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L. León Bianchi, M.V. Galmarini, D. García-Burgos, M.C. Zamora

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Time-intensity and reaction-time methodology applied to the dynamic perception and liking of bitterness in relation to body mass index

León Bianchi, L.^{ab}, Galmarini, M.V.^{ab}, García-Burgos, D.^c & Zamora, M.C.^{ab}

^a*Facultad de Ingeniería y Ciencias Agrarias, Pontificia Universidad Católica Argentina (UCA). Av. Alicia Moreau de Justo 1500, C1107AFF Buenos Aires, Argentina*

^b*Member of Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Godoy Cruz 2290, C1425FQB Buenos Aires, Argentina*

^c*Department of Psychology, Clinical Psychology and Psychotherapy, University of Fribourg, 2 Rue de Faucigny, CH-1700 Fribourg, Switzerland*

Corresponding author: María Clara Zamora

e-mail: czamora@uca.edu.ar

Address: Av. Alicia Moreau de Justo 1300, C1107AAZ Buenos Aires, Argentina

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Abstract

There are very few studies which have considered perception temporality when relating perceived intensity and hedonic responses in relation to body mass index (BMI; kg/cm²). The aim of the present study was to determine the relationship between BMI with the dynamic perception and liking of bitter tasting solutions. For this purpose, two different categories of bitter products were applied: 6-n-propylthiouracil (PROP) solutions (0.010, 0.032 and 0.060 mmol/L) and commercial beverages (coffee, *yerba mate* infusion and grapefruit juice). The proposed methodology to evaluate perception and hedonic response was based on the measurement of reaction-time (R-T) and multiple-sip time-intensity (T-I) registers in people with a high BMI (25 < BMI < 30; overweight group) and a normal BMI (<25; normal-weight control group). The multiple-sip evaluation to describe perception of PROP solutions and liking of beverages was used as a more ecologically valid laboratory methodology to simulate a situation of usual consumption. In this sense, working with a multiple-sip design helped confirm that bitter taste has a cumulative effect since in every case the sip effect was significant when evaluating the maximum intensity; this effect was more important as the bitterness increased. Regarding the body weight group comparisons, the normal BMI group perceived bitter taste more intensely and the time to react to it was shorter (faster reaction) for both PROP solutions and the three beverages. Interestingly, even though the high BMI group rated the bitter taste as less intense, they had a lower level of acceptance than normal BMI. This result suggests that the hedonic rather than the sensory component might be playing a crucial role in the perception of bitter taste in individuals with high BMI.

Keywords: acceptance; bitter taste; overweight; reaction-time; time-intensity

1. Introduction

High consumption of fruits and vegetables has been associated with a reduced risk of mortality with an average reduction of 5% for each additional serving a day (6% for fruit and 5% for vegetables; Wang, Ouyang, Liu, Zhu, Zhao, Bao & Hu, 2014). Indeed, the consumption of fruits and vegetables has been reported to reduce obesity, risks of cancer, cardiovascular disease, stroke, Alzheimer disease, cataracts, and some of the functional declines associated with aging (Liu, 2003; Boeing, *et al.*, 2012). However, some of these foods such as citrus fruits, cruciferous and green leafy vegetables are bitter (Drewnowski & Gomez-Carneros, 2000) and generally disliked due to the instinctive rejection of the bitter taste (Steiner, 1979).

The relationship between the sensitivity to bitterness and BMI has been suggested to play an important role in obesity. Subjects with a higher sensitivity to bitter tasting foods might be more likely to elicit a stronger rejection and avoidance of unpalatable healthy bitter substances involved in human appetite control and weight regulation (e.g., enhancing satiety; Slavin & Lloyd, 2012).

Gaudette and Pickering (2013) reviewed bitter taste as an important modulator of consumers' behavior; they analyzed new functional ingredients, including polyphenolics, employed in the creation of novel functional foods looking to reduce their aversive bitter taste. Bitterness presents temporal distinctive characteristics since it requires more time to reach maximum intensity in the oral cavity, and it takes longer to return to baseline compared with, sweet taste (Guinard, Hong & Budwig, 1995). The perceived intensity of bitterness can also increase upon repeated ingestion (Guinard, Pangborn & Lewis, 1986). This may be particularly important when consuming beverages such as red wine and beer, where the presence of polyphenolics (red wine) and isohumulones (beer) may lead to the increasing bitterness (cumulative effect) of these beverages over the length of an ingestion session (Guinard, Hong, Zoumas-Morse, Budwig & Russell, 1994; Noble, 1994). Overall, the increase in bitterness of bitter-tasting beverages over repeated ingestion may negatively impact their

acceptance, as excessively bitter-tasting foods are generally undesirable to the consumer (Drewnowski & Gomez-Carneros, 2000; Lesschaeve & Noble, 2005).

In order to overcome methodological issues and better understand the impact of bitterness perception and BMI, advanced methodology that takes into account the temporal changes in taste perception has been suggested. For example, García-Burgos and Zamora (2013) using Time-Intensity (T-I) methodology did not find any effect of BMI status on sensory response to bitter intensity of chocolate and grapefruit drink. In this work, participants were classified into two groups: low BMI ($BMI < 20$) and high BMI ($BMI > 23$). Moreover, consuming a beverage is not a matter of a single sip and it implies sensory, physiological and psychological phenomena with time (Delarue & Blumenthal, 2015; Rocha-Parra, García-Burgos, Munsch, Chirife, & Zamora, 2016; Galmarini, Loiseau, Visalli & Schlich, 2016; Thomas et al., 2017). Therefore, to further explore the sensory and hedonic experience, dynamic changes over several sips in perception and acceptance of bitter taste should be considered. In this sense, it remains to be explored whether the evaluation of the cumulative bitter sensation in multiple sips might be a more sensitive method to detect differences in bitterness perception across different BMI status.

