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Selecting and Training a Panel to Evaluate the Metallic Sensation of Meat

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The objective was to select and train assessors to evaluate metallic sensations in beef. The detection threshold of $FeSO_4.7H_2O$ was determined using the method of limits. Twenty-six undergraduates participated in the test, and a single detection threshold was determined. Candidates with a threshold lower than or equal to the general value threshold were accepted. Next, the performance of the accepted candidates was evaluated using meat from the *semimembranosus* muscle of bovines at an internal cooking temperature of 65, 72 or 75°C. The metallic sensation in the meat was evaluated using the ranking test and an unstructured 9.0 cm line scale. The extremes of the scale have the expression not perceptible and highly perceptible. The assessors were selected to help the team analyze the discriminative power, reproducibility and overall use of scale. The results demonstrated that the processes for selecting and training assessors to evaluate the metallic sensation in meat were adequate.

Keywords: beef, ferrous sulfate, threshold, metallic sensation

Introduction

The term "quality," when referring to meat, consists of many factors, such as appearance, color, juiciness, odor, flavor and texture (Sacks *et al.*, 1988; Wood *et al.*, 2003). However, other factors, such as metallic sensation, also affect the quality of the meat (Kubberod *et al.*, 2002; Walshe *et al.*, 2006).

Several papers have investigated the nature of a meat-related metallic sensation resulting from iron compounds. Various papers report that a metallic sensation may be multimodal, including taste and olfactory sensations (Schiffman, 2000; Zacarias *et al.*, 2001; Lim and Lawless, 2005a; Stevens *et al.*, 2006; Epke and Lawless, 2007; Epke and McClure, 2009; Hong *et al.*, 2010; Laughlin *et al.*, 2011). Some divalent salts, such as those of iron, copper and zinc, are described as having a metallic taste or flavor (Lim and Lawless, 2005a; Hong *et al.*, 2010; Laughlin *et al.*, 2011). This is particular to ferrous sulfate ($FeSO_4.7H_20$), which has been proposed as a reference standard in the training and monitoring of selected assessors during food sensory evaluation (ISO 3972-1991, Lim and Lawless, 2005a; Lim and Lawless, 2005b).

Perception of food aroma, texture or taste is a dynamic phenomenon and a classical method of sensory evaluation that quantifies the sensory response using the threshold concept. According to Lawless and Heymann (1999), one the earliest measured characteristics of human sensory functions was the detection threshold. The detection threshold is an energy level below which the sensation is not produced by the stimulus and that above this level the sensation remains.

The threshold concept is an important instrument in sensory evaluation (Meilgaard *et al.*, 2007, Lawless and Heymann, 1999). However, this approach also presents some difficulties. For example, in the method of limits, the threshold is defined as the value that is positively reported in 50% of the trials. However, in some forced-choice methods, it is the value for correctly discriminating at 50 above the chance level of performance (ASTM, 1991; Lawless and Heymann, 1999).

The success or failure of the panel development process depends on the strict criteria and procedures used to select and train the panel (Meilgaard *et al.*, 2007). In addition the

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aim of this study was to establish a procedure for evaluating the metallic sensation in beef using the threshold concept criteria for selecting and training assessors, associated with the use of meat samples cooked at different temperatures.

Materials and Methods

Subjects Twenty-six undergraduates from the Federal University of Rio Grande participated in the study; they had reported no prior taste problems. The average age of the subjects was twenty-five. None of the subjects received any prior training regarding metallic sensation perception.

Stimuli Eight concentrations of ferrous sulfate ($FeSO_4.7H_2O$) between 0.0005 and 0.064 g/L (Table 1) were chosen based on a logarithmic function. All stimuli were reagent grade and were dissolved in water. Solutions were made every 24 h before the application test. The solutions were presented as 20 mL samples in a 60-mL odorless plastic cup labeled with a random three-digit number. They were kept at constant room temperature (approximately 21°C). These samples were sipped and expectorated.

Procedure

Evaluation of detection threshold The detection threshold and single threshold were evaluated by applying the method of limits (ABNT, 1994b).

The evaluation took place in a total of four sessions. Each candidate participated in two sessions on the same day, the other two being held in a time interval of no more than a week. In each session, there was a series of samples representing increasing concentrations of ferrous sulfate. As described in item 2.2, a log series of concentrations was used.

The samples in the series were presented in order of physical concentration. The subject judged the presence or absence of a metallic sensation. The ascending series and the descending series were given alternatively. Each series was continued until the judgment changed (from no to yes or vice versa). The starting point was varied among successive series of presentation. Blanks (zero concentration) were used in the series. The subjects cleaned their mouths with water between each stimulus.

