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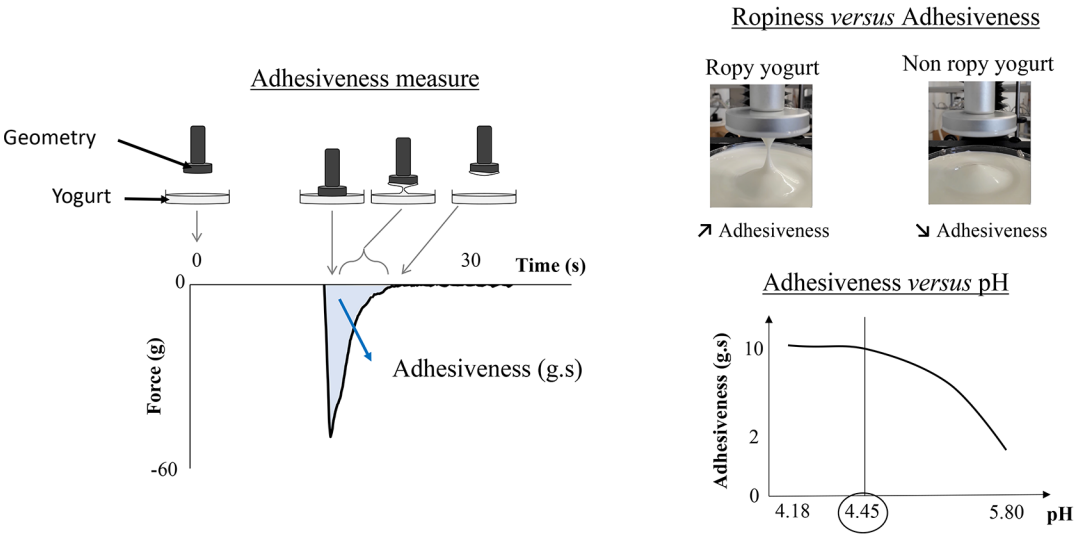
Technical Note
Dairy Foods

A methodological approach to assess the ropy character of stirred acid dairy gels based on the measure of adhesiveness

Siwar Nahali,[1] Audrey Gilbert,[1] Charlotte Marchand,[1] Marie-Hélène Lessard,[1] Donna Miller,[2] Sébastien Fraud,[3] Steve Labrie,[1] and Sylvie L. Turgeon[1]*

Graphical Abstract

Potential and limits of adhesiveness measurement to assess ropiness of acid dairy gels



Summary

This study investigated the potential and limits of adhesiveness measurement to assess ropiness of acid dairy gels. The adhesiveness of commercial yogurts was significantly affected by yogurt type. Whatever the material or the diameter of the probes, the results had the same tendency. The relationship between final pH and adhesiveness was studied. Adhesiveness increased when pH decreased to reach a plateau at pH <4.45. This method can be an instrumental tool for starter selection in an industrial context due to its simplicity and the availability of a texturometer in industrial facilities.

Highlights

- A simple textural method, adhesiveness, can be used to measure yogurt ropiness.
- Similar adhesiveness trends were found with different probes (diameter and material).
- Strain expression of ropiness in dairy gels can be detected using adhesiveness.

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The list of standard abbreviations for JDSC is available at adsa.org/jdsc-abbreviations-24. Nonstandard abbreviations are available in the Notes.

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Abstract: This work aims to evaluate the potential and limits of adhesiveness measurement using a texturometer to assess the ropiness of acid dairy gels for starter selection. Commercial yogurts of various formulations and textures were used to assess the ability of adhesiveness to detect ropiness and to compare performance of different probes. Chemically acidified gels using different concentrations of glucono-delta-lactone (GDL) were tested to determine the effect of pH on adhesiveness. In addition, acidified (GDL) milk containing 3 exopolysaccharide-producing adjunct strains was produced to evaluate the detection of ropy strains using adhesiveness, compared with an inoculation loop stretching test. The adhesiveness of commercial yogurts was mainly affected by its formulation. Visually ropier commercial yogurts with high protein and fat contents presented higher adhesiveness. Globally, adhesiveness analysis was not affected by probe material, but larger probes resulted in higher adhesiveness values. The addition of adjuncts increased the adhesiveness of GDL gels compared with the controls. Results between inoculation loop stretching test and adhesiveness were similar, except for *Lactobacillus delbrueckii lactis* LMA-1511, which exhibited high adhesiveness but no threading with the loop stretching test. When varying GDL concentration, adhesiveness increased with decreasing pH until pH 4.45, and remained stable until pH 4.18. In the pH zone from 4.18 to 4.45, differences in adhesiveness could indicate the presence of a ropy strain. This method is promising for industries as a quality control or screening method for starter cultures due to its simplicity and the availability of texturometers.

