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Miracle fruit: An alternative sugar substitute in sour beverages

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High sugar consumption has been related to several chronic diseases and thus, many alternative sweeteners have been extensively researched. However, there is still controversy regarding the harmful effects of their consumption, mainly regarding the use of artificial sweeteners, controversy which increases the demand for natural sweeteners, such as miracle fruit. This tropical plant grows in West Africa is named for its unique ability of changing a sour taste into sweet. Therefore, this study aimed to characterize the temporal profile of miracle fruit and assess its sugar substitute power in sour beverages through time-intensity and temporal dominance of sensations tests. For this, unsweetened lemonade and lemonades with sugar, sucralose and previous miracle fruit ingestions were evaluated. We noted that the dynamic profile of lemonade ingested after miracle fruit ingestion indicates that it seems to be a good sugar substitute, since it provides high sweetness intensity and persistence, reduced product sourness and an absence of aftertastes. The miracle fruit also provided a sensory profile similar to that of sucralose, an established and recognized sugar substitute. The results of this study provide important information for future applications of miracle fruit as a sugar substitute in sour beverages, providing an alternative use for a natural substance as a sweetening agent.

1. Introduction

The harmful effects related to high sugar consumption has been a matter of great public and scientific interest. These adverse effects have been associated with the development of obesity and the risk of several chronic diseases, like Type 2 diabetes and cardiovascular diseases (Hu, 2013; Jeong, Gilmore, Bleakley, & Jordan, 2014; Malik & Hu, 2015; Pineli et al. 2016). As a result, many alternative sweeteners have been extensively researched and their consumption has gained much popularity due to their reduced costs. Moreover, according to according to Gardner et al. (2012) nonnutritive sweeteners could facilitate reductions in added sugar intake and weight loss/control, promoting beneficial effects on related metabolic parameters. However there is still controversy regarding the harmful effects of consuming some sweeteners, mainly in relation to the use of artificial sweeteners (Fitch & Keim, 2012; Suez et al. 2014; Burke & Small, 2015; Pepini, 2015; Hu, 2013). Moreover, Suez et al. (2014) demonstrate that consumption of commonly used non-caloric artificial sweetener formulations drives the development of glucose intolerance through induction of compositional and functional alterations to the intestinal microbiota. Therefore, the general public has shown interest in the use of natural substances as sweeteners (Philippe, Mey, Anderson, & Ajikumar, 2014).

Miracle fruit (*Synsepalum dulcificum*) has been studied as a sweetness enhancer. It is an indigenous tropical plant growing in West Africa, named for its unique ability to change a sour taste into sweet. The active compound in miracle fruit is Miraculin, a single polypeptide chain having a molecular weight of 24,600 kDa and two sugars linked to two amino acid residues (Theerasilp & Kurihara, 1988) that bind adjacently to sweet receptor cells on the tongue, activating them in response to a low pH (Kurihara, 1992). This effect lasts until the miraculin is diluted and eliminated by saliva. Miraculin, itself, has no taste, but stimulates a sweet taste estimated to be 400,000 times sweeter than sucrose on a molar basis (Kurihara & Beidler, 1968). According to Kurihara and Beidler (1968), the miracle fruit effect occurs when the miraculin is bound to taste cell membranes near the sweet receptor site. The receptor membrane undergoes a structural change in the presence of protons (H+), causing the sugar part of the miraculin molecule to

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bind to the sweet receptor site in the membrane, thereby evoking a strong sensation of sweetness. In other words, the basis of the sweetness-inducing behavior under acidic conditions is the pH-dependent conformational changes of the receptor membrane that detects the sweet sensation (Kurihara & Beidler, 1968; Misaka, 2013).

With the taste modification function, miracle fruit has great potential to be applied in food as an alternative sweetener or taste modifier to mask undesirable sour tastes in food products (Wong & Kern, 2011). Moreover, it showed high antioxidant activity (Du, Shen, Zhang, Prinyawiwatkul, & Xu, 2014). Studies have investigated developing miraculin into an alternative sweetener (Bartoshuk, Gentile, Moskowitz, & Meiselman, 1974; Wong & Kern, 2011; Igarashi et al. 2013), its molecular mechanisms (Bartoshuk, 1987; Dzendolet, 1969; Paladino, Costantini, Colonna, & Facchiano, 2008, 2010; Yamamoto et al. 2006), and the engineering of other plants to produce miraculin (Sun, Cui, Ma, & Ezura, 2006; Sun, Kataoka, Yano, & Ezura, 2007; He at al. 2015). However, more studies are needed to characterize and compare the miracle fruit sensory profile to sucrose and other sweeteners in order to better elucidate its sugar substitute power.

