Rheological and sensory performance of a protein-based sweetener (MNEI), sucrose, and aspartame in yogurt

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ABSTRACT

Sweeteners and flavors are generally added to yogurt to make them more palatable. However, the addition of these ingredients may affect the fermentation process of yogurt as well as its physical and sensory characteristics. Consumers prioritize yogurt products that are “natural.” A modified single-chain form of the natural sweet protein monellin extracted from the fruit of *Dioscoreophyllum cumminsii*, called MNEI, could be a useful alternative to artificial sweeteners. The aim of the present work was to evaluate new rapid sensory methods in combination with rheology to assess the viability of using MNEI to develop sweetened yogurts without the calories of sugar. We studied the gelation and cooling kinetics of 4 yogurt samples (unsweetened or sweetened with MNEI, aspartame, or sucrose) by using a rheometer. Furthermore, the 4 yogurts, with and without addition of a flavoring agent, were characterized from a sensory perspective using a combination of 2 rapid sensory methods, ultra flash profile and flash profile. Rheological results showed that, when added at typical usage levels, aspartame, sucrose, and MNEI did not generally affect the yogurt fermentation process or its rheological properties. Sensory results demonstrated that texture attributes of yogurts with aspartame and sucrose were strongly linked to sweetness and flavor perception, but this was not true for MNEI-sweetened yogurts. In contrast to results obtained from samples sweetened with sucrose and aspartame, MNEI protein did not sweeten the yogurt when added before fermentation. This study highlights the enhancing effect of flavor on sweetness perception, supporting previous reports that noted synergistic effects between sucrose or aspartame and flavors. Hence, future studies should

be conducted to determine how sweet proteins behave in yogurt when added after fermentation.

Key words: sugar replacement, sweet protein, gelation, flash profile

INTRODUCTION

Yogurt is a fermented milk produced by *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*, both of which must be viable and available in substantial amounts until the date of minimum durability, the “best before” date of consumption (CODEX, 2003). Yogurt and sour milk products have emerged to be one of the most important types of fermented dairy products (Ramírez-Sucre and Vélez-Ruiz, 2013; Gahrui et al., 2015), gaining global recognition as healthy foods due to their nutritional content and beneficial properties (Sanders et al., 2007; Aswal et al., 2012; Gahrui et al., 2015) and creating an \$85 billion market in 2015, which is expected to continue to grow (Euromonitor, 2015).

Sweeteners and flavors are generally added to make yogurts more palatable (Pinheiro et al., 2005; Ramírez-Sucre and Vélez-Ruiz, 2013; Aubrey, 2015). The natural compound generally associated with sweet taste is sucrose. Consumers prioritize yogurt products that are “natural” and sweet without the calories of sugar (Pinheiro et al., 2005; Sharma, 2014; Euromonitor, 2015).

Aspartame is one of the most commonly used artificial sweeteners in the food industry (Shankar et al., 2013), due to its sweetness profile, which is very similar to that of sucrose despite a slightly higher and lingering sweetness at the end (Di Monaco et al., 2014). However, recent controversies regarding the use of artificial high-intensity sweeteners, together with the increasing concern for well-being and health, have driven the use of natural high-intensity sweeteners (Moure et al., 2006; Simonsohn, 2012; Hill et al., 2014).

Monellin is a natural protein sweetener isolated from the fruit of the serendipity berry, *Dioscoreophyllum cum-*

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minsi (Inglett and May, 1969). The interest in monellin is due to the fact that it is the sweetest naturally occurring substance, up to 3,000 times sweeter than sucrose (Hung et al., 1999; Picone and Temussi, 2012); however, it is quite unstable to temperature increases (Kim et al., 1989; Tancredi et al., 1992). A modified form of monellin, MNEI (obtained from the covalent binding of the 2 polypeptide chains A and B through a bridge peptide, Gly-Phe) exhibits enhanced stability to changes in both temperature and pH (Esposito et al., 2006; Di Monaco et al., 2013; Leone et al., 2016).

