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RESEARCH ARTICLE

Electroencephalography and acceptance test to assess sodium reduction in tomato sauce: an exploratory research

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ABSTRACT

The association of excessive salt consumption with development of noncommunicable diseases has created initiatives for the reformulation of processed foods aiming to reduce the salt content. A neurophysiological and sensory approach provides valuable information to ensure that a reformulated product retains its acceptability to consumers. This study evaluated consumer perception during the ingestion of tomato sauce which was low in sodium by using an electroencephalogram and hedonic acceptance scale. Monosodium glutamate promoted a large increase in cortical activity and also generated a higher rate of acceptance. Disodium inosinate also promoted increased electrical activity and had a similar acceptance to sodium chloride. The sample containing potassium chloride without flavor enhancer was the one that generated the least electrical activity and acceptance. The addition of flavor enhancers — umami taste promoters — masks the sensory defects of KCl and this effect seems to be related to increased electrical activity in brain regions related to taste and reward/pleasure.

Keywords: Electrophysiology; Umami; Salt substitutes; Food choice; Neural response; Salt reduction

Practical Application: Neural and sensory response data on salt substitution aim to provide information on how to reduce sodium in a product without compromising its acceptance by the consumer.

INTRODUCTION

Diets with frequent consumption of processed foods are related to a high intake of salt, sugar, and saturated fats. This dietary habit is strongly related to the development of noncommunicable diseases (NCDs) such as hypertension, diabetes mellitus, chronic kidney disease, cancer, heart attack, and stroke (Beaglehole et al., 2011). Several health agencies around the world such as WHO, ANVISA, AWASH, Health Canada, and Public Health England propose guidelines aimed at reducing excessive salt consumption by means of educational policies, an increased consumption of potassium, and reformulating industrialized foods (Joffres et al., 2007; Nilson et al., 2012; Ogbu and Arah, 2016; Public Health England, 2017; Webb et al., 2017; Webster et al., 2015; World Health Organization, 2010).

Some foods have been established as a priority for salt reduction due to high sodium content provided per portion. These include sausage meats, bread, dairy products and sauces like: tomato sauce, ketchup, soy sauce, and mayonnaise (WHO and PAHO, 2013). However, it is not enough to reduce the sodium of the formulation; it is necessary to replace it in order to not reduce consumer product acceptance. As a result, strategies like sensory evaluation and neurophysiological approaches yield valuable insights for preserving the commercial success of a product (Tomadoni et al., 2018).

In the field of sensory evaluation, descriptive methods have been widely used in the reformulation of food (Maurice, 2020; Silva et al., 2018). However, such methods are not specialized in verifying the affective response of a food modification process, and so, it is very common to perform

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acceptance tests with a hedonic scale (Aaltonen et al., 2020; Conroy et al., 2018; Felicio et al., 2016).

In this scenario, new approaches such as electroencephalography (EEG), can be used to aggregate information related to food consumption and acceptance. The technique has already been used successfully in the analysis of neural stimulation of flavored gum, discrimination of sweet and salty stimuli, as well as in the acceptance of beer and sweetened solutions (Andersen et al., 2019; Gonzalez Viejo et al., 2019; Hashida et al., 2005; Yagyu et al., 1998). However, there are no reports in the literature on the use of EEG in products with sodium reduction and the addition of flavor enhancers.

The EEG is a low-cost, non-invasive method of monitoring cortical electrical activity (Nunez and Srinivasan, 2009). EEGs have been successfully used to evaluate the behavior of neural stimulation of flavorless and flavored chewing gum, discrimination of sweet and salty stimuli in aqueous solutions, acceptance of beer, and sweetened solutions with sucrose and sweeteners (Andersen et al., 2018; Gonzalez Viejo et al., 2018; Hashida et al., 2005; Yagyu et al., 1998).

This study used EEG and a modified acceptance test to explore the neuronal response of individuals when consuming conventional tomato sauce in terms of sodium content, ie, salted only with sodium chloride (NaCl) and also tomato sauces with 50% substitution of sodium by potassium chloride (KCl) - mixed or not with monosodium glutamate (MSG) and disodium inosinate (IMP).

MATERIALS AND METHODS

Material

Tomato sauce

The tomato sauce samples were elaborated in the Laboratory of Food Technology at the Federal University of Alfenas. The sauce ingredients can be seen in Table 1 and the concentrations of salts used in Table 2. The concentration of sodium substitutes and flavor enhancers used in this paper were defined in a previous study that determined the appropriate concentrations through sensory evaluation (Tavares-Filho, 2020).

