Comparison of brain activity while tasting of passion fruit juice sweetened with caloric and non-caloric sweeteners.

Discrimination of different sweeteners

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Abstract

Sweetener type can influence sensory properties and consumer's acceptance and preference for low-calorie products. An ideal sweetener does not exist, and each sweetener must be used in situations to which it is best suited. Aspartame and sucralose can be good substitutes for sucrose in passion fruit juice. Despite the interest in artificial sweeteners, little is known about how artificial sweeteners are processed in the human brain. Here, we evaluated brain signals of 11 healthy sub-jects when they tasted passion fruit juice equivalently sweetened with sucrose (9.4 g/100 g), sucralose (0.01593 g/100 g), or aspartame (0.05477 g/100 g). Electroencephalograms were recorded for two sites in the gustatory cortex (i.e., C3 and C4). Data with artifacts were disregarded, and the artifact-free data were used to feed a CNN. Our results indicated that the brain responses distinguish juice sweetened with sucrose from juice sweetened with aspartame and sucralose.

Keywords: sucrose, sucralose, aspartame, electroencephalography (EEG), convolutional neural network (CNN).

Introduction

Sugar has been the main sweetener in human diet for centuries. It represents a high percentage of the human daily energy consumption, but it offers little additional nutritional value (Bassoli and Merlini, 2003; Caballero, 2013; Zorn et al., 2014). Given that replacing sugar with non-caloric (or low-calorie) sweeteners have become popular among consumers seeking to lose or to maintain weight. Moreover, foods with the same sweetening capacity might be perceived differently due to their caloric content (Frank et al., 2008; Hill et al., 2014). Additionally, the brain might respond distinctly to perceptually similar and identical tastes (Andersen et al., 2019).

A satisfactory method for collecting an artifact-free EEG signal has not been developed yet, but there are important results relating gustatory stimuli to evoked potential parameters in humans (Andersen et al., 2019; Hashida et al., 2005; Jacquin-Piques et al., 2016; Linforth, 2000; Mouillot et al., 2020; Ohla et al., 2012). In addition, from the food science standpoint, several aspects must be considered when it comes to taste perception, including the hypothesis that the taste perception threshold is related not only to the sensitivity of the sensory organ—in this case, the tongue—but also to a cognitive process in the brain (Huang et al., 2006; Naim et al., 2002; Okamoto and Dan, 2007).

Any study involving equi-intense sweeteners (i.e., sweeteners used in equivalent amounts) must consider how sweeteners influence sensory properties and consumer's acceptance and preference for low-calorie products (Pinheiro et al., 2005). An ideal sweetener does not exist, so each sweetener is appropriate for specific situations (Nabors, 2002). Passion fruit is a popular tropical fruit that has an important commercial variety named yellow passion fruit, which is used to prepare juice that requires sweetening (Deliza et al., 2005). Aspartame and sucralose can be good substitutes for sucrose in passion fruit juice (Rocha and Bolini, 2015b, 2015a).

For this study, we have hypothesized that EEG can distinguish the stimuli resulting from the consumption of drinks sweetened with caloric or non-caloric sweeteners. To test this hypothesis, we have compared brain signals acquired in response to the consumption of passion fruit juice sweetened with sucrose (caloric sweetener), sucralose, or aspartame (low-calorie sweeteners).

Materials and Methods

The methods used herein were divided into three main sections: (1) Participant selection, (2) acquisition of EEG signal from the selected group, and (3) signal processing and CNN (Convolutional Neural Network) training and tests. Our aim was to use a CNN to determine differences between stimuli.

Stimuli

The passion fruit juices were prepared in the laboratory; unsweetened passion fruit pulp (DeMarchi™) was employed. The following ratio was used: one part of pulp to two parts of water. The juice samples were sweetened with sucrose (União™), aspartame (Ajinomoto™), or sucralose. Pure water was considered as reference. The samples were prepared one day before the experiment, stored at 4–6 °C, and tested at room temperature.

The sweetener concentration in the passion fruit juice was chosen according to Rocha and Bolini's study (Rocha and Bolini, 2015b, 2015a), who stated that 9.4/100 g was an ideal sucrose concentration according to consumer's acceptance. They also stated that the equivalent aspartame and sucralose concentrations in passion fruit juice were 0.0547 g/100 g and 0.0159 g/100 g, respectively.