According to Lawless and Heymann (1999), the consumer acceptability of different sugar substitutes depends on the similarity of their time profile to that of sucrose. Moreover, the replacement of sucrose by alternative sweeteners can produce product sensory profile changes (Souza et al. 2013). Sucralose is the only commercial sweetener derived from sucrose and is an intense sweetener made by selective substitution of the hydroxyl groups of sucrose with chlorine (Binns, 2003). According to Ketelsen, Keay, and Wiet (1993), sucralose has a taste profile very close to that of sucrose, presenting a very low level of bitterness and sourness. Therefore, in assessing miracle fruit as a sugar substitute, it is interesting to compare its sensory profile with sucrose and sucralose profiles.

Temporal methodologies such as Time–intensity (TI) and Temporal Dominance of Sensations (TDS) have proven to be efficient techniques to characterize and monitor the sensory profile of different products with substituted ingredients (i.e. sugar or salt), providing information about the temporal evolution and intensity of different sensations and off-flavors developed during food consumption (Melo, Bolini, & Efraim, 2007; Rodrigues, Golçalves, Pereira, Carneiro, & Pineiro, 2014; Souza et al. 2013). Thus, the aim of this study was to characterize the temporal profile of miracle fruit and assess its sugar substitute power in sour beverages.

2. Experimental

The study was reviewed and accepted by the Ethics Committee in Human Research of the Federal University of Lavras and it was performed in accordance with the Declaration of Helsinki.

2.1. Materials

The following is a list of materials uspred for sample preparation in this study: lemons, sucrose, sucralose (Nutramax®) and Frooties® brand dried miracle fruit.

2.2. Preparation of lemonade

Lemonades were prepared in a proportion of 3:1 (water: lemon) (w/w) with lemons characterized as medium size = 6.87 ± 0.21 cm x 6.12 ± 0.27 cm; outer skin color: L* = 41.39 ± 3.12 a* = -14.29 ± 2.50 b* = 24.81 ± 2.01 c* = 28.64 ± 1.14 Hue = 119.86 ± 2.23; and pulp color: L* = 45.20 ± 1.64 a* = -4.76 ± 1.37 b* = 11.84 ± 1.34 c* = 12.77 ± 1.23 Hue = 110.82 ± 1.93. The lemons were squeezed using a Walita RI2745® brand juicer. Thereafter, water and the sweetener (in the case of the treatments with sucrose or sucralose) were added. The lemonade was then stirred with a glass rod for 5 min. The prepared lemonade was standardized at a pH = 2.43 ± 0.20 and solid soluble (SS) = 2.1 ± 0.25 °Brix. Thus, four samples were established for this study varying the sweetener addition as presented in Table 1.

2.3. Sensory analysis

Sensory analyses were performed at the sensory analysis laboratory of the Federal University of Lavras with subjects that did not have any restriction to the products analyzed and they did not used nose clips during the sensory tests.

2.4. Ideal test

The ideal sugar concentration in lemonade was determined using sensory tests with an ideal scale (Just about-right-scale), using a linear scale with 9 cm, anchored by “less sweet than ideal” and “sweeter than ideal” at the end points and “just right” in the center. During the analysis, 75 consumers with a minimum lemonade consumption frequency of once a week evaluated the samples and recorded their responses (ideal sweetness scores) on a Just about-right-scale (Lawless & Heymann, 1999) based on how perfect these samples were regarding the sweetness using the method reported by Vickers (1988). The tested samples were determined by pre-test, in which the sucrose concentrations were defined varying from 0 to 30% sucrose (0%, 7.5%, 15%, 22.5%, 30%). Samples were served in plastic cups labeled with randomly selected 3 digit numbers, in a balanced order according to Walkeling and MacFie (1995).

The responses were converted into numerical values and analyzed by regression analysis, using the Sensomaker software (Pinheiro, Nunes, & Vietoris, 2013).

2.5. Time-intensity (TI)

2.5.1. Selection

We recruited seventeen individuals that were available and interested in participating in the research (individuals were part of the group of consumers that performed the Ideal test) to participate in the selection stage. The individuals had experience in sensory

Table 1
Lemonade samples preparation.

Samples	Preparation
Unsweetened Lemonade	Lemonade without any sweetener addition
Lemonade sweetened with sugar	Lemonade with added sugar in the concentration determined by the ideal test (134 g/L lemonade or 0.39 mol/L lemonade);
Lemonade sweetened with sucralose	Lemonade with added sucralose, based on the ideal concentration of sugar determined by the ideal test (0.022% - 0.22 g/L lemonade or 5.53×10^{-4}/L lemonade). A sweetness power of 600 was considered (Withers et al. 2016).
Miracle fruit ingestion followed by unsweetened lemonade	300 mg of spray-dried miracle fruit (content determined by pretests) was served 5 min before unsweetened lemonade intake. The panelist was instructed to place the miracle fruit on their tongue and roll it around very slowly until completely dissolved.

analysis and consumed lemonade at least once a week.

