

Development of a Mathematical Model and Statistical Procedure for the Analysis of Multiple Time Intensity Curves of Sweet Beverages

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Abstract: The aim of the present study is to develop a mathematical model devoted to analyze the Multiple Time Intensity Curve (MTIC) and to establish a statistical methodology appropriate to draw conclusions about how to vary the taste sensation perceived at successive sips during time. It was designed a software in Turbo Pascal language, MTIC.exe, running under the DOS operating system. It allows an adequate acquisition of data to assess the MTIC. To model experimental MTICs it is proposed a function $I = f(t)$ that represents the real action of ingesting a drink. A mechanistic approach could be able to interpret all physicochemical and physiological events during sensory testing. We developed a model representative of the MTICs dependent of six parameters. It was analyzed, step by step, all the phenomena occurring while registering the curve. That is, since the judge puts the solution in his mouth, until the completion of recording after repeating four sips. A function $I = f(t, a, b, k_0, t_1, v_1, h)$ describes temporal changes in perceived sweet intensity.

Keywords: Dynamic oral perception, modeling human responses, software data acquisition, successive time-intensity curves, taste

INTRODUCTION

Often, a static test is not enough to describe all changes in the intensity of taste, hence the importance of monitoring the intensity of a specific sensory property as a function of time. These curves describe dynamically the taste perception. In dynamic profiles, traditional curves comprise a single exposure to the stimulus under study, but a more adjusted view to real taste world is to record the continuous sensory response, using Multiple Time Intensity Curve (MTIC). In this sense, Courregelongue *et al.* (1999) evaluated soy milk and they described the astringency of the samples by its significant latency. After they perform repeated sips, astringency builds up in mouth. Repeated sips evoked an MTIC, so the authors suggest future studies considering the rate of onset of the attribute in sequential sips.

Another way to clarify the multiple dynamic description of each product includes modeling of MTIC. This approach should take into account the nature of the attribute to be assessed and to determine if the evaluation produces successive physiological adaptation or sensitization, since each of these phenomena will result in a different pattern for MTIC. A decreasing trend (lower successive peaks with lower maximum intensity and lower area under the curve) is

observed if taste or smell are stimulated (concentration detectors) explaining thus sensory adaptation. Instead, MTIC which show an increasing trend (successive peaks with higher peak intensity and/or higher area under the curve) are obtained if the sensor is a kind of mass detector as trigeminal system explaining the sensitization to repeated stimulations. In Fig. 1, there is schematized one MTIC with its main parameters.

Garrido (1997) and Garrido *et al.* (1999, 2001) proposed a model for the treatment of simple TI curves whose advantage is that, once the final estimates of the parameters were found, the curve can be reconstructed by replacing the values of the parameters on the proposed function.

The objective of this research is to develop a mathematical model devoted to analyze the MTIC and to establish a statistical methodology appropriate to draw conclusions about how to vary the taste sensation perceived at successive sips during time. For this general purpose computer software was developed first to record the MTIC, a software in R language was produced to obtain estimates of the parameters of the nonlinear model. This model is a mechanistic one, which analyze step by step the events occurring during the record of a MTIC.

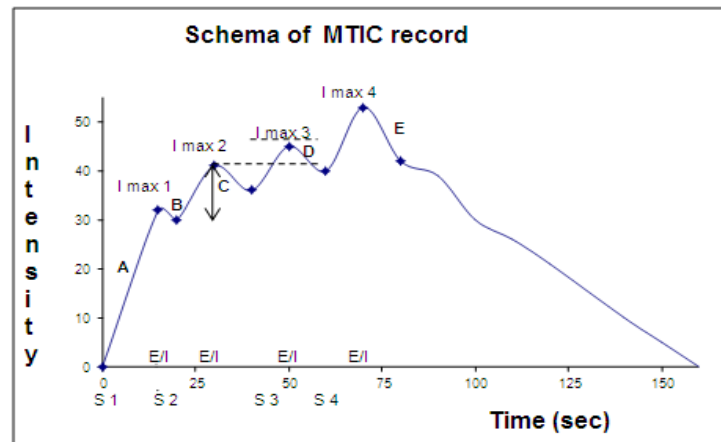


Fig. 1: Common parameters extracted from the MTICs

A: Rate of appearance of the attribute; B: Rate of disappearance of the intensity at first sip (S1); C: Change of intensity in the second sip (S2); D: Intensity variation between the second and third sip; E: Extinction rate in the last sip; Imax: Maximum perceived intensity corresponds to the maximum of the curve, for every sip ($i = 1, 2, 3, 4$); Si: Sip i^{th} ($i = 1, 2, 3, 4$); E/I: The moment when the evaluator spits and swallows