Table 1: Tomato sauce standard basis formulation

Ingredients	(%)
Canned peeled tomatoes (Fiamma®, Ottaviano - Italy)	60
Tomato sauce (Fiamma®, Ottaviano - Italy)	26.15
Fresh onion	10
Fresh garlic	0.75
Crystal Sugar (União®, Sertãozinho - Brazil)	1.25
Extra virgin olive oil (Hojiblanca®, Madrid - Spain)	0.5
Cornstarch (Maizena®, Moji-Guaçu - Brazil)	1.25
Dried oregano	0.025
Dried basil	0.025
Dried chive	0.025
Dried parsley	0.025
Total	100%

Table 2: Set of samples used as a gustatory stimulus

Sample name	Composition
NaCl	Sauce basis + 0.87% sodium chloride
KCl	Sauce basis + 0.58% potassium chloride + 0.435% sodium chloride
MSG	Sauce basis + 0.58% potassium chloride + 0.435% sodium chloride + 0.6% monosodium glutamate
IMP	Sauce basis + 0.58% potassium chloride + 0.435% sodium chloride + 0.03% disodium inosinate

Methods

Participants

Twenty tasters were recruited to compose the panel in the Federal University of Alfenas medical school, through dissemination in the information networks of the university. The recruitment conditions consisted of consuming tomato sauce (at least once a week) and availability of time to participate in the sessions.

After screening, nine volunteers (five men and four women) were recruited to participate in the study. The exclusion criteria for participation in the study were: people who were ill or using medication for chronic illness, smokers, pregnant, chemical dependents, minors, people with any taste disorders, people presenting allergies to any component of the formula or having already presented any adverse reaction to examinations similar to an EEG. Participants were instructed to not ingest water or food for at least two hours before the examination.

Stimulating taste

The taste stimuli consisted of four different samples of tomato sauce. The constitution of each sample can be seen in Table 2. Distilled water was used as neutral stimulus solution (baseline) to promote the return of EEG basal stimulus and to wash participants' mouths after each stimulation.

Sensory evaluation

After the electroencephalography sessions, the participants were sent to a rest room where they took a 30-minute break and then were sent to the acceptance test. The acceptance test (Stone et al., 2012) only evaluated the flavor attribute to be most affected by the replacement of sodium chloride. The participants received the samples in a sequential monadic manner, in transparent 50 mL disposable plastic cups, encoded with three random numbers. Participants were also provided with water and unsalted crackers for palate cleansing (Esmerino et al., 2017). Acceptance was determined using a 9cm unstructured linear hedonic scale (Stone et al., 2012), anchored at its ends, on the left "I disliked it extremely" and on the right "I liked it extremely". All samples will be evaluated using complete block balancing (Wakeling and MacFie, 1995).

Procedure

The participants performed a test session where each sample was stimulated for 2 minutes, preceded and succeeded by a stimulus with a neutral solution of distilled water for 1 minute. Before the test, a verbal explanation was given about the procedure, the positioning of electrodes, how to keep the sample in the mouth, and how to maintain posture during the procedure.

The EEG procedure was adapted from studies of Hashida et al., (2005) and Park et al., (2011). The sessions were held in a comfortable chair. After positioning the electrodes, EEG recording began, with patients remaining at rest for 1 minute until the stabilization of their brain waves. Then, participants received stimulus first with distilled water, followed by the sample, distilled water again, the sample, and so on until the sample set was finished. All samples were evaluated using complete block balancing (Wakeling and MacFie, 1995). During stimulation, testers were instructed to neither swallow the samples nor open their eyes for 20 seconds. After the EEG was executed, a pause of 30 minutes was taken and the samples were again presented together on the hedonic scale.

Electrodes and data collection

EEG data were recorded at positions Cz, FP1, FP2, T3, and T4 according to the system 10-20, with a sensitivity of 7uV / mm and 60Hz filter. The taste-related stimuli, as well as the decision-making system, shares the following areas: prefrontal cortex, cingulate gyrus, and temporal cortex.

Results analysis

The electroencephalographic records were analyzed by detailed visual inspection performed by clinical neurophysiologists in the field of electroencephalography. Initially, the spectra were read in isolation and then palate readings were compared with baseline readings.