The selection stage was performed in two steps. The first step was the basic tastes identification test according to ISO 8586 (2012). The second step evaluated discrimination ability (Meilgaard, Civille, & Carr, 2006), applying triangular tests with two sucrose solutions at concentrations of 0.1 and 0.2% w/w. These samples were defined in paired comparison tests in which there was a significant difference at 1%. The results were assessed using the sequential analysis of Wald (Amerine, Pangborn, & Roessler, 1965) where from the defined parameters ($P = 0.30$, $p1 = 0.70$, $\alpha = 0.10$ and $\beta = 0.10$) the Wald graph was constructed and judges were selected or rejected according to the number of correct tests. Therefore, twelve panelists (nine women and two men, between 20 and 35 years of age – average = 27 ± 4.6) were selected to perform the TI and TDS tests.

2.5.2. Training session

The selected panelists were introduced to the time–intensity test procedure to become familiar with the methodology. They performed pretests with three lemonade samples with different sucrose contents in three replicates using the Sensomaker software (Pinheiro et al., 2013). An analysis of variance (ANOVA) was applied for each panelist and each parameter (Imax – Maximum intensity, Plateau - running time of maximum intensity, and Area – area under the curve) for each attribute (sweetness and sourness) separately, to evaluate their discriminating capability ($P < 0.30$) and repeatability ($P > 0.05$). Moreover, the team consensus was also evaluated to ensure the team was trained.

2.5.3. Final session

Twelve selected individuals performed Time-Intensity tests in triplicate in four sections for each attribute for the four lemonade samples (unsweetened lemonade, lemonade with sugar, sucralose and miracle fruit). The attributes evaluated were sweetness and sourness and the samples were presented in a monadic way, using a balanced complete block design (Walkeling & MacFie, 1995). The samples were served in plastic cups labeled with randomly selected 3 digit numbers, in a balanced order according to Walkeling and MacFie (1995). The presentation order of the samples was also randomized among subjects. The panel members were then instructed to drink the lemonade (30 ml) all at once and immediately start the evaluation for 50s, using the mouse to record the intensity of the attribute through a graphic interface in the form of a 10-point scale, with 0 meaning no perception and 10 signifying an extreme perception of the attribute (Cardello, Silva, & Damásio, 2003) on the Sensomaker software scale (Pinheiro et al., 2013). Thus, the intensity of the attribute was evaluated during and after the ingestion in order to quantify "after tastes".

2.6. Temporal dominance of sensations (TDS)

According Pineau et al. (2009), when a confidence interval of a proportion based on a normal approximation is calculated, it is recommended that $np\,(1\text{-}p)>5$, n being the number of trials and p the probability of success. In this study $p = 0.2$, so the minimum number of evaluations should be $n = 5/[0.2 \times (1-0.2)] = 32$. Thus, twelve selected panelists (the same individuals that performed TI analysis) performed the TDS tests in triplicate, totaling 36 evaluations. After the selection stage, the panel was introduced to the TDS module of SensoMaker (Pinheiro et al., 2013) and instructed that the dominant sensation is that perceived to have the greatest clarity and predominance; i.e., the most striking perception at a given time (Pineau et al., 2009). Thus, the definite tests were performed for the four samples of lemonade (unsweetened lemonade, lemonade with sugar, sucralose and miracle fruit).

The attributes involved in the TDS sensory test were sweet, sour, bitter, unpleasant, and no taste, which were earlier defined by a focus group (Lawless & Heymann, 1999). The samples were presented in disposable white plastic cups coded with three-digit numbers in monadic order, following a balanced order (Walkeling & MacFie, 1995). After instructions were presented, each panelist was asked to drink the lemonade sample (approximately 30 ml) all at once and immediately start the evaluation for 50 s, selecting the dominant sensation that was experienced during and after the lemonade ingestion (i.e over 50 s), in order to also quantify the "after tastes".

2.7. Statistical analysis

ANOVA (sources of variation: samples, panelists and sample*-panelist interactions) was performed with all Time-intensity data and Tukey's test was applied to compare the averages of samples using the Sensomaker software (Pinheiro et al., 2013). The mean TI curves were also computed. The data of the mean curve of every panelists in every replications were presented in graphic form (through calculation parameters) using the Microsoft Excel 2012. In the graphs, the horizontal axis denoted time, while the vertical axis displayed the intensity values.

The TDS curves were computed according methodology described by Pineau et al. (2009) using SensoMaker software (Pinheiro et al., 2013). Briefly, two lines are drawn in the TDS graphical display: the "chance level" and the "significance level". The "chance level" is the dominance rate that an attribute can obtain by chance and the "significance level" is the minimum value this proportion should equal, in order to be considered significant (Pineau et al. 2009). The calculations were done based on the confidence interval of a norm proportion based on a normal approximation according to Pineau et al. (2009). Three TDS parameters (DRmax - maximum dominance rate, TDRmax - time at which the maximum dominance occurs, and Plateau - duration of the attribute, i.e., the time range over which the dominance rate is equal to or higher than 90% of the maximum dominance rate.) were also computed.

