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MULTIPLE TIME-INTENSITY ANALYSIS: SWEETNESS, BITTERNESS, CHOCOLATE FLAVOR AND MELTING RATE OF CHOCOLATE WITH SUCRALOSE, REBAUDIOSIDE AND NEOTAME

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ABSTRACT

The objective of this research was to compare the dynamic sensory profile (multiple time-intensity analysis) of milk chocolate formulated with different sweeteners. Eight different milk chocolates were formulated as follows: four milk chocolates (sweetened with sucrose, sucralose, rebaudioside and neotame) and four soy-based chocolates, using soy extract as milk replacement. The multiple time-intensity analysis tested the following four attributes by evaluating them in separate instances with an intensity reference for each attribute: sweetness, bitterness, chocolate flavor and melting rate. Twelve assessors evaluated the samples with four repetitions according to the time required to evaluate each attribute. The collected curve parameters were statistically analyzed by analysis of variance, Tukey's test and principal components analysis. The samples and attributes were evaluated individually, and their results were simultaneously represented graphically by an overlay of curves to visualize the results. The neotame formulation of milk chocolate and sucralose soy-based chocolate presented multiple time-intensity results with parameter curves that were not significantly different ($P > 0.05$) from those of the sucrose control. The other sweeteners may also be interesting alternatives in product development, especially in chocolate formulation for dietetic purposes.

PRACTICAL APPLICATIONS

Time-intensity analysis is different from conventional descriptive analysis because it allows one to verify changes in the perception of an attribute over time. The sensory results obtained in this study are useful for researchers and product developers working with different sweeteners in food, especially in chocolate products. The time-intensity method was an important tool used to determine the concentrations of sweeteners providing the same sweetness as the sucrose sample. This study considered the multiple perceptions, analyzed by temporal characteristics, involved in producing quality diet and light milk chocolates. Multiple time-intensity analysis graphically represents dynamic profiles by overlaying curves of collected attributes (register of intensity of one determined sensory characteristic during the consumption of a product) on two or more sensory attributes of a specific sample.

INTRODUCTION

Considered a worldwide epidemic, obesity occurs independently of economic and social conditions, and it has shown a significant increase in the last 20 years. However, it would be difficult to explain this fact based on genetic changes occurring in this time span. Thus, the main mechanisms involved in the development of obesity have been related to environmental factors, such as poor eating habits and reductions in daily caloric expenditure (Pereira *et al.* 2003).

Another worldwide concern is the incidence of lactose intolerance, which has been increasing in people who are 31 to 40 years old. However, there is a gradual decrease in lactose intolerance incidence after the age of 40 as evidenced by the capacity of the intestinal mucous to adapt to repeated aggression, thus better tolerating the continuous ingestion of lactose. In addition, there is no significant difference in the incidence of lactose intolerance between male and female individuals with percentages of 42.68 and 44.94, respectively (Pereira and Furlan 2004).

For this reason, the formulation of products by replacing sucrose and/or lactose and the knowledge of their sensory properties are of high interest to food science research to develop increased product options for people who want or need to consume this type of food.

Researches of products with sweeteners to replace sucrose is very relevant, and they are an important scientific contribution to the knowledge to sensory science because the sweetener potency and respective sensory performance can presents variations in different products in function of matrix food (Heikel *et al.* 2012; Di Monaco *et al.* 2013).

Studies on products with sweeteners to replace sucrose are relevant, and they contribute to the knowledge of sensory science because the sweetener potency and respective sensory performance can present variations in the food matrix of different products (Heikel *et al.* 2012; Di Monaco *et al.* 2013).

A previous study compared different chocolate formulations focusing on thickening agents, and the results showed no significant difference at the 5% level between chocolates produced with only polydextrose and two other formulations produced with combinations of this polymer with polyols, such as maltitol and lactitol. In this previous study, the following sensory attributes were analyzed by 30 judges: aroma, hardness, melting in the mouth and flavor (Gomes *et al.* 2007).

The time-intensity method has been used for the last 25 years as an important tool because it allows comparison of the perception of sensory characteristics in a dynamic manner and can be applied to several food products with different objectives (Giovanni and Guinard 2001; Guinard *et al.* 2007; Palazzo *et al.* 2011; Drake and Drake 2011). For example, Melo *et al.* (2007) applied time-intensity analysis in their research on the intensity and time of the perception of sweetness in the production of equi-sweet diabetic milk chocolates. Using time-intensity methodology, Gwartney and Heymann (1994) concluded that the intensity and duration of burn, bitterness and cooling of menthol is dependent upon concentration, and they also showed that the cooling and burn perception of I-menthol is more intense and lasts longer than d-menthol. Allison and Chambers (2000) used the same methodology to verify that caffeine may enhance the sweet taste of noncaloric sweeteners through neurological interactions in the oral cavity.

Palazzo and Bolini (2009) in research with raspberry gelatin, showed an extension for the classic time-intensity analysis, named Multiple Time-Intensity Analysis (MTIA) that represents graphically the dynamic profiles by curves overlay of attributes collected (register of intensity of one determined sensory characteristic during the consumption of a product) of two or more sensory attributes of a specific sample.

MTIA must be applied in studies to replace ingredients in each type of product, because this allows to show clearly that the substitution of materials promote the modification of the time-intensity profile during the time of consumption differently in each attribute analyzed, and each kind of food. This information is not supplied by a classic sensory descriptive analysis, but using MTIA, it is possible to verify when the start of the perception of maximum intensity or decay of important attributes to consumer in specific products. This information supplied by MTIA allows the choice of ingredients and their concentration that could contribute to perception of high (or low) intensity and total time of duration of sensory attributes that is previously of knowledge in accordance to preferences of consumers. This type of results can contribute to production of competitive foods, with sensory characteristics of intensity and time of duration of sensory characteristics that can have influence to the consumer's decision of purchase and choice among market products.

Sweeteners are each time more studied, because while many mechanisms were discovered, the foods are a complex system and the interactions of chemical compounds can modify the flavor sensation and sweetener power (Bolini *et al.* 1999; Palazzo *et al.* 2011; Souza *et al.* 2011).