The addition of certain ingredients such as sugar and sweeteners to yogurt may affect its fermentation process as well as syneresis and apparent viscosity, which may in turn affect the yogurt's overall sensory consumer acceptance (Lee and Lucey, 2010).

Rheological measurements can be used to characterize the coagulation of casein micelles (Peng et al., 2009), which correspond to an increase in elastic modulus (G'), loss modulus (G''), and complex dynamic viscosity (η^*) during fermentation (Bensmira et al., 2010), and can be used as indicators of texture perception in yogurt (Tabilo-Munizaga and Barbosa-Cánovas, 2005; Ciron et al., 2012).

Delivering the same sugar-like sweetness is a challenging task as high-intensity sweeteners generally exhibit an unusual sensory characteristic; for example, delay in sweetness perception, long sweetness persistency, or a lingering aftertaste (De Melo et al., 2007; De Marchi et al., 2009; Moraes and Bolini, 2010), and most consumers are not willing to sacrifice a product's sensory attributes in exchange for its health benefits (Portmann and Kilcast, 1998; Civille and Oftedal, 2012; Miele et al., 2017a). To overcome the high cost and time-consuming aspects of traditional sensory methods, a modified version of the flash profile method, including a preliminary session consisting of the ultra flash profile method, has been developed to investigate different food products such as pasta, polenta sticks, cakes, and wine (Di Monaco et al., 2015; Liu et al., 2016). Flash profile is a rapid, descriptive sensory method based on a combination of free-choice term selection and comparative ranking evaluation (Delarue, 2014).

Many studies have combined rheology and sensory analysis to examine the effects of various factors such as composition on yogurt gel formation and texture (Folkenberg et al., 2006; Guggisberg et al., 2009; Ciron et al., 2012).

The aim of the present work was to evaluate new rapid sensory methods in combination with rheology to assess the viability of using MNEI to develop sweetened yogurts without the calories of sugar. To do so,

we studied the gelation and cooling kinetics of 4 yogurt samples (unsweetened, or sweetened with MNEI, aspartame, or sucrose) by using a rheometer. Furthermore, sensory attributes of the 4 yogurts, with and without the addition of a flavoring agent, were characterized using a combination of 2 rapid sensory methods, ultra flash profile and flash profile.

MATERIALS AND METHODS

Yogurt Ingredients

The materials used were whole UHT milk (33 g of protein and 36 g of fat per liter; Sterilgarda Alimentari S.p.A., Mantua, Italy), mineral water (22 mg/L fixed mineral residual, Sant'Anna, Vinadio, Italy), citric acid (minimum 99.5% purity, Sigma Aldrich, St. Louis, MO), sucrose (Eridania, Bologna, Italy), aspartame (granular PSL E951, Tillmanns s.p.a., Milan, Italy), sweet protein MNEI (provided by the Department of Chemistry, University of Naples Federico II, Naples, Italy; Leone et al., 2016), mixed berry flavor (Arda Natura s.r.l., Piacenza, Italy). *Streptococcus thermophilus* (ST), previously isolated from commercial starter YC-350-CH (Chr. Hansen, Hoersholm, Denmark), were stored at -20°C in appropriate broths with 25% (vol/vol) sterile glycerol.

Preparation of MNEI

A citric acid solution of pH 2.8 to 2.9 was prepared by dissolving 0.1 g of citric acid in 100 mL of Sant'Anna mineral water. The MNEI was then dissolved completely in the citric acid (1:1) using a vortex mixer (IKA MS3 digital, IKA-Werke GmbH and Co. KG, Staufen, Germany) and stored overnight at 60°C before addition to milk for preparation of milk and yogurt.