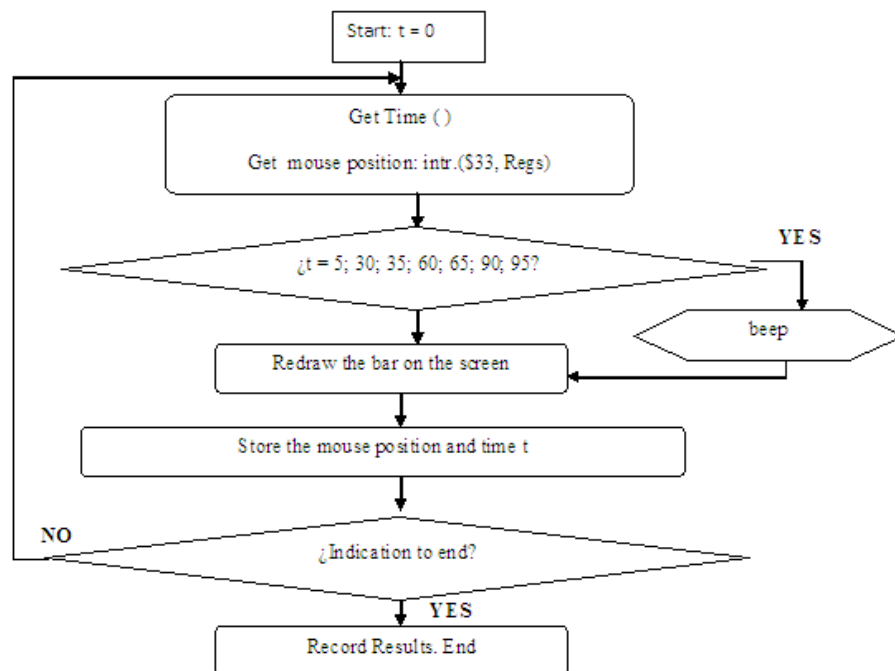


Fig. 2: Flow diagram for the program MTIC.exe

METHODOLOGY

Software development for MTIC acquisition data: It was designed a software in Turbo Pascal language, MTIC.exe, running under the DOS operating system. It allows an adequate description of the MTIC. Turbo Pascal language facilitates the use of operating system functions for defining the parameters of the mouse such as: horizontal and vertical mouse position, button status, among others. Figure 2 shows the corresponding flowchart.

The graphical interface of MTIC.exe is a highlighted bar on the monitor, contained in a rectangle 20 pixels high and 600 wide, having reference marks at 0, 25, 50, 75 and 100%, of the scale respectively. The position of the right end of the bar is variable and scrolls jointly with the hand movements made by the assessor which drives the mouse.

The sampling data can be previously configured with the software MTIC.exe. In this research it

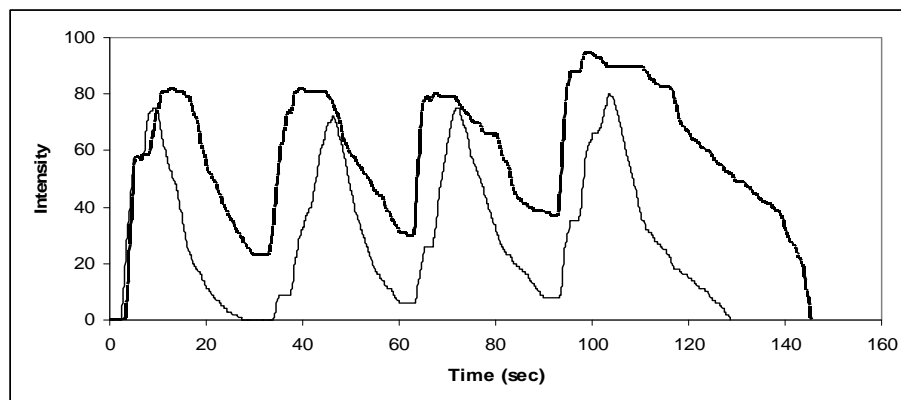


Fig. 3: MTICs obtained by two different trained judges who evaluate 8.4% W/V dextrose into ethanolic solutions at 8% V/V

picks up 10 data/sec, measuring time values in hundredths of a second. The design allows for four successive records. It was established a tasting time of 5 sec and a pause of 30 sec between successive stimuli. A linear transformation is then applied to these data to bring the intensity range (0, 100). The data are stored in a file in ASCII format, so they can be imported from other programs for further analysis. Sips were set at 0, 30, 60 and 90 sec and short beeps sound at 5, 35, 65 and 95 sec, respectively, to tell the assessor that eliminates the oral content. When the assessor completes each MTIC, rinsed his mouth with distilled water. The task was completed by 10 trained judges who evaluate 8.4% W/V dextrose into ethanolic solutions at 8% V/V.

Measurement scale: For determination of sweetness it was used dextrose in water solution as the reference standard stimulus. Participants worked with a current scale of 0 to 100, with five landmarks corresponding to the values: 0, 25, 50, 75 and 100. For the training of judges, triangle tests were conducted which proved discrimination between sweetness levels. The following references were used: for 50% of the scale, a solution of 4.2% W/V of dextrose in distilled water stained with tartrazine, for 75% of the scale, a solution of 8.4% W/V of dextrose in distilled water stained with tartrazine.

Recording curves: MTIC showed several relative maxima corresponding to the I_{MAX} perceived after each sip. Several parameters (Fig. 1) establish the sequential changes of intensity and a more fully kinetic study of a given sensory property.