Regarding the García-Burgos and Zamora's (2013) study, potential differences were observed at the beginning of the T-I curves, in which low BMI subjects started the curves with zero values (scale 0-100) while approximately 32% of high BMI subjects started with values higher than 30 during the evaluation of the more bitter product. Would it be possible to consider that high BMI subjects can perceive bitter taste faster? To explore such a possibility R-T methodology may be an appropriate measure (Bonnet, Zamora, Buratti, & Guirao, 1999; Guirao & Zamora, 2000). R-T to taste is defined as the minimum time required by a subject to report any taste changes after onset of taste stimulation at low concentration levels (Bonnet et al., 1999). T-I and R-T methods give complementary information (Galmarini, Zamora & Chirife, 2009) since T-I is a quantitative, dynamic method using a scale while R-T is a qualitative rather

than quantitative test (bitter - no bitter). Moreover, they reflect different moments of perception: R-T methodology shows what happens at the beginning of the tasting while T-I methodology depicts the rest of the tasting, allowing the research to explore the entire process of tasting during ingestion.

The aims of the present work were to analyze the relationship between BMI and: a) the perception of bitter taste, b) the hedonic response to bitter beverages. The methodology proposed to evaluate perception and hedonic response was based on the T-I and R-T methodologies of bitter PROP solutions and, three beverages (grapefruit juice, coffee and yerba mate infusions) in people with a high BMI ($25 < \text{BMI} < 30$; overweight group) and a normal BMI (< 25 ; normal-weight control group). If being overweight has any connection with the rejection of bitter-tasting foods, it would be expected to find a higher bitter intensity by T-I measure and lesser R-T values of bitter taste in high BMI compared to normal BMI group. In this line, perceived bitter intensity should increase at the end of the third sip by cumulative effect, and this effect would decrease the liking of the beverages, especially in high BMI condition.

2. Materials and Methods

Participants

A number of 66 healthy adults from the Pontificia Universidad Católica Argentina (UCA; 34 females, 32 males; 18 to 61 years old) participated in the study. They were asked to report their height and weight, afterwards two groups were formed based on the calculated BMI: normal BMI, consisting of lean subjects ($\text{BMI} < 25$; mean 21.2; $n = 34$; mean age 28.0); and high BMI, encompassing participants that were within the overweight range ($25 < \text{BMI} < 30$; mean 29.0; $n = 32$; mean age = 38.0). The BMI ranges corresponded to the World Health Organization classification according to the nutritional status of adults (OMS, 2003). Exclusion criteria included aversions, smoking (more than 5 cigarettes per week; Sato, Endo & Tomita, 2002), illnesses, a

history of eating disorders, diabetes and allergy to the products to be tested in the study. Also, people who described themselves as being on weight-loss diets or actively losing weight were excluded since this might be associated to bias when reporting sensory and affective perceptions of stimuli or might influence the relationship between bitter responsiveness and body weight (Tepper & Ullrich, 2002).

Tasted samples

PROP solutions

Three concentrations of PROP (Sigma Chemical Company, St Louis, USA) were used: 0.010, 0.032 and 0.060 mmol/L (belonging to the regular PROP series for taste detection thresholds; e.g., Drewnowski, Henderson & Shore, 1997). All solutions were prepared with distilled water one day before testing.

Beverages

Three beverages (Table 1) were selected based on their different bitter compounds and bitter intensity: coffee (*La Virginia*, Argentina), *yerba mate* infusion (*La Tranquera*, Argentina) and grapefruit juice (*Citric*, Argentina). The coffee and *yerba mate* infusions were prepared the day of the tasting as follows: coffee (three sachets, 7 g each) and *yerba mate* (two sachets, 3 g each) were put in 200 mL of mineral water at 90 ± 2 °C for 5 minutes. Both infusions were tasted at 45 ± 2 °C. Grapefruit juice was stored at 4 °C and served directly from the original packaging at 20 ± 2 °C. The beverages were selected for their bitter level; the intensity (high, medium and low) was evaluated prior to the experiment by a trained panel of UCA using a non-structured scale of 15 cm.

-Insert Table 1 about here-

Evaluation procedure

The experiment consisted of two sessions of 90 minutes each (one per week). In the first session, the participants evaluated bitter intensity of three PROP concentrations by T-I (first) and R-T procedures. In the second session, participants evaluated liking and intensity of the three bitter-tasting beverages using first a T-I liking procedure; then, a R-T liking procedure; and, finally, a bitter intensity by T-I procedure. In all the cases, the T-I procedure was carried out over multiple sips.

Some training was given to the participants regarding the use of T-I and R-T. The training was focused on the fact that these methods require certain motor skills from the participants (when to start the evaluation, how to follow a dynamic scale). However, it should be noted that most of the expected responses were hedonic. Participants were expressing how much they liked the different beverages along a time period (T-I) and had to decide whether they liked the product or not. Considering the hedonic aspect of the response, it is natural not to over train subjects.