3. Results

3.1. Ideal test

The results of the ideal test showed a significant difference ($F_{value\ (2,\ 293)} = 79.39$ and $p_{valu} = 0.000001$) among the lemonade samples with different concentrations of sugar (saccharose), indicating that the samples differ in relation to the ideal sweetness. Therefore, to correlate the ideal sweetness with ideal sugar content in the lemonade, the regression model was adjusted. The linear model ($Y = 0.133X+2.726$) fitted the data with a coefficient of determination of 0.95 (Fig. 1).

By setting the average score for the ideal scale at 4.5 (y in the regression equation), the amount of sugar (x in the regression equation) to be added to the lemonade was calculated and found to be 13.4% (i.e. 134 g/L lemonade or 0.39 mol/L limonade). The high sugar content considered ideal by consumers is one more indication of the need to reduce sugar consumption, using sugar substitutes. This information is relevant, since consumers are used to eating foods with high sugar content and the consumption of sugar-sweetened beverages has been growing in Brazil as well as in other parts of the world. Consumption of such products has been related to several chronic diseases (Basu, McKee, Galea, & Stuckler, 2013; Malik, Pan, Willett, & Hu, 2013; Pereira et al., 2015).

Thus, to evaluate the miracle fruit power as sugar substitute, we established the following samples: control-unsweetened lemonade

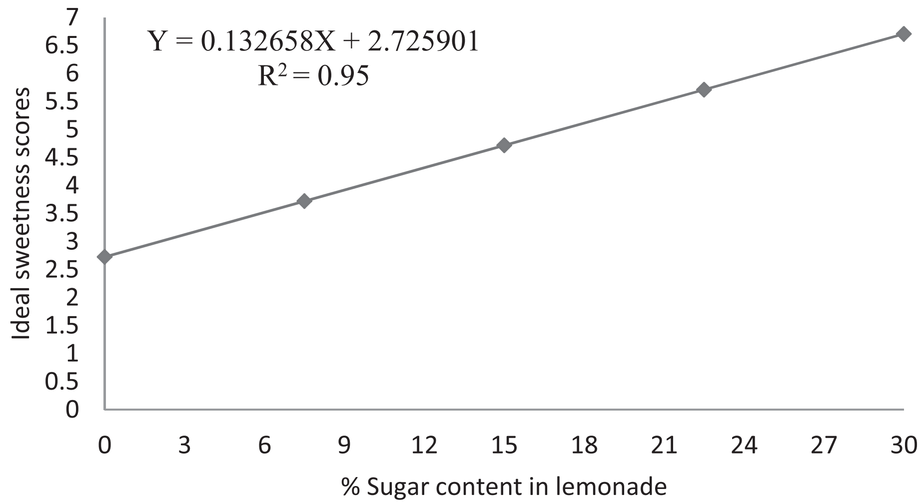

Fig. 1. Regression analysis results obtained in the sweetness ideal test for lemonade. X axis represents the sucrose content (%) in lemonade and Y represents the scores obtained for the Ideal sweetness test (0- much less sweet than ideal; 4,5- ideal sweetness; 9- much sweeter than ideal).

(without sweetener); lemonade at an ideal sugar content determined by Ideal test (13.4% - 134 g/L lemonade or 0.39 mol/L limonade); lemonade with sucralose content based on equivalence of ideal sugar content and considering its sweetness power of 600 times (Souza et al. 2013) more than sugar (0.022% - 0.22 g/L lemonade or 5.53 × 10^{-4}/L limonade); and 300 mg of miracle fruit ingestion before unsweetened lemonade intake. The miracle content was determinate following the producer recommendation, and performing a focus group, in which the participants evaluated different miracle fruit contents and verified which one most approached the lemonade sweetened with the optimal concentration of sucrose.

3.2. Time-intensity (TI)

Sugar can influence sweetness and sourness attributes in lemonade. Thus, to evaluate the sweeteners time-intensity profiles, sweetness and sourness attributes were evaluated.

Time-intensity curves for the sweetness attribute obtained for the unsweetened lemonade and lemonades with different sweeteners (sugar, sucralose and miracle fruit) are presented in Fig. 2. Fig. 2 shows that all the evaluated sweeteners (sugar, sucralose and miracle fruit) had an effective sweet effect on lemonade and have a similar time-intensity profile in relation to the sweet flavor.

The ANOVA indicates that there is a significant difference among the samples regarding TI parameters (Imax – Maximum intensity ($F_{value(3,96)} = 159.63$ and $p_{value} = 3.51xe^{-37}$), Plateau - running time of maximum intensity ($F_{value(3,96)} = 4.55$ and $p_{value} = 0.005$), and Area – area under the curve ($F_{value(3,96)} = 153.97$ and $p_{value} = 1.48xe^{-36}$). This indicates that the samples differ in relation to their temporal sweetness profiles. Thus, the means of time intensity parameters and the results of Tukey test are presented in Table 2.