Figure 3 shows for a given stimulus the MTICs obtained experimentally by two different judges. One record shows that the minimum intensity that

reaches each curve before the next sip is zero, or very closes to this value, as if the sensation disappeared completely before each sip. While the other judge does not show such disappearance of sweetness. That is, the rate of extinction is much more abrupt in the first case. The null $t_{plateau}$ for one of the judges can also be observed. Also, one can see that the latency and the rate of appearance of sensation, for the first section of the curve, are similar for both judges.

Computation of parameters: To calculate initial parameters a program was developed through which starting from the data curve, all parameters are automatically obtained. The program was carried out in a spreadsheet in Excel (2003).

Output of the adjustment program: To find the estimates of proposed parameters, statistical package R 2.11 (R Project for Statistical Computing, Versión 2.11) (2010) (Ritz and Streibig, 2008) was used the nonlinear model which uses R, estimates the parameters by the least squares using the Newton Full-Type algorithm (Dennis and Schnabel, 1983). It is an iterative method which approximates the function $f(t_i, \theta)$ by a 2nd order Taylor polynomial (Adams, 2009).

In order to study the quality of the fit, a program was devised in R language: the parameter estimates, the Sum of Squared Residuals (SSR), the iteration in which SSR reached the lowest value, the asymptotic correlation matrix of parameter estimates, the Residual Mean Square (RMS), the asymptotic standard deviation (standard error), a list of all observations (observed value (Y_i), the value predicted by the model (\hat{y}_i), the residue ($Y_i - \hat{y}_i$), the standard deviation of the predicted value, Cook's distance, the standardized residue, the graph of residuals ($Y_i - \hat{y}_i$) for each value of the

independent variable and the frequency polygon of residuals were obtained.

RESULTS AND DISCUSSION

Physicochemical theoretical background to model MTICs: It is necessary to describe the events that take place in the sensory judge. First, it is possible to analyze the physicochemical events at successive steps.

Oral cavity: The purpose is to determine the concentration of stimulus in the mouth at each time t during the registration period of the curve.

Considering:

t = Time since the judge placed the stimulus in their mouth (s)
 $C(t)$ = Concentration of stimulus in the mouth at the time t (mol/mL)
 $n(t)$ = Number of moles of stimulus in the mouth at t
 $V(t)$ = Volume in the mouth at the instant t (mL)

Determine first separately $V(t)$ and $n(t)$ and then calculate $C(t)$ by the ratio:

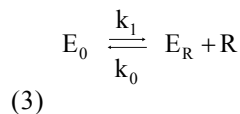
$$C(t) = n(t)/V(t) \quad (1)$$

To calculate the concentration $C(t)$ it is necessary to divide $n(t)$ by the expression of $V(t)$ yielding:

$$C(t) = \begin{cases} C_0 & \text{if } km \leq t \leq d + km \text{ with } k = 0, 1, 2, 3 \\ C_0 e^{-v_1(t-(d+km))} & \\ C_0 e^{-v_1(t-(d+3m))} & \text{if } d + km < t \leq (k+1)m \text{ with } k = 0, 1, 2 \\ C_0 e^{-v_1(t-(d+3m))} & \\ \text{if } t > d + 3m & \end{cases} \quad (2)$$

Taste receptors: At this level, the molecules diffuse into the solution, towards the area of cells where there are the sweet taste receptors. There is a localized concentration of molecules and, once in contact with the receptors, they are combined with them.

The diffusive mechanism may be represented by the following equation which is assumed to be reversible:



where,

E_0 = Initial concentration of molecules of encouragement in the mouth
 E_R = Concentration of molecules in contact with receptors
 R = Free receptors
 k_0, k_1 = Diffusion rates to and from the receptors

The mechanism represented by Eq. (3) indicates that sweet molecules diffused in the mouth and from the area where there are the receptors at k_0 and k_1 rates. Assume that the reaction corresponds to a first order kinetics and therefore is represented by the following differential equation:

$$\frac{d[E_R]}{dt} = k_1 \cdot [E_0] - k_0 \cdot [E_R]$$

where,

$[E_0]$: The concentration of stimulus in the mouth, which Birch *et al.* (1980) assumes that is constant

The process considered in this study is a dynamic process and, therefore, $[E_0]$ is the function $C(t)$ expressed in (2). We obtain the following differential equation replacing $[E_0]$ for $C(t)$ and solved:

$$\frac{d[E_R]}{dt} + k_0 \cdot [E_R] = k_1 \cdot C(t) \quad (4)$$

Since the function $C(t)$ is divided into eight sections, according to the values of t , each differential equation must be solved separately. After solved for the upstream and downstream sections of the function, for ease of notation we define:

$$P(t) = \frac{1}{k_0 - v_1} \left[k_0 \cdot e^{-v_1(t-(d+\lceil \frac{t}{m} \rceil m))} - v_1 \cdot e^{-k_0(t-(d+\lceil \frac{t}{m} \rceil m))} \right] \quad (5)$$

where,

$$\left\lceil \frac{t}{m} \right\rceil = \begin{cases} 0 & \text{if } t < m \\ 1 & \text{if } m < t < 2m \\ 2 & \text{if } 2m < t < 3m \\ 3 & \text{if } t > 3m \end{cases}$$

Furthermore, the following constant is defined:

$$K = \frac{1}{k_0 - v_1} \left[k_0 \cdot e^{-v_1(m-d)+k_0m} - v_1 \cdot e^{k_0d} \right] - e^{k_0m} \quad (6)$$