The criteria to apply the two temporal methods over conventional scaling, was in response, to the following question proposed by Lawless & Heymann (2010): Is some aspect of the temporal profile likely to be related to consumer acceptability?

The experiment was approved by the Ethics Committee of the *Pontificia Universidad Católica Argentina*.

Bitterness intensity measurement by T-I over multiple sips

All the acquisition of T-I data was done using the I-T module of the software SensoMaker v1.8 (Federal University of Lavras, Brazil). Before starting the test, participants were instructed on how to use the evaluation software by tasting the most concentrated PROP solution (0.06mM) in only one sip. They were also asked to rank the PROP solutions from the lowest to the highest concentration before doing the T-I evaluation.

During the T-I evaluation each participant took a total of three consecutive sips of the same solution (presented in three separate cups of 7.5 mL each, at 20 °C ± 2

°C), taking one sip (5 mL) every 20 seconds (s), and registering a continuous rating for 60 s. The timing of sample sips was managed by a timer on the screen; in this way time was standardized *a priori* for the panel. The cursor was always visible on the continuous scale during the rating. The specific instructions given to the subjects regarding the temporal intensity task were as follows: *“You will receive three cups of the same sample and the task is to evaluate bitter intensity through a 60 s period. Press the start button while you put the entire content of the first cup in your mouth and evaluate the level of bitter intensity moving the cursor along a linear scale on the screen using a mouse. When the timer indicates 20 s, put the content of the second cup of the sample in your mouth and continue the evaluation. At 40 s, put the content of the third cup of the sample in your mouth and continue the evaluation until 60 s.”* Mineral water was used for rinsing between concentrations but not between sips. The participants took a 10-minute break between tasting each concentration to avoid saturation of the receptors. The experiment was conducted in an individual booth (at $22 \pm 2^\circ \text{C}$) equipped with a computer for data acquisition. The software provided the T–I curves as well as the parameters maximum intensity reached (I_{max} ; 0–10) and area under the curve (AUC; representing the overall bitterness perception of the stimuli over the total time of recording). The same procedure was done for the evaluation of beverages’ bitterness intensity in the second session.

R-T to bitterness

R-T measurements were performed using the equipment developed by Guirao & Zamora (2000) and modified according to Najún Zamora (2016). This equipment consisted of a pumping system, a computerized interface and a push button. It allowed the delivery of a standardized amount of a chosen PROP solution directly into the mouth of the participant and the registration of the time lapse between sample delivery and subjects’ response. Stimuli presentation time was of 400 milliseconds (ms), with inter-stimuli intervals of 3000 ms. Once the stimulus was delivered, the subject had to

press the push button as soon as a taste different from that of water was identified according to a “go/no-go” procedure. This response was registered by the software recording the time elapsed between stimuli pumping and button pressing.

Participants were first trained on the technique and use of the equipment with distilled water. During the training subjects had to press the button as soon as they felt water on their tongue surface. In this way, they got acquainted with the technique while their individual Touch Reaction Time (TRT) was obtained. An amount of 4 mL of the samples was pumped directly into the mouth of the assessor. Each concentration was tested in a separate sequence of eight stimuli, four times a same PROP concentration (four replications) and four times distilled water, presented in random order. Rinsing with distilled water between samples was mandatory. The participants took a 10-minute break between tasting each concentration sequence to avoid saturation of the receptors and attention loss due to boredom.

T-I liking over multiple sips

The participants evaluated the affective value of coffee, *yerba mate* infusion and grapefruit juice through time with the T-I method over three consecutive sips (10mL each, served in separate cups). Data was acquired with the software SensoMaker v1.8. The procedure was similar to the method described above for bitter T-I measurement over multiple sips. But, for this test, the specific instructions given to the subjects were: *“You will receive three cups of a same sample and you will evaluate the liking through a 60 s period responding to the question: How do you like this beverage now? For that you will put the entire content of the first cup of the sample in your mouth, and at the same time press the start button and evaluate the level of liking using the mouse to move the cursor along the line scale on the screen. When the timer indicates 20 s, put the content of the second cup of the sample in your mouth and continue the evaluation. At 40 s, put the content of the third cup of the beverage in your mouth and continue the evaluation of liking until 60s”*. Beverages were served in transparent plastic containers

and the order of presentation was balanced among the 62 participants. Participants evaluated the three beverages in the same session; they had a 10-minute break between each beverage for resting and rinsing their mouth with mineral water.

R-T to liking

An additional function was added to the modified reaction time equipment (Najún Zamora, 2016). This new version was endowed with a “YES” and a “NO” button. The specific instructions given to the subjects regarding the task were: *“You will evaluate if you like the beverages or not, so as soon as you perceive the stimulus you will have to decide whether you like it or not and press the YES or the NO button as soon as possible”*. An amount of 4 mL of sample was pumped directly into the mouth of the assessor. Once the stimulus was delivered and the subject pressed the corresponding button, the software recorded two responses: the time (elapsed between pumping and button pressing) and the “Yes” or “No” choice. Each beverage was tested in a separate sequence of six stimuli, three times a same sample (three replications) and three times distilled water, presented in random order (e.g., water-juice-juice-water-juice-water); rinsing with distilled water between samples was mandatory. The participants took a 10-minute break between each beverage tasting sequence. The order of beverage presentation was balanced among 62 participants.