The data obtained in the acceptance test were submitted to Variance Analysis (ANOVA) and Fisher tests, when the occurrence of significant differences among averages were verified ($p \leq 0.05$) (Meilgaard et al., 2016; Stone et al., 2012).

RESULTS AND DISCUSSION

The study explored changes in electrical response of the cerebral cortex in the temporal and frontal regions. The frontal region of the prefrontal cortex acts in the primary reinforcement system, which includes taste, touch, processing of texture, and recognition of facial expression. Therefore, it plays a fundamental role in the affective process that determines whether a food will be accepted or not (Rolls, 2017). Another important brain region in this process is the primary gustatory area (G area), positioned at the transition between the parietal operculum and the cerebral cortex insula, including the temporal cortex borders. This is a region with high responsiveness to gustatory stimuli (Kobayakawa et al., 1996).

The complex phenomenon of food choice, the sensation of satiety, and the pleasure promoted by food seems to be strongly involved with these cortical regions. This region is also related to the factors that lead to excessive consumption of foods rich in fats, sugars, and salt, which are considered to be high palatability foods (Kenny, 2011). Two pathways are elucidated involving this process: the pathway that controls the expression of metabolic signs of hunger and satiety, and another involved with stimulation of the reward system (Sclafani and Ackroff, 2003).

The standard NaCl sample, which contained sodium chloride exclusively as a saltiness substance, caused moderate changes in brain activity (Fig. 1 and Table 3), as recorded at five electroencephalogram positions, with a higher intensity in the pre-frontal region detected by Fp1 and Fp2 electrodes. Individually, sodium chloride activates a small region of the primary gustatory area (Chiaraviglio, 1984), but in this study, the stimulation appeared to be more intense and covered a larger area of the prefrontal cortex, both for NaCl and for the other samples. This can be explained mainly by the presence of various aromatic substances that promote taste and scent in tomato sauce (Markovic et al., 2007), as well as the affective memory of tomato sauce since this is widely consumed in a wide range of dishes.

The KCl sample stimulated the cortex less than the others. The electroencephalogram only traced mild and short-changes (Fig. 2 and Table 3). Plata-Salaman et al. (1996), when comparing the stimuli of different substances in neurons isolated from the insular and opercular cortex of monkeys, found that the stimulus pattern of KCl was more similar to bitter substances such as quinine and magnesium chloride (MgCl) than with NaCl. This shows that, although the KCl produced salty taste, its bitterness stimulation overlapped in the studied neurons.

According to Peng et al. (2015), the stimulation of bitterness and its cortical response affects consumption behavior because bitter taste is evolutionarily characteristic of poisonous substances, its stimulation seems to somehow