Yogurt Preparation

For the starter preparation, 2% each of *Lactobacillus bulgaricus* (LB) and ST were preinoculated in an appropriate culture media and grown until optical density reached 2.2 to 2.4. The day after, an inoculum of 4% of the culture mix (40 mL/L) was prepared and used for yogurt preparation. Milk was heated to 37°C before the addition of other ingredients. Plastic tubes containing 16 mL of yogurt mix were then incubated at 43°C for 7 h (until reaching a pH of 4.6 to 4.4, based on preliminary experiments) and cooled immediately to about 20°C before refrigerated storage (Surono and Hosono, 2011). The amount of sweetener used was based on pre-

vious publications (Di Monaco et al., 2016; Miele et al., 2017b) and on preliminary iso-sweetness concentration determination in milk (data not shown) according to the procedure used by Di Monaco et al. (2014). The 8 formulations are summarized in Table 1.

Rheological Characterization of Yogurt

Yogurt gel formation of 16 mL of milk mixture was monitored using a Haake Mars 60 Rheometer (Thermo Scientific, Waltham, MA) with a cup-and-bob geometry consisting of coaxial cylinders (inner diameter 25.08 mm; outer diameter 27.2 mm). During fermentation, the mixture was held at 43°C for 2 h and then subjected to small deformation oscillation for 5 h at a frequency of 1 Hz and with an applied strain of 0.5%, which did not disrupt the development of the gel network (Dejmek, 1987; Gunasekaran and Mehmet, 2000) as verified by preliminary strain and frequency sweep tests (data not shown). After a total fermentation time of 7 h, oscillation measurement was continued during cooling from 43° to 4°C; G' was measured every 10 min and time of gelation (T_{gel}) was arbitrarily defined as the first point when $G' > 1$ Pa (Lucey et al., 1997; Peng et al., 2009). Three replications of each rheological measurement for each sample were performed. pH was measured before fermentation, when $G' > 1$ Pa, and after 7 h using an HI2211 pH/ORP meter (Hanna Instruments, Woonsocket, RI). Each measurement was conducted in 3 replicates.

Degree of Syneresis

As proposed by Hassan et al. (2015), 25 g of yogurt was centrifuged at $1,600 \times g$ for 20 min at 4°C (ALC Multispeed Refrigerator Centrifuge PK 131R, ALC International, Milan, Italy) and the degree of syneresis was determined as follows:

$$\text{Syneresis (\%)} = \frac{\text{weight of collected supernatant}}{\text{weight of yogurt sample}} \times 100. \quad [1]$$

Three replicates were performed for each treatment.

Sensory Evaluation

Panel. Eight judges (2 men and 6 women), students aged 23 to 29 yr (mean age 26 yr), participated in the sensory tests performed in the Sensory Laboratory of the Department of Agricultural studies (University of Naples-Federico II). They were selected for their discriminating abilities and were previously trained in the sensory procedure.

Procedure. After refrigerated storage overnight, 16-mL samples of all formulations (Table 1; serving temperature ~9°C), each coded with a 3-digit random number, were given simultaneously to the judges for sensory evaluation. The assessors were instructed to rinse their mouths thoroughly with water in between samples. Assessors evaluated the yogurt samples by using a modified approach of the flash profile method, as proposed by Liu et al. (2016). During the first session, the judges were asked to position each sample in a bidimensional space using Fizz software (version 2.45G; Biosystèmes, Couternon, France) according to their differences and similarities in such a way that the more similar they were, the closer they would be on the screen (Perrin et al., 2008; Liu et al., 2016). They were also instructed to input noted attributes next to each sample. All generated attributes were then pooled into a global attribute list. During the second session, all samples were presented again to the judges while they compared their individual attribute list with that of the global list to choose up to 10 of the most relevant attributes. Furthermore, the judges had to define each attribute and specify the evaluation technique used. In