Data analysis

Sample size calculation was based on exploring the relationship between BMI (2 groups) and the perception and hedonic response to bitter beverages (3 measurements) using the software G*Power (version 3.13). Assuming two-sided tests with $\alpha = 0.05$, $\beta = 0.05$, and effect size $f = 0.50$, the required sample size would be 66 to ensure a power >0.95 , $F[2, 128] = 3.067$. In the visual analysis of individual curves, four participants were inconsistent in the $I_{\max}$ values and the ranking given to the PROP

solutions, this is why their data was not included in the data analysis and results section, obtaining a final $n=62$.

When the most concentrated PROP solution (0.06mM) was tested in only one sip, it was noted that the maximum intensity was reached after 20s for both BMI groups. This meant that when evaluating the solutions in multi-sips, the second sip was taken before the maximum bitter intensity was attained. This observation would also apply to hedonic responses according to the studies of Veldhuizen, Wuister & Kroeze (2006). These authors evaluated both intensity and hedonic reactions to a citrus beverage, finding that the time to reach the maximum intensity response was shorter than time to reach the maximum hedonic response. Therefore, the procedure of three consecutive sips every 20s, had the advantage to obtain curves with a more consistent shape.

The parameters I_{max} and AUC were obtained at an individual level for each participant by product and these were then averaged across the panel. Differences in T-I parameters for PROP concentrations (I_{max} and AUC) were analyzed using 2 BMI x 3 PROP concentrations x 3 Sip mixed-factorial ANOVAs. Beverages were analyzed using 2 BMI x 3 beverages x 3 Sip mixed-factorial ANOVAs. Cumulative effect of bitter taste was analyzed by the increasing of I_{max} among sips ($p < .05$).

The average of individuals TRT values for each participant were subtracted from their R-T values, to evaluate only gustatory response, dismissing motor and cognitive individual differences. Obtained values of RT-TRT equal or close to 0 were dismissed, since they showed that subjects' responses were coming earlier than the actual stimuli. On the other hand, responses longer than 5000 ms were automatically stopped by the software and were not taken into consideration. These R-T values (RT-TRT) were analyzed using 2 BMI x 3 PROP concentrations mixed-factorial ANOVAs. Beverages were analyzed using 2 BMI x 3 beverages mixed-factorial ANOVAs. The "Yes" or "No" choice responses obtained during the liking R-T procedure were analyzed by Chi-Square Test of Independence to determine if there was a significant

relationship between BMI groups. The post hoc comparisons were carried out by Tukey test. For all analyses, $p < .05$ was considered significant.

3. Results

Bitterness T-I assessment of the PROP solutions

$I_{\max}$ results for the multi-sip evaluation of different PROP concentrations is presented in Fig. 1. ANOVA test (Table 2) showed significant differences ($p < .001$) for PROP concentration, BMI and sip; but no significant differences were obtained for the two-way interaction and three-way interaction. As expected, maximum perceived intensity of bitter taste increased over successive sips and as PROP concentration increased ($p < .05$). This difference became more evident as the stimulus became more intense confirming the cumulative effect of bitter taste intensity. On the other hand, in every case, the solutions were perceived as bitterer by those subjects with a normal BMI; the cumulative effect was also higher in the normal BMI group (Fig. 1). It should be noted that, $I_{\max}$ was reached after 20 seconds for both BMI groups (data not shown). This means that when evaluating the solutions in multi-sips, the second sip was taken before the maximum bitter intensity from the first sip was attained, what would have probably favored a cumulative effect of the bitter taste.

-Fig. 1 about here-

-Insert Table 2 about here-

For AUC (Table 2), no differences were obtained for BMI and sip, but there were differences for PROP concentration. Although differences were not significant for BMI, the intermediate concentration of PROP (0.032mM) showed a higher gap between the BMI groups ($\text{AUC normal BMI} - \text{AUC high BMI} = 13.3$) than the other concentrations (8.0 in both cases). Likewise, no significant differences were obtained for the two-way interaction, and three-way interaction.

Bitterness R-T assessment of the PROP solutions

A similar behavior was observed when analyzing the R-T results (Fig. 2). The factors BMI and PROP concentration were significant, but there was no significant interaction BMI*PROP concentration) (Table 2). Those subjects with a normal BMI always detected the bitter taste before (they were faster than) those with a high BMI. Also, the higher the PROP concentration, the faster the bitter taste was detected, finding significant differences between concentrations 0.010 mM (3883 and 4327msec for normal and high BMI, respectively) and 0.06 mM (3510 and 4043 msec for normal and high BMI, respectively) ($p < .05$).

-Fig. 2 about here-

Bitterness T-I assessment of the beverages

Fig. 3 shows the results obtained for the perceived $I_{\max}$ of bitterness over the three sips for the three beverages (coffee, *yerba mate* infusion and juice) and the two BMI groups. ANOVA (Table 2) results showed that there was a significant effect of BMI, beverage and sip ($p < .001$). No significant differences were obtained for the two-way interaction, and the three-way. The only significant interaction was for BMI*beverage revealing that differences in bitter perception between normal and high BMI were not the same for the three types of drink. For the sip effect, post hoc comparisons showed that, regardless of the stimulus, there was a cumulative effect increasing the perception of bitterness over the three sips for the two BMI groups, as previously observed for PROP solutions. According to the $I_{\max}$, the juice was the less bitter beverage, then the mate and the most bitter was the coffee ($p < .05$).