Therefore, $[E_R]$ can be written as follows:

$$[E_R] = \begin{cases} \frac{k_1 \cdot C_0}{k_0} [1 - e^{-k_0 t}] & \text{if } 0 \leq t \leq d \\ \frac{k_1 \cdot C_0}{k_0} [P(t) - e^{-k_0 t}] & \text{if } d < t < m \\ \frac{k_1 \cdot C_0}{k_0} [1 - e^{-k_0 t} + K \cdot e^{-k_0 t}] & \text{if } m \leq t \leq d + m \\ \frac{k_1 \cdot C_0}{k_0} [P(t) - e^{-k_0 t} + K \cdot e^{-k_0 t}] & \text{if } d + m < t < 2m \\ \frac{k_1 \cdot C_0}{k_0} [1 - e^{-k_0 t} + K \cdot (1 + e^{k_0 m}) \cdot e^{-k_0 t}] & \text{if } 2m \leq t \leq d + 2m \\ \frac{k_1 \cdot C_0}{k_0} [P(t) - e^{-k_0 t} + K \cdot (1 + e^{k_0 m}) \cdot e^{-k_0 t}] & \text{if } d + 2m < t < 3m \\ \frac{k_1 \cdot C_0}{k_0} [1 - e^{-k_0 t} + K \cdot (1 + e^{k_0 m} + e^{k_0 2m}) \cdot e^{-k_0 t}] & \text{if } 3m \leq t \leq d + 3m \\ \frac{k_1 \cdot C_0}{k_0} [P(t) - e^{-k_0 t} + K \cdot (1 + e^{k_0 m} + e^{k_0 2m}) \cdot e^{-k_0 t}] & \text{if } t > d + 3m \end{cases} \quad (7)$$

It is noted that: $[E_R]_{(t=0)} = 0$ and $\lim_{t \rightarrow \infty} [E_R] = 0$

The meaning is that the concentration in the area of the receptors is zero when the time starts to be measured and also after the last sip.

It can be seen that the expression of $[E_R]$ has the same shape for upward intervals, on the one hand and for downward intervals on the other hand. Additionally, to facilitate the notation, we define the following function:

$$H(t) = \begin{cases} 1 & \text{if } 0 \leq t \leq d \\ P(t) & \text{if } d < t < m \\ K \cdot e^{-k_0 t} + 1 & \text{if } m \leq t \leq d + m \\ P(t) + K \cdot e^{-k_0 t} & \text{if } d + m < t < 2m \\ K(1 + e^{k_0 m}) \cdot e^{-k_0 t} + 1 & \text{if } 2m \leq t \leq d + 2m \\ P(t) + K(1 + e^{k_0 m}) \cdot e^{-k_0 t} & \text{if } d + 2m < t < 3m \\ K(1 + e^{k_0 m} + e^{k_0 2m}) \cdot e^{-k_0 t} + 1 & \text{if } 3m \leq t \leq d + 3m \\ P(t) + K(1 + e^{k_0 m} + e^{k_0 2m}) \cdot e^{-k_0 t} & \text{if } t > d + 3m \end{cases} \quad (8)$$

Using this function in the expression of $[E_R]$ given by (7), we obtain:

$$[E_R] = \frac{k_1 \cdot C_0}{k_0} [H(t) - e^{-k_0 t}] \quad (9)$$

Which is the general expression for the concentration of stimulus in contact with receptors.

Brain and perceived intensity: Ennis (1992), in a study of kinetic models for the sweet intensity generated in the brain, describes the process of binding molecules to receptors by the following reaction:



ER = Concentration of receptors occupied with Stimulus

R = Concentration of free receptors

k_2, k_3 = Rates of association and dissociation between stimulus and receptor

It uses the theory developed by Michaelis-Menten. Rosen (1972) and Lehninger (1981) to perform the calculations required for the function describing the intensity of the sensation that the brain generates at time t .

The association constant stimulus-receptor (K) was defined by Beidler (1971) as follows:

$$\frac{[ER]}{[E_R] \cdot [R]} = K = \frac{k_2}{k_3}$$

The dissociation constant stimulus-receptor (K_S) (Birch *et al.*, 1980) is:

$$K_S = \frac{[E_R] \cdot [R]}{[ER]} \quad (11)$$

K_S is the inverse of the association constant stimulus-receptor (K).

The total number of sweet taste receptors (R_0), is constant. These can be empty or occupied by stimulus, thus:

$$R_0 = [R] + [ER] \quad (12)$$

We call S to taste sensation generated by the brain:

$$S = \frac{[ER]}{R_0} \quad (13)$$

As it is necessary to obtain an expression for $[E_R]$, is:

$$S = \frac{[E_R]}{K_S + [E_R]}$$

The next step is to find a function of time to assess the sensation generated by the brain (S), for this reason, it is defined:

$X(t)$ = intensity of the sweet sensation generated by the brain at time t during the development of the MTIC. $X(t)$ has the same expression as S :

$$X(t) = \frac{[E_R]}{K_S + [E_R]}$$

If we replace the expression for $[E_R]$ obtained in (9) we get:

$$X(t) = \frac{H(t) - e^{-k_0 \cdot t}}{a + H(t) - e^{-k_0 \cdot t}} \quad (14)$$

where, a is a constant:

$$a = \frac{K_S \cdot k_0}{k_1 \cdot C_0}$$

The proportion of occupied receptors given by $X(t)$ is a number ranging between 0 and 1, since a is greater than 0.