Participant Selection

A total of 105 volunteers were included in this study. The volunteers comprised students, teachers, and employees aged 19–55 years, recruited on campus. They did not have diabetes, smoke, or use medications that affect taste or cognitive processes. Preference was given to volunteers that were used to consuming passion fruit juice (or that at least had no aversion to the fruit taste).

Each volunteer received samples containing 30 mL of passion fruit juice sweetened with different amounts of sugar (i.e., 4.7, 7.05, 9.4, 11.75, and 14.1 g). The samples

88 were placed in disposable cups and numbered randomly between 0000 and 9999 (e.g.,
89 A7932). The participants received samples in a randomized sequence of concentrations
90 and had to answer the following question: "How much sugar did you have in your
91 juice?" Answers were collected in a 9.0-cm visual scale (VAS). VASs are input
92 mechanisms that allow users to specify a value within a predefined range. The
93 volunteers were instructed to consider the centre of the scale as ideal sweetness, 0 as
94 less sweet than ideal, and 9 as sweeter than ideal. Our objective was to select
95 individuals with a perception of sugar ideal sweetness as close as possible to 9.4 g/100 g
96 and, among these individuals, to select those that had good ability to order the samples
97 according to the sugar concentration.
98 The volunteers were informed about the nature and aims of the experiments and
99 provided informed consent. The study was approved by the Ethics Committee of the
100 Animal Science and Food Engineering College (FZEA) of the University of São Paulo
101 (USP) (CAAE 59017516.6.0000.5422).
102 **EEG recording**
103 EEG signals were acquired from 11 individuals (both sexes) while they were tasting
104 passion fruit juice. The individuals had been selected according to item 'Participant
105 selection' and had signed an informed consent form (ICF).
106 EEG was carried out non-invasively on the scalp surface; the participants wore an EEG
107 cap. The signal was acquired at positions C3 and C4 in the primary gustatory cortex
108 (Hashida et al., 2005; Kobayakawa, 1999) as defined by the international 10–20
109 electrode disposal system (Jasper and H., 1958). This electrode position was chosen on
110 the basis of the study by Kabayakawa et al. (1999), who showed magnetic fields
111 recorded from the brain (i.e., MEG) in response to two tastants: 1 M NaCl and 3 mM

saccharin, one of which was more activated in the central area of the head at positions C3 and C4. The ground electrodes were placed on the participant's ear lobes, and a reference electrode was placed on the forehead. The signals were sampled at 512 Hz by using an iCelera digital portable electroencephalograph. Each recording lasted 16 seconds.

The volunteers were accommodated inside a Faraday cage, and the EEG signal started being recorded when the volunteer drank the solution (juice). The volunteers were instructed to keep their eyes closed and not to move during the recordings. Each volunteer received eight 30-mL samples for evaluation. The samples were placed in disposable cups and numbered randomly between 0000 and 9999 (e.g., A7932). The samples were offered in duplicate in the following order: water (reference), passion fruit juice sweetened with sucrose, passion fruit juice sweetened with sucralose, and passion fruit juice sweetened with aspartame. Each volunteer participated on three different days of the experiment (repetitions). In the intervals between the sweetened samples, the participants received sparkling water to clean residues from their palate and to reduce other interferences.

Signal processing and CNN training

The signal was processed by using the Python programming language and the Pandas, NumPy, and SciPy libraries. These libraries are employed to solve mathematical and scientific problems, including signal processing. Initially, the data were inspected, and the recordings with many artifacts were excluded from the database. The remaining data consisted of signals from 68 experiments lasting 16 seconds each, recorded in two channels (i.e., C3 and C4). These signals came from one of four stimuli (i.e., sucrose, sucralose, aspartame, or water) and were captured across three days of repetitions per

volunteer. The data were bandpass-filtered from 8 to 40 Hz, and each signal was divided into two-second segments with a 0.1 second stride. This means that 16-second segments (512 samples per second) were resampled in 9520 vectors at a length of 1028. The EEG dataset and the processing code that supported the findings of this study are available on GitHub (https://github.com/Atzingen/EEG_Sweetners.).

