 ^a	0.48 $\pm$ 0.02 ^a	0.60 $\pm$ 0.05 ^a	7.97 $\pm$ 0.40 ^{ab}
GTY ₁₀	1.22 $\pm$ 0.16 ^{abc}	6.10 $\pm$ 0.80 ^{ab}	0.48 $\pm$ 0.02 ^a	0.58 $\pm$ 0.06 ^{ab}	7.87 $\pm$ 0.50 ^a
GTY ₁₅	1.30 $\pm$ 0.13 ^a	5.87 $\pm$ 0.79 ^{ab}	0.47 $\pm$ 0.02 ^a	0.60 $\pm$ 0.05 ^a	7.66 $\pm$ 0.27 ^a
PTY ₅	1.06 $\pm$ 0.05 ^b	5.53 $\pm$ 0.82 ^{ab}	0.49 $\pm$ 0.03 ^a	0.52 $\pm$ 0.03 ^b	8.91 $\pm$ 0.86 ^b
PTY ₁₀	1.12 $\pm$ 0.13 ^{bc}	5.46 $\pm$ 0.76 ^{ab}	0.50 $\pm$ 0.05 ^a	0.58 $\pm$ 0.07 ^{ab}	8.60 $\pm$ 0.89 ^{ab}
PTY ₁₅	1.11 $\pm$ 0.13 ^{bc}	5.26 $\pm$ 0.96 ^b	0.56 $\pm$ 0.05 ^b	0.58 $\pm$ 0.04 ^{ab}	8.04 $\pm$ 0.72 ^{ab}

^{a-c}Mean values in columns followed by different letters are significantly different at $P < 0.05$.

¹NY = natural yogurt (without tea); GTY₅, PTY₅, GTY₁₀, PTY₁₀, GTY₁₅, PTY₁₅ = yogurts with, respectively, 5, 10, or 15 mL/100 mL of green tea or Pu-erh tea infusion.

²Data from the BET experiment from our previous study (Najgebauer-Lejko et al., 2014); other data are from original studies.

atures (from 8–25°C). The addition of both tea extracts to the yogurt composition significantly decreased relaxation times in all studied temperatures in comparison to NY. Only the PTY_{5%} samples had slightly longer T_2 than the NY samples for temperatures 15°C, 20°C, and 25°C (Figure 3 a). Decrease in water mobility (T_2 time) was also observed in NMR studies performed by Harbourne et al. (2011) on skim milk acidified with glucono delta-lactone upon addition of tannic acid and gallic acid. Additionally, T_2 values decreased gradually as concentration of tea extracts increased for a given temperature. It should be noted that shorter spin-spin relaxation times were observed for yogurts with GT compared with PT supplementation. Generally, reducing the T_2 relaxation time indicates lower mobility of water molecules in a proximity of proteins in yogurt. It does not result from the moisture content, which was very similar for all tested yogurts (on average $87.64 \pm 0.56\%$), but it may be due to structural changes in the gel matrix that are consequences of the yogurt fortification. Water located in a protein network modified by bioactive ingredients derived from teas moves more slowly compared with the unmodified matrix. This is also consistent with the study of Harbourne et al. (2011), who observed that the water molecules were more tightly bound within the acidified milk gel matrix with increasing concentration of added tannic acid. The microstructural changes are also evident from distribution of T_2 (small figures enclosed at Figure 3 a). We not only found the T_2 shortening, highlighted as the peak shift, but we also observed a significant reduction in the width of the T_2 distribution for yogurts with additives compared with NY. The narrowing of T_2 distributions can be an indicator of a different ordering of water caused by additives. Moreover, the wide distribution of T_2 for natural yogurt may point out more water components, which remain in contact with each other; this may also imply complex rheological properties. Reducing of the water mobility and changing its distribution as a result of tea addition may be connected with the higher yield stress and hardness determined for GTY in comparison to PTY and the NY. The stabilizing effect of the GTY on structure observed in the flow studies is consistent with the T_2 values as a function of temperatures, especially for the 10 and 15% tea concentrations. Indeed, their T_2 values were not changed significantly as a function of temperature (Figure 3 a). For GTY_{5%}, T_2 increased with the temperature more visibly. This exceptional behavior was also noticed in the shear experiment. On the other hand, adding PT extract to yogurt also deformed the structure. However, it had a less consolidated character, as demonstrated by lower yield stress and hardness values, as well as fluid-like

Figure 3. The (a) spin-spin and (b) scaled spin-lattice relaxation time dependence on temperature (T) for all GTY and PTY samples at different concentrations. Inside (a) the T_2 distributions for 8°C were shown. GTY = yogurt with green tea infusion; PTY = yogurt with Pu-erh tea infusion; NY = natural yogurt with no infusion; T_2 = spin-spin relaxation time; T_1 = spin-lattice relaxation time; b_c = scaling factor.

behavior. In a looser PTY structure, the water moves faster and has longer T_2 relaxation times.

Values of T_1 depended on the amount of hydration water and the nature of its binding to proteins, thus also on changes in structure and conformation of proteins. Shortening of T_1 for all tea yogurts in comparison to the NY was observed in all temperatures. Additionally, we observed that the larger the addition of a given tea,

the shorter the T_1 time was. This indicated a reduction in molecular mobility, probably due to an increase in the amount of water interacting with yogurt proteins modified as a result of supplementation. To show the effect of the given type of tea on spin-lattice relaxation, T_1 values were multiplied by scaling coefficient b_C , described in the materials and methods (Eq. 6), and presented as a function of temperature (Figure 3 b). Shorter scaled relaxation times were observed for yogurts with the addition of GT than with the addition of PT. This demonstrated the greater availability of binding sites in the created structure for interaction with water in GTY compared with PTY. Both tea types differ greatly in their composition; in particular, they are characterized by different profiles of the polyphenolic compounds (Supplemental Figure S1, <https://doi.org/10.3168/jds.2020-19032>), which are known to interact with milk proteins, namely proline-rich casein. Green tea is richer in catechins, while PT is mainly composed of more polymerized polyphenols (e.g., theabrownins). The results of NMR studies can be a sign of a greater effect of GT ingredients (catechins) on proteins that are an integral part of the yogurt structure.

CONCLUSIONS

The plain yogurts as well as those supplemented with tea extracts were thixotropic fluids, exhibiting shear-thinning behavior. The NY demonstrated rheological properties that could not be subjected to temperature-scaling. The addition of GT or PT extracts changed the mechanical properties of yogurts. Primarily, the apparent viscosities of these yogurts could be scaled in relation to the temperature (8, 10, 15, 20, 25°C) and tea concentration (5, 10, 15%), which is indicative of the structural stabilization of the products. This stability was also confirmed in NMR studies, which revealed that tea addition to yogurt base in a concentration-dependent manner caused lower mobility of the water molecules within the gel network. The NMR technique, together with the shear viscosity and texture examinations, was a relevant tool to show the differences in the internal structure and the mechanical properties of the yogurt gel upon supplementation with GT or PT extracts. Both types of tea produced yogurts with different physical characteristics: lactic acid fermentation of milk with GT formed more adhesive and slightly harder gels with more tightly bound water molecules, while softer structure of PTY coincided with exclusively viscous character of these products. This knowledge can be very useful during development of novel dairy products with not only bioactive properties, as supported by many reports devoted to health-promoting

properties of tea, but also with improved texture and more stable protein structure.

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