In a study using raspberry gelatin, Palazzo and Bolini (2009) used an extension of the classic time-intensity analysis, named MTIA, which graphically represents dynamic profiles by overlaying curves of collected attributes (the intensity of a sensory characteristic during the consumption of a product) on two or more sensory attributes of a specific sample.

MTIA must be applied in studies on the replacement of ingredients in each type of product because MTIA clearly shows that the substitution of materials promotes the modification of the time-intensity profile during consumption differently for each attribute and food type analyzed, and this information is not supplied by a classic sensory descriptive analysis. MTIA identifies when the consumer perception of maximum intensity or decay of important attributes occurs in specific products. The information supplied by MTIA allows the selection of ingredients and the concentrations that contribute to the perception of high (or low) intensity and total duration of sensory attributes according to consumer preferences. These results can contribute to the production of competitive foods with sensory characteristics for intensity and duration that can influence the consumer's decision to purchase a certain product.

Sweeteners are increasingly being studied because foods are a complex system and the interactions of chemical compounds can modify the flavor sensation and sweetener effects (Bolini *et al.* 1999; Souza *et al.* 2011; Palazzo *et al.* 2011).

There is a growing interest in alternative nonnutritive sweeteners (Simons *et al.* 2008). Alternatives to sucrose serve a number of purposes, including caloric, carbohydrate or sugar intake control; assistance with weight control or reduction; and aid in the management of diabetes. If eaten as part of a healthy diet plan or combined with exercise, sweet foods can be eaten with moderation. Foods with sweeteners to replace sucrose can be appealing and assist in weight loss or weight maintenance for nondiabetic consumers. These products often also have fewer carbohydrates, which can promote health for diabetic consumers (Melo *et al.* 2010).

Melo *et al.* (2010) verified that nondiabetics and diabetics have different expectations regarding sugar claim, sweetener type and calorie reduction, which is likely due to their different nutritional needs. In addition, consumer testing has shown different acceptance means for conventional, diabetic and reduced-calorie milk chocolates among nondiabetics and diabetics. A natural sweetener (stevia) has been shown to be appealing to both consumer groups, but this natural sweetener does not meet consumer expectations of either group when tasted in chocolates. Diabetic consumers are more accepting than nondiabetic consumers of diabetic and diabetic/reduced-calorie milk chocolates. Thus, alternative products must be developed and labeled according to the specific consumer groups they are intended for.

The leaves picked from the plant known as *Stevia rebaudiana* (Bert.) Bertoni (Asteraceae), or simply stevia, contain sweetening glycosides, including steviosides, rebaudiosides and dulcosides (Fernandes *et al.* 2001). The advantages of the sweetening glycosides are based on their noncaloric characteristics, that is, they are not metabolized or fermented by the organism. Moreover, these sweetening glycosides are also noncarcinogenic, and they remain stable at a wide range of pH values and temperatures (Cândido and Campos 1996).

Neotame is a high-potency nonnutritive sweetener, and it is used in different products to replace sucrose (Prakash *et al.* 2001). Neotame has a clean sweet taste close to that of sucrose with none of the undesirable bitter or metallic aftertastes associated with other well-known artificial sweeteners (Nofre and Tinti 2000).

MATERIALS AND METHODS

The following eight samples were prepared: four different milk chocolates including a traditional one (sweetened only with sucrose) and three other samples differing only with respect to their sweetener, namely Sucralose (SPLENDA Micronized Powder, Johnson and Johnson, New Brunswick, NJ), Rebaudioside (Enliten 300000, Corn Products, Mogi Guaçu, Sao Paulo, Brazil) and Neotame (additive supplier SweetMix, Sorocaba, Sao Paulo, Brazil). As shown in Table 1, the sucrose of the diet chocolates was replaced by Polydextrose (Litesse, Danisco, Jundiaí, Sao Paulo, Brazil) and Erythritol (Zerose 16961, Cargill) (17:26), as bulking agents, and the three high intensity sweeteners. In the other

TABLE 1. RECIPES USED IN TRADITIONAL AND DIET MILK CHOCOLATES

Ingredients (%)	MSA	MSU	MRE	MNE	SSA	SSU	SRE	SNE
Sucrose	43.00	–	–	–	43.00	–	–	–
Cocoa butter	21.30	21.30	21.30	21.30	22.30	22.30	22.30	22.30
Cocoa mass	14.00	14.00	14.00	14.00	14.00	14.00	14.00	14.00
Dry milk	12.00	12.00	12.00	12.00	–	–	–	–
Nonfat dry milk	9.00	9.00	9.00	9.00	–	–	–	–
Soy lecithin	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
PGPR*	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Vanilla flavor	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Soy extract	–	–	–	–	20.00	15.80	15.80	15.80
Sucralose	–	0.075	–	–	–	0.075	–	–
Rebaudioside	–	–	0.215	–	–	–	0.215	–
Neotame	–	–	–	0.005	–	–	–	0.005
Polydextrose	–	17.00	17.00	17.00	–	21.20	21.20	21.20
Erithritol	–	26.00	26.00	26.00	–	26.00	26.00	26.00

* PGPR (polyglycerol polyricinoleate).

Coded chocolates: MNE, milk neotame; MRE, milk rebaudioside; MSA, milk sucrose; MSU, milk sucralose; SER, soy rebaudioside; SNE, soy neotame; SSA, soy sucrose; SSU, soy sucralose.

four samples, soy extract (Provesol PC, Olvebra, Porto Alegre, Rio Grande do Sul, Brazil) was used to replace the milk powder.

Sample Preparation

The chocolates were produced by conventional method by mixing in a Kitchen-Aid planetary mixer (model K5SS; Kitchen-Aid, St. Joseph, MI), refining in a Draiswerk GMBH three-roll refiner (Draiswerke GmbH, Manheim Waldo, Mahweh, NJ), conching in a Friwessa longitudinal conch (ACMC, Bohemia, NY), molding in polycarbonate molds and cooling in a Siaht tunnel cooler (Siaht, Jundiaí, SP, Brazil). All chocolates were batch produced (700 g) in the Cereal and Chocolate Research Center (ITAL/Campinas/Brazil).