When the vectors with length *1028* were used as inputs for the CNN, they were structured as a network consisting of three parallel convolution processes merged into a fully two-layer-deep connected network.

The deep learning model was built by using an open-source Python library, i.e., Keras, which runs in a TensorFlow background. Optimal performance (e.g., number of neurons on a given layer and number of filters and kernel size on the convolution layer) was determined by using manual fine adjustment of the hyperparameters (e.g., number of neurons per fully connected layer and number of filters and kernel size for convolutional layers).

The last network layer contained four neurons, which were responsible for mapping four possible results of an entry (water, sugar, aspartame, or sucralose). A categorical cross-entropy loss function was used.

Training was accomplished by using an Adam algorithm with two-thirds of the data, while the test was performed with the remaining one-third.

Finally, performance evaluation metrics, such as accuracy, precision, recall and F1 score were calculated as follows.

$$accuracy = \frac{TP+TN}{TP+T \quad +FP+FN} \tag{1}$$

$$precision = \frac{TP}{TP+FP} \tag{2}$$

$$recall = \frac{TP}{TP+FN} \quad (3)$$

$$F1\ score = \frac{2(precision)*(recall)}{(precision)+(recal\ )} \quad (4)$$

where TP (true positive), FP (false positive), TN (true negative) and FN (false negative) are technical terms for binary classifier. Specific, TP is the positive samples correctly classified, FP is the positive samples misclassified, TN is the negative samples correctly classified and FN represents the negative samples misclassified.

Results and Discussion

Participant selection

We selected the groups according to two criteria, namely, preference for the sample with a sucrose concentration of 9.4 g/100 g and good ability to order the sample concentrations. This means that the selected participants indicated values around 4.5 cm on the VAS, which was equivalent to a sucrose concentration of 9.4 g/100 g. Furthermore, they were able to order all the concentrations properly. Of the individuals considered fit, 11 agreed to participate in the brain signal acquisition stage.

Figure 1-a illustrates the scale presented to the participants. Figure 1-b shows participant A, who placed all the samples in the correct sequence of concentrations and chose the sample with sugar concentration of 9.4 g/100 g as his favourite. He was selected for the next step. Participants B and C were not selected. Participant B (Figure 1-c) placed the samples correctly, but his preferred sample was not the sample with sugar concentration of 9.4 g/100 g. Although participant C (Figure 1-d) preferred the sample with a sugar concentration of 9.4 g/100 g, he was not able to order the samples correctly.

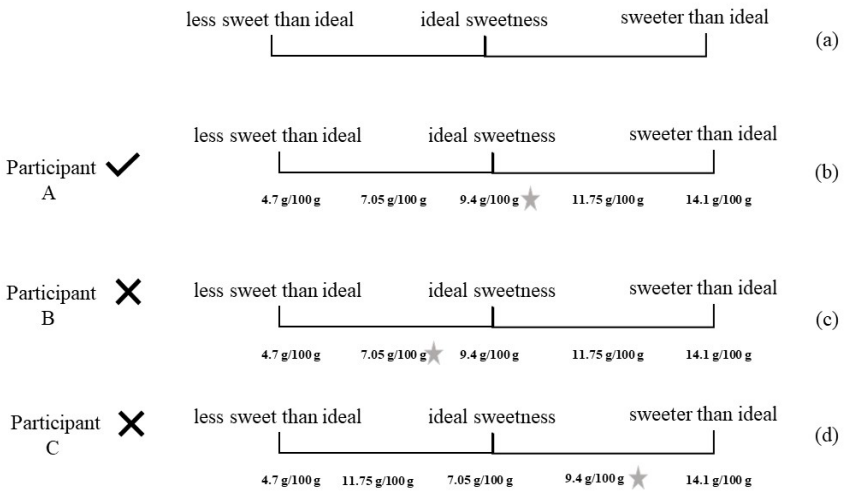

Figure 1: (a) Participant selection form. (b) Participant selection form from a selected participant. (c) Participant selection form from a discarded participant. (d) Participant selection form from a discarded participant. The star placed on the right side illustrates the preferred sample. In the original form, the volunteer marked the sample number on the scale. For didactic purposes, these codes were replaced with the sample concentration in the figure.