Table 1. Yogurt formulations

Sample	Milk (mL)	Starter culture (mL)	Sweetener (g)	Citric acid (mL)	Flavor (mL)	Water (mL)
Control	1,000	40	—	30	—	10
Sucrose	1,000	40	30	30	—	10
Aspartame	1,000	40	0.28	30	—	10
MNEI ¹	1,000	40	0.03	30	—	10
Control + flavor	1,000	40	—	30	10	—
Sucrose + flavor	1,000	40	30	30	10	—
Aspartame + flavor	1,000	40	0.28	30	10	—
MNEI + flavor	1,000	40	0.03	30	10	—

¹MNEI = modified single-chain form of the natural sweet protein monellin extracted from the fruit of *Dioscoreophyllum cumminsii*.

the third session (ranking test), the judges ranked the intensity of each attribute for each sample according to their individual vocabulary lists. This ranking procedure was repeated twice over 2 consecutive days. Yogurt samples for the sensory evaluation were prepared in 2 batches: one for sessions 1 and 2 and the other for the 2 replications of the ranking test. The MNEI-sweetened yogurt with flavor (**MNEI flav 2**) was included in the sample test twice as a hidden control.

Data Analysis

Rheological and syneresis data were subjected to one-way ANOVA and post hoc Duncan's test ($\alpha = 0.05$) using SPSS (version 17.0; SPSS Inc., Chicago, IL). Ranking data given by each judge for each individual attribute were used to build individual matrices (samples $\times$ attributes). For samples with equal rank orders (tied samples), a mean value was used. The flash profile data set was also averaged over the 2 individual repetitions. Individual matrices were assembled and analyzed by generalized Procrustes analysis (**GPA**) to obtain a sample consensus map and loadings of individual attributes configurations (XLSTAT version 2014 5.03, Addinsoft, New York, NY).

RESULTS

Rheological Characterization of Yogurt

The gelation kinetic profile of microbially acidified milk is shown in Figure 1. After 2 h at 43°C, the G' value remained low (~ 0.1 Pa), characteristic of a liquid-like behavior. This was followed by an increase in G' to a value >1 Pa between 240 and 260 min, when the highest change in G' was observed, indicating the formation of a 3-dimensional gel structure. For the control and sweetened with MNEI samples, this turning point of G' was observed at 240 min, followed by aspartame (time $t = 250$ min) and sucrose ($t = 260$ min), all occurring at a pH of ~ 5.2 . After gelation, G' continued to increase but started to plateau from approximately $t = 330$ min. Upon cooling, G' increased, first linearly at about 12 Pa/°C, and then exponentially at a maximum rate of about 127 Pa/°C (Figure 2).

Based on Table 2, the addition of sweeteners did not significantly affect ($\alpha = 0.05$) the rheological properties of the yogurt during fermentation at 43°C and cooling to 4°C, except for the sample with sucrose, which differed significantly from the control but not from the yogurts sweetened with MNEI or aspartame ($P = 0.003$). The yogurt with sucrose registered the lowest G' throughout the fermentation and cooling steps. The

final pH of the yogurt sweetened with sucrose was significantly higher than that of the control ($P = 0.009$) but not compared with MNEI- or aspartame-sweetened samples ($\alpha = 0.05$).

Degree of Syneresis

The overall average syneresis in the samples was $23 \pm 1.7\%$, but the addition of the sweeteners did not significantly affect ($\alpha = 0.05$) the degree of syneresis in the yogurt samples after overnight refrigerated storage (results not shown).

Sensory Evaluation

Diversity of Attributes Generated and Used by the Panel. During session 1, the judges generated 5 to 14 attributes, resulting in a total of 81 attributes and 35 unique attributes (Table 3).

After the panelists were given the chance to compare their own lists with the global attribute list, each panelist narrowed their own attribute list to between 6 and 10 attributes, reducing the total attributes used for the flash profile to 59 with 2 unique attributes. After attribute comparison by the judges, the most-used attributes in both generations were sweetness, presence of air bubbles, sourness, creaminess, strawberry odor, fruity flavor, and bitterness (Tables 4 and 5). In addition, the judges highlighted astringency and berry flavor after comparing their own lists with that of the global attribute list (Table 5). In both cases, the panel seemed to discriminate samples first by taste followed by mouthfeel and odor.