From Table 2 we observed that the maximum sweetness intensity varies significantly ($p \leq 0.05$) among the samples, with sugar (Imax = 6.49) reaching approximately four times more in relation to unsweetened lemonade (Imax = 1.59), and sucralose (Imax = 5.76) and miracle fruit (Imax = 4.98) reaching 3.6 and 3.1 times respectively. However, in relation to the duration of sweet sensation (Plateau parameter), we observed that sucralose and miracle fruit showed higher values (21.22 and 21.18 respectively) in relation to sugar (16.7). Moreover, instead of a higher area under

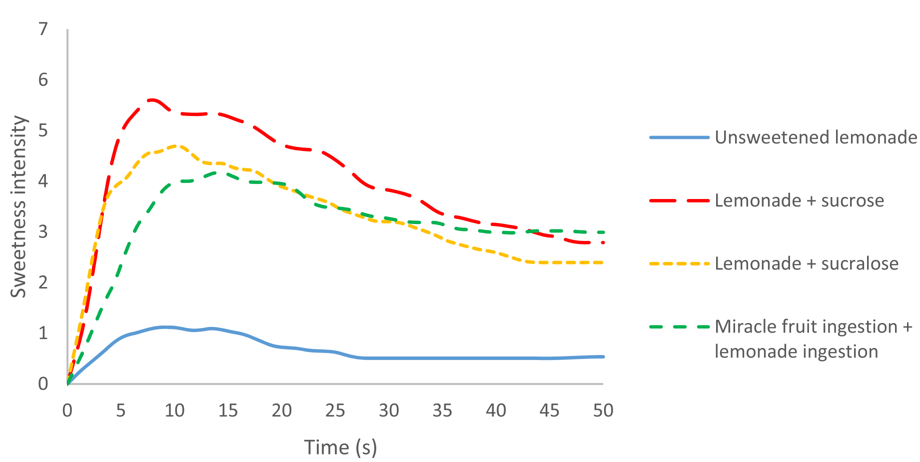

Fig. 2. Time-intensity curves obtained for sweetness intensity over time (50 s) for unsweetened lemonade, lemonade with sucrose (13.4% w/w), lemonade with sucralose (0.022% w/w) and miracle fruit ingestion (300 mg) followed by lemonade ingestion.

Table 2
Means of sweetness time-intensity parameters for evaluated lemonades.

Samples	Imax	Plateau	Area
Unsweetened Lemonade	1.59 ± 1.02 a	14.74 ± 7.71 ab	37.45 ± 18.31 a
Lemonade sweetened with Sugar	6.49 ± 1.76 d	16.7 ± 7.31 a	197.97 ± 79.22c
Lemonade sweetened with Sucralose	5.76 ± 1.98 c	21.22 ± 6.67 b	168.85 ± 74.25 b
Miracle fruit ingestion followed by unsweetened lemonade	4.98 ± 2.00 b	21.18 ± 8.12 b	159.95 ± 78.15 b

*Means followed by the same letter in the column did not differ significantly ($p \leq 0.05$) each other by Tukey test. Imax – Maximum intensity, Plateau - running time of maximum intensity, and Area – area under the curve.

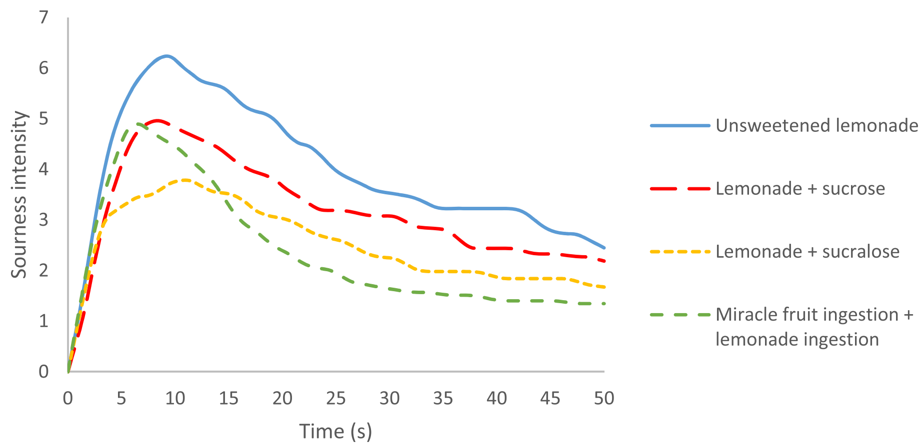

Fig. 3. Time-intensity curves obtained for sourness intensity over time (50 s) for unsweetened lemonade, lemonade with sucrose (13.4% w/w), lemonade with sucralose (0.022% w/w) and miracle fruit ingestion (300 mg) followed by lemonade ingestion.

the curve value obtained for sugar (197.97), similar values were verified for sucralose and miracle fruit (168.85 and 159.95).

The evolution of sourness intensity attribute over time for the lemonade samples (unsweetened lemonade, lemonade with sugar, sucralose and miracle fruit) was also investigated (Fig. 3) in the same way as for the sweet attribute.