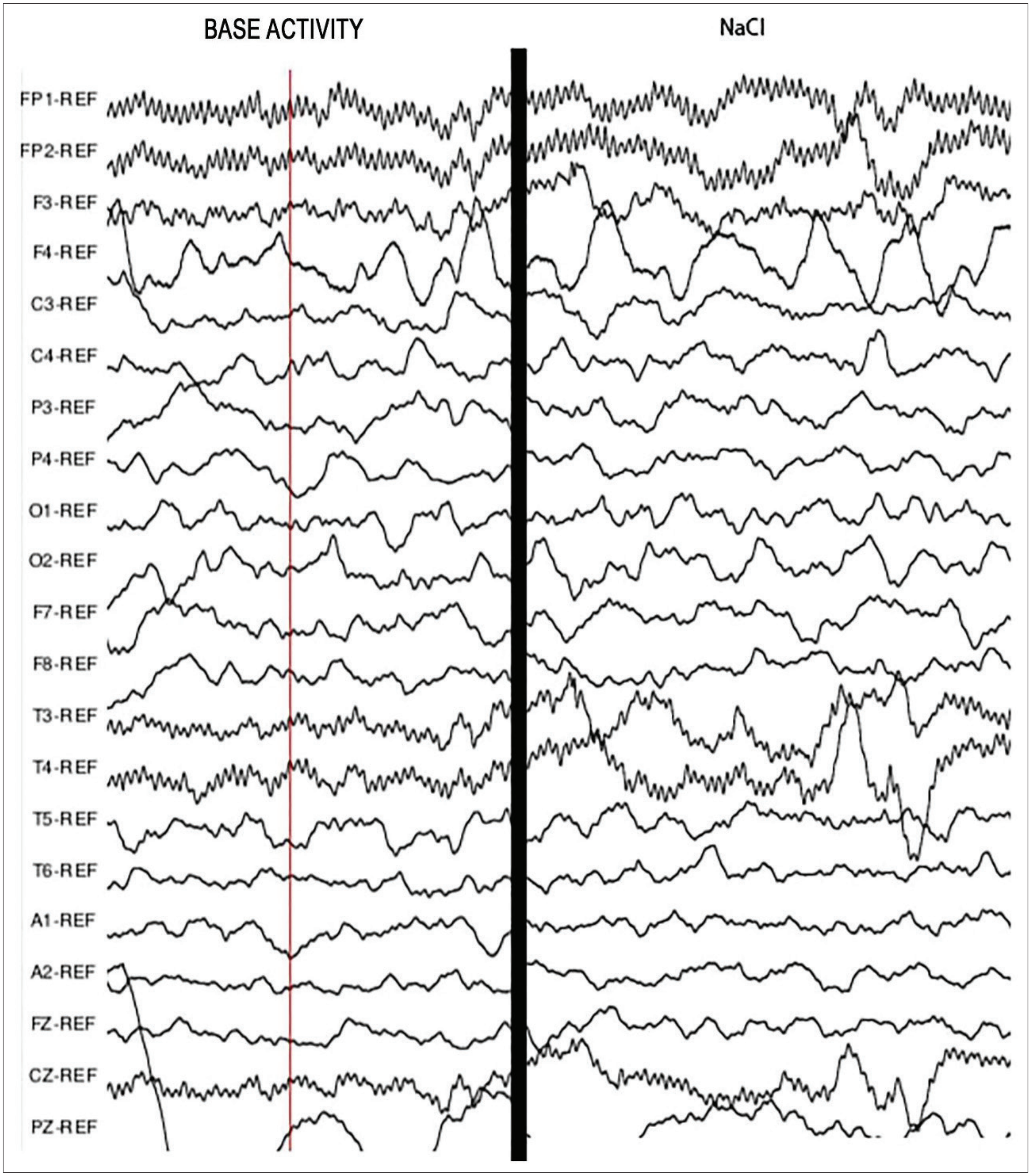

Fig 1. Base activity compared to NaCl. Presence of little activity with small change of wavelength, mainly in temporal and frontal regions to the right

inhibit pleasure when ingested. Among the basic tastes, bitter is the one that has the least acceptance among consumers. Moreover, sweet stimuli (best acceptance) and bitter (worse acceptance) are differentiated more rapidly by the brain, which correlates with the hedonic evolutionary characteristic of acceptance or rejection of each substance, respectively (Wallroth and Ohla, 2018).

MSG and IMP promoted a strong increase in brain electrical activity in the five regions studied, as evidenced in Fig. 3 and Table 3. “Umami” (typical of monosodium glutamate and inosinate) and sweet (glucose) stimuli, displayed greater activity of the anterior parts of the insular cortex, frontal operculum, and orbit-frontal cortex when compared to a solution containing only the major ionic components of saliva. In addition, De Araujo et al. (2003) found that a psychophysical effect, known as “synergism for umami substances,” is reflected in the activity of the orbit-frontal cortex, and given the range of flavors present in spicy tomato sauce, this synergistic effect seems to have occurred. De Araujo et al., (2003), also exhibited this synergy. This finding exemplifies the process of a complex interaction between the structural properties of gustatory stimuli and their palatability, and the increased palatability promoted by the addition of MSG to food.

The action of glutamate and disodium inosinate ions can promote taste improvement in tomato sauce containing KCl as a partial substituent for NaCl, mainly by the signaling at T1R1 / T1R3 receptors. Umami taste is mediated by multiple receptors, including taste receptors T1R1 + T1R3, and metabotropic glutamate receptors (mGluR) (Yasumatsu et al., 2015). The mGluR occur mainly in the posterior region of the tongue and is are