Sensory Characterization of Yogurt Samples Using Ultra Flash Profile and Flash Profile. A consensus test was performed to check whether the consensus configuration was a true consensus. Judges' responses were highly replicable and GPA significantly reduced the variance and was suitable for data treatment because the quartile was beyond the confidence interval (data not shown). Furthermore, GPA was used to generate the consensus map of the samples (Figure 3a) and the global attribute map (Figure 3b).

In Figure 3a, the configuration of each sample by each judge and consensus points are displayed. It can be seen that judges evaluated samples similarly and, thus, consensus was a good representation of individual sample configuration. The samples were more differentiated along the x-axis, which explained most of the observed variance (71.61%). The shorter the distance between samples, the more similar they were judged to be. The sample sweetened with MNEI was positioned close to that of control, whereas samples sweetened with

Figure 1. Elastic modulus, G' , versus time during milk fermentation at 43°C of yogurt samples sweetened with sucrose, aspartame, or MNEI (modified single-chain form of the natural sweet protein monellin extracted from the fruit of *Dioscoreophyllum cumminsii*) or unsweetened (control). Mean results of 3 independent trials $\pm$ standard deviation.

aspartame and sucrose were positioned far from them. Likewise, with the addition of flavoring, the 2 samples sweetened with MNEI (MNEI flav and MNEI flav 2) were correctly identified as being equal to each other and both were positioned close to that of added flavor, whereas aspartame + flavor and sucrose + flavor were positioned farther from the MNEI and control samples.

The attributes generated per judge (Table 5) were transformed into a global attributes map (Figure 3b). Regarding sensory attributes that discriminated among the samples, the judges agreed on most of the attributes used: sourness, sweetness (intensity), presence of air bubbles, astringency, creaminess, fruity flavor, and strawberry odor.

Table 2. Effect of sweetener addition on the rheological parameters measured during yogurt fermentation for 7 h at 43°C and cooling to 4°C¹

Parameter ²	Control	Sucrose	Aspartame	MNEI ³
t_{gel} (min)	258 $\pm$ 13 ^{ab}	283 $\pm$ 21 ^b	267 $\pm$ 15 ^{ab}	250 $\pm$ 17 ^a
G'_{gel} (Pa)	3.21 $\pm$ 2.80 ^a	2.36 $\pm$ 1.13 ^a	4.77 $\pm$ 0.45 ^a	3.94 $\pm$ 3.62 ^a
pH_{gel}	5.21 $\pm$ 0.00 ^a	5.25 $\pm$ 0.08 ^a	5.25 $\pm$ 0.00 ^a	5.24 $\pm$ 0.08 ^a
G'_{end} (Pa)	254 $\pm$ 22 ^b	189 $\pm$ 51.06 ^a	223 $\pm$ 11.71 ^{ab}	226 $\pm$ 37.41 ^{ab}
pH_{end}	4.56 $\pm$ 0.02 ^a	4.66 $\pm$ 0.06 ^b	4.63 $\pm$ 0.03 ^{ab}	4.56 $\pm$ 0.06 ^a
$G'_{4°C}$ (Pa)	2,204 $\pm$ 32 ^b	1,798 $\pm$ 352 ^a	1,799 $\pm$ 80 ^{ab}	1,998 $\pm$ 223 ^{ab}

^{a,b}Means within a row with different superscript letters indicate significant differences ($\alpha = 0.05$).

¹Mean results of 3 independent trials $\pm$ SD.

² t_{gel} = time (min) at which elastic modulus (G') > 1 Pa; G'_{gel} = G' at t_{gel} ; pH_{gel} = pH at which $G' > 1$ Pa; pH_{end} = pH after 7 h of fermentation at 43°C; G'_{end} = G' after 7 h of fermentation at 43°C; $G'_{4°C}$ = G' after cooling from 43° to 4°C.