The equi-sweet concentrations were determined previously using time-intensity analysis. The closest equivalents to sucrose were 0.075% sucralose, 0.215% rebaudioside and 0.005% neotame sweetener (Palazzo *et al.* 2011).

The chocolate's conching was divided into three different phases (Table 2) aiming at maximum elimination of moisture and formation of color compounds, flavor compounds and rheological properties. In the dry phase, approximately 12% total fat was incorporated into the mass allowing for maximum moisture elimination (approximately 0.5%), evaporation of volatiles (such as acetic acid) and formation of different aromatic compounds.

Intense mechanical work occurred during the plastic phase and was adjusted by the increase in mixing speed plus the mixing time. This flavors formation of the characteristic flavor and aroma compounds, and it contributes to the rheological adjustment by decreasing the viscosity of the mass. The rest of the fat was incorporated into the mass at this stage.

The last stage, which is known as liquid conching, occurred when the emulsifiers, such as soybean lecithin or polyglycerol polyricinoleate (PGPR), were added to the mixture, resulting in the final flavor and texture of the product.

The conching and mixing of each formulation performed were exactly the same procedure.

The eight formulation samples were packaged as bars and stored in a room with controlled humidity, pressure and temperature until stabilization for sensory analysis.

TABLE 2. CONCHING PARAMETERS USED IN TRADITIONAL AND DIET MILK CHOCOLATES

Phases	Speed (rpm)	Temperature (C)	Time (min)
Dry conching	48	40	10
Plastic conching	80	60	420
Liquid conching	80	60	60

Sensory Analysis

Chocolate samples (4 g) of the same size (1.5 × 1.5 × 1.0 cm) were presented in disposable plastic beakers coded with a three-digit number. Tests were performed in individual air-conditioned booths (22C) in the Laboratory of Sensory Science and Consumer Research of the School of Food Engineering (UNICAMP/Campinas/Brazil) and evaluated under white light, thus ensuring comfort and privacy for the panelists. A complete balanced block design was used (Macfie *et al.* 1989; Stone *et al.* 2012), and the samples were presented sequentially in a monadic way.

Time-Intensity Analysis

Subjects were recruited among UNICAMP undergraduates, graduates and employees, who presented themselves as consumers of milk chocolate and showed interest in becoming members of the sensory group to be trained. The panelists were first preselected, and those approved were trained to use time-intensity analysis of flavors and tastes (TIAFT) software (Bolini 2012).

In the preselection, several subjects were submitted to Wald's sequential analysis using triangle tests (Amerine *et al.* 1965) to select people with high discrimination ability. In these tests, the subjects received two milk chocolate samples sweetened with a significant difference of 0.1%, predetermined by a paired-comparison test with 60 subjects. The variable values used in Wald's sequential analysis for the selection were as follows: $r0 = 0.45$, $r1 = 0.70$, $a = 0.05$ and $b = 0.05$. Twenty-five subjects were prescreened as potential panelists.

Training Session

Training for the formation of sensory memory and equalization among the panelists was performed by direct contact of the individuals with the reference of maximum intensity for each attribute (sweetness, bitterness, chocolate flavor and melting rate). The panel was trained in six 1 h training sessions to perform the TI trials.

The time-intensity parameters of interest were Imax (maximum intensity recorded by the judge), Timax (time at which the maximum intensity was recorded), Area (area of time × intensity curve) and Ttot (total duration time of the stimulus) (ASTM 1999).

The data collection for the time-intensity analysis was carried out on a computer using a dynamic sensory profile using TIAFT, which was developed at the Laboratory of Sensory Science and Consumer Research of the School of Food Engineering (Bolini 2012).

The standardized conditions for analysis of the four attributes were as follows: (1) judge's wait time, 10 s; (2) time with sample in the mouth, 15 s; (3) time after swallowing, 50 s; and (4) intensity scales, 9.

Selection of Subjects for Time-Intensity Analysis

The 25 preselected and trained judges analyzed the eight samples with respect to sweetness, bitterness, chocolate flavor and melting rate in four repetitions using the time-intensity method. The experimental samples were used during attribute intensity, panelist selection and training sessions. The 20 selected panelists were further trained, and samples were evaluated using previously determined references in four replications. From panelist selection to data collection, the samples were presented monadically on disposable plates bearing appropriate three-digit codes using a balanced block design (Macfie *et al.* 1989). Sensory analyses were performed in individual air-conditioned (22C) booths with white light. Crackers and taste-free water were provided for palate cleansing.

After the training sessions, the samples were analyzed by all subjects for all four attributes. The samples were presented in a monadic way with four repetitions, and the panelists evaluated the sample using a computer mouse to record the intensity of the attribute on the scale according to the time. The software shows a continuous scale marked with 10 points with numbers (0 to 9) on the screen. The mouse cursor slides freely so that the trained assessor can continuously indicate the perceived intensity as a function of time. The continuous scale is horizontal, and it has 10 vertical lines indicating the numbers 0 through 9. The vertical scale is labeled such that 0 corresponds to none (far left), 4.5 corresponds to moderate (middle) and 9 corresponds to strong (far right). Data are continuously collected by the software from the start until conclusion of the test. A plot and table with each tenth of a second of analysis and its corresponding intensity were generated.

The software time manager is pre-established for each food analyzed. In the present analysis, all conditions were standardized with the same time for the four attributes. Upon hearing the first signal given by the computer (10 s) after pressing start, the panelist placed the whole sample in their mouth and indicated the intensity of the particular sensory attribute on the scale using the mouse. Upon hearing the second signal (15 s), the panelist swallowed the sample, and a third signal (50 s) indicated the end of the test.

In the selection of the judges for the TI assessment, analysis of variance (ANOVA) was applied for each panelist for each attribute separately, and 20 panelists were selected to participate according to their discriminating capability ($P < 0.30$) and repeatability ($P > 0.05$). Moreover, an individual consensus was also considered (Damásio and Costell 1991) as verified in relation to each parameter curve separately for each attribute.

