ABSTRACT

To successfully substitute sucrose for sweeteners, further studies must be carried out based on previous knowledge of sweetener concentration to determine the equivalent sweetness of such compounds. In this work, sweetness equivalence of strawberry-flavored yogurt with different sweeteners and/or their combinations (aspartame, acesulfame-K, cyclamate, saccharin, stevia, and sucralose) and yogurt sweetened with 11.5% w/w sucrose was measured using the sensory magnitude estimation method. The sweetness concentrations equivalent to strawberry yogurt sweetened with 11.5% w/w sucrose in the tested sweeteners were 0.072% w/w for aspartame, 0.042% w/w for aspartame/acesulfame-K (2:1), 0.064% w/w for cyclamate/saccharin, 0.043% w/w for cyclamate/saccharin (1:1)/stevia (1.8:1) and 0.30% w/w for sucralose. These results can promote the use of different sweetener combinations in strawberry-flavored yogurt, specially acesulfame-K and stevia, once they produce more pleasing in this product.

PRACTICAL APPLICATIONS

This study provides some useful information, since there is no data in the literature about sweetness equivalence of sweeteners in yogurt, but only in simpler matrices such as pure water, juices, coffee, and teas. The use of stevia blend presented several advantages such as increased sweetening power, demonstrating the potential of this natural sweetener. The magnitude estimation method has been successful in this study, being an important tool for development of new low-calorie products. It may be noted that when evaluating different types of food using the same kinds of sweeteners, these promote distinct characteristics and that reflect directly on the sensory quality of the final product. Thus, such studies generate important information for the food industries working with dietetic food.

INTRODUCTION

The consumption of low-calorie foods and noncaloric sweeteners has been rapidly increasing. With increased consumer interest in reducing sugar intake, a great number of sweeteners during the last decade have triggered the development of new sugar-free products. Changes in eating habits and lifestyle are mainly due to the incessant search for health (Pinheiro et al. 2005). These products play an important role in the diets of patients with diabetes, in addition to being an alternative that health-conscious people use to avoid diseases caused by high sucrose intake, such as obesity and tooth decay, or simply to maintain physical fitness (Cardello et al. 1999; Castro and Franco 2002).

Attempting to meet this public’s expectation, the food industry has been introducing into the market low-calorie
products containing different types of sweeteners used as sucrose substitutes. The category of low sugar yogurt represents a large market share. However, some restrictions are imposed, associated to the use of some sweeteners, possibly due to the lack of information about their sensorial characteristics (Reis 2007).

Various types of sweeteners are allowed for consumption. Salts of saccharine and cyclamate, aspartame, sucralose, acesulfame-K and stevia leaf extract are permitted by the Brazilian legislation (Brasil 1995) to be used in dietetic foods and beverages with defined quantities of acceptable daily intake. These sweeteners have specific sensory characteristics of sweet taste intensity and persistence and the presence of a residual bitter taste. These characteristics can also differ based on temperature, acidity, sweetener concentration and chemical composition of the food product (Schiffman et al. 1995, 2000; Cardoso et al. 2004).

With a large number of sweeteners available, each one can be used in the situations they are best suited for, and limitations of individual sweeteners can be overcome by using them in blends (Meyer 2002; Nabors 2002). The use of sweetener blends presents several advantages such as increased stability of sweetness, synergism effect that increases sweetening power, reduced costs, improved sweetness quality, which results in the reduction of undesirable effects of some combined sweeteners and production of sweeteners with sucrose-like effects (Lim et al. 1989; Lindley 1991; Portmann and Kilcast 1998).

In order to substitute sucrose successfully, it is necessary to know previously sweetener concentrations that would be used and their sweetness equivalency related to sucrose. One of the most utilized methodologies to obtain this information is magnitude estimation and the graphic presentation of the normalized results using Steven’s power function (Stone and Oliver 1969; Moskowitz 1970; Bonnans and Noble 1993; Marcellini 2005; Cardoso and Bolini 2007).

The magnitude estimation method has been successfully used by many researchers to determine the relative sweetness of different sweeteners under different systems (Cardello et al. 1999; Cardoso et al. 2004; Marcellini 2005; Cardoso and Bolini 2007).