³MNEI = modified single-chain form of the natural sweet protein monellin extracted from the fruit of *Dioscoreophyllum cumminsii*.

Figure 2. Elastic modulus, G' , versus temperature during cooling from 43°C to 4°C after fermentation of yogurt samples sweetened with sucrose, aspartame, or MNEI (modified single-chain form of the natural sweet protein monellin extracted from the fruit of *Dioscoreophyllum cumminsii*) or unsweetened (control). Mean results of 3 independent trials $\pm$ standard deviation.

It is possible to characterize the samples by comparing panels a and b of Figure 3. The control sample, placed to the right along the first dimension (F1), was characterized by judges as sour, astringent, bitter, creamy, and less sweet than the other samples. It was also correctly described as having an inherent intense cheese flavor without fruity/berry flavor or odor. The presence of air bubbles seemed to be the discriminating attribute in samples sweetened with MNEI, whereas “sweetness” was not correlated with samples prepared

with MNEI. The samples sweetened with aspartame and sucrose were perceived as sweet. In the unsweetened flavored control sample, strawberry odor was perceived more than fruity/berry flavor. In contrast, the flavored samples sweetened with aspartame or sucrose were perceived as sweeter than the same samples without flavoring agent and had the most intense fruity/berry flavor and odor.

DISCUSSION

Rheological Characterization of Yogurt During Fermentation

During yogurt fermentation, milk sugar lactose is converted into lactic acid. The resulting decrease in pH lowers the net negative charge of the casein particles and the colloidal calcium phosphate, which binds the casein micelles together (Walstra, 1990; Horne, 1998). The colloidal calcium phosphate is leached out into the serum and, at a certain pH (i.e., ~5.2; Table 2), micelle coagulation is initiated. Then, as pH reaches the isoelectric point of casein (pH 4.6–4.7), maximum curd firmness is obtained, entrapping the fat globules and residual serum (Afonso and Maia, 1999). The metabolic

Table 3. Number of attributes generated and used by the panel for session 1 (ultra flash profile) and session 2 (flash profile)

Panelist	Ultra flash profile	Flash profile
Judge 1	6	9
Judge 2	10	6
Judge 3	9	7
Judge 4	14	10
Judge 5	5	4
Judge 6	13	7
Judge 7	12	8
Judge 8	12	8
Total number of attributes	81	59
Total number of unique attributes	35	22

Table 4. List of attributes generated and frequency of use by the panel during session 1 (ultra flash profile)

Attribute	Frequency
Creaminess	8
Sweetness (intensity)	7
Sourness	7
Presence of air bubbles	5
Bitterness (intensity)	4
Fruity flavor	3
Strawberry odor	3
Overall flavor	2
Presence of syneresis (visual)	2
Smoothness	2
Density	2
Homogeneity	2
Global odor	2
Milk odor	2
Saltiness	2
Unknown aftertaste	2
Watery	2
Strawberry flavor	2
Berry flavor	2
Cheese flavor	2
Bitter aftertaste	2
Sweet aftertaste	2
Sour aftertaste	2
Whiteness	1
Bitter (persistence)	1
Sweet (persistence)	1
Yeast flavor	1
Lemon flavor	1
Fruity aftertaste	1
Viscosity	1
Adhesiveness	1
Fluidness	1
Astringency	1
Excessive salivation	1
Dryness in the mouth	1
Total elicited attributes	81

activity of lactic acid bacteria, which promotes the association of casein micelles by lowering pH, corresponds to an increase in G' during fermentation (Bensmira et al., 2010), as observed in yogurt samples during fermentation in the current study (Figure 1). All samples produced the typical gelation kinetic profile of a microbially acidified milk (Lucey and Singh, 1997; Haque et al., 2001; Kristo et al., 2011).