Yogurt quality depends on its texture and sensory attributes such as creaminess, which is a complex and multidimensional attribute that consumers appreciate and improves the perception of the overall quality of the product (Upadhyay et al., 2020). Creaminess is often associated with ropiness induced by lactic acid bacteria (**LAB**) producing ropy exopolysaccharides (**EPS**) (Folkenberg et al., 2006; Hassan, 2008). Ropiness is denoted as a threadlike texture (Mende et al., 2016). Although the mechanism remains uncertain, ropiness and gel viscoelastic properties often correlate (Mende et al., 2016) and ropy EPS are generally localized at the interface between serum pores and protein network (Hassan, 2008). Several physical methods have been proposed to evaluate the ropy character. Some methods are used on strain culture before fermentation, such as the loop stretching test on colonies on agar medium (Mende et al., 2016). Strains are considered ropy when the thread measures >5 mm (Mäkelä and Korkeala, 1992). This method evaluates the potential of strains to produce ropy EPS but is a poor tool for the prediction of ropy expression in yogurt, as it highly depends on growth conditions (medium and temperature; Mäkelä and Korkeala, 1992). Other empiric methods allow differentiation of ropy strains during yogurt fermentation by determining the time necessary for a given amount of fermented milk, taken before gelation, to flow through a funnel (Gentès et al., 2011). However, this method cannot be applied directly to the final yogurt. Extensional properties allow determination of thread formation and were used to assess ropiness in yogurt using a texturometer (Hess et al., 1997; Surber et al., 2020) or a capillary extensional rheometer (Surber et al., 2020). Hess et al. (1997) determined the extensibility (in mm) based on the time required to break the formed thread between the probe and the sample, and reported that the longer the time before rupturing, the ropier the yogurt. Surber et al. (2020) found direct positive correlations between the yogurt thread stretchability (thread length) measured in a texturometer, the breakup time, extensional viscosity obtained with an extensiometer (Haake CaBER rheometer, Thermo Haake, Germany), and the apparent shear viscosity. Using a texturometer only, other attributes are available such as adhesiveness, which could also inform on the product extension behavior and has been associated with the ropy character of yogurt (Toba et al., 1990; Marshall and Rawson, 1999). No precise adhesiveness method with defined limits has been used for ropiness evaluation.

Adhesiveness refers to the force or work needed to detach the product from the geometry or probe surface (Wang and Hartel, 2021). Yogurt adhesiveness was measured by single compression tests (Akalın et al., 2012), 2 successive compressions (textural profile analysis, **TPA**; Mudgil et al., 2017), or back-extrusion tests (Marshall and Rawson, 1999). In TPA, the probe withdrawal after the first compression is used to measure adhesiveness, but the product may not separate completely from the probe, which can produce erratic results. A single compression test may be more relevant (Wang and Hartel, 2021). Moreover, adhesiveness depends on the probe material used (surface roughness, hydrophobicity, and