Table 3: Representing the consensus obtained by visual inspection of the EEG

		Substance	Position				
			Fz	Fp1	Fp2	T3	T4
Participants	1	NaCl	-	-	-	↑	-
		KCl	-	-	-	-	-
		MSG	↑↑	↑↑↑	↑↑	↑↑↑	↑↑
		IMP	↑↑	↑↑↑	↑↑	↑↑↑	↑↑
	2	NaCl	-	-	↑	-	-
		KCl	-	-	↑	-	-
		MSG	-	-	↑↑↑	-	-
		IMP	-	-	↑↑↑	-	-
	3	NaCl	-	-	↑	-	-
		KCl	-	-	↑	-	-
		MSG	-	-	↑	-	-
		IMP	-	-	↑	-	-
	4	NaCl	-	-	↑	-	↑
		KCl	-	-	↑	-	↑
		MSG	↑↑	↑↑	↑↑↑	↑↑	↑↑↑
		IMP	↑↑	↑↑	↑↑↑	↑↑	↑↑↑
	5	NaCl	-	-	-	-	-
		KCl	-	-	-	-	-
		MSG	↑↑	↑↑↑	↑↑	↑↑↑	↑↑
		IMP	↑↑	↑↑↑	↑↑	↑↑↑	↑↑
	6	NaCl	-	-	↑↑	-	-
		KCl	-	-	-	-	-
		MSG	-	-	↑↑↑	-	↑↑↑
		IMP	-	-	↑↑↑	-	↑↑↑
	7	NaCl	↑	↑↑	↑	↑↑	↑
		KCl	-	-	-	-	-
		MSG	↑	↑↑	↑	↑↑↑	↑↑
		IMP	↑	↑↑	↑	↑↑↑	↑↑
	8	NaCl	-	-	-	↑	↑
		KCl	-	-	-	↑	-
		MSG	-	-	-	↑↑↑	↑↑↑
		IMP	-	-	-	↑↑↑	↑↑
	9	NaCl	-	↑↑	-	-	-
		KCl	-	↑↑	-	-	-
		MSG	-	↑↑↑	-	-	-
		IMP	-	↑↑↑	-	-	-

Legend: Arrows pointed up indicate an increase in the electrical activity of the brain. The number of arrows indicates the intensity of this increase (↑ = Discrete; ↑↑ = Moderate, ↑↑↑ = High).