ANOVA results for the parameter AUC (Table 2) showed the same results in terms of significant main effect of BMI, beverage and sip ($p < .001$). No significant differences were obtained for the two-way interactions, and the three-way interaction. The only significant interaction was also BMI*beverage as in $I_{\max}$. Mean values of AUC for each beverage between BMI (normal and high, respectively) were: 115.7 ± 10.4 and

76.0 $\pm$ 9.1 for coffee, 80.3 $\pm$ 8.1 and 60.0 $\pm$ 7.4 for yerba mate, 46.6 $\pm$ 5.2 and 35.0 $\pm$ 6.3 for juice.

-Fig. 3 about here-

T-I liking assessment of beverages over multiple sips

The $I_{\max}$ of the liking ratings given for the three beverages over the consecutive sips, comparing the two groups, is presented in Fig.4. The ANOVA (Table 3) of the parameter $I_{\max}$ for liking data showed that the factors BMI beverage and sip were significant at $p<.001$, while there were no significant interactions. It can be observed that for all the beverages and all the sips a higher acceptance was given by the normal BMI group, which also rated the beverages as more bitter than the high BMI group ($p<.05$). Of all the three beverages, the most accepted one was the grapefruit juice, which happened to be also the least bitter (Fig. 3). Moreover, it can be observed that acceptance increased from sip to sip, a more important change between sip 1 and 2 than sip 2 and 3 for the high BMI group in yerba mate and coffee ($p<.05$).

-Fig. 4 about here-

-Insert Table 3 about here-

The parameter AUC (Table 3) was also analyzed in terms of an ANOVA. The same as for $I_{\max}$ was observed: the factors BMI, beverage and sip were significant at $p<.001$, while there were no significant interactions. The normal BMI group, who perceived the beverages as more bitter than the high BMI group, also showed a higher acceptance for these beverages ($p<.05$).

Liking R-T assessment of beverages

ANOVA results (Table 3) of liking R-T showed that there was a significant effect of the beverage and of the BMI, but there was no significant interaction. Each beverage

was tested three times, but the repetitions order did not show differences in the decision of the participants.

Grapefruit juice was the one which had the smallest values of liking R-T in comparison to the other two beverages ($p < .05$). Fig. 5 shows the liking R-T results that are in agreement with those obtained with R-T to bitter perception (Fig. 2). It can be observed that normal BMI group replied equally or faster than the high BMI. Significant differences were found for yerba mate, which in terms of liking was somewhat in between coffee and grapefruit juice. For this beverage, the normal BMI group gave their liking response faster than the high BMI group ($p < .05$).

-Fig. 5 about here-

The results obtained from measuring the liking through pressing the “Yes” or the “No” button as soon as possible, showed no significant differences between both BMI groups for the three beverages. The “Yes” choice was selected for coffee 43.9% and 42.9% ($\chi^2 = 0.888$), for yerba mate 62.3% and 53.6% ($\chi^2 = 0.219$), and for grapefruit juice 82.5% and 85.7% ($\chi^2 = 0.538$) in normal compared to high BMI, respectively. When the acceptance of the beverage was higher (maximum liking level), the percentage of the “Yes” option increased in both BMI groups at the same level.

4. Discussion

The main hypothesis of the current study was, that if being overweight has any connection with the rejection of bitter foods, it would be expected to find that the group of people with high BMI ($25 < \text{BMI} < 30$; overweight group) would perceive a higher intensity of bitter by T-I measure than normal BMI, and lesser R-T values of bitter taste. However, the results obtained were very consistent in demonstrating the opposite of what was expected, that the normal BMI group gave bitterness intensity ratings as more intense and reacted faster than the high BMI group. This was observed both in PROP solutions as well as in the three beverages.

The review of Shizukuda et al. (2018) showed that the associations between bitterness perception and anthropometrics are conflicting. Previous researches have shown a positive correlation between bitter taste and obesity, one indicated that this correlation is influenced by the subject's age, one suggested a negative correlation, and two found no association. In the line of the results of the present paper, Choi & Chang (2015), using PROP in the test, reported that subjects with a better perception of bitterness had a lower BMI. Simchen, Koebnick, Hoyer, Issanchou & Zunft (2006) suggested that the sensitivity to bitterness is related to body weight and age. In subjects younger than 65 years, bitterness perception among overweight individuals ($BMI > 28 \text{ kg/m}^2$) was less than that reported by subjects of normal weight ($BMI < 28 \text{ kg/m}^2$). On the other hand, overweight subjects older than 65 years were more sensitive to bitterness.

For T-I measures, some sensitive differences were observed between PROP solutions and the beverages. In the case of PROP solutions, the higher differences between BMI were observed in $I_{\max}$, while AUC values gives the idea as a global bitter perception because no differences were found among sips. However, for the beverages both measures ($I_{\max}$ and AUC) showed a similar sensitivity; it may indicate that the flavor of these beverages contributed to the perception of each sip as a different event.

Working with a multiple-sip design, helped confirm that bitter taste has a cumulative effect, since in every case the sip effect was significant when evaluating the $I_{\max}$ and this effect was more important as the bitterness intensity increased. Also, what became clear from these results was that the rating of bitter taste was different according to the different BMI. In every case, there was a significant BMI effect, showing that people with a BMI higher than normal rated the stimulus as less bitter and had longer R-T. PROP solutions were rated as bitterer by those subjects with a normal BMI. In the case of beverages, in the least bitter one (grapefruit juice), practically no differences can be found in the maximum intensity between the two BMI groups. But,

as the stimulus became more intense (*yerba mate* infusion and then coffee), the curves expressing the ratings of the two BMI groups grew further apart, observing the biggest difference for coffee after the third sip where this beverage is rated as bitterer by the normal BMI group.