Signal processing and CNN training

Figure 2 shows the network architecture that achieved the best performance. The best number for *n* was 20, as can be seen by the input layer shape. The other three fully connected layers had 16, 16, and 64 neurons. Its dropout optimal value was 0.2.

We used this optimal architecture to train the CNN with 70 % of the data, while we employed the remaining 30% for the test.

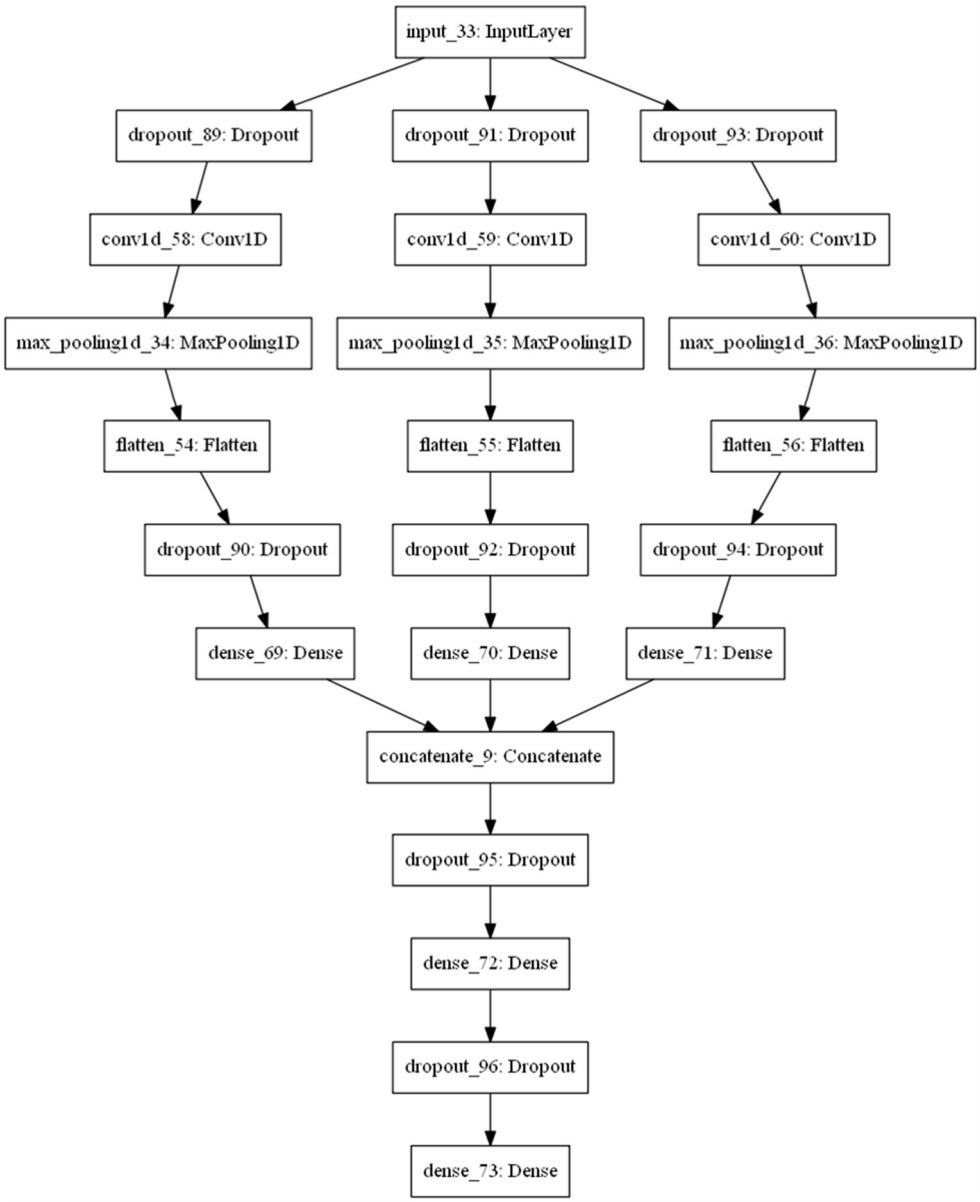

Figure 2: The convolutional neural network (CNN) architecture that achieved the best performance.