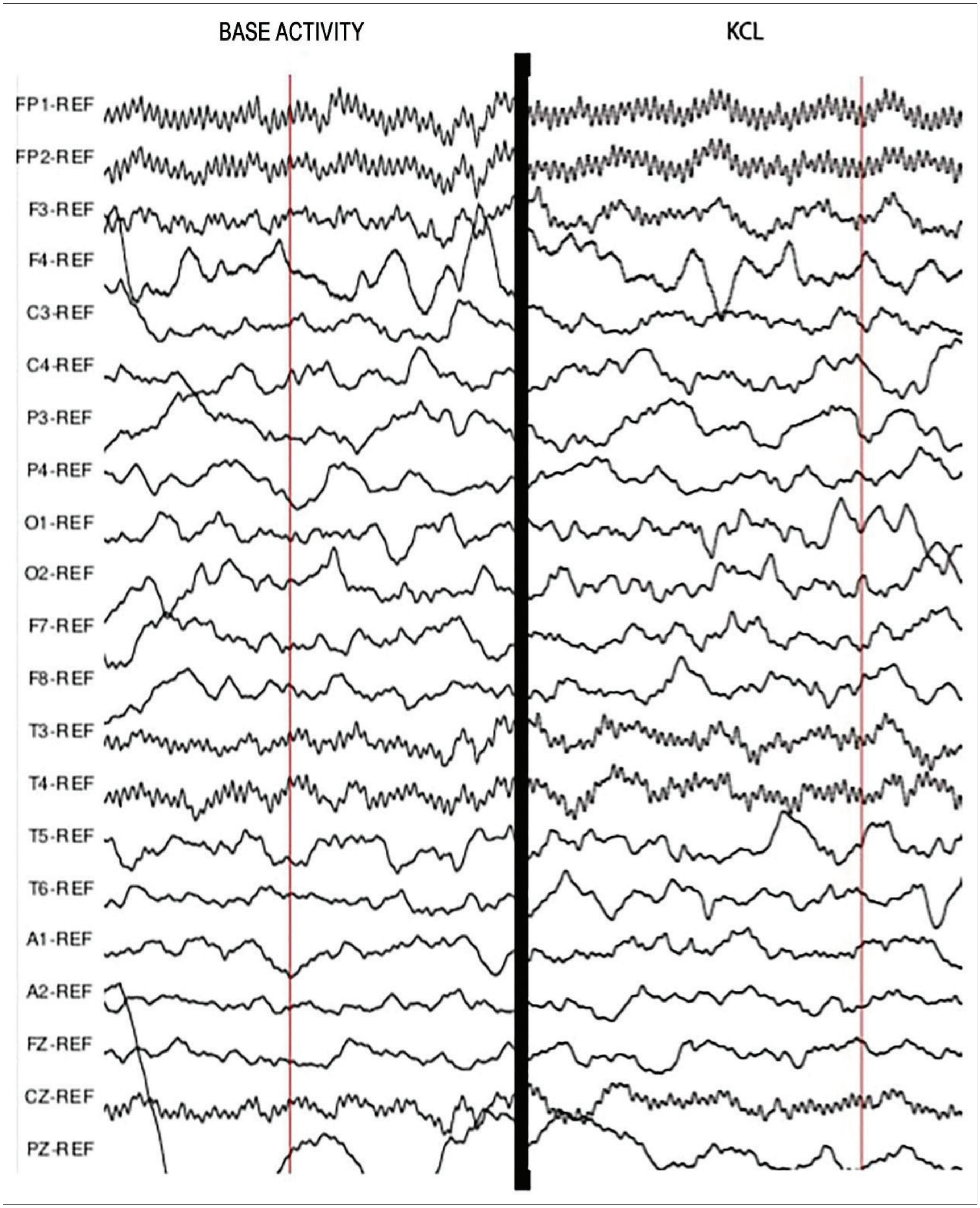

Fig 2. Base activity compared to KCl. Practically unchanged activity in regions studied, compared to baseline activity.