A subject assigned a value of one when there was a stim-

Table 1. Results of the limits test for twenty-six subjects.

$FeSO_4.7H_2O$ (g/L)	0	0.0005	0.001	0.002	0.004	0.008	0.016	0.032	0.064	Individual Threshold $FeSO_4.7H_2O$ (g/L)
Log $FeSO_4.7H_2O$ Concentration	–	−3.30	−3.00	−2.69	−2.39	−2.09	−1.79	−1.49	−1.19	
Subjects										
1	0	0	0	0	0	1	1	1	1	0.0056
2	0	0	0	1	1	1	1	1	1	0.0014
3	0	0	0	0	0	0	1	1	1	0.011
4	0	0	0	0	0	1	1	1	1	0.0056
5	0	0	0	1	1	1	1	1	1	0.0014
6	0	0	0	0	0	0	0	0	1	0.045
7	0	0	0	0	1	1	1	1	1	0.0028
8	0	0	0	0	0	0	1	1	1	0.011
9	0	0	0	0	0	0	1	1	1	0.011
10	0	0	0	0	0	0	1	1	1	0.011
11	0	0	0	0	0	1	1	1	1	0.0056
12	0	0	0	0	0	0	1	1	1	0.011
13	0	0	0	0	0	1	1	1	1	0.0056
14	0	0	0	0	1	1	1	1	1	0.0028
15	0	0	0	0	0	1	1	1	1	0.0056
16	0	0	0	1	1	1	1	1	1	0.0014
17	0	0	0	0	1	1	1	1	1	0.0028
18	0	1	1	1	1	1	1	1	1	< 0
19	0	1	1	1	1	1	1	1	1	< 0
20	0	0	0	0	1	1	1	1	1	0.0028
21	0	0	1	1	1	1	1	1	1	0.0007
22	0	0	0	0	1	1	1	1	1	0.0028
23	0	0	0	1	1	1	1	1	1	0.0014
24	0	0	0	1	1	1	1	1	1	0.0014
25	0	0	0	1	1	1	1	1	1	0.0014
26	0	0	0	0	0	0	1	1	1	0.011
Frequency noticed	0	2	3	9	14	19	25	25	26	
Proportion	0	0.08	0.11	0.35	0.54	0.73	0.96	0.96	1.00	

Stimulus perceived: value 1;
Stimulus not perceived: value 0.

ulus. If there was no metallic sensation detected, a value of zero was assigned.

The best estimate threshold for each subject was the geometric mean of the concentration at which the last incorrect choice occurred and the next higher concentration. General threshold values were calculated by regression, plotting the proportions for the stimuli values against the logarithm concentrations of the $FeSO_4.7H_2O$. Point estimations of the threshold were made based on the percent correct at each concentration level. The threshold values are the stimulus values that were perceived 50% of the time (ASTM, 1991). Subjects with a detection threshold less than or equal were accepted.

Evaluation of the capability to discriminate metallic sensation in meat using the ranking test The accessors' abilities to discriminate a metallic sensation in meat were evaluated using a ranking test (ABNT, 1994a) and the *semimembranosus* muscle from the Crioula Lageana bovine. Meat samples were grilled on aluminum foil strips to an internal temperature of 65, 72 and 75°C. The samples were served to each subject on plates in a random order. Each piece of steak measured 1.5 × 1.5 × 1.5 cm. The steaks were collected twenty-four hours post-mortem from Crioula Lageano bovine carcasses, which were slaughtered in a commercial manner. Steaks were vacuum-packaged and placed in a freezer at −30°C. The steaks were removed from the freezer and placed in a cooler at 3°C ± 1°C to thaw for approximately twenty-four hours. The sensory evaluation was performed in four repetitions in four different sessions. The results were evaluated by the Friedmann test and by using the Neweel and MacFarlane (1987) table.

Performance Evaluation of the subjects accepted

Criteria for Evaluation Performance The performance of the accepted subjects was evaluated using semimembranosus muscle from the Crioula Lageana bovine that was cooked to an internal temperature of 65°C, 72°C or 75°C. The samples were prepared and served under the same conditions described in item 2.3.2. The sensory analysis of metallic in the samples was evaluated using an unstructured 9.0 cm line scale. The extremes of the scale have the expression not perceptible and highly perceptible. A reference sample of distilled water was given to subjects for the zero (not perceptible) on the scale, while a ferrous sulfate solution (0.064 g/L) was used for the nine (highly perceptible). The subjects were evaluated with each sample analysis, repetition and team concordance, according to an evaluation of variance (Power *et al.*, 1984), with two variation sources (sample and repetition) for metallic sensation and for each subject. F_{sample} and $F_{repetition}$ values were obtained for each subject.