Fig. 3 demonstrates that miracle fruit performed in a manner similar to sugar and sucralose, reducing sourness in lemonade. Moreover, among the sweeteners used (sugar, sucralose and miracle fruit) after 15 s of lemonade ingestion, miracle fruit was the sweetener that reduced the intensity of lemonade sourness the most. A significant difference among the samples regarding the TI parameters (Imax – Maximum intensity ($F_{value(3,96)} = 38.50$ and $p_{value} = 2.00xe^{-16}$), Plateau - running time of maximum intensity ($F_{value(3,96)} = 4.55$ and $p_{value} = 0.005$), and Area – area under the curve ($F_{value(3,96)} = 24.37$ and $p_{value} = 8.29xe^{-12}$) were observed through ANOVA, indicating that the lemonades differ in relation to their temporal sourness profiles. Thus, the Tukey test was applied (Table 3).

Table 3 shows the unsweetened lemonade reached a significantly ($p \leq 0.05$) higher sourness intensity, while the miracle fruit, sugar and sucralose did not differ significantly from each other in relation to the maximum intensity of sourness. However, sucralose and sugar presented higher sourness duration (Plateau = 17.99, 19.78) and area under the curve (142.84, 160.70) than the miracle fruit (Plateau = 12.84 and Area = 135.66), indicating that the sour sensation lasted longer when the lemonade was sweetened with them in relation to miracle fruit.

3.3. Temporal dominance of sensations (TDS)

TDS profiles and the graphic representation of dominance duration time for significant sensations of unsweetened lemonade and lemonade with the sugar, sucralose and miracle fruit sweeteners are presented in Fig. 4.

The TDS parameters (DRmax - maximum dominance rate, TDRmax - time at which the maximum dominance occurs, and Plateau - duration of the attribute, i.e., the time range over which the dominance rate is equal to or higher than 90% of the maximum dominance rate) were also obtained.

In Fig. 4, we noted that sour sensation was significantly dominant, detected over the 40 s of lemonade ingestion with a maximum dominance rate of 0.63 (Table 4), i.e., 63% of the panelists elected the sour sensation as dominant at around 10 s and the

Table 3
Means of sourness time-intensity parameters for evaluated lemonades. N = 3.

Samples	Imax	Plateau	Area
Unsweetened Lemonade	6.84 ± 2.65 a	16.95 ± 7.24 ab	200.90 ± 102.99 c
Lemonade sweetened with Sugar	5.34 ± 2.68 b	19.78 ± 7.68 b	160.70 ± 79.07 b
Lemonade sweetened with Sucralose	4.99 ± 1.99 b	17.99 ± 9.17 b	142.84 ± 70.07 ab
Miracle fruit ingestion followed by unsweetened lemonade	5.99 ± 2.38 ab	12.84 ± 5.62 a	135.66 ± 61.22 a

*Means followed by the same letter in the column did not differ significantly ($p \leq 0.05$) each other by Tukey test. Imax – Maximum intensity, Plateau - running time of maximum intensity, and Area – area under the curve.

duration of the sour sensation dominance was 35 s. The lemonade prepared with sugar had the sweet sensation as dominant at the start of the analysis (DRmax = 0.6) until 8 s, but the sour sensation had more predominance, with a dominance duration of 30 s. On the other hand, the lemonade prepared with sucralose and the miracle fruit ingestion followed by the unsweetened lemonade ingestion showed the sour sensation as a significant dominant sensation until 10 and 11 s respectively. The sweet sensation perceived as dominant during the rest of the analysis, plus, the miracle fruit, obtained a greater dominance duration for this attribute (33 s – miracle fruit and 28 s - sucralose) (Table 4).