Thus, this study aimed to determine the sweetness equivalence of aspartame, sucralose and combinations of cyclamate and saccharin, cyclamate, saccharin and stevia and of aspartame with acesulfame-K in strawberry-flavored yogurt sweetened with 11.5% w/w sucrose.

**MATERIALS AND METHODS**

**Sweeteners**

The sweeteners utilized were sucrose (analytical grade), aspartame (NutraSweet, Chicago, IL), stevia leaf extract (Steviafarma, Paraná, Brazil), cyclamate and saccharin (M.Cassab, São Paulo, Brazil); sucralose (Splenda Johnson, Fort Washington, PA) and acesulfame-K (Lowçucar, Paraná, Brazil).

**Strawberry-Flavored Yogurt Processing**

The yogurt was processed using pasteurized whole milk (3.0% fat) in two stainless steel fermentation tanks with 50 L capacity. The processing was conducted with three repetitions.

The milk was heated to 85°C and maintained at this temperature for 15 min. Subsequently, the temperature was reduced to 42–45°C and the lactic culture *Streptococcus salivarius* ssp. *thermophilus* and *Lactobacillus delbruechii* ssp. *bulgaricus* (Christian Hansen, Brazil) was added (0.02% final concentration) to initiate the fermentation process. After 4–5 h, the product reached 0.7–0.75% lactic acidity (expressed in terms of lactic acid) and pH 4.5–4.6. At this point, the mixture was cooled at 25°C to stop the fermentation process. Then, 1.2% strawberry pulp with seeds (Ritter Alimentos SA, RS, Brazil) was added to the yogurt and gently mixed. The product was packed into 1,000 g polyethylene containers and maintained under refrigeration (4°C). The sweeteners were added after the strawberry-flavored yogurt processing.

**Preselection of the Taster Team**

Preselection was performed by the Wald sequential probability ratio test (Wald 1947) using two yogurt samples: yogurt sweetened with 11.5% w/w sucrose (sample A) and yogurt sweetened with sucrose 11.5% w/w plus aspartame 0.25% w/w (sample B). To confirm whether the samples were statistically different, a paired comparison test was first performed with 30 panelists by presenting them the two yogurt samples. The samples presented a significant difference at 0.5% of probability, and thus were used during the taster selection phase.

To select the sensory panelists with the Wald sequential method (Wald 1947), a series of triangular tests was conducted in which the candidates were offered the two yogurt samples: A (containing 11.5% w/w sucrose) and B (containing 11.5% w/w sucrose + 0.25% w/w aspartame). Each taster performed no more than three triangular tests per day so as to preserve their sensory ability.

The parameters used in the sequential analysis were: $p_0 = 0.45$ (maximum unacceptable ability), $p_1 = 0.75$ (minimum acceptable ability), $\alpha = 0.10$ (likelihood of accepting a candidate without sensory acuity) and $\beta = 0.10$ (likelihood of rejecting a candidate with sensory acuity).

Based on these parameters, a graph was obtained delimiting the following three regions: acceptance, indecision and
rejection of the sensory panelists. The sensory panelists were selected according to the number of triangular tests conducted and the cumulative number of correct judgments.

Sweetness Equivalence Determination

Preliminary Tests. Preliminary tests were conducted to define the concentrations of each sweetener to be used in the strawberry-flavored yogurt. In this phase, the strawberry-flavored yogurt was processed (as described in item 2.2) once, only for this test. The importance of these tests was to define the concentrations of the sweeteners to be added to the yogurt in order to obtain sweetness intensities lesser and greater than that of the yogurt with 11.5% sucrose.

During the preliminary tests, some sweeteners were observed to be unable used alone due to their undesirable flavor, sweetness absence, accentuated bitterness or change in the characteristic flavor of the yogurt. Thus, some probable combinations among all the sweeteners were tested to minimize such undesirable effects. Some combinations were empirically defined until a product with more pleasing flavor was obtained.