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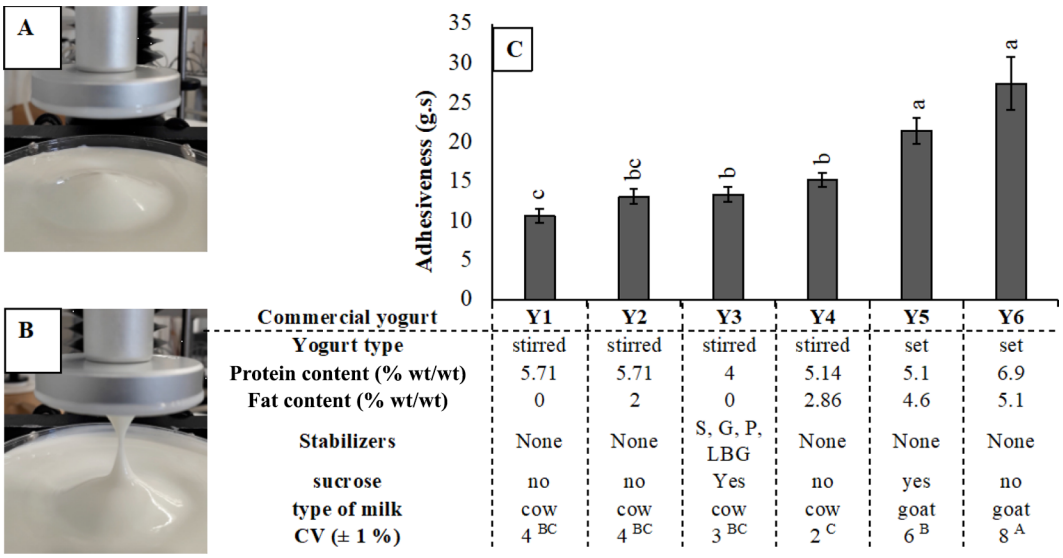

Commercial yogurt	Y1	Y2	Y3	Y4	Y5	Y6
Yogurt type	stirred	stirred	stirred	stirred	set	set
Protein content (% wt/wt)	5.71	5.71	4	5.14	5.1	6.9
Fat content (% wt/wt)	0	2	0	2.86	4.6	5.1
Stabilizers	None	None	S, G, P, LBG	None	None	None
sucrose	no	no	Yes	no	yes	no
type of milk	cow	cow	cow	cow	goat	goat
CV (± 1 %)	4 ^{BC}	4 ^{BC}	3 ^{BC}	2 ^{C}	6 ^{B}	8 ^{A}

Figure 1. Visual threading of non-ropy commercial yogurt Y1 (A) and ropy commercial yogurt Y6 (B) during a 3-cm extension at 40 mm·s^{-1} using a cylindrical probe (45 mm in aluminum; TA-XT Plus). Commercial yogurt composition and adhesiveness (C; g·s) obtained with the geometry 45-P (diameter 45 mm in Perspex). Bars represent SE (n = 3). CV = adhesiveness coefficients of variation per triplicate. Lowercase (a–c) and uppercase (A–C) letters indicate significant differences ($P < 0.05$). S = starch; G = gelatin; P = pectin; LBG = locust bean gum.

so on; Wang and Hartel, 2021), and a wide variety of geometries with different diameters and materials has been used (Delikanli and Ozcan, 2017; Mudgil et al., 2017).

Adhesiveness can be modulated by different formulation factors. For instance, high protein (Domínguez-Soberanes et al., 2001) and fat contents (Sandoval-Castilla et al., 2004) increase adhesiveness, while stabilizers show various effects depending on their nature (Mudgil et al., 2017; Mudgil et al., 2018). The EPS was reported to increase adhesiveness Broadbent et al. (2003). Other factors related to strain such as optimal temperature of growth, acidification rate, and postacidification may also modulate adhesiveness, but their effect has not been thoroughly documented.

A simple instrumental method allowing characterization of ropiness would support the industry in its quality control efforts and the strain selection involved in research and innovation. Sensory analysis, the gold standard to determine creaminess, cannot be systematically performed as it is time consuming. The commercial extensiometer (Haake CaBER rheometer) or alternatives are sophisticated devices.

In this study, a methodological approach using a texturometer was tested to evaluate adhesiveness as a tool to assess the ropiness of acid dairy gels. First, the method was tested on commercial yogurts to determine the effect of different geometries (material and diameter). Then, several factors that could modulate adhesiveness measurement were studied using chemically acidified gels such as the addition of mesophilic adjunct strains, the incubation temperature (30°C or 37°C), and the final pH (from 4.18 to 5.80).

Three different batches of 6 commercial yogurt samples were chosen to represent a variety of compositions and textures (Figure 1) and were analyzed between 8 and 46 d before their expiration date. All commercial yogurts were stirred manually with a spoon (standardized procedure) and were placed in a water bath at 15°C at least 1 h before measurement.