The lowest final G' value in the sample sweetened with sucrose was in contrast to the expected effect of increasing the total solids of milk (Sodini et al., 2004), such as by the addition of sucrose and sweeteners; that is, an increase in G' , perceived thickness (Lee and Lucey, 2010), and mechanical firmness of yogurt (Oliveira and Damin, 2003).

Likewise, the relatively higher final pH of the yogurt sweetened with sucrose seems to deviate from the established ability of bacteria to use sucrose if added at <3 g/L as a source of nutrition, promoting their proliferation and thus facilitating pH reduction (Kim

et al., 2009). But at >5 g/L (Kim et al., 2009), sucrose can hamper microbial activity due to an osmotic effect (Béal and Helinck, 2014). This is why, despite the standard usage level for sucrose of 0.3 g/L in fermented dairy foodstuffs that has been shown to not affect bacterial growth (Béal and Helinck, 2014), it is still recommended to add sweetening agents after fermentation.

In this study, the weaker milk gel of sucrose-sweetened yogurt with a reduced G' value may have arisen due to reduced protein aggregation (Brennan and Tudorica, 2008; Lazaridou et al., 2008, 2014), which depends heavily on the concentration and molecular and structural features of the added substance (Ramirez-Santiago et al., 2010; Corredig et al., 2011; Repin et al., 2012).

Aspartame and MNEI did not appear to affect the gelation and cooling kinetics of the yogurt, perhaps because their concentrations were low compared with that of sucrose. The concentration of sucrose was about 100 and 1,000 times that of aspartame and MNEI, respectively.

Degree of Syneresis

A higher degree of syneresis is generally associated with a weak gel characterized by the presence of larger pore size and the propensity toward casein particle rearrangement in the network of gelled coagulum (Lee and Lucey, 2004, 2006). Although the gel rheology of sucrose-sweetened yogurt was significantly different

Table 5. List of attributes generated and frequency of use by the panel during session 2 (flash profile)

Attribute	Frequency
Sweetness (intensity)	7
Presence of air bubbles	6
Astringency	6
Sourness	5
Creaminess	4
Strawberry odor	4
Berry flavor	4
Bitterness (intensity)	3
Fruity flavor	3
Cheese flavor	2
Strawberry flavor	2
Sweet aftertaste	2
Viscosity	2
Adhesiveness	1
Watery	1
Milk odor	1
Global odor	1
Sweetness (persistence)	1
Saltiness	1
Bitter aftertaste	1
Sour aftertaste	1
Fruity aftertaste	1
Total elicited attributes	59

from that of the control, the difference was small enough that the degree of syneresis in the sucrose-sweetened yogurt did not differ significantly from the control other samples. This confirms that, in general, the addition of sweeteners did not affect the fermentation kinetics of yogurt during its production, despite small differences observed between the control and sample with sucrose.

Sensory Evaluation

Taste is the primary factor that consumers consider in a variety of food categories (Tuorila and Cardello, 2002; Urala and Lähteenmäki, 2004; Verbeke, 2006). In the current study, panelists seemed to first discriminate yogurt samples by taste attributes. The diversity of the attributes generated and used by the panel during sessions 1 and 2 showed that the ultra flash profile method helped judges form an initial impression of a sample's characteristics and was able to elicit more discriminant attributes in flash profile while still being consistent with the most discriminant attributes in both scenarios. The simultaneous serving procedure facilitates better discrimination because it allows judges to have a direct comparison among samples (Delarue and Sieffermann, 2004). The comparison between their own attributes and the global list gave judges the chance to adapt their own list, thereby improving overall agreements on attributes. This reduction in the number of attributes used is desirable for the application of flash profile because too many attributes can blur the results (Tekin, 2014).