For visualization, Figure 3 presents the confusion matrix for this dataset. In the confusion matrix, the horizontal axis is the predicted label, and the vertical axis is the true label. The elements on the diagonal represent the numbers of correctly classified samples.

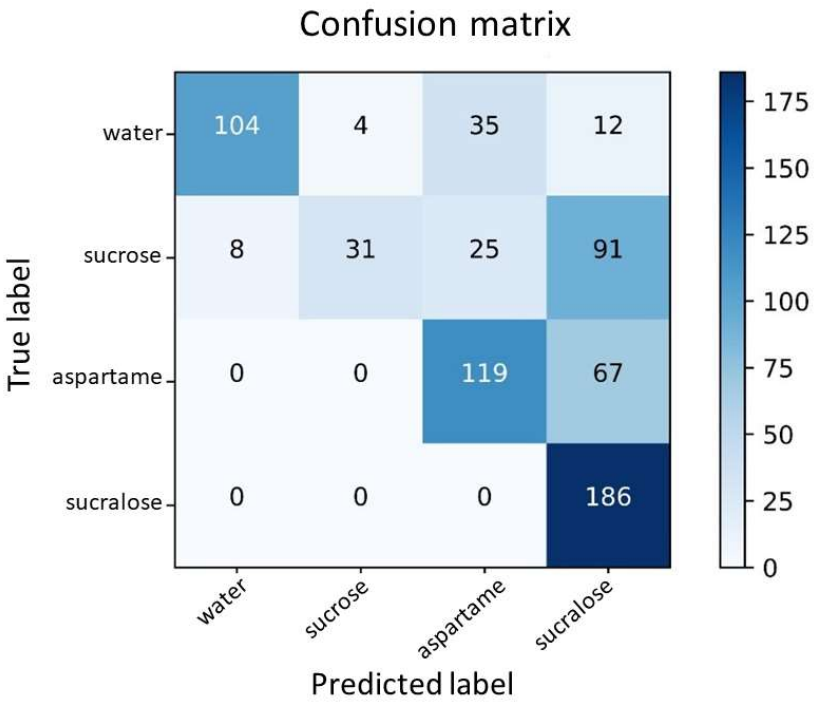

Figure 3: Confusion matrix for the classes water, sucrose, sucralose, and aspartame.

In Figure 3, for the water class, 104 samples were correctly predicted as water (67.1%), whereas 35 samples (7.7%) were incorrectly predicted as aspartame and 4 samples (2.6%) were incorrectly predicted as sucralose. For the sucrose class, 31 samples (20.0%) were correctly predicted as sucrose, whilst 25 samples (16.1%) were incorrectly predicted as aspartame, and 91 samples (58.7%) were incorrectly predicted as sucralose. As for the aspartame class, 119 samples (64.0%) were correctly classified, while 67 samples (36.0%) were incorrectly predicted as sucralose. Concerning the sucralose class, 186 samples (100.0%) were correctly classified. The main difficulty lay in the classes of water and sucrose.

Table 1 lists the metrics results for the four stimuli (classes). As a result, the average metrics were: 0.823 for accuracy, 0.750 for precision, 0.628 for recall and 0.611 for f_1 score. There are no studies using CNN in similar databases for comparison purposes,

but these values are compatible with those obtained for studies involving brain signals to assess emoticons (YIN ZHONG) and cerebral dominance (TORAMAN).

When we consider reference (water) only, the classification in Figure 3 indicated that the average identification accuracy is 91,4% compared to an overall classification of 82.3%. This work uses a drink instead of a solution and, in a first study of this nature, it was important to be able to distinguish a critical reference well.

Table 1: Results of CNN classification performance for the four stimuli (classes).