Acidic gels were produced using reconstituted nonfat dry milk (**RNFM**) powder (Dairy America, Fresno, CA) rehydrated at 13.71% wt/vol milk solids and 3.52% wt/vol protein. The RNFM (900 mL) was mixed for 1 h at speed 1.5 (arbitrary unit) at room temperature and pasteurized using a Thermomix (Thermomix TM6 Vorwerk, Wuppertal, Germany). The temperature was increased to 95°C in 18 min and kept at 95°C for 5 min. The RNFM was then cooled in an ice-water bath until it reached the incubation temperature. A sterilized (121°C, 15 min) sucrose solution (40% wt/vol) was added to RNFM to reach a 4% sucrose concentration. In the first set of experiments, RNFM tempered at 30°C or 37°C was added with 1.4% (wt/vol) of glucono-delta-lactone (**GDL**, Sigma-Aldrich, St. Louis, MO). The RNFM was uninoculated (**CTRL**) or inoculated with an adjunct LAB strain (10^5 cfu/mL) and was incubated at the optimal growth temperature (**OGT**) of the adjunct. The 3 adjuncts *Lactobacillus delbrueckii* ssp. *lactis* **LMA-1511** and *Lactobacillus crispatus* **LMA-1517** (OGT of 37°C), and *Leuconostoc lactis* **LMA-1515** (OGT of 30°C) were tested. The CTRL were incubated at either 30°C or 37°C. In a second set of experiments, RNFM aliquots were added with 0.6%, 1.0%, 1.4%, 1.8%, or 2.2% GDL (wt/vol, Alfa Aeser, Haverhill, MA). For both sets of experiments, RNFM aliquots were gently mixed right after GDL addition (and inoculation if applicable) by shaking the bottle manually in a circular way for 1 min. The mixture was separated into aliquots of 35 mL and incubated in a thermostatic bath at either 30°C or 37°C. After 24 h, the gels were cooled down (ice bath, 20 min) and kept at 15°C (40 min, thermostatic bath). Before adhesiveness measurement, gels were stirred using an overhead stirrer (Caframo Ltd., Georgian Bluffs, ON, Canada) with a 1-cm blade at 100 rpm for 30 s, and simultaneously moved upward and downward 10 times. After addition of GDL and adjunct strain, a sample of 5 mL of each inoculated batch was placed in a thermostatic bath at OGT for 24 h to measure the pH continuously (InPro-3100

sensor probe, Transmitter multi-parameter M300, Metter-Toledo GmbH, Switzerland).

Adhesiveness was measured in triplicate with a TA-XT Plus (Texture Technologies Corp., Hamilton, ME) equipped with a 5-kg cell using similar sample preparation and extension procedures as Surber et al. (2020). Adhesiveness was measured instead of threading. Samples (30 ± 1 g, 15°C) were transferred into a Petri dish (polystyrene, D = 94 mm, height = 16 mm) fixed on the platform of the instrument. The geometry was placed at 33 mm from the bottom of the Petri dish, lowered down at 5 mm/s to enter the gel, and settled at 3 mm from the bottom for 20 s. The geometry was then withdrawn to its initial position at 40 mm/s. Adhesiveness (g·s) was calculated as the area obtained from the force versus time curve generated by Exponent software (Texture Technologies Corp.; Le et al., 2011; Wang and Hartel, 2021). Acidic gels were analyzed using a 40 mm diameter aluminum geometry (**40-A**). The effect of probe materiel and diameter was tested on commercial yogurts using 40-A, 45 mm diameter aluminum (**45-A**), or 45 mm diameter Perspex (**45-P**) cylindrical geometries.

The ropy phenotype of LAB strains was measured using a modified loop stretching test (Mäkelä and Korkeala, 1992). A 24-h preculture at OGT without oxygen of each strain was made in de Man, Rogosa, and Sharpe (**MRS**) broth (GranulCult^{R}, Merck KGaA, Germany), diluted (7-log) in sterile 0.1% (wt/vol) peptone water (Bacto Peptone, BD Difco, Le pont de Claix, France), plated on MRS agar (Bacto Agar, BD Difco) supplemented with 4% (wt/vol) sucrose, and incubated 72 h at OGT. Three isolated colonies of each Petri dish were touched with a sterile inoculation loop to measure the thread formed. Three repetitions were done.