Given the relative positioning of the samples (Figure 3a) and the discriminant attributes used by the judges (Figure 3b and Table 5) for taste, odor, and flavor attributes, the samples sweetened with sucrose and aspartame were mainly characterized by sweet and fruity attributes, whereas the unsweetened (control) and the MNEI-sweetened yogurts were characterized by sour and dairy attributes. This was expected because when sweetness was not perceived, the judges had to focus their attention on other discriminant attributes.

Considering that yogurts sweetened with different sweeteners generally had the same textural attributes and that MNEI seems to be able to impart sweetness in milk but not in yogurt, we can hypothesize that the mechanism of sweetness release of MNEI is not governed by texture but might be due to its interaction with the food matrix, causing a modification in its configuration that consequently affects its sweet taste perception, as has been indicated in previous studies (Di Monaco et al., 2016; Miele et al., 2017b). Moreover, MNEI has been shown to be stable at pH 2.9 to 7 (Spadaccini et al., 2001, 2015; Rega et al., 2015) and the sweetness perception of a sweet protein (such as curculin) may be

prolonged in an acidic food system because the divalent cations of saliva do not bind to the hydrophobic end of the sweet taste receptor responsible for the sweetness perception (Kurihara, 1992). But this does not seem to apply to MNEI even though yogurt is an acidic food matrix. To maintain the sweetness of MNEI, its positive surface charge and 3-dimensional structure need to be retained (Esposito et al., 2006; Xue et al., 2009; Leone et al., 2016). It is possible that MNEI was affected by the hydrophobic and coulombic interactions that milk proteins undergo as the casein precipitate, as milk becomes slightly acidic during fermentation (Lucey and Singh, 1997; Lee and Lucey, 2004, 2010). This is in agreement with previous findings indicating that, despite its enhanced stability compared with its natural precursor monellin, MNEI is still affected by pH changes that can promote unfolding, aggregation, and precipitation with resulting loss of sweetness (Esposito et al., 2010; Aghera et al., 2011; Rega et al., 2015).

Meanwhile, the observed enhancement of the sweetness and fruity/berry flavor and odor in the flavored samples sweetened with either aspartame or sucrose confirms previous reports of synergistic effects between certain sweeteners and flavors such as in the case of aspartame and berries (Glória, 2003; King et al., 2003).

It has long been accepted that the general physical, microstructural, and rheological attributes of acid milk gels can be used as predictors of their general sensory characteristics (Lucey, 2002). Using dynamic linear viscoelastic measurements, it has been ascertained that weak gel's rheological properties and sensory thickness are directly proportional (Baines and Morris, 1987; Richardson et al., 1989; Hill et al., 1995), which in turn has a general inverse effect on strength of observed flavor and taste (Christensen, 1980; Baines and Morris, 1987; Malkki et al., 1993), as observed in the current experiment. Furthermore, flavor detection is highly related to sweetness, which is negatively affected by a product's shear-thinning behavior (Abson et al., 2014). However, even if rheological results showed small differences among sweeteners, these differences were lost after coagulum breaking, and textural attributes did not discriminate among samples. Only for MNEI the "air bubbles" attribute was cited by 6 of 8 judges. This finding indicated a strong ability of the MNEI protein to incorporate air during coagulum rupture and to retain air in the matrix over time (the samples were tasted the next day).

CONCLUSIONS

Correlations among rheology, physical structure, and sensory characteristics of yogurts were examined to better evaluate how the type and amount of sweetener

can affect yogurt fermentation and its physical properties, as well as sweetness and flavor perception. When added at typical usage levels, sweeteners do not generally affect the yogurt fermentation process. Combining rheology and sensory characterization is a powerful means of evaluating the feasibility of innovative methods to develop sweetened yogurts without the calories of sugar. Through the use of the ultra flash profile and flash profile methods, we demonstrated that although texture can be strongly linked to sweetness and flavor perception, this does not hold for the sweet protein MNEI. Indeed, MNEI seemed to be unable to impart sweetness in yogurt when added before fermentation. Hence, future studies should be conducted to better understand how sweet proteins behave in yogurt when added after fermentation.

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