Evaluation of Four Attributes by Time-Intensity Analysis

The panelists evaluated four attribute intensities during the time assessment. The following attributes were analyzed: sweetness, bitterness, chocolate flavor and melting rate of the chocolates. The samples were monadically presented with four repetitions by recording the intensity of the attribute over time. All references are described in Table 3. Each one of the attributes was analyzed separately.

The method utilized was the classic single time intensity with an extension. The four attributes were collected one at a time in four repetitions by the same group of assessors. The conditions for each of the four attributes were exactly the same as follows: (1) pieces of same shape and weight; (2) waiting time to put the sample in the mouth (10 s); (3) time of residence of the sample in the mouth (15 s) before swallowing; and (4) time of test until the total extinction of attribute (50 s). All assessors presented discrimination power verified by pF_{sample} (<0.030), reproducibility $pF_{repetition}$ (>0.05) and consensus with other members. Based on these results, a representation of means for each curve for each attribute was proposed, and the curves were then superimposed. According to ASTM (2013), various curves can be

TABLE 3. ATTRIBUTES ANALYZED IN THE TIME-INTENSITY ANALYSIS AND RESPECTIVE REFERENCES USED IN TRAINING THE JUDGES

Attributes	Definitions	References
Sweetness	Refers to sucrose	None: - Strong: Milk chocolate (Hershey's)
Bitterness	Refers to caffeine in aqueous solution	None: - Strong: Dark chocolate 50% cocoa (Garoto)
Chocolate flavor	Characteristic flavor of milk chocolate in bar	None: - Strong: Milk chocolate (Nestlé)
Melting rate	Amount of time required for solid chocolate turn into liquid while moving the tongue	None: - Fast: Milk chocolate with fast melting rate (Hershey's)

summarized by modeling the shape of the time-intensity curve, and in this case, a consensus curve is formed by plotting the model predictions. The model predictions are then calculated using the estimated panel parameters from the model fit separately for each attribute (Overbosch 1986; Dijksterhuis and Eiler 1997).

This method was used in research with raspberry-flavored gelatins and was named multiple time-intensity (Palazzo and Bolini 2009). The method shows results with the superposition of curves with different attributes in the same graphic. This representation is mentioned in ASTM (2013) as a way to represent the application of different attributes at the same time because the conditions are exactly standardized to the fundamentals of research published by Dijksterhuis and Eiler (1997) and Overbosch (1986).

Drake and Drake (2011) applied the same fundamentals to represent the time-intensity curves in studying salt. This representation was important to verify that equivalent sodium concentrations of different salts have distinct salty taste intensities and time-intensity profiles, thus demonstrating that it may be possible to substitute some sea salts for table salt in foods to help lower the sodium content.

Statistical Analysis

The parameters obtained by time-intensity curves in relation to each attribute were evaluated separately by ANOVA, Tukey's test averages ($P > 0.05$) and principal components analysis (PCA). The statistical software used was the Statistical Analysis System (SAS 2012).

RESULTS AND DISCUSSION

Twenty judges were selected to perform the analyses of sweet taste, bitter taste, chocolate flavor and melting in the mouth during four repetitions. The data were statistically analyzed and included in tables with the mean values obtained for the eight chocolates with respect to the maximum time and intensity occurring for each stimulus as well as the total duration and area under the curve.

Table 4 shows a comparison of the means obtained by the samples for sweetness, and the characteristic curves of the time-intensity analysis recorded for the four attributes (multiple time-intensity representation) are shown graphically in Fig. 1 (A to H). The means of the parameters for each sample were used to construct the curves. The same method was used for the stimuli of sweetness, chocolate flavor, bitterness and melting in the mouth. Figure 1 shows the graphic representation of MTIA, which verifies the dynamic profile for each different formulation (Fig. 1: graphics A to H correspond to each one of the formulations). The MTIA is a practical and simple way to express the results because of all them were previously standardized. These graphics should be jointly observed with the respective data analysis (ANOVA and Tukey's tests for each corresponding sensory attribute studied).

The ANOVA showed that there was no significant difference among any of the milk chocolate samples for the Area parameter at $P \leq 0.05$. An analysis of the efficiency of substituting the sucrose in the milk chocolates showed that there was greater similarity with the results obtained with neotame, which did not differ significantly from sucrose, for the attributes of Timax, Imax and Ttot. Moreover, the maximum intensity values were 6.5 and 7.0 for the chocolates made with neotame and sucrose, respectively. For the chocolates made with soybean extract, the Imax means were

TABLE 4. TIME-INTENSITY RESULTS FOR SWEETNESS

Products	TImax	Imax	Ttot	Area
MSA	24.41^{cd}	7.02^{a}	46.55^{d}	199.99^{a}
MSU	27.02^{b}	6.34^{bc}	52.08^{abc}	180.58^{abc}
MRE	29.43^{a}	6.04^{cd}	53.82^{ab}	181.86^{abc}
MNE	23.49^{d}	6.65^{ab}	45.32^{d}	201.73^{a}
SSA	24.05^{cd}	5.48^{e}	49.84^{c}	155.68^{d}
SSU	24.28^{cd}	6.58^{b}	50.36^{c}	188.86^{ab}
SER	25.12^{bcd}	6.70^{ab}	51.10^{bc}	176.98^{bcd}
SNE	25.73^{bc}	5.86^{de}	54.33^{a}	164.66^{cd}
MDS	2.01	0.40	3.02	21.97

Means with a same superscript letter are not significantly different at a 5% level.

Imax, maximum intensity recorded by the judge; MDS, minimum significant difference in *Tukey's test* ($P \leq 0.05$); MNE, milk neotame; MRE, milk rebaudioside; MSA, milk sucrose; MSU, milk sucralose; SER, soy rebaudioside; SNE, soy neotame; SSA, soy sucrose; SSU, soy sucralose; Timax, time at which the maximum intensity was recorded; Ttot, total duration time of the stimulus.