Commercial yogurts followed a factorial plan with 2 factors (6 yogurts, 3 probe sizes) and were carried out in 3 repetitions according to a split-plot design with the yogurts as the main plot. For acidic gels, 2 separate experimental plans were realized: the first plan (1.4% GDL, with or without adjunct) followed a randomized incomplete block design with 4 repetitions at 30°C and 3 repetitions at 37°C; the second plan (without adjunct, different GDL concentration), followed a factorial design of 2 factors (5 GDL concentrations, 2 incubation temperatures) and 4 repetitions following a split-plot with incubation temperature as the main plot. For the stretching loop test, a fully randomized plan was followed over 3 repetitions. All datasets were analyzed using the mixed procedure of SAS v9.4 (SAS Institute Inc., Cary, NC). To satisfy model assumption, some data needed corrections (heteroskedasticity issues). Normality was checked using the Shapiro-Wilk statistic, and homogeneity of variance was verified using residual plots. Correlations between different physical variables were calculated using Pearson correlation. Results are presented as means ± SE. Adhesiveness measurements repeatability was documented using the CV per triplicate (Shechtman, 2013).

Commercial yogurts (**Y1** to **Y6**) had a wide range of protein and fat contents and differed in milk type and stabilizer content (Figure 1). Adhesiveness was significantly affected by the commercial yogurt type depending on the geometry used for measurements ($P < 0.01$). Except for Y3, the probes had very similar trends and only the results using 45-P geometry are represented (Figure 1C). When extended, visually ropier yogurts such as Y6 created a thread (Figure 1B), unlike visually non-ropy yogurts such as Y1 (Figure 1A). As expected, the extension measured for ropy yogurts had higher adhesiveness values, compared with non-ropy ones (Toba et al., 1990). The visually ropiest yogurts Y5 and Y6 that also contained high protein and fat contents presented the highest adhesiveness values. Although having different formulations, yogurts Y1, Y2, Y3, and Y4 showed similar adhesiveness values (Figure 1C). Yogurt formulation is known to affect adhesiveness as TPA showed a >2× increase in adhesiveness when stirred yogurt protein content increased from 2.8% to 3.7% using skim milk ultrafiltration retentates (Domínguez-Soberanes et al., 2001). Similarly, a simple compression test showed a 4× higher adhesiveness when increasing set yogurt milk solid nonfat (8% to 11%) (Le et al., 2011). This aligns with the 50% higher yogurt extensibility found when increasing solid content (10% to 14%; Hess et al., 1997). The presence of EPS-producing strains can modulate the effects of protein and solid contents on adhesiveness in both set and stirred yogurt (Domínguez-Soberanes et al., 2001). Although the effects of adding stabilizers vary between studies, starch and gelatin generally increase yogurt adhesiveness (Sandoval-Castilla et al., 2004; Mudgil et al., 2018) and perceived ropiness (Ares et al., 2007). Other factors such as storage period and proteolytic activity may affect ropiness but were not controlled in this study.

Adhesiveness is generally measured using geometries of diverse diameters (25–40 mm) and materials. In this study, the 3 different probes showed adhesiveness results with very similar trends for all yogurts (all correlations between probes >0.85), except for 40-A, which could not differentiate Y3 from Y1 (results not shown). This difference is probably due to the higher contact area between the yogurt and the probes with larger diameter (45-A or 45-P). Larger surface area would involve a higher quantity of yogurt sample into the measurements helping to differentiate more subtle differences between samples. Both 45 mm geometries generally gave higher adhesiveness results than 40-A. Adhesiveness obtained with 45-P tended to be higher than 45-A, but it was significant only for Y4 and Y6 (results not shown). The yogurt probably adhered slightly differently due to distinctive probe surface properties (Wang and Hartel, 2021). The adhesiveness method could be used to distinguish between yogurts with visually different ropiness, whatever the diameter and geometry material used.

The CV of adhesiveness measurement depended on the yogurt type ($P < 0.001$; Figure 1) and were under 5% for most yogurts except for Y5 and Y6 (most adhesive and visually ropiest yogurts). For better repeatability, protocols may need more than triplicates when measuring yogurt with high ropiness (Domínguez-Soberanes et al., 2001)

The ability of adhesiveness measurements and the loop stretching test allowed differentiation of the impact of EPS produced by adjuncts. The adhesiveness was significantly increased in the presence of adjuncts ($P < 0.01$, Table 1), and *L. delbrueckii* ssp. *lactis* LMA-1511 and *L. crispatus* LMA-1517 were the strains with the highest ropiness phenotypes in dairy gels.