At the time of starting the curve ($t = 0$), $H(0) = 1$, where $X(0) = 0$. This satisfies the condition at the initial instant, where the brain does not perceive sweetness.

Moreover, $\lim_{t \rightarrow +\infty} H(t) = 0$ implies that, $\lim_{t \rightarrow +\infty} X(t) = 0$ with the passage of time after the last tasting, sweetness disappears (extinction time).

Model development: With this methodology it is possible to characterize taste stimuli and to study mechanisms of production of sweetness, bitterness or astringency of products (Francois *et al.*, 2006). To model experimental sweet MTICs it is necessary to find a function $I = f(t)$ that represents, the real action of ingesting a drink. A mechanistic approach could be able to interpret all events in sensory testing. That is, since the judge puts the solution in his mouth, until the completion of recording after repeating four sips.

We developed a model representative of the MTICs dependent of six parameters. It was analyzed, step by step, all the phenomena occurring while registering the curve (Núñez, 2011). Specifically:

- We calculated the concentration of stimulus in the mouth as a function of time considering the time to spit and saliva flow.
- It was postulated that tastants diffuse into the area where there are the taste receptors and the concentration in that area was calculated.
- Assuming that the perceived taste intensity generated by the brain is given by the ratio of receptors occupied with tastant molecules it was calculated the function that measures the perceived intensity by the brain at each time t .
- Taking into account the judge' interpretation of the measurement scale and the delays that occur in the process of transferring information from the receptors to the brain and from the nervous motor system to the hand that moves the mouse, we postulate a function that gives perceived intensity versus time and which depends on six parameters:

$$I = f(t, a, b, k_0, t_l, v_l, h)$$

The intensity recorded by the judge is zero at the beginning, from t_l (latency time) must be modified with the corresponding delay and multiply by 100, as the scale of measurement was between 0 and 100, The maximum scale value corresponds to the situation in which all receptors were occupied. To find the perceived intensity, multiply by 100, since $X(t)$ is the percentage of occupied receptors. Defined accordingly:

$$I(t) = \begin{cases} 0 & \text{if } 0 \leq t \leq t_l \\ 100 \cdot X(t - t_l) & \text{if } t > t_l \end{cases} \quad (15)$$

where, $X(t)$ is the function:

$$X(t) = \frac{H(t) - e^{-k_0 t}}{a + H(t) - e^{-k_0 t}} \quad (16)$$

And

$$I(t) = \begin{cases} 0 & \text{if } 0 \leq t \leq t_l \\ 100 \cdot \frac{H(t - t_l) - e^{-k_0(t - t_l)}}{a + H(t - t_l) - e^{-k_0(t - t_l)}} & \text{if } t > t_l \end{cases} \quad (17)$$

where, $H(t)$ calculated at $t - t_l$ is:

$$H(t - t_l) = \begin{cases} 1 & \text{if } 0 \leq t - t_l \leq d \\ P(t - t_l) & \text{if } d < t - t_l < m \\ K \cdot e^{-k_0(t - t_l)} + 1 & \text{if } m \leq t - t_l \leq d + m \\ P(t - t_l) + K \cdot e^{-k_0(t - t_l)} & \text{if } d + m < t - t_l < 2m \\ K(1 + e^{k_0 m}) \cdot e^{-k_0(t - t_l)} + 1 & \text{if } 2m \leq t - t_l \leq d + 2m \\ P(t - t_l) + K(1 + e^{k_0 m}) \cdot e^{-k_0(t - t_l)} & \text{if } d + 2m < t - t_l < 3m \\ K(1 + e^{k_0 m} + e^{k_0 2m}) \cdot e^{-k_0(t - t_l)} + 1 & \text{if } 3m \leq t - t_l \leq d + 3m \\ P(t - t_l) + K(1 + e^{k_0 m} + e^{k_0 2m}) \cdot e^{-k_0(t - t_l)} & \text{if } t - t_l > d + 3m \end{cases} \quad (18)$$

The function $H(t)$ is constant and equal to 1 until $t = d$ and decreasing for $d < t < m$. As $H(t)$ starts decreasing from $t = d$, the perceived intensity $I(t)$ starts to decrease from $t - t_1 = d$. Therefore, the decrease occurs from time $t = t_1 + d$. Similar decrements occur on the other downstream sections of the curve.

$H(t)$ begins to grow from $t = m$ and the perceived intensity $I(t)$ will start to grow from $t - t_1 = m$. Therefore growth occurs from time $t = t_1 + m$. Similarly, this increase occurs on the other upstream portions of the MTIC.