Yogurt Sample Preparation for the Magnitude Estimation Test. The yogurt samples evaluated by the sensory panelists were prepared following the way: the yogurt was processed as described above, once the five concentrations of each sweetener or combinations were defined, the samples were prepared and weighed on an analytical scale (the concentration was expressed in % w/w); the sweeteners were mixed into the yogurt with a glass rod. Table 1 shows the five concentrations of each sweetener or sweeteners blends used for the magnitude estimation test.

Training the Panelists to Utilize the Sensory Magnitude Scale. Fifteen tasters were selected by the Wald sequential analysis (Wald 1947) and trained to use the sensory magnitude scale with samples of different sweetness intensities to measure sweetness equivalence.

The training entailed a thorough explanation of the methodology, correct use of the scale and verifying whether the samples prepared had sweetness intensities perceived as greater or lesser than the reference sample (11.5% w/w sucrose).

During training, the panelists evaluated four samples, one being the reference (yogurt with 11.5% w/w sucrose), coded with the letter R. The reference sample was designated with the intensity of 100 (an arbitrary value), followed by a random series of samples with intensities both less and greater than the reference intensity. Three sweetener blends were utilized in this phase: cyclamate/saccharin/stevia, cyclamate/saccharin and aspartame/acesulfame-K.

The subject had to estimate the sweetness intensity of the unknown samples relative to the reference. For example, if a sample is two times sweeter than the reference, it should receive an intensity of 200; if the sample is half sweet, the intensity should be 50, and soon. They were instructed not to rate the samples’ intensity as zero.

Final Evaluation of the Yogurt Samples. The yogurt samples added to the different sweeteners or their combinations were presented to panelists in individual cabins under white light, coded with three-digit random numbers at 4–6°C and arranged in complete blocks.

In each session (for each yogurt processed), six samples were presented to the panelists, one being the reference (R) and the others, five different concentrations of the same sweetener or combinations of sweeteners (Table 1). The panelists taste each sample three times.

Data Analysis of the Sensorial Magnitude Estimation Method. The concentration/sensory stimulus curves for each sweetener correspond to a power function (Eq. 1):

\[ S = aC^n \]  

where \( S \) is the sensation perceived through a stimulus, \( C \) is the concentration of the stimulus and \( a \) and \( n \) are the parameters of the estimated model.

For data analysis, the values of sweetness magnitude estimated (\( S \); grades given to each yogurt sample in relation to the reference) were expressed using geometric average. For each yogurt sample, the \( S \) and \( C \) values were plotted in log-log scale

<table>
<thead>
<tr>
<th>Sweeteners</th>
<th>Concentration (% of mass)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>5.870 8.210 11.500 16.100 22.540</td>
</tr>
<tr>
<td>Aspartame</td>
<td>0.068 0.109 0.174 0.278 0.445</td>
</tr>
<tr>
<td>Aspartame/acesulfame-K (2:1)</td>
<td>0.012 0.019 0.030 0.048 0.077</td>
</tr>
<tr>
<td>Sucralose</td>
<td>0.014 0.023 0.036 0.058 0.092</td>
</tr>
<tr>
<td>Cyclamate/saccharin (2:1)</td>
<td>0.050 0.070 0.098 0.137 0.192</td>
</tr>
<tr>
<td>Cyclamate/saccharin (2:1)/stevia (1.8:1)</td>
<td>0.028 0.039 0.055 0.077 0.106</td>
</tr>
</tbody>
</table>
(base 10), thereby obtaining a graph with the lines of each yogurt. The linear regression model was adjusted to the observed data for each yogurt sample with the sweetener or combination of sweeteners, estimating the parameters $a$ and $n$, and obtaining the power functions for each sample.

**RESULTS AND DISCUSSION**

**Selection of Sensory Panelists by the WALD Sequential Method**

The graph obtained from the parameters defined for the sequential method is presented in Fig. 1. Through seven triangular tests, an adequate number of sensory panelists (12) was obtained and placed in the acceptance zone. However, tests were continued to obtain a greater number of selected panelists because the dropout level in sensory tests is relatively high. Thus, after 12 triangular tests, 15 panelists were selected.

After selection, the panelists were trained to utilize the magnitude scale, although they were already duly prepared to apply the magnitude estimation test because the sequential analysis is considered more rigorous than the others.