Ropiness assessment obtained with both loop stretching test and adhesiveness measurement showed the same tendency except for *L. delbrueckii* ssp. *lactis* LMA-1511 (Table 1). The results of the loop stretching test depend on the medium used (Mäkelä and Korkeala, 1992) suggesting that the adhesiveness method applied on yogurts is better suited to ropy strain selection. The increases in gel adhesiveness could be related to ropy EPS produced during fermentation (Domínguez-Soberanes et al., 2001). However, in ad-

Table 1. Effect of adjunct strain addition on acid dairy gel adhesiveness and pH, and loop stretching results of strains incubated at their optimal growth temperature (OGT) on MRS agar medium[1]

Strain (T°C)[2]	Adhesiveness (g·s) measured at 15°C	Thread length at OGT (mm)	pH
CTRL (30)	8.56^{D}	ND[3]	4.48^{AB}
LMA-1515 (30)	9.42^{BC}	5.11^{B}	4.60^{A}
CTRL (37)	8.89^{CD}	ND	4.58^{A}
LMA-1511 (37)	10.51^{A}	0.89^{C}	4.29^{B}
LMA-1517 (37)	10.23^{AB}	27.33^{A}	4.31^{B}
SE	<0.32	0.55	<0.06

$^{A–D}$Mean values in columns with different superscripts are significantly different ($P < 0.05$).

[1]Values are reported as means ± SE (gels incubated at 37°C: n = 3; gels incubated at 30°C: n = 4).

[2]Incubation temperature (°C) of GDL gels with adjunct strains indicated in parentheses.

[3]ND = not detected.

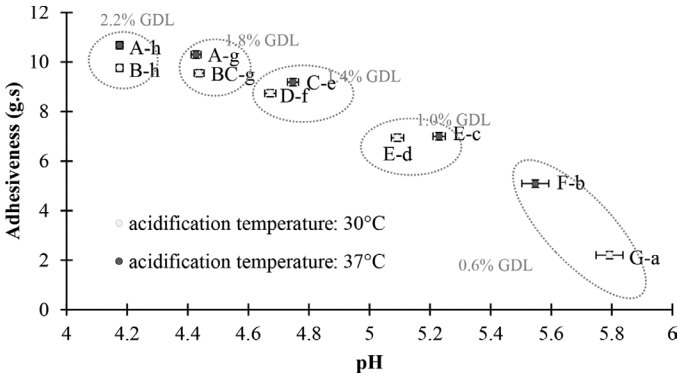

Figure 2. pH and adhesiveness (g·s) obtained for acid dairy gel acidified at 30°C or 37°C using 0.6%, 1.0%, 1.4%, 1.8%, or 2.2% (wt/wt) glucono-δ-lactone (GDL) without adjunct strains. Bars represent SE (n = 4). Different uppercase letters (A–G) indicate significant differences ($P < 0.001$) between acid dairy gel adhesiveness. Different lowercase letters (a–h) indicate significant differences ($P < 0.05$) between acid dairy gel pH.

dition to their EPS production, adjuncts could probably modulate the pH, which could affect the ropiness. For instance, Gentès et al. (2011) detected higher product ropiness at pH 5.5 using the funnel method, whereas Surber et al. (2020) found no major effect of pH on product extensibility. To our knowledge, no study described the combined effect of EPS and pH on yogurt gel adhesiveness. The pH of *Ln. lactis* LMA-1515 did not differ from its CTRL. However, both *L. delbrueckii* ssp. *lactis* LMA-1511 and *L. crispatus* LMA-1517 had lower pH than their CTRL (Table 1).