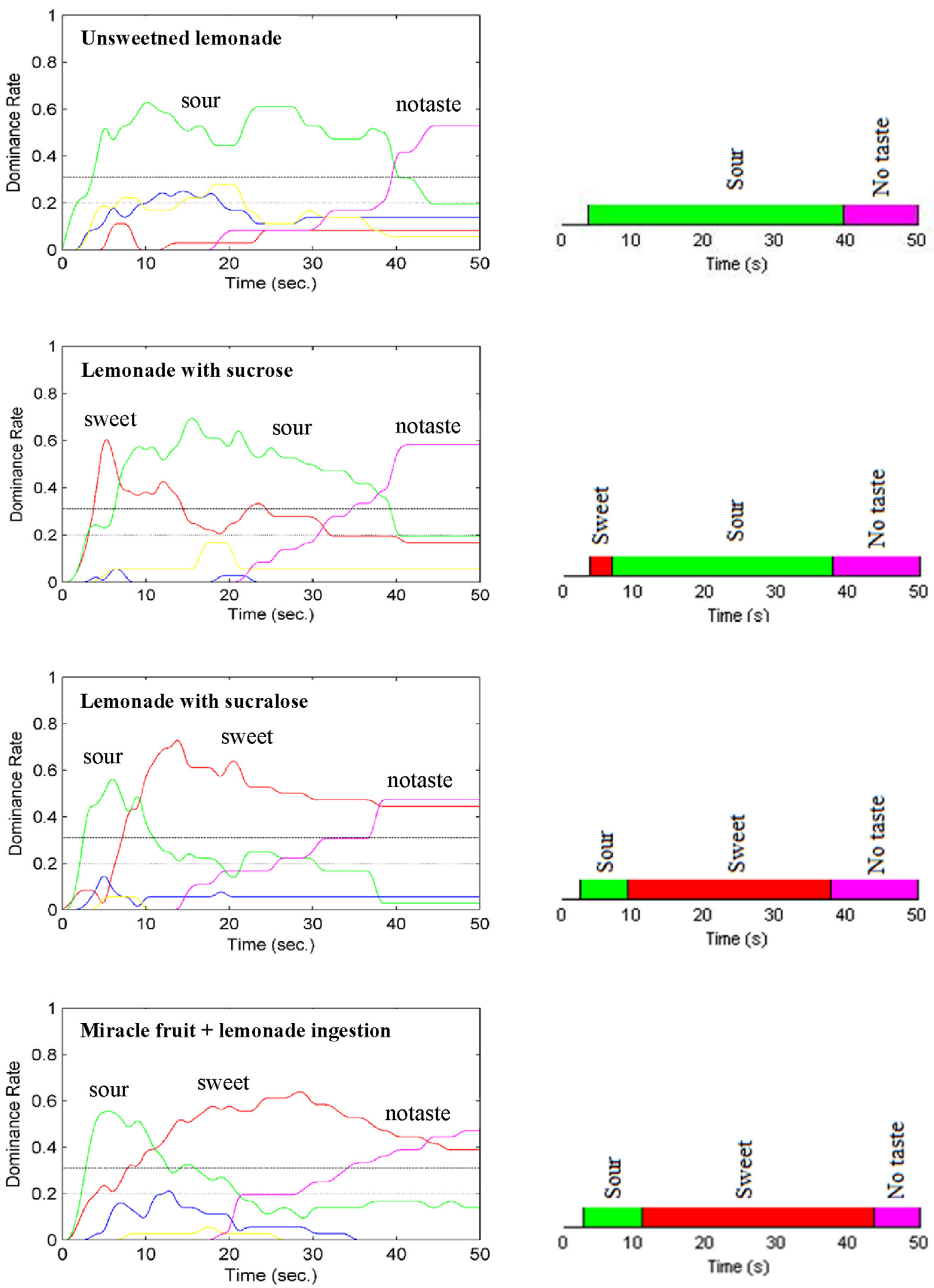

Fig. 4. TDS curves and graphic representation of dominance duration time for significant sensations over time (50 s) for unsweetened lemonade, lemonade with sucrose (13.4% w/w), lemonade with sucralose (0.022% w/w) and miracle fruit ingestion (300 mg) followed by lemonade ingestion.

Table 4
TDS parameters obtained for unsweetened lemande and lemonades with sugar, sucralose and miracle fruit.

Lemonades	TDS Parameter	Sweet	Sour	Bitter	Unpleasant	No taste
Unsweetened Lemonade	DR_max	0.11	0.63	0.25	0.28	0.53
	T_max	6.50	10.20	14.5	18.50	44.50
	Plateau	1.80	19.80	7.20	3.50	6.90
Lemonade sweetened with Sugar	DR_max	0.60	0.69	0.06	0.17	0.58
	T_max	5.30	15.50	6.50	17.50	49.60
	Plateau	1.30	7.00	0.80	3.00	10.10
Lemonade sweetened with Sucralose	DR_max	0.73	0.56	0.14	0.06	0.47
	T_max	13.80	6.00	5.00	5.50	38.50
	Plateau	3.40	1.80	0.80	3.80	12.20
Miracle fruit ingestion followed by unsweetened lemonade	DR_max	0.64	0.56	0.21	0.06	0.47
	T_max	28.50	5.50	12.70	17.50	48.50
	Plateau	14.30	5.50	2.00	1.20	6.30

DRmax - maximum dominance rate, TDRmax - time at which the maximum dominance occurs, and Plateau - attribute duration, i.e., the time span over which the dominance rate is equal to or higher than 90% of the maximum dominance rate.

4. Discussion

4.1. Time-intensity (TI)

Misaka (2013) suggests that once humans taste miraculim, it binds to hT1R2–hT1R3, the sweet taste receptor and subsequently acts as an agonist every time a sour solution is tasted. The TI results demonstrated that the miracle fruit had an effective effect on sourness perception reduction, besides contributing to the sweet taste of lemonade (Fig. 2). Bartoshuk et al. (1974) and Yamamoto et al. (2006) also noted that the miracle fruit works by reducing the acidity and intensifies the sweetness of acid products; and Wong and Kern (2011) observed in their study that miracle fruit can successfully improve the sweetness of a low sugar popsicle to a magnitude that is similar to a sugar sweetened popsicle, without subsequent energy compensation for the absent calories. Moreover, the ingestion of 300 mg of miracle fruit before lemonade ingestion presented a sweet profile similar to sucralose (Fig. 2), one of the most used sugar substitutes, as it resulted in a taste profile very close to that of sucrose (Cadena et al. 2013; Withers, Barnagaud, Mehring, Ferris, & Thomson, 2016; Zorn, Alcaire, Vidal, Giménez, & Ares, 2014).