The subjects were selected for F_{sample} values having significance at $p \leq 0.30$ and $F_{repetition}$ not being significant at $p > 0.05$, and for means in concordance with the team (mean values of each subject and team mean for each evaluated attribute).

Equivalence Evaluation of the Metallic Sensation in Meat with the Reference Ferrous Sulfate The equivalence of metallic sensation was evaluated by analyzing ferrous sulfate concentrations using an unstructured 9.0 cm line scale from zero (not perceptible) to nine (highly perceptible). A linear regression was developed with ferrous sulfate concentration (dependent variable) and sensory response (independent variable). The metallic sensation equivalent in ferrous sulfate should be calculated using the sensory response values obtained from the subjects who tasted the meat.

Results and Discussion

Threshold Detection Determination The results for general detection and single threshold were obtained for twenty-six subjects using the $FeSO_4.7H_2O$ solutions; data were submitted to the limits test, and results are shown in Table 1. The general detection threshold was estimated by equation 1, where the independent variable is the logarithm concentration of $FeSO_4.7H_2O$, and the dependent variable is the sensory response (proportion). The regression model described for equation 1 presented correlation of $r = 0.9855$, and this result demonstrated that the model had high linear dependence between concentration and panelists' responses.

$$Y = 1.6561 + 0.4856\ x \qquad \text{Eq. 1}$$

The value of the general detection threshold calculated by equation 1 (0.0041 g/L − $FeSO_4.7H_2O$) was obtained from the subjects evaluated by this study. It is different, in absolute value, from data which are presented by other authors (Shiffman, 2000; Zacarias *et al.*, 2001; Gonzalez *et al.*, 1998; Lim and Lawless, 2005a; Lim and Lawless, 2006; Epke and Lawless, 2007). However, approximated to that indicated by ISO (1991), which indicate the concentration of 0.00475 g/L, which is perceptible. According to literature the measure of threshold values is defined differently by various psychophysical methods and is therefore not a fixed value that has meaning beyond the exact procedure used to determine it (ASTM, 1991; Lawless and Heymann, 1999).

Several studies have measured the detection threshold. However, the studies varied in terms of test methods, nasal occlusion condition, aqueous sample volumes, water types and statistical methods of analysis, where all of these variables can affect the resulting threshold values (Lim and Lawless, 2005a; Lim and Lawless, 2006; Stevens *et al.*, 2006; Epke and Lawless, 2007; Epke and McClure, 2009). Investigations regarding the influence of water quality on the sensitivity of taste for metallic sensation have been performed (Gonzalez *et al.*, 1998; Shiffmann, 2000; Zacarias *et al.*, 2001; Lawless *et al.*, 2005; Hoehl *et al.*, 2010). The results of the study by Hoel *et al.* (2010) indicate that there is a difference in the identification of basic tastes and metallic sensations, and corresponding thresholds depending on the water in which substances are diluted. The results of this current study suggest that sensation sensitivity for metallic sensations can be measured most effectively in water that is low in mineral content. Therefore, investigators should direct attention to water quality, especially when analysis of metallic sensation is important. Deionized or distilled water has been suggested because it ensures constant composition (Lawless *et al.*, 2005). However, the pH of the deionized water is below neutral and therefore generates a bitter taste or metallic sensation (Whelton *et al.*, 2007). In 2006, Lim and Lawless estimated the detection threshold of a 0.02757 g/L ferrous sulfate solution using deionized water. This value is approximately 6.7 times more than the value from this paper, when a limit method was employed. The experiment of Lim and Lawless (2006) was expanded to include factors similar to our study, including an aqueous sample volume of 20 mL and open nasal passages, which allows subjects to use their sense of smell.

According to several authors, the $FeSO_4.7H_2O$ at low concentrations can be discriminated from water even in the absence of retronasal cues (Schiffman, 2000; Lim and Lawless, 2005a, Lim and Lawless, 2006, Epke and Lawless, 2007). These studies examined which metallic sensations from ferrous sulfate solutions are primarily detected with a retronasally-perceived sensation because it is effectively decreased by nasal occlusion. However, we used distilled water. The value thresholds in the literature were obtained with different types of water. For example, deionized water was used in the Shiffman (2000) study and 0.0078 g/L mineral water was used by Gonzalez *et al.* (1998). Zacarias *et al.* (2001) showed that water quality does influence the threshold of detection for copper salts. They evaluated the detection threshold of salts, while taking into consideration the conditions of the aqueous sample volumes (sample size 20 mL), nasal opening and two different water types, deionized distilled and uncarbonated mineral. The results clearly demonstrated an influence of water. An increased threshold value was obtained when deionized distilled water was used. Moreover, Zacharias (1979) only worked with distilled water and found a ferrous sulfate value threshold of 0.0033 g/L. This concentration is significantly lower than the concentration obtained by Lim and Lawless (2006) and more similar to our result (0.0041 g/L). These results support the importance of standardizing the procedure for metallic sensation evaluation.