TABLE 5. TIME-INTENSITY RESULTS FOR CHOCOLATE FLAVOR

Products	TImax	Imax	Ttot	Area
MSA	23.78^{a}	6.75^{a}	48.77^{ab}	157.42^{a}
MSU	23.31^{a}	5.60^{b}	48.29^{abc}	134.79^{bcd}
MRE	23.84^{a}	6.36^{a}	47.12^{abc}	149.43^{ab}
MNE	25.33^{a}	5.49^{bc}	49.52^{ab}	129.69^{cde}
SSA	24.60^{a}	4.71^{d}	46.29^{bc}	116.40^{de}
SSU	22.91^{a}	5.04^{cd}	49.93^{a}	123.48^{cde}
SER	24.89^{a}	5.46^{bc}	50.28^{a}	136.77^{bc}
SNE	23.13^{a}	4.85^{d}	44.78^{c}	114.58^{e}
MDS	2.46	0.53	3.59	19.36

Means with a same superscript letter are not significantly different at a 5% level.

Imax, maximum intensity recorded by the judge; MDS, minimum significant difference in *Tukey's test* ($P \leq 0.05$); MNE, milk neotame; MRE, milk rebaudioside; MSA, milk sucrose; MSU, milk sucralose; SER, soy rebaudioside; SNE, soy neotame; SSA, soy sucrose; SSU, soy sucralose; Timax, time at which the maximum intensity was recorded; Ttot, total duration time of the stimulus.

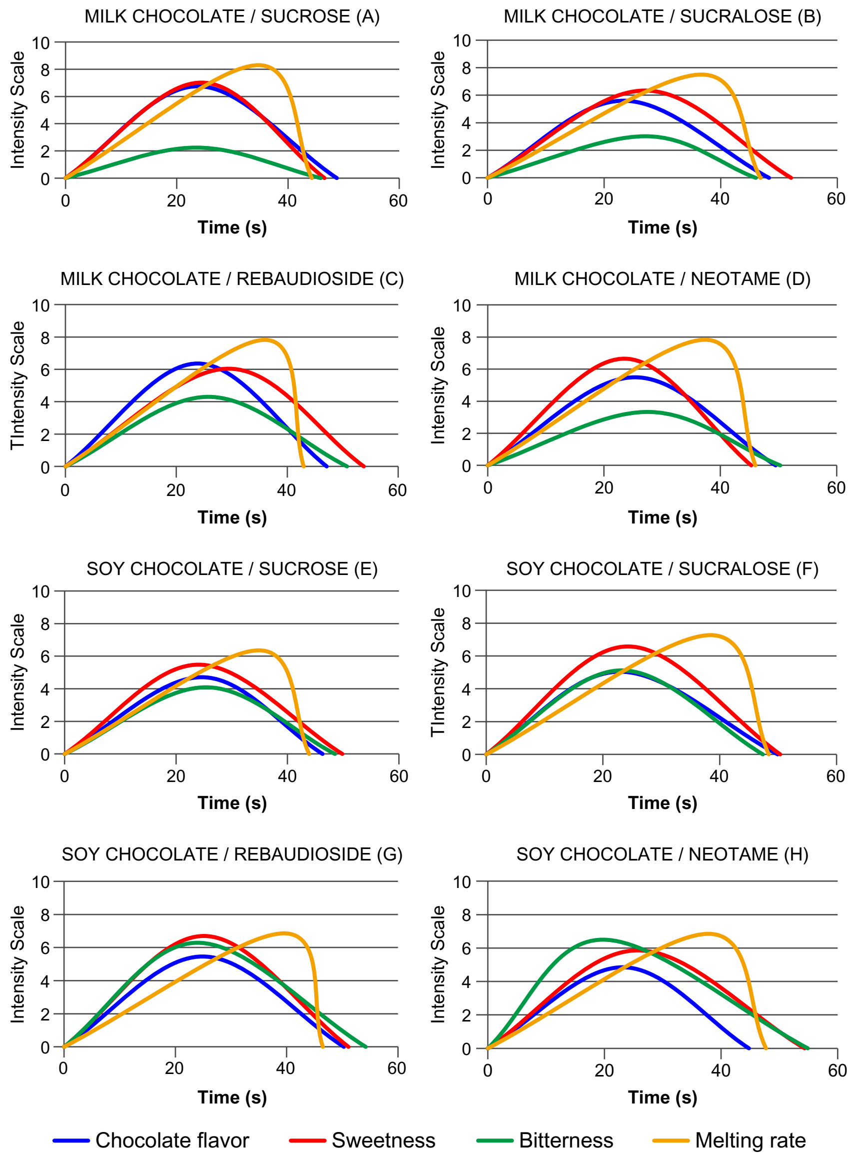

FIG. 1. MULTIPLE TIME-INTENSITY OF MILK CHOCOLATE SWEETENED WITH SUCROSE (A), SUCRALOSE (B), REBAUDIOSIDE (C) AND NEOTAME (D) AND SOY CHOCOLATE SWEETENED WITH SUCROSE (E), SUCRALOSE (F), REBAUDIOSIDE (G) AND NEOTAME (H)

5.86 and 5.48 for chocolates sweetened with neotame and sucrose, respectively (with no significant difference between them at 5%).

With respect to chocolate flavor (Table 5), rebaudioside in the milk chocolate did not differ significantly when compared to sucrose for any of the parameters analyzed. For the soy-based chocolates, the sweetener sensorially closest to sucrose was neotame. There was no significant difference among any of the milk chocolate samples and those made with soy with respect to the Timax parameter at $P < 0.05$.

Sucralose was ideal to replace sucrose in relation to bitterness (Table 6), mainly with respect to the total duration time during the analysis.

The parameters of Area and Ttot showed no significant difference at $P < 0.05$ among any of the samples of milk chocolate and soy chocolate in relation to melting in the mouth. The time-intensity curves in relation to melting rate did not differ among the samples of milk chocolate, thus showing the potential for substitution with a sweetener with respect to the time for the chocolate to dissolve in the mouth before ingestion (Table 7).