It would be possible to associate this lower intensity rating of bitterness to a higher acceptance of bitter foods. However, the results of the present work indicated a higher acceptance of the three bitter beverages studied in the normal BMI group, which rated the bitter taste as more intense. These results were not as expected, because the group of people with high BMI (>25 and <30 ; overweight group) presented lower intensity ratings of the bitter taste by T-I measure than normal BMI, and longer R-T values. Moreover, bitter taste increased at the end of the third sip by cumulative effect, and this effect did not decrease the liking of the beverages. These results are in accordance with Garcia-Burgos and Zamora (2013), who evaluated the relationship between the facial reactions, to the food bitterness and BMI, and reported evidence of high levels of disgust in facial expressions for bitterness in participants with a high BMI.

This showed that people with a normal BMI probably eat more vegetables and bitter products not because they have a reduced perception of this taste but because they actually like it. Duffy & Bartoshuk (2000), did not find a consistent association between genetic taste measures and bitter beverages acceptance. One of the reasons for this might be that patterns of food acceptance (and possibly food selection) may reflect a genetic predisposition, and not necessarily an unwillingness to make dietary changes (Duffy & Bartoshuk, 2000). Indeed, genetic variations in PTC/PROP sensitivity have been found to determine human food likes and dislikes (Ly & Drewnowski, 2001).

Results of liking R-T for *yerba mate* infusion, in which the maximum difference between BMI groups was observed, showed that the normal BMI group gave their liking response faster than the high BMI group. This is in agreement with T-I results, since the more something is liked, the faster the answer is given (Finlayson, King, & Blundell, 2008). The “Yes” or “No” acceptance response shown, for *yerba mate*

infusion, a trend for higher acceptance in normal BMI (62.3%) than in high BMI group (53.6%). This result is also in agreement with Finlayson et al. (2008) who stated that when a category was selected more frequently, it was also chosen more rapidly. The grapefruit juice was the beverage with the higher T-I liking and with the smaller reaction time to liking, so it can be interpreted that the higher the acceptance, the shorter the reaction time.

Interestingly the novelty of the present paper was to demonstrate through two different methodologies that, even though the high BMI group rated the bitter taste as less intense, they had a lower level of acceptance than the normal BMI and this had not yet been reported to date. In summary, although BMI is a complex variable for which aversive reactions explain only a small portion, hedonic (appetitive or aversive) over-responding may be one factor contributing to the susceptibility to weight gain also through avoidance of health-promoting food.

The present work had some limitations in relation to PROP taster status and BMI, which may be predictive of food preference and eating behavior (Donaldson, Bennett, Baic & Melichar, 2009). The review of Tepper, Melis, Koelliker, Gasparini, Ahijevych & Tomassini Barbarossa (2017) describes common factors that can influence the characterization of the PROP tasters status, such as age, gender, obesity and smoking. It would be interesting to expand the sample in order to balance all variables, and to analyze their influence over BMI.

Conclusion

R-T gives complementary measurements to T-I evaluations, both in perception and liking. They reflect different moments of perception: R-T shows what happens at the beginning of the tasting while T-I depicts the rest of the tasting during the entire process of intake. Furthermore, the cumulative bitter sensation in multiple sips was more sensitive to detect differences in bitterness intensity across different BMI status.

Even though the high BMI group rated the bitter taste less intensely, they had a lower level of liking than the normal BMI. This result indicated that the hedonic component was also involved in the perception of bitter taste in individuals with high BMI. Additional research is therefore needed to examine affective mechanisms that control dietary selection and food consumption, given the increasing incidence of obesity in the population.

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ACCEPTED MANUSCRIPT

Figure captions

Fig. 1. Maximum bitter intensity ($I_{\max}$) over successive sips (s1, s2, s3) for PROP solutions (0.010, 0.032, 0.060 mM). Data expressed as mean $\pm$ SEM. Comparison of $I_{\max}$ between BMI groups (normal and high) were all significant ($p < 0.05$, Tukey post-hoc test).

Fig. 2. Reaction Time (R-T; msec) for PROP concentration (0.010, 0.032, 0.060 mM). Data expressed as mean $\pm$ SEM. Different letters in every column indicate differences in R-T between BMI groups at each concentration (normal and high) ($p < .05$, Tukey post-hoc test).

Fig. 3. Maximum bitter intensity ($I_{\max}$) over successive sips (s1, s2, s3) for beverages (grapefruit juice, coffee and yerba mate infusions). Data expressed as mean $\pm$ SEM. Comparison of $I_{\max}$ between BMI groups (normal and high) were all significant ($p < .05$, Tukey post-hoc test).

Fig. 4. Maximum liking level ($I_{\max}$) over successive sips (s1, s2, s3) for beverages (grapefruit juice, coffee and yerba mate infusions). Data expressed as mean $\pm$ SEM. Comparison of $I_{\max}$ between BMI groups (normal and high) were all significant ($p < .05$, Tukey post-hoc test). Lowercase letters show differences among sips for normal BMI, and capital letters for high BMI group.

Fig. 5. Reaction Time to liking (R-T; msec) for for beverages (grapefruit juice, coffee and yerba mate infusions). Data expressed as mean $\pm$ SEM. Different letters in every beverage indicate differences in R-T between BMI groups (normal and high) ($p < .05$, Tukey post-hoc test).