Values d and m are fixed, where d is the time elapsed until the judge spits the sample and m is, the time that elapses until the judge turns to put the solution in his mouth. When the judge makes their judgments, measurement has some inertia, at maximum and minimum perceived intensity, he also suffers a delay to start recording the decrease and increase in intensity. This fact introduced two new parameters b : time when the judge indicated that the intensity begins to decrease and h : time when the judge indicated that the intensity begins to grow. That is:

$$b = t_1 + d \quad (19)$$

$$h = t_1 + m \quad (20)$$

If we write $H(t - t_1)$ as a function of the parameters b and h , instead d and m , $H(t - t_1)$ is expressed as follows:

$$H(t - t_1) = \begin{cases} 1 & \text{if } t_1 \leq t \leq b \\ P(t - t_1) & \text{if } b < t < h \\ K.e^{-k_0(t-t_1)} + 1 & \text{if } h \leq t \leq b + m \\ P(t - t_1) + K.e^{-k_0(t-t_1)} & \text{if } b + m < t < m + h \\ K(1 + e^{k_0 m}).e^{-k_0(t-t_1)} + 1 & \text{if } m + h \leq t \leq b + 2m \\ P(t - t_1) + K(1 + e^{k_0 m}).e^{-k_0(t-t_1)} & \text{if } b + 2m < t < 2m + h \\ K(1 + e^{k_0 m} + e^{k_0 2m}).e^{-k_0(t-t_1)} + 1 & \text{if } 2m + h \leq t \leq b + 3m \\ P(t - t_1) + K(1 + e^{k_0 m} + e^{k_0 2m}).e^{-k_0(t-t_1)} & \text{if } t > b + 3m \end{cases} \quad (21)$$

Replacing $H(t - t_1)$ from (21) into Eq. (17) we obtained $I(t)$ as Eq. (22) being b and h the parameters given into Eq. (19) and (20).

If we change the parameter values, different curves are obtained but all are from the same family, i.e., with a similar shape. For curves simply replace the values of the parameters in the expression for $I(t)$ given by Eq. (22).

Figure 4 can display a graphical representation of this function, $I = f(t)$, with a shape similar to the MTICs shown in Fig. 3:

$$I(t) = \begin{cases} 0 & \text{if } 0 \leq t \leq t_1 \\ 100 \cdot \frac{1 - e^{-k_0(t-t_1)}}{a + 1 - e^{-k_0(t-t_1)}} & \text{if } t_1 < t \leq b \\ 100 \cdot \frac{P(t-t_1) - e^{-k_0(t-t_1)}}{a + P(t-t_1) - e^{-k_0(t-t_1)}} & \text{if } b < t \leq h \\ 100 \cdot \frac{K \cdot e^{-k_0(t-t_1)} + 1 - e^{-k_0(t-t_1)}}{a + K e^{-k_0(t-t_1)} + 1 - e^{-k_0(t-t_1)}} & \text{if } h < t \leq b+m \\ 100 \cdot \frac{P(t-t_1) + K \cdot e^{-k_0(t-t_1)} - e^{-k_0(t-t_1)}}{a + P(t-t_1) + K \cdot e^{-k_0(t-t_1)} - e^{-k_0(t-t_1)}} & \text{if } b+m < t \leq m+h \\ 100 \cdot \frac{K(1 + e^{k_0 m}) \cdot e^{-k_0(t-t_1)} + 1 - e^{-k_0(t-t_1)}}{a + K(1 + e^{k_0 m}) \cdot e^{-k_0(t-t_1)} + 1 - e^{-k_0(t-t_1)}} & \text{if } m+h < t \leq b+2m \\ 100 \cdot \frac{P(t-t_1) + K(1 + e^{k_0 m}) \cdot e^{-k_0(t-t_1)} - e^{-k_0(t-t_1)}}{a + P(t-t_1) + K(1 + e^{k_0 m}) \cdot e^{-k_0(t-t_1)} - e^{-k_0(t-t_1)}} & \text{if } b+2m < t \leq 2m+h \\ 100 \cdot \frac{K(1 + e^{k_0 m} + e^{k_0 2m}) \cdot e^{-k_0(t-t_1)} + 1 - e^{-k_0(t-t_1)}}{a + K(1 + e^{k_0 m} + e^{k_0 2m}) \cdot e^{-k_0(t-t_1)} + 1 - e^{-k_0(t-t_1)}} & \text{if } 2m+h < t \leq b+3m \\ 100 \cdot \frac{P(t-t_1) + K(1 + e^{k_0 m} + e^{k_0 2m}) \cdot e^{-k_0(t-t_1)} - e^{-k_0(t-t_1)}}{a + P(t-t_1) + K(1 + e^{k_0 m} + e^{k_0 2m}) \cdot e^{-k_0(t-t_1)} - e^{-k_0(t-t_1)}} & \text{if } t > b+3m \end{cases} \quad (22)$$