A comparison of the soy-based chocolates containing sucrose and those sweetened with neotame showed no significant difference between any of the parameters evaluated at $P < 0.05$.

Data collected for the four attributes of each sample were used in PCA as shown in Figs. 2 to 5.

Of the total variations found among the samples for sweet flavor, 62.3% was explained by the first axis (PCI) with all the Imax and Area parameters contributing

TABLE 6. TIME-INTENSITY RESULTS FOR BITTERNESS

Products	Timax	Imax	Ttot	Area
MSA	23.52[b]	2.24[e]	45.88[d]	69.74[d]
MSU	27.07[a]	3.02[d]	46.01[d]	74.83[d]
MRE	25.77[ab]	4.30[c]	50.79[b]	123.36[bc]
MNE	27.50[a]	3.33[d]	50.30[bc]	78.41[d]
SSA	25.36[ab]	4.09[c]	48.51[bcd]	119.79[c]
SSU	23.22[b]	5.12[b]	47.39[cd]	146.58[b]
SER	23.99[b]	6.29[a]	54.20[a]	208.54[a]
SNE	19.74[c]	6.50[a]	54.87[a]	221.79[a]
MDS	2.97	0.64	2.97	24.96

Means with a same superscript letter are not significantly different at a 5% level.
Imax, maximum intensity recorded by the judge; MDS, minimum significant difference in *Tukey's test* ($P \leq 0.05$); MNE, milk neotame; MRE, milk rebaudioside; MSA, milk sucrose; MSU, milk sucralose; SER, soy rebaudioside; SNE, soy neotame; SSA, soy sucrose; SSU, soy sucralose; Timax, time at which the maximum intensity was recorded; Ttot, total duration time of the stimulus.

TABLE 7. TIME-INTENSITY RESULTS FOR MELTING RATE

Products	Timax	Imax	Ttot	Area
MSA	34.68[b]	8.30[a]	44.27[ab]	136.05[ab]
MSU	36.70[ab]	7.50[abc]	46.83[ab]	139.47[ab]
MRE	35.96[b]	7.82[ab]	42.97[b]	141.65[ab]
MNE	37.37[ab]	7.83[ab]	46.04[ab]	153.93[a]
SSA	34.86[b]	6.36[d]	43.89[ab]	117.99[b]
SSU	38.42[ab]	7.27[bc]	48.41[a]	132.64[ab]
SER	39.51[a]	6.86[cd]	46.50[ab]	138.79[ab]
SNE	37.84[ab]	6.85[cd]	47.71[ab]	129.62[ab]
MDS	4.25	0.81	4.94	29.43

Means with a same superscript letter are not significantly different at a 5% level.
Imax, maximum intensity recorded by the judge; MDS, minimum significant difference in *Tukey's test* ($P \leq 0.05$); MNE, milk neotame; MRE, milk rebaudioside; MSA, milk sucrose; MSU, milk sucralose; SER, soy rebaudioside; SNE, soy neotame; SSA, soy sucrose; SSU, soy sucralose; Timax, time at which the maximum intensity was recorded; Ttot, total duration time of the stimulus.

positively to the variability associated with this axis. A percentage of 88.7% was obtained for the total variation explained by the PCA (Fig. 2).

The proximity of the milk chocolates containing sucrose and those sweetened with neotame (MSA and MNE) was clear, as both were characterized by the vectors of Area and Imax. The same association was observed between the soy-based chocolates (SSA and SNE) indicating that substitution with neotame with respect to sweetness is effective.

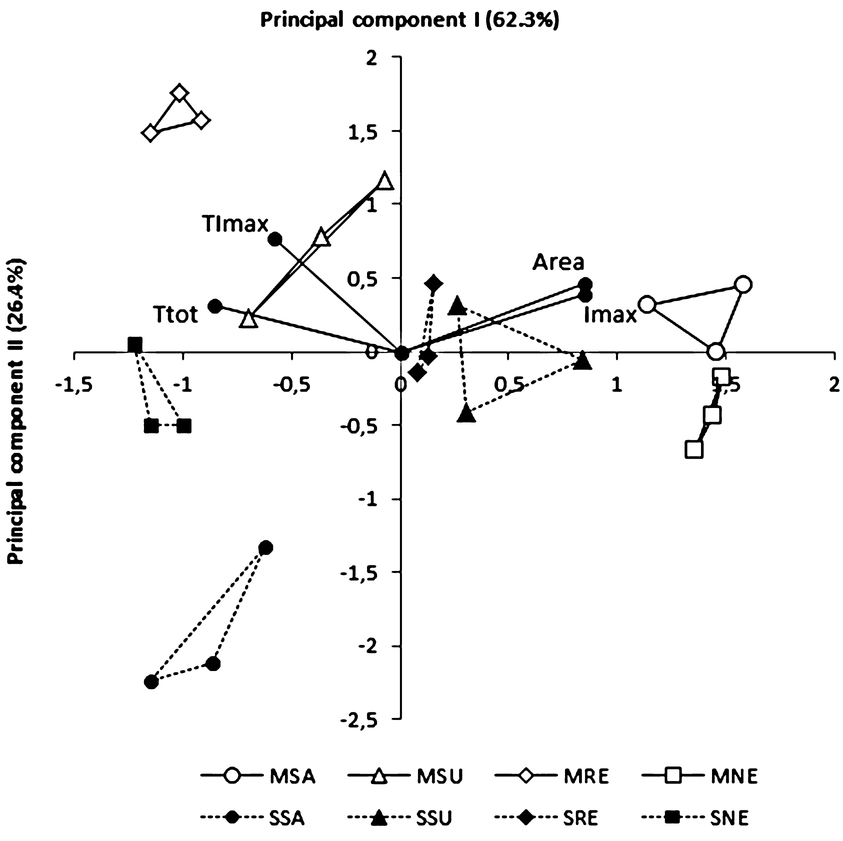

FIG. 2. PRINCIPAL COMPONENT ANALYSIS OF MILK AND SOY CHOCOLATE SWEETENED WITH SUCROSE, SUCRALOSE, REBAUDIOSIDE AND NEOTAME FOR THE ATTRIBUTE SWEETNESS