**Sweetness Equivalence**

The preliminary tests showed that the sweeteners cyclamate, saccharin, stevia and acesulfame-K could not be added purely to strawberry-flavored yogurt. This result was already expected for cyclamate and saccharin. Although displaying a high sweetening power, the latter also has a metallic flavor and bitter taste that intensify with increased concentration, while the former has a lesser sweetening power with but also a bitter residual taste that can be minimized when associated to saccharin. Thus, the ability of the cyclamate and saccharin association to mask the undesirable taste of both was confirmed while simultaneously increasing the sweetening power of the former (Cândido and Campos 1996; Cardoso and Bolini 2007). The ratio established for these sweeteners was 2:1, which is a similar ratio to that of a highly commercialized table sweetener in Brazil, and a ratio that has been already used in other studies (Cardello et al. 1999, 2000, 2001; Cardoso et al. 2004).

The sweeteners stevia and acesulfame-K were tested in their pure form at different concentrations. Stevia was found to alter the color and flavor of the strawberry-flavored yogurt. Acesulfame-K presented a rather accentuated bitter taste during tasting. According to Horne et al. (2002), acesulfame-K presents a bitter taste after ingestion, more intense and lasting longer than that of aspartame or alitame. Tunaley et al. (1987) found it difficult to measure the sweetness intensity of stevia and acesulfame-K due to the perception of other stimuli, such as high bitter taste and astringency, respectively. Marcellini (2005) did not obtain a satisfactory result measuring sweetness equivalence using stevia in pineapple juice, attributing this to the high bitterness produced by the sweetener. All of these observations are coherent with the results obtained by Cardello et al. (2000), who verified that it was impossible to measure sweetness equivalence of stevia in its pure form compared with the aqueous solutions with concentrations superior to 10% w/w of sucrose due to the elevated bitter taste.

Due to the above-mentioned difficulties in the isolated use of such sweeteners, it was satisfactory testing sensory combinations that could be used in strawberry-flavored yogurt. Thus, stevia was mixed with cyclamate/saccharin (2:1), combinations already utilized in table sweeteners. Acesulfame-K was mixed with aspartame in a proportion of 1:2, a combination also used by a commercial brand of “light” strawberry-flavored yogurt. An acesulfame-K–aspartame blend has a sweet taste and its degree of sweetness is approximately 300–400 times higher than of sucrose, depending on the concentration used (Pinheiro et al. 2005).

To define the concentrations of the sweeteners, preliminary tests were performed based on their sweetening intensity and concentrations already defined in studies conducted by Cardoso et al. (2004). However, these tests showed that the sweeteners did not produce sweetness sensations in the strawberry-flavored yogurt. Emphasizing the difficulty found in measuring such concentrations is important because the power of such sweeteners is defined individually and separately, and is expressed relative to aqueous sucrose solutions.

The relationship between the sweetness intensities and the sweeteners’ concentrations are represented in logarithmic scale in Fig. 2. These results allowed the determination of the concentrations of each sweetener or combinations of
sweeteners equivalent to the sucrose concentration (11.5% w/w) in strawberry-flavored yogurt.

The positioning of the curves indicates the sweetening intensity of each sweetener. When comparing the curves of sweetener combinations, one observes that the aspartame and acesulfame-K combination increased sweetening intensity compared with pure aspartame. This synergism effect between the two sweeteners was verified in various studies. For instance, the use of pure acesulfame-K produces an elevated bitter taste (Nabors 2002). The same occurred in the cyclamate, saccharin and stevia combination, in contrast to cyclamate/saccharin. Thus, the effect of synergism among the sweeteners was confirmed. What makes their combinations interesting is how they present superior equivalent sweetness, reduce costs and in most products, they produce more pleasing flavors (Lindley 1991).

Based on the data obtained through the magnitude estimation method, the power functions for each sweetener or sweetener combinations were obtained, and consequently, the parameters $a$ and $n$ are presented in Table 2. The literature does not include power values of these sweeteners in strawberry-flavored 11.5% w/w sucrose yogurt sweetened. Thus, a parallel was made with data found in the literature for other products.