Table 1. Characteristics of the commercial beverages

Beverages	pH	Protein (%)	Carbohydrate (%)	Caffeine (%)	Bitter Intensity
Coffee	4.7	0.0	20.0	28.0	11.2
Yerba Mate	5.9	0.0	0.0	3.8	8.1
Grapefruit juice	3.2	0.6	6.5	0.0	2.7

Note: Nutritional data was obtained from the product packaging and is expressed on product weight basis. Bitter intensity is the mean result obtained by the panel using a non-structured scale (15 cm).

Table 2. ANOVA *F*-values for the evaluation of bitterness of PROP solutions (R-T and the T-I parameters $I_{\max}$ and AUC) and for the commercial beverages (T-I parameters $I_{\max}$ and AUC).

Factors and interactions	PROP solutions			Beverages	
	$I_{\max}$	AUC	R-T	$I_{\max}$	AUC
PROP /Beverages (df=2)	126.506***	134.092***	5.893**	154.590***	132.848***
BMI (df=1)	35.087***	1.710 ^{ns}	26.333***	82.140***	45.510***
Sip (df=2)	25.265***	0.218 ^{ns}	-----	28.774***	37.842***
PROP /Beverages *BMI (df=2)	2.386 ^{ns}	1.979 ^{ns}	0.123 ^{ns}	13.374***	7.049***
PROP /Beverages *sip (df=4)	1.597 ^{ns}	0.197 ^{ns}	-----	0.630 ^{ns}	1.969 ^{ns}
BMI*Sip (df=2)	1.672 ^{ns}	0.257 ^{ns}	-----	1.300 ^{ns}	2.388 ^{ns}
PROP /Beverages *BMI*sip (df=4)	0.294 ^{ns}	0.190 ^{ns}	-----	0.292 ^{ns}	0.456 ^{ns}

df = degrees of freedom

***p<0.001; **p<0.01

ns = not significant

Table 3. ANOVA *F*-values of liking evaluations of beverages by Time-intensity (parameters $I_{\max}$ and AUC) and R-T to liking.

Factors and interactions	Beverages		
	$I_{\max}$	AUC	R-T
Beverages (df=2)	42.770***	29.786***	8.315***
BMI (df=1)	28.261***	16.149***	7.246**
Sip (df=2)	8.816***	30.138***	-----
Beverages *BMI (df=2)	0.212 ^{ns}	0.873 ^{ns}	0.231 ^{ns}
Beverages *sip (df=4)	0.386 ^{ns}	1.852 ^{ns}	-----
BMI*Sip (df=2)	0.131 ^{ns}	0.174 ^{ns}	-----
Beverages *BMI*sip (df=4)	0.274 ^{ns}	0.494 ^{ns}	-----

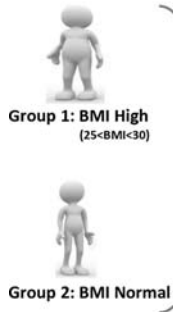
df = degrees of freedom

*** $p < 0.001$; ** $p < 0.01$

ns = not significant

Highlights

- Perception and liking of bitter solutions were explored in relation to BMI.
- Reaction-Time and multiple-sip time-intensity methods were used.
- High BMI group perceived bitterness as less intense and reacted to it later than normal BMI group.
- A lower liking of the three bitter beverages was observed in the high BMI group.

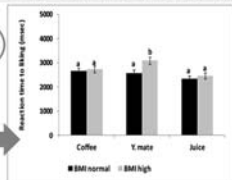
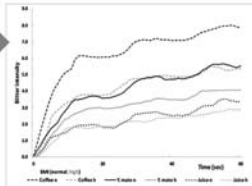


Multi Sip Time-Intensity (T-I)