Selection and Training

Evaluation of the capability to discriminate a metallic sensation in meat by a ranking test The use of panelists as measuring devices is analogous to using any scientific instrument to elicit measurements of specific parameters for products under study. The instrument is selected for its capability to provide the desired measurement as accurately and consistently as possible. In this case, certain criteria are involved in selecting sensory parameters (Lawless and Heyman, 1999; Meilgaard *et al.*, 2007).

The individual threshold estimated for each subject is clear in Table 1. Fourteen subjects were selected (2, 5, 7, 14, 16, 17, 18, 19, 20, 21, 22, 23, 24 and 25). Specifically, all subjects with a detection threshold less than or equal to the general detection threshold (0.0041) were accepted.

The ranking test is less demanding in terms of organization for data capture and processing (Meilgaard *et al.*, 2006). Moreover, the sorting familiarizes the team with product to be tested (McEwan *et al.*, 2003).

The rank sum differences obtained by fourteen selected subjects using *semimembranosus* muscle samples with internal cooking temperatures of 65°C, 72°C and 75°C are expressed in Table 2. In the ranking test, subjects received three or more samples, which were to be arranged in order of intensity or degree for some specified attribute. For each subject, the sample ranked first was given a "1." The second sample was assigned a "2" and so on. The ranked numbers of each sample were summed, and the resulting rank sums indicated the overall rank order of the samples (Meilgaard *et al.*, 2007). The Friedman's test was recommended (ABNT, 1994a) for data analyses (Friedman, 1937), and results were evaluated using the Neweel and MacFarlane (1987) table. We can calculate the value of the test statistic by tabulating the scores as shown in Table 2 and by calculating the rank sums for each sample (column sums). If this value exceeds the upper – α critical to the Neweel and Macfarlane (1987) table, we can conclude that significant differences exist among the samples. The data showed that the sample cooked

Table 2. Rank sum scores for the evaluation of metallic sensation in three meat samples cooked at different temperatures.

	Sample Temperature		
	65°C	72°C	75°C
Rank sums	72[a]	51[b]	51[b]
Difference versus 65°C	–	21	21
Difference versus 72°C	21	–	–

Different letters account for rank sum significance at the 5% level (LSD rank: 12,83 according; Newell and MacFarlane, 1987).
Number of subjects: 14

at 65°C was perceived differently (at 5% significance) from the samples cooked at 72°C and 75°C. This finding demonstrated that certain subjects were able to identify the metallic sensation. Moreover, it showed that an internal cooking temperature of 65°C serves as an adequate reference temperature for training metallic sensationing subjects.

Evaluation Subject Performance Evaluating a subject's performance in sensory analysis is an important step toward demonstrating that results from a sensory team are consistent. Several studies have investigated methods for measuring and screening sensory assessor performance (Naes, 1998; Rossi, 2001). Moreover, the results from the team as a single unit are the basis for decision making on the sensory quality of products. However, poor performance by individual assessors will unavoidably result in a lower performance of the team (McEwan *et al.*, 2003).

The subjects' ability to discriminate between samples and the repetition of their work were examined using Analysis of variance (ANOVA) of two factors (sample and repetition) for each subject (Damasio *et al.*, 1992; Queiroz, 2001; Naes *et al.*, 2010). Tables 3 and 4 summarize the results obtained when fourteen subjects evaluated metallic sensation in meat cooked to an internal temperature of 65°C, 72°C or 75°C. Assessment involved an unstructured 9.0 cm line scale from

Table 3. Significance level (p) for the subjects regarding their sample discrimination (F_{sample}).

Subjects	Sample temperature		
	65°C	72°C	75°C
2	0.000183	0.013114	0.000183
5	0.000183	0.004983	0.000183
7	0.000183	0.004983	0.000183
14	0.072162	0.029844	0.000930
16	0.002471	0.175835	0.043546
17	0.000183	0.436613	0.000183
18	0.000183	0.004764	0.000190
19	0.000184	0.518292	0.000188
20	0.000183	0.935154	0.000183
21	0.000183	0.170855	0.000183
22	0.000291	0.008707	0.000184
23	0.000183	0.288223	0.000183
24	0.311471	0.556588	0.877319
25	0.547843	0.300693	0.060887

Subjects with F_{sample} p value ≤ 0.30 were selected;
Repetition: 4

Table 4. Significance level (p) to analyze the repeatability of the subjects ($F_{repetition}$).