In relation to the angular coefficient ($n$) values, different results were verified by Cardoso et al. (2004) in cold tea (6C) sweetened with 8.3% w/w sucrose. The authors found superior angular coefficient values for sucrose, sucralose, aspartame and cyclamate/saccharin (2:1) compared with the yogurt in the present study. Therefore, it can be concluded that sweetness perception was faster in tea than in the 11.5% w/w sucrose strawberry-flavored yogurt. This study confirms the influence of the product’s characteristics on the properties of sweetness perception of the sweeteners.

The concentration values of each sweetener that must be added to the strawberry-flavored yogurt to promote the same sweetness sensation found in the sucrose yogurt are presented in Table 3. These flavors were calculated using the power functions obtained for each sweetener.

In accordance with Table 3, the lowest amount of sweetener needed to promote the same sweetness equivalence of

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**TABLE 2. RESULTS OF MAGNITUDE ESTIMATION TEST TO DETERMINE THE EQUI-SWEET OF THE SWEETENERS IN RELATION TO SUCROSE 11.5%**

<table>
<thead>
<tr>
<th>Sweeteners</th>
<th>Power function</th>
<th>$n$</th>
<th>$a$</th>
<th>$R$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>$S = 0.705 \ C^{1.9745}$</td>
<td>1.9745</td>
<td>$-0.1518$</td>
<td>0.92</td>
</tr>
<tr>
<td>Sucralose</td>
<td>$S = 1,239.08 \ C^{0.7529}$</td>
<td>0.7529</td>
<td>3.0931</td>
<td>0.94</td>
</tr>
<tr>
<td>Aspartame</td>
<td>$S = 534.56 \ C^{0.6863}$</td>
<td>0.6863</td>
<td>2.7280</td>
<td>0.96</td>
</tr>
<tr>
<td>Aspartame/acesulfame-K (2:1)</td>
<td>$S = 15,502.44 \ C^{1.634}$</td>
<td>1.634</td>
<td>4.1904</td>
<td>0.98</td>
</tr>
<tr>
<td>Cyclamate/saccharin (2:1)</td>
<td>$S = 1,808 \ C^{1.1011}$</td>
<td>1.1011</td>
<td>3.2572</td>
<td>0.98</td>
</tr>
<tr>
<td>Cyclamate/saccharin (2:1)/stevia (1.8:1)</td>
<td>$S = 4,671.97 \ C^{1.2648}$</td>
<td>1.2648</td>
<td>3.6695</td>
<td>0.92</td>
</tr>
</tbody>
</table>

$S =$ sensory stimulus perceived, $C =$ sweetener concentration (% of mass), $n =$ slope, $a =$ intercept, $R =$ Pearson correlation coefficient.
11.5% sucrose in strawberry-flavored yogurt occurred when sucralose was used. On the other hand, aspartame and cyclamate/saccharin (2:1) had to be added in greater concentrations. For cyclamate/saccharin/stevia and aspartame/acesulfame-K, the amount added was practically the same: 0.043 and 0.042, respectively.

In the present study, the amount necessary to produce the same sweetness intensity found in yogurt sweetened with 11.5% sucrose was much higher that the values measured by Cardoso et al. (2004) in tea with 8.3% sucrose and by Cardello et al. (1999) in aqueous solutions containing 10% sucrose. In contrast to Cardello et al. (1999), the sweeteners in this study were added to yogurt, which is a more complex matrix with various constituents interfering in the sweetness equivalence. Cardoso and Bolini (2007) measured the sweetness equivalence of aspartame, sucralose and 2:1 cyclamate and saccharin combination in peach nectar compared with 10% sucrose sweetened nectar and found for nectar, the amount of sweetener necessary to produce the same sweetness in sucrose was higher than in tea and water. This result was also verified in the present study.

The sweetener potency, or sweetening power, was defined as being the number of times a compound is sweeter, based on its equivalent sweetness to sucrose. In this study specifically, the sweetener potency was defined according to Eq. (2):

\[ P_{ed} = \frac{C_{ed}}{11.5} \]  

(2)

where \( P_{ed} \) is sweetener potency and \( C_{ed} \) is the sweetener concentration equivalent to yogurt with 11.5% sucrose.