To distinguish between the effect of EPS produced by adjunct and pH on adhesiveness, a second experiment using acidic gels with various GDL concentrations (0.6%–2.2%) was carried out. The GDL hydrolysis generates both gluconic acid and lactone, slowly acidifying the milk, the final gel pH depending on the final concentration of gluconic acid (Lucey et al., 1998). The interaction between GDL concentration and incubation temperature controlled the final gels' pH ($P < 0.0001$). In addition, the acidification rate observed with acidification curves (data not shown) increased with incubation temperature and GDL concentration, reaching a constant pH after approximately 20 h. Increasing GDL concentrations from 0.6 to 1.4% (wt/vol) decreased pH (Figure 2). For 0.6% GDL, pH was higher at 30°C than at 37°C. Then, up to 1.4% GDL, pH was lower at 30°C. For higher concentrations (1.8% and 2.2%) final pH depended only on the GDL concentration. This can be explained by a higher hydrolysis rate of GDL at higher temperature and concentration (Denin-Djurdjević et al., 2002). However, the pH difference obtained at 30°C or 37°C for 0.6% GDL samples has no equivalent in the literature. This surprising result may come from a higher pH variability induced by the small quantity of GDL used. In any case, the pH targeted (≈5.7) is not in the typical pH range targeted for fermented milks.

Adhesiveness depended on incubation temperature and GDL concentration interactions ($P < 0.0001$). It increased with increasing GDL concentrations to reach a plateau at pH <4.45. Gels incubated at 30°C had a globally lower adhesiveness than those incubated at 37°C (Figure 2). In literature, lower incubation temperature has been associated with slower gel formation and homogeneous structure, which reduced gel elasticity (Lucey et al., 1997), whereas higher temperature caused faster acidification and coarser networks (Bringe and Kinsella, 1993), which could affect adhesiveness. In this study, the 30°C and 37°C CTRL in the experimental plans with adjuncts were similar (pH and adhesiveness) but differed in pH and adhesiveness when varying GDL concentration and temperature. This is probably due to a difference in variability between the experimental plans, even though the CV (<5%) per triplicate for acidic gels did not depend on strain addition, incubation temperature, or GDL concentration.

All samples (pH ≤ 5.5) were gelled except the 30°C sample with 0.6% GDL (pH = 5.80) that stayed liquid, which was expected (Lucey et al., 1997). When pH decreased, adhesiveness increased, and for a given incubation temperature, adhesiveness was the same at pH values between 4.18 and 4.45. Adhesiveness variations in this range of pH would be due to factors other than pH. Yogurt pH strongly depends on the acidifying and postacidifying properties of yogurt starters. Thus, when comparing the adhesiveness of yogurts produced with different starters, if the pH of the product is out of the linear zone (>4.45) adhesiveness may be affected by both the dairy gel pH and the presence of ropy EPS from the starter. Since *Ln. lactis* LMA-1515 gel and its CTRL had similar pH (Table 1), the higher gel adhesiveness reflects a ropier behavior. The pH of *Lactobacillus delbrueckii* ssp. *lactis* LMA-1511 and *L. crispatus* LMA-1517 gels was under 4.45; as their CTRL was higher than 4.45, it is not possible to attribute their higher gel adhesiveness to a ropier behavior or a lower pH.

The potential of adhesiveness measurement to assess ropiness for starter selection was investigated. Probe composition and diameter followed the same trend, and the adhesiveness method was able to differentiate between gels added with different adjuncts. The relationship between milk gel pH and adhesiveness was highlighted. When using this method as a strain screening tool, pH should be closely monitored. Further studies to specify the pH zone where adhesiveness is linear as well as the influence of formulation and shearing processes (high *vs* low shear) should be conducted. It is used directly on the final product and could be easily implemented in industrial facilities for quality control or strain selection during an innovation process.

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Notes

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Nonstandard abbreviations used: 40-A = 40 mm diameter aluminum; 45-A = 45 mm diameter aluminum; 45-P = 45 mm diameter Perspex; CTRL = chemically (GDL) acidified dairy gel without strain addition; EPS = exopolysaccharides; GDL = glucono-delta-lactone; LAB = lactic acid bacteria; LMA-1511 = *Lactobacillus delbrueckii* ssp. *lactis* LMA-1511; LMA-1515 = *Leuconostoc lactis* LMA-1515; LMA-1517 = *Lactobacillus crispatus* LMA-1517; MRS = de Man, Rogosa, and Sharpe; OGT = optimal growth temperature; RNFM = reconstituted nonfat dry milk; TPA = textural profile analysis; Y1–Y6 = commercial yogurts.