	Accuracy	Precision	Recall	F_1 score
Water	0,9135	0,9286	0,6710	0,7790
Sucrose	0,8123	0,8857	0,2000	0,3263
Aspartame	0,8138	0,6648	0,6398	0,6521
Sucralose	0,7507	0,5225	1,0000	0,6863

When we compare general performance with reference performance, mainly f_1 score that consider recall and precision measures simultaneously we can deduce that misclassifications in sweeteners classes are greater that that in reference classe. Analysing Figure 3 in more detail, suggests that the network is more sensitive to the low-calorie sweetener classes than to the sucrose class. By evaluating the false positives (FP) of the classification, aspartame can be predicted to be sucralose, but not sugar. In turn, sucralose had no false positives. The most surprising result is that sucrose can be classified both as sugar and low-calorie sweetener. In other words, evaluated low-calorie sweeteners had not been confused with the caloric sweetener, but the caloric

238 sweetener can be confused with the low-calorie sweetener. Andersen et al. (2019)
239 observed that similar tastes that are consciously indistinguishable can result in different
240 brain cortical activations. A similar result was obtained when gustatory evoked
241 potentials (GEPs) were used to assess the brain response to sucrose, aspartame, and
242 stevia in humans (Mouillot et al., 2020). The authors stated that, although sucrose,
243 aspartame, and stevia led to the same taste perception, the GEPs showed that cerebral
244 activation by these different sweet solutions had different recordings. They suggested
245 that, besides the difference in taste receptors and cerebral areas activated by these
246 substances, neural plasticity and changes in the synaptic connections related to sweet
247 innate preference and sweet conditioning could explain the differences in cerebral
248 gustatory processing after sucrose and sweetener activation. The results presented
249 herein showed that this may be true.
250 Unlike Andersen et al. (2019) and Mouillot et al. (2020), in this study we evaluated a
251 beverage (i.e., passion fruit juice) with good acceptance after it is sweetened with
252 aspartame or sucralose (Rocha and Bolini, 2015b, 2015a) as a stimulus instead of
253 evaluating a sweet solution. This may explain FP rates in classification. During tasting,
254 the stimuli came not only from the sweet solution, but also from the other sensory
255 characteristics of the beverage.
256 Apart from using a beverage instead of a solution and from dispensing a taste delivery
257 system (Andersen et al., 2019; Frank et al., 2008; Jacquin-Piques et al., 2016;
258 Kobayakawa et al., 1996; Kobayakawa, 1999) that is common in this kind of
259 experimental design, we only placed two electrodes near the gustatory cortex. While
260 this is not a new strategy (Cincotti et al., 2002; Costa and Cabral, 2000; Hashida et al.,
261 2005), it is uncommon (Andersen et al., 2019; Frank et al., 2008; Jacquin-Piques et al.,

2016; Kobayakawa et al., 1996; Kobayakawa, 1999) and has advantages and disadvantages. The main disadvantage is that it reduces the amount of data and makes some feature extraction techniques unfeasible. On the other hand, developing commercial applications based on a single EEG channel, such as the applications used by Hashida et al. (2005) and Silva et al. (2005), is easy.

Andersen et al. (2019) observed that response discrimination by between-participant qEEG is inferior to discrimination by within-participant qEEG. Therefore, we can improve the results of this study by increasing the number of samples tested per individual and also the number of individuals. This would allow us to train the network for each participant, for instance. However, this approach would involve the use of a taste delivery system to facilitate the increase in the number of samples.

This fact does not invalidate the main contribution of this study, which corroborates recent studies (Andersen et al., 2019; Frank et al., 2008; Mouillot et al., 2020) stating that similar stimuli, despite being consciously indistinguishable, may result in different cortical responses. Moreover, this study included a beverage instead of a solution and used only two monitoring positions (i.e., C3 and C4) over the gustatory cortex.

Conclusions

We have compared brain signals acquired in response to the consumption of passion fruit juice sweetened with sucrose (caloric sweetener), sucralose, or aspartame (low-calorie sweeteners). We used the artifact-free data to feed a CNN. The results indicated that the brain responses can distinguish the juice sweetened with sucrose from the juice sweetened with aspartame and sucralose.

Author's Contributions

Conceptualization: Silva, A.C.S, Costa, E.J.X., Miñano, H.L.A. **Data acquisition:** Ortega, N. F., Miñano, H.L.A. **Data analysis:** Silva, A.R. **Design of Methodology:** Silva, A.C.S, Costa, E.J.X; Atzingen, G.V. **Software development:** Atzingen, G.V. **Writing – original draft:** Silva, A.C.S. **Writing -review & aditing:** Silva, A.C.S., Costa, E.J.X; Atzingen, G.V., Miñano, H.L.A. **Funding acquisition**: Silva, A.C.S., **Project administration:** Silva, A.C.S.

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