According to Koizumi et al. (2011) and Misaka (2013), the ability of miraculim to modify taste perception from sour to sweet depends on the pH. Miraculin is likely to be in equilibrium between being an agonist and an antagonist at acidic and neutral pH, respectively. Furthermore, according to Igarashi et al. (2013), commercial sour liquids that mainly contain citric acid, products like lemonade, are more effective than acetic acid-based liquids in eliciting a perception of sweetness after the miracle fruit application.

Yamamoto et al. (2006) noted in their study that the response latency to citric acid after miracle fruit was essentially the same as that to sucrose. As for the peripheral mechanism of taste-modifying action of miracle fruits, miraculin stimulates sweet receptors under acidic conditions, i.e., acid information is not converted to sweet information, but both acid and sweet information are conveyed through the taste nerves to the brain. This explains the good results obtained in this study for the use of miracle fruit as a sugar substitute for sour beverages (i.e. lemonade). However, future studies regarding the time-intensity profile of other sour products with predominance of other acids after miracle fruit ingestion should be conducted.

4.2. Temporal dominance of sensations (TDS)

According to Souza et al. (2013) and Zorn et al. (2014), the replacement of sucrose by alternative sweeteners can produce changes in the sensory profile of the product as noted for the lemonade sweetened with sucralose and that sweetened by the effect of miracle fruit. However, similar TDS profiles were observed between them.

The substitution of sucrose by other sweeteners is a challenge for the food industry and researchers, because in addition to the sweet taste, other sensory attributes may be modified (Cadena et al. 2013). Bitterness and metallic off-flavors have been one of the most common problems of low-calorie sweeteners (Cardoso & Bolini, 2008; Du Bois & Prakash, 2012; Souza et al. 2013). In relation to these aspects, sucralose has been considered as the sweetener that best substitutes sucrose, since it provokes less sensory alterations in the product (Brito & Bolini, 2010; Souza et al. 2013; Zorn et al., 2014) as also noted in the present study. However, sucralose is among the artificial sweeteners and some toxic effects related to its heating have been investigated (Dong, Liu, Zhang, Gao, & Zheng, 2013). Moreover, a high demand for natural sweetening agents has grown (Philippe et al. 2014). In line with that, miracle fruit also presented great results regarding the lemonade sensory profile, since it showed a profile similar to sucralose and unpleasant tastes were not significantly detected. Furthermore, Pimentel, Madrona, and Prudencio (2015) suggested that the intensity and persistence of the sweet taste and the presence or absence of aftertastes are key factors for acceptance of products with sweeteners by consumers, which indicates the power of miracle fruit as a sugar substitute.

Due to the relation of high sugar ingestion and chronic diseases, regulators place pressure on the food industry to decrease the sugar content in their products (Food Standards Agency, 2008; Nestle, 2013). A typical example of a product consumed in large volumes that contains high sugar levels are sweetened beverages. Therefore sugar alternatives, with lower caloric content, are under continuous development and scrutiny to become the ideal sugar replacement (Withers et al. 2016). Through this study, we observed that miracle fruit seems to be a great sugar substitute in sour beverages, as it is a natural product that confers a sweet flavor and reduces sourness, besides presenting a sensory profile similar to sucralose. However, the product (e.g. lemonade) must not be sweetened with miracle fruit (e.g. mixing miracle fruit with the lemonade). It must always be consumed before the unsweetened beverage, once it is necessary the contact of miraculin with the membrane of taste cells, to promote a structural change in the membrane receptor, causing the sugar part of the miarculin molecule to bind to the sweet receptor site in the membrane, evoking the sweet sensation (Kurihara & Beidler, 1969; Misaka, 2013). But it is not necessary to wait between tasting miracle fruit and consuming a sour liquid (Kurihara &

Beidler, 1969). Moreover, it is important highlight that miraculin can affect other aspects of a meal, i.e., although lemonade is sometimes consumed alone, it may be part of a meal in which other sour things (such as pickles) would have substantially changed flavor profiles. Therefore, future studies assessing consumer perceptions and regarding better sensory characterization of the effects of miracle fruit on different products should be carried out.

5. Conclusion

Time-intensity and temporal dominance of sensations profiles of lemonade ingested after miracle fruit ingestion indicates that it seems to be a good sugar substitute in sour beverages, as it presents great intensity and persistence of the sweetness, reduces the product sourness and presented no aftertaste, besides providing a sensory profile similar to that of sucralose, an established and recognized sugar substitute. However, future studies of the miracle fruit effects on different products assessing consumer perception and regarding sensory characterization by other methodologies should be conducted.

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