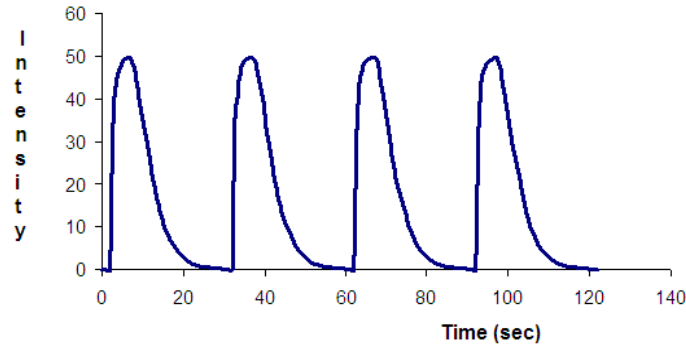
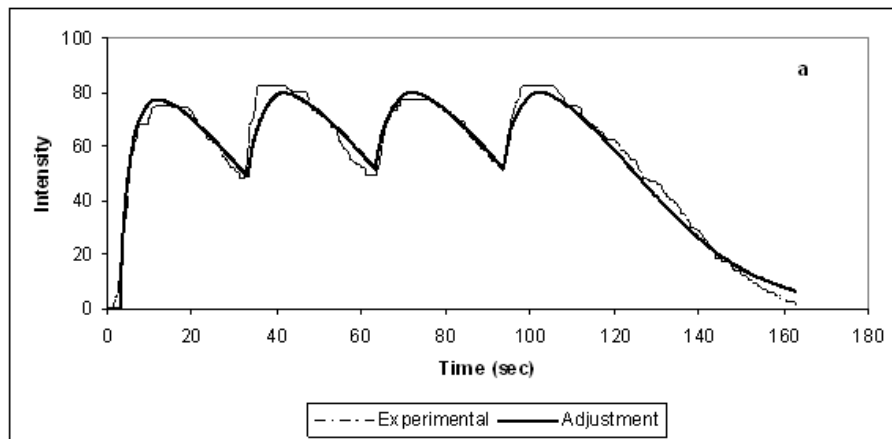


Fig. 4: Theoretical curve $I = f(t)$ corresponding to the Eq. (22) with parameter values: $a = 1$, $b = 7$, $k_0 = 1$; $t_1 = 2$, $v_1 = 0.3$, $h = 32$



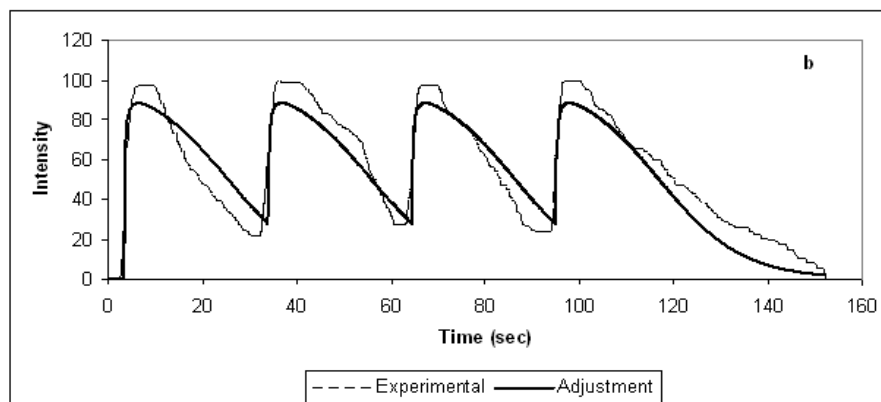


Fig. 5: a) Experimental and adjusted MTIC to the best record, b) experimental and adjusted MTIC to the worst record

Therefore, the assumption that the reactions correspond to first order kinetics was adequate.

Non lineal adjustment to the model: For each experimental MTIC we have to search the estimates of these 6 parameters. Nonlinear fitting models should be used.

It is essential to have a vector of initial parameters to begin the process. They require good initial estimates of parameters to ensure convergence and reduce the number of iterations. Also to avoid falling into a saddle point, since this point has derivatives that are zero but does not correspond to a minimum.

Applicability of the model to describe sweet MTICs: Although no mathematical models have been developed for the use of the MTIC, some statistical methodologies were used to analyze the experimental data. Guinard *et al.* (1986a) found that Table 1: Comparisons of individual MTICs parameters and computed output of curve adjustments

| Parameters | Judge 1 | Judge 2 |
|-------------------------|----------|-----------|
| a^* | 0.12 | 0.12 |
| b^* | 9.50 | 5.30 |
| k_0^* | 0.07 | 0.91 |
| t_i^* | 3 | 3.60 |
| v_i^* | 0.37 | 0.11 |
| h^* | 33.20 | 34 |
| SSR [#] | 18667.39 | 143134.80 |
| $n^{\#}$ | 1478 | 1379 |
| RMS [#] | 12.63 | 103.80 |
| Iterations [#] | 103 | 113 |

*: Estimates of parameters; #: Measures of curve adjustment

the total duration of astringency increases for wine plus tannic acid, while the maximum intensity does not change when the models were evaluated with Multiple Times Intensity Curves (MTICs). Also Courregelongue *et al.* (1999) assessed the effect of viscosity, sucrose and oil in perceived astringency during consumption of soy milk using a sequential IT procedure. In this case, the judges sucked 4 samples, ensuring that the perceived Maximum

Intensity (IMAX) of astringency increases significantly with successive sips and that the time to reach IMAX decreases from sip 1 to 3, but is longer in the fourth sip. In determining MTICs for beers it was proved that the duration of bitterness increased significantly between the first and subsequent sips and that duration, the peak intensity and the time to reach maximum intensity increase significantly with the concentration of iso- α -acids but are not affected by the time between sips (Guinard *et al.*, 1986b).