Liking Multi Sip T-I

Reaction-Time to bitter taste

Reaction-Time to liking



Graphics Abstract

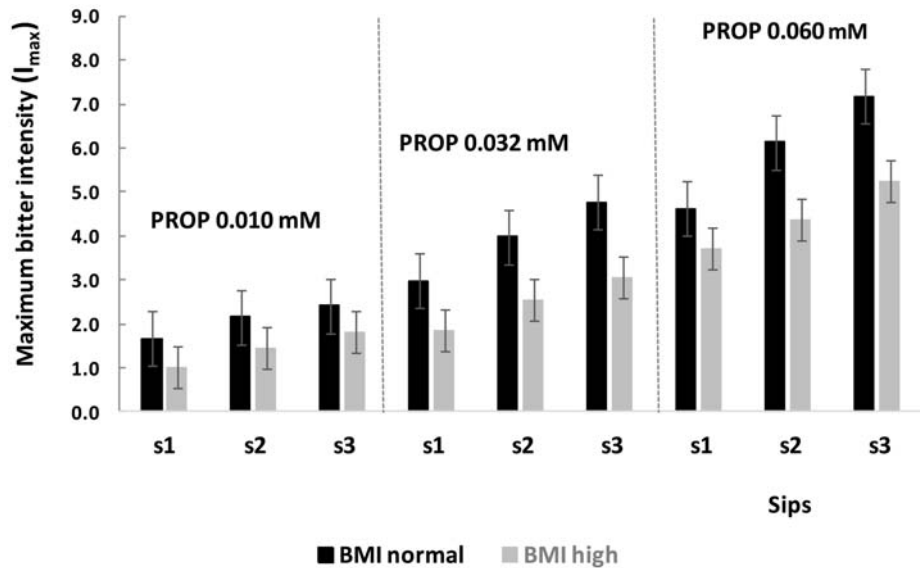


Figure 1

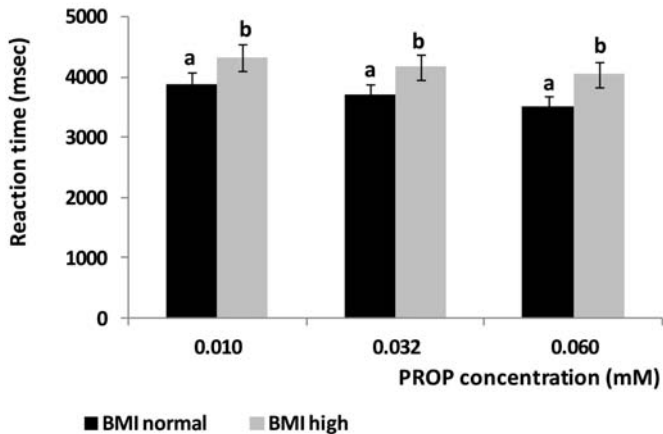


Figure 2

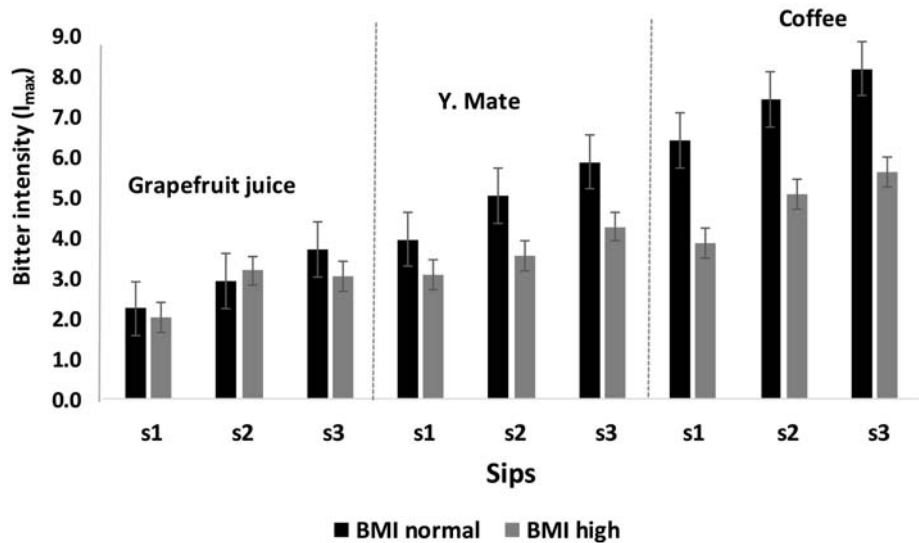


Figure 3

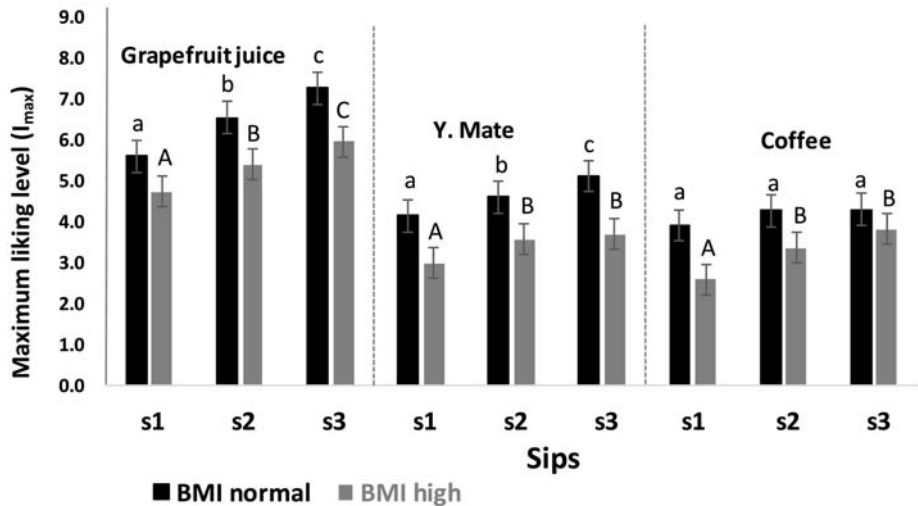


Figure 4

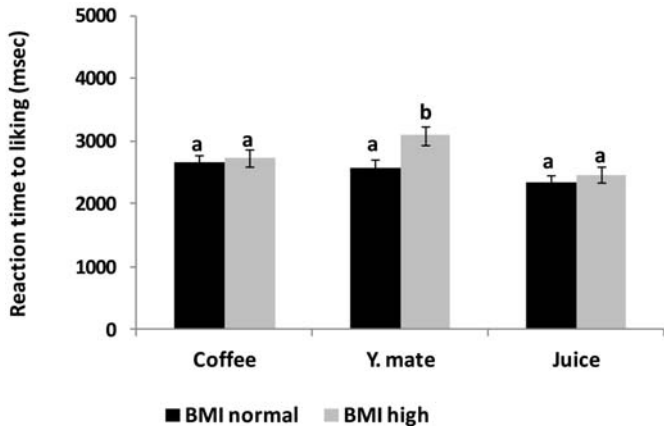


Figure 5