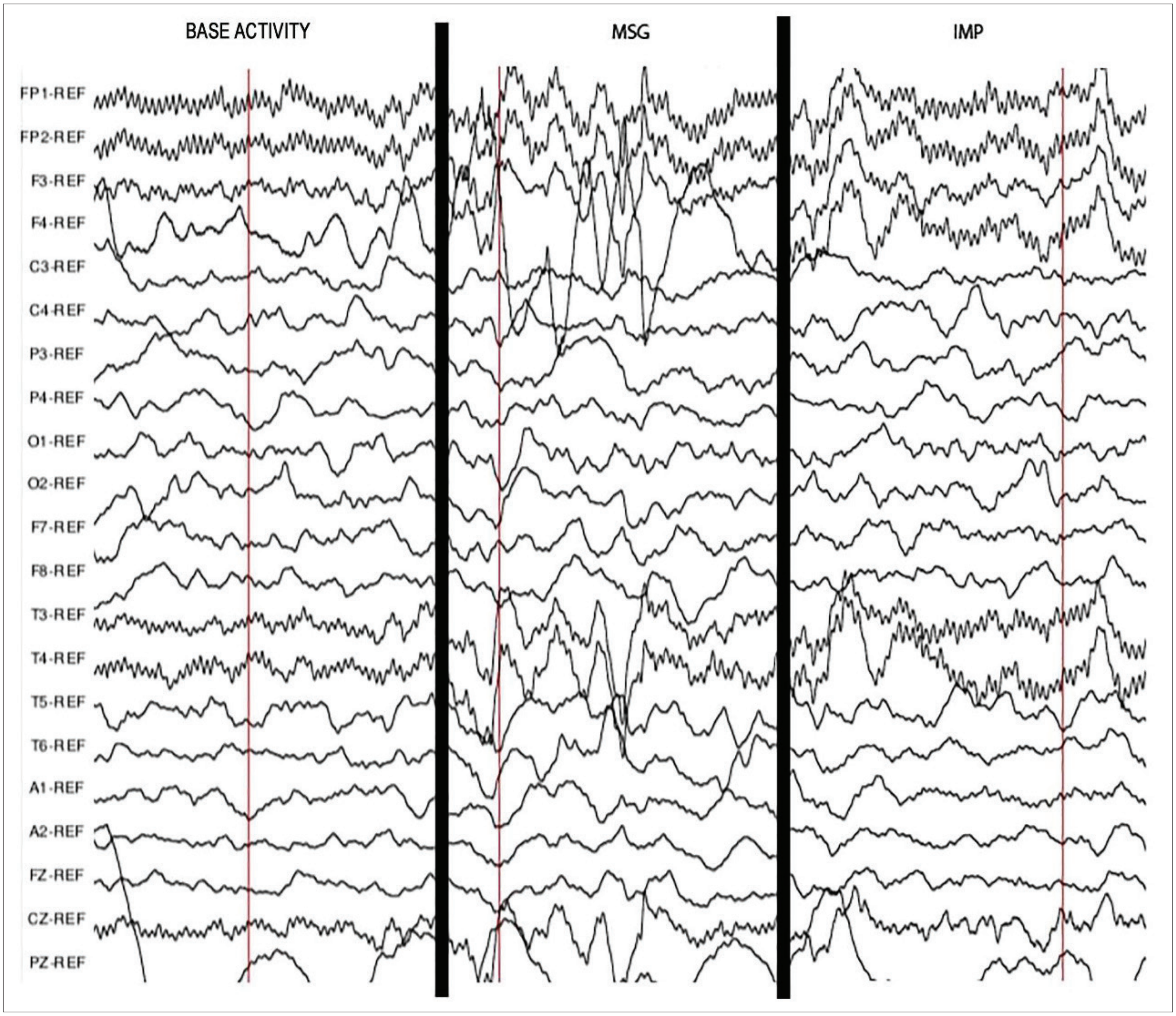

Fig 3. Basic activity compared to MSG and IMP. Presence of great alteration of the wave amplitude, with greater activity in temporal lobes.

associated with a distinction between umami and other flavors, whereas the T1R1 / T1R3 signal occurs mainly in the anterior region of the tongue and plays an important role in preference behavior (Dang et al., 2019).

Individual variation exists in the perception of basic tastes by humans (Melis and Barbarossa, 2017) and for umami tastes (Singh et al., 2010). Individuals can be classified into High-Taster and Low-Tasters according to their sensitivity (Han et al., 2018). This phenomenon may explain the discrepancy of the cortical electrical response of Participant 3 in relation to the others.

Acceptance tests showed that MSG presented a higher mean of acceptance (Table 4). Monosodium glutamate promotes umami taste, and has an appetite-increasing property by improving the palatability of food (Rogers and Blundell, 1990), even in individuals with impaired palates (Schiffman, 1998). When used in fish burgers, fried bread, soup, and chicken broth, monosodium glutamate also promoted increased consumer acceptability (Chi and Chen, 1992; Maheshwari et al., 2017; Quadros et al., 2015), NaCl and IMP did not differ between themselves in an acceptance test. This result showed that disodium inosinate was able to improve the flavor of potassium

Table 4: Fisher's mean test for means of acceptance

Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups
MSG	6.456	0.471	5.484	7.428	A
NaCl	4.350	0.471	3.378	5.322	B
IMP	4.344	0.471	3.372	5.316	B
KCl	2.144	0.471	1.172	3.116	C

chloride-containing dressing. Campagnol et al. (2011) and Dos Santos et al. (2014) have demonstrated that the use of disodium inosinate is efficient in reducing sensory defects caused by the replacement of NaCl with KCL.

The sample containing KCl presented the lowest acceptance mark, distinguishing it from all others. This result is associated with KCl sensory defects, such as bitter and metallic tastes, when used in large proportions. Wu et al., (2015) reported that replacing NaCl with KCl in proportions greater than 40% without adjuvant strategies promotes serious sensory defects.

The sample size (“N”) of the study was its main limitation, mainly in relation to the results observed in the acceptance with the hedonistic scale. Future studies can be performed with a larger number of participants, using not only the “flavor” attribute of the acceptance test, but also appearance, aroma, texture, and overall impression.

CONCLUSIONS

The addition of monosodium glutamate and disodium inosinate increased the cortical stimulation and the acceptance test scores, resulting from the ingestion of tomato sauces containing potassium chloride. Thus, it seems that high scores on the acceptance test may be related to greater stimulation of the frontal and temporal cortex.

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Author contributions

Tavares-Filho E.R, Esmerino E.A, Santos-Junior V.A: designed the study, conducted the tests, collected/analyzed the data and drafted the manuscript. Tavares, E.J.S. conducted sensory tests, collected data, and drafted the manuscript. Bolini H.M.A and Castro R.A.O. worked on the study design process, analyze the results, edited the draft, and reviewed all documents critically, and approved the final manuscript for submission to the Journal.

The present study was approved by the Research Ethics Committee of the Federal University of Alfenas-MG, under the number of accepted opinion 2398232.

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