Schiffman *et al.* (2003) determined that the sweetness intensity decreases more slowly, when intakes are reiterated to binary or ternary combinations in relation to those single sweeteners and they link these results with a synergistic phenomenon governing the intensity of these mixtures.

An advantage of the model proposed in Eq. (22) is that it allows to reconstruct the curve, from the values of the parameters, by $I = f(t, a, b, k_0, t_i, v_i, h)$. So far no exist other models that allow drawing the curve from the values of the parameters.

As an example we present the results of two experimental curves and their respective adjustments applying Eq. (22) these curves are from different judges (Table 1 and Fig. 5a). The model also allows describing adequately the experimental curves with some defects, as shown in Fig. 5b.

CONCLUSION

We have examined MTICs and propose a model to describe the change in perceived intensity of sweetness as a function of time. Another aim of this research was to develop the statistical methodology appropriate to draw conclusions about how to vary the taste sensation perceived at successive sips during time. Furthermore, it was designed a software for MTIC acquisition data.

The physicochemical theoretical background to model MTICs includes an analysis of events at successive steps:

- Oral cavity, where the purpose was to determine the concentration of stimulus in the mouth at each time t during the registration period of the curve
- Taste receptors, where it was obtained the general expression for the concentration of stimulus in contact with receptors
- Brain and perceived intensity where it was found a function of time to assess the sensation generated by the brain at time t during the development of the MTIC

Finally we developed a model representative of the MTICs, dependent of six parameters. It was analyzed, step by step, all the phenomena occurring while registering the curve. An advantage of the model proposed in Eq. (22) is that it allows to reconstruct the curve, from the values of the parameters, by $I = f(t, a, b, k_0, t_1, v_1, h)$. The model allows fitting experimental data from different judges and also allows describing adequately the experimental curves with some defects.

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REFERENCES

- Adams, R.A., 2009. Calculation. Pearson Education, Madrid, Spain (Spanish).
- Beidler, L.M., 1971. In: Handbook of Sensory Physiology. Chemical Senses, Part 2, Taste. Springer Verlag, Berlin, 4: 200.
- Birch, G.G., Z. Latymer and M. Hollaway, 1980. Intensity/time relationships in sweetness: Evidence for a queue hypothesis in taste chemoreception. *Chem. Sens.*, 5: 63-78.
- Courregelongue, S., P. Schlich and A.C. Noble, 1999. Using repeated ingestion to determine the effect of sweetness, viscosity and oiliness on temporal perception of soymilk astringency. *Food Qual. Prefer.*, 10: 273-279.
- Dennis, J.E. and R.B. Schnable, 1983. Numerical Methods for Unconstrained Optimization and Nonlinear Equations. Prentice Hall, Englewood Cliffs.
- Ennis, D.M., 1992. Kinetic models os sweet taste. *Trends Food Sci. Tech.*, 3: 169-172.
- Francois, N., C. Guyot-Declercq, B. Hug, D. Callemien, B. Govaerts and S. Collin, 2006. Beer astringency assessed by time-intensity and quantitative descriptive analysis: Influence of pH and accelerated aging. *Food Qual. Prefer.*, 17: 445-452.
- Garrido, D., 1997. Development of statistical methods for sensory analysis of intensity-time curves. Ph.D. Thesis, Faculty of Pharmacy and Biochemistry, University of Buenos Aires, Argentine (Spanish).
- Garrido, D., R. Cossalter and A.M. Calviño, 1999. Methodology for recording computerized time-intensity sensory attributes of foods. *Int. J. Inform. Technol. (Spanish)*, 10: 15-20.
- Garrido, D., A.M. Calviño and G. Hough, 2001. Averaging time-intensity taste data by a parametric model. *Food Qual. Prefer.*, 12: 1-8.
- Guinard, J.X., R.M. Pangborn and M.J. Lewis, 1986a. The time-course of astringency in wine upon repeated ingestion. *Am. J. Enol. Viticulture*, 37: 184-189.
- Guinard, J.X., R.M. Pangborn and M.J. Lewis, 1986b. Effect of repeated ingestion on temporal perception of bitterness in beer. *ASBC J.*, 44: 28-32.
- Lehninger, A.L., 1981. Biochemistry, the Molecular Basis of Cell Structure and Function. Omega SA Edn., Barcelona (Spanish).
- Núñez, M., 2011. Development of statistical methods for analyzing time-current curves for successive gustatory stimulations. Ph.D. Thesis, Faculty of Pharmacy and Biochemistry, University of Buenos Aires (Spanish), Argentine.
- R 2.11 (R Project for Statistical Computing, Versión 2.11), 2010. Free Software Developed by Bell Laboratories under the terms of the Free Software Foundation and the GNU (Spanish). General Public License.
- Ritz, C. and J.C. Streibig, 2008. Nonlinear Regression with R. Springer, USA.
- Rosen, R., 1972. Foundations of Mathematical Biology: Dubcellular Systems. Academic Press. New York, Vol. 1.
- Schiffman, S., E.A. Sattely-Miller, B.G. Gram, J. Zervakis, H.H. Butchko and W.W. Stargel, 2003. Effect of repeated presentation on sweetness intensity of binary and ternary mixtures of sweeteners. *Chem. Sens.*, 28: 219-229.