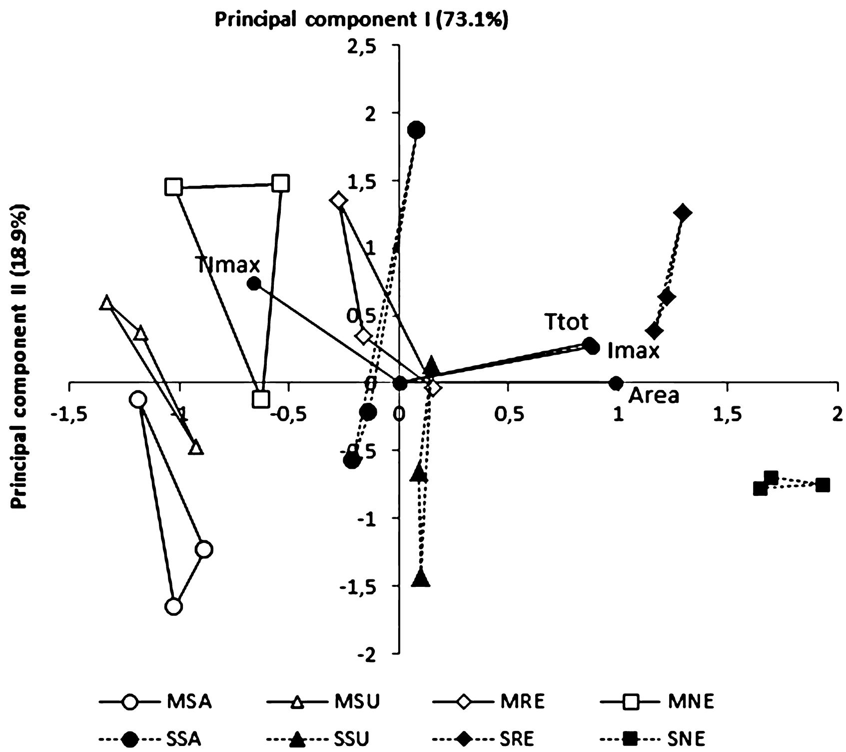

FIG. 3. PRINCIPAL COMPONENT ANALYSIS OF MILK AND SOY CHOCOLATE SWEETENED WITH SUCROSE, SUCRALOSE, REBAUDIOSIDE AND NEOTAME FOR THE ATTRIBUTE OF BITTERNESS

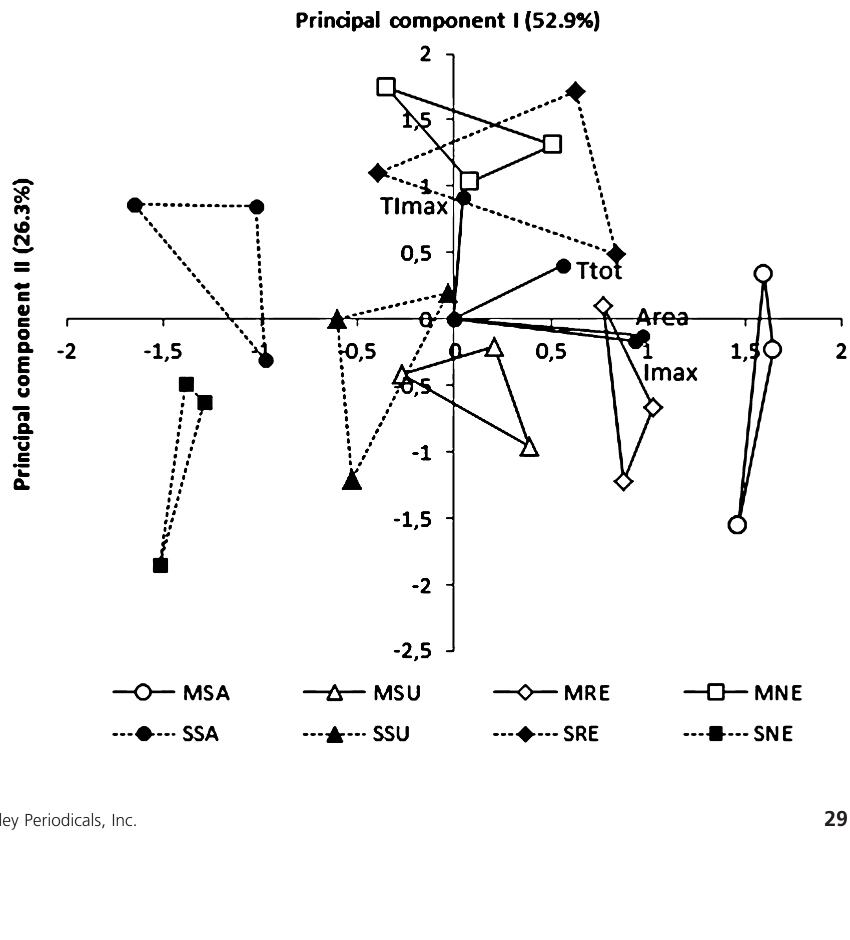

FIG. 4. PRINCIPAL COMPONENT ANALYSIS OF MILK AND SOY CHOCOLATE SWEETENED WITH SUCROSE, SUCRALOSE, REBAUDIOSIDE AND NEOTAME FOR THE ATTRIBUTE OF CHOCOLATE FLAVOR