For all sweeteners or combinations analyzed, the sweetener potency was measured according to Eq. (2) as shown in Fig. 3. Sucralose had the highest sweetener potency (388.08). These results were also found by Moraes and Bolini (2010) in coffee beverage at 9.5% of sucrose (635), Cardoso and Bolini (2007) who found a value of 629 in peach nectar and by Cardoso et al. (2004) where sucralose was 679 times sweeter than sucrose at 8.3% in hot tea and 554 times in iced tea.

Aspartame presented itself as being the lowest sweetener power (160.41). This value is close to the one found by Cardoso and Bolini (2007) in peach nectar at 10% of sucrose and found a potency of 185. In hot tea, equivalent to 8.3% of sucrose, Cardoso et al. (2004) determined a potency of 163. Aspartame presented itself as being 187 times sweeter than sucrose in instant coffee with 9.5% of sucrose (Moraes and Bolini, 2010).

There was practically no difference between the aspartame/acesulfame-K (273.14) and the cyclamate/saccharin/stevia combinations (266.76). These associations were satisfactory as they increased significantly the sweetener potency and they also can promote the use of acesulfame-K and stevia in strawberry-flavored yogurt.

The cyclamate/saccharin/stevia combination presented greater sweetening power (266.76) than cyclamate/saccharin (179.75). Thus, a synergy effect occurred when stevia was added. The same effect was observed by Iop et al. (1999) in powdered desserts.

Moraes and Bolini (2010) found that cyclamate/saccharine mixture (2:1) has a sweetening power of 280 in instant coffee with 9.5% of sucrose; this value is close to the one found by Cardoso et al. (2004) of 272 in hot tea equivalent to 8.3% of sucrose; by Cardoso and Bolini (2007) of 280 in peach nectar equivalent to 10% of sucrose; and by Umbelino (2005) of 223 in mango juice equivalent to 8.0% of sucrose and 220 in mango nectar equivalent of 7.5% of sucrose.

Schiffman et al. (1995) obtained values of 385 for sucralose, 107 for aspartame and 59 for stevia, compared with the aqueous 10% sucrose solution.

It is important to verify the difference in sweetening power when the product is more complex, i.e., when other ingredients, such as fat, proteins, acids, carbohydrates, etc., are involved. When a sweetener or sweetener combinations is added to the food product, one must consider the various interactions among the sweeteners and the food ingredients promoting a change in the sweetener potency. In addition to yogurt ingredients, other factors must be considered, such as the concentration of the sucrose solution to be compared, pH, acidity and temperature.

For this study, the concentration of the solution to be compared was 11.5% sucrose for the strawberry-flavored yogurt. Increased concentration of the sucrose solution decreases the sweetening power (Schiffman et al. 1995; Cardello et al. 1999).

The influence of temperature at consumption was verified by Schiffman et al. (2000). The authors observed that aspartame sweetening power is lower in aqueous solutions at a temperature of 6°C than at 50°C. However, Cardoso et al.

Bonnans, S. and Noble, A.C. 1993. Effect of sweetener type on the perception of sweetness in orange juice, lemon juice and fruit ice creams. Stampanoni (1993) studied the influence of citric acid content on the perception of sweetness in orange juice, lemon juice and fruit ice creams. Bonnans and Noble (1993) studied the influence of sucrose concentration (8, 10 and 12%) and aspartame at the same equivalence (0.06, 0.07 and 0.08%) in aqueous solutions at different citric acid concentrations (0.075, 0.15 and 0.225%). The authors concluded that increased acid concentration decreased sweetness intensity for all sucrose and aspartame levels.

**CONCLUSION**

The magnitude estimation method allowed the determination of sweetness equivalence of aspartame, sucrose, aspartame/acesulfame-K (2:1), cyclamate/saccharin (2:1) and cyclamate/saccharin (2:1)/stevia (1.8:1) in strawberry-flavored yogurt compared with traditional yogurt (11.5% sucrose). The magnitude estimation method allowed the determination of sweetness equivalence of aspartame, sucralose, aspartame/acesulfame-K (2:1), cyclamate/saccharin (2:1) and cyclamate/saccharin blend as compared to sucrose at different concentrations. Plant Foods Hum. Nutr. 54, 119–130.

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