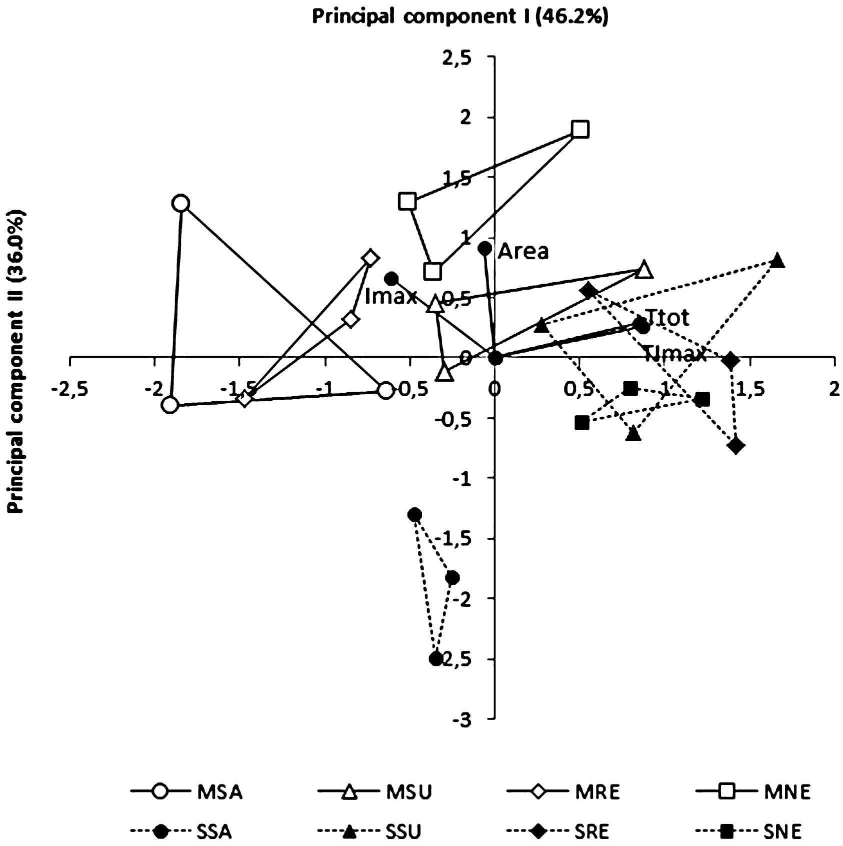

FIG. 5. PRINCIPAL COMPONENT ANALYSIS OF MILK AND SOY CHOCOLATE SWEETENED WITH SUCROSE, SUCRALOSE, REBAUDIOSIDE AND NEOTAME FOR THE ATTRIBUTE OF MELTING RATE

Moreover, 52.9% of the variation among the samples in relation to chocolate flavor was explained by the first axis (PCI), and all the parameters contributing positively to the variability associated with this axis. The Imax and Area attributes contributed more to the variability associated with this axis (Fig. 3).

The Timax attribute showed the greatest positive value on the second axis (PCII) with a percentage of 26.3%.

Similar to the sweet stimulus, the same proximity between neotame and sucrose was observed for the soy-based chocolates (SSA and SNE) in relation to the bitter taste (Fig. 4). For the milk chocolates, however, the rebaudioside sweetener was the one closest to sucrose (MRE and MSA) with both being characterized by the vectors of Area and Imax.

With respect to sucralose, the time-intensity profile behavior was observed for both of the chocolates to which this sweetener was added (SSU and MSU). For the dairy chocolates, the rebaudioside sweetener was the closest to sucrose for chocolate flavor as illustrated in the PCA with both being characterized by the vectors represented by Imax and Area. There was a clear separation between the milk chocolates located in the negative part of axis I, accounting for 73.1% of all the variation explained by the graph. In contrast, the soy-based chocolates were located on the positive side of the graph in the quadrant characterized by the presence of the vector for the maximum intensity of bitter taste.

The substitution of sucrose by sucralose was shown to be the most effective because it was closest to that of traditional chocolate (MSA and MSU). The soy-based diet chocolates were characterized by the Timax vector, which showed the longest time between placing the product in the mouth and complete melting (Fig. 5).

The figures of PCA should tell us which of the parameters of the T-I curve were relevant to provide a perceptual map of the chocolate with different sweeteners as well as to establish the variability among the respective analyzed attribute. Calvino and Garrido (2000) described the same observation with PCA of the time-intensity parameters of sweetness curves to different sweeteners (sucrose, aspartame, D-tryptophan and thaumatin) in solution of distilled water.

Studies showed the importance of food matrix and dispersion elements in the intensity of flavors and tastes. According Lawless (1986), interactions between sensory modalities are complex, ranging from inhibition of taste and odor sensations by trigeminal irritation, to relative independence of tastes from odor stimulation and independence of odors from tastes.

Comparing taste thresholds and intensities between an aqueous phase and an emulsion system, results obtained by Thurgood and Martini (2010) suggest that, depending on the type of ingredient used, the presence of a lipid phase in an emulsified form can alter the sensory perception of foods.

The graphs of the MTIA (Fig. 1) showed that the stimuli of sweet taste and chocolate flavor showed behavior characteristics as a function of time providing the desired sensation for this type of product. In contrast, bitterness showed a small accentuated profile in relation to the other stimuli and was clearly visible in the case of the milk chocolates. However, this behavior was different for the soy-based chocolates, which were characterized by bitterness, and in some samples, bitterness was higher than sweet taste and chocolate flavor.

Melting in the mouth started soon after the peaks of maximum intensity for the other stimuli, showing a curve sloping to the right. Nevertheless, the soy-based chocolates maintained profiles similar to those of the milk chocolates, thus providing positive results for the substitution of this ingredient as a function of melting during chewing.

The chocolates sweetened with sucralose were nearly equivalent with no significant differences among the parameters ($P < 0.05$) in the time-intensity curves of sweetness, chocolate flavor and melting rate in the dairy medium and soybean extract.

CONCLUSIONS

Neotame presented distinct results when comparing its use in milk chocolates with that in soy-based chocolates, and its intensity for sweet taste and chocolate flavor was greater in the samples containing soybean extract than the samples containing dairy. In the milk chocolates, however, neotame provided a sensory dynamic sensory profile with parameter curves closer to those sweetened with sucrose.

With respect to rebaudioside, there was an increase in the intensity of bitterness in relation to traditional chocolate and a displacement of the sweetness curve in relation to that of chocolate flavor. Moreover, there was an intensification of the chocolate flavor in the milk chocolate samples sweetened with rebaudioside.

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