Subjects	Sample temperature		
	65°C	72°C	75°C
2	0.824679	0.370025	0.824679
5	0.552317	0.552317	0.847690
7	0.388896	0.649451	0.552317
14	0.270485	0.738119	0.552317
16	0.552317	0.441099	0.738119
17	0.000481	0.000566	0.001125
18	0.649451	0.441099	0.441099
19	0.000001	0.000004	0.000029
20	0.003003	0.000222	0.240921
21	0.738119	0.000070	0.824679
22	0.192657	0.370025	0.552317
23	0.819384	0.738119	0.552317
24	0.000000	0.000106	0.000188
25	0.000000	0.000106	0.000188

Subject with $F_{repetition}$ p value ≥ 0.05 were selected;
n: 4

zero (not perceptible) to nine (highly perceptible). We can examine the F_{sample} results for each subject in relation to each sample (Table 3) and the $F_{repetition}$ results for each subject in relation to each sample (Table 4). The subjects with a significant value of F_{sample} ($p < 0.30$) and with no significance for $F_{repetition}$ ($p > 0.05$) were selected. Eight subjects were chosen (2, 5, 7, 14, 16, 18, 22, and 23). The subjects who showed a reduced ability for sample discrimination also had diminished repetition.

Table 5 shows the value for samples attributed by the selected team. In each column and in each line, mean values with different letters are significantly different ($p \leq 0.05$) using Tukey test. Five subjects (2, 5, 7, 14 and 16) were able to determine differences in the samples and to present repeatable data (Table 4), besides not presenting any significant differences in mean values of each sample. However, the subjects who were not selected to form the evaluation team can attain this qualification through subject training with the scale used.

Table 6 shows that the ability of five chosen subjects to detect ferrous sulfate was significantly different ($p \leq 0.05$, calculated with the Tukey test) between the sample with an internal cooking temperature of 65°C and the other samples. In Table 6, it is also possible to see the equivalence between the metallic sensation of the meat and the amount of ferrous sulfate estimated by Equation 2, which was obtained with the data presented in the Table 7. This table examines the sensory response related to the metallic sensation of the ferrous sulfate solutions. The regression model described above presents a correlation of r = 0.9689, and this value indicates that a strong relationship was obtained with the model, where the independent variable is the logarithmic concentration of

Table 5. Mean values for samples attributed by team selected.

Subject	Sample temperature		
	65°C	72°C	75°C
2	7.20^{aA}	2.75^{aB}	2.90^{aC}
5	7.17^{aA}	2.77^{aB}	3.20^{aC}
7	7.12^{aA}	2.85^{aB}	3.05^{aC}
14	7.17^{aA}	2.70^{aB}	3.00^{aC}
16	7.27^{aA}	2.80^{aB}	3.15^{aC}
18	6.85^{acA}	3.65^{bdB}	2.72^{bC}
22	7.80^{bA}	3.17^{cB}	2.72^{bC}
23	6.30^{cA}	3.65^{dbB}	2.67^{bC}

[abcd] Within a column, mean values with different letters are significantly different ($p \leq 0.05$).
[ABCD] Within a line, mean values with different letters are significantly different ($p \leq 0.30$)

Table 6. Equivalence of metallic sensation for the meat with a ferrous sulfate solution.

Sample temperature (°C)	Sensory response in beef	Equivalence in $FeSO_4.7H_2O$ (g/L)
65	7.18^{a}	0.045
72	2.77^{b}	0.013
75	3.06^{b}	0.015

[ab] Within a column, mean values having different letters are significantly different ($p \leq 0.05$).
n: 5

Table 7. Sensory response for the metallic sensation to $FeSO_4.7H_2O$.

$FeSO_4.7H_2O$ (g/L)	Mean	p $F_{repetition}$
0.000	0.00^{a}	–
0.004	1.00^{b}	0.449482
0.008	2.08^{c}	0.255839
0.016	4.26^{d}	0.182069
0.032	6.13^{e}	0.922848
0.064	9.00^{f}	–

[abcdef] Within a column, mean values having different letters are significantly different ($p \leq 0.05$).
n: 5

$FeSO_4.7H_2O$, and the dependent variable is the sensory response.

$$y = 0.9123 + 137.0668x \quad \text{Eq. 2}$$

The observed mean values of the metallic sensation are 7.2, 2.7 and 3.1 for samples at an internal cooking temperature of 65°C, 72°C, and 75°C, respectively. There is a noticeable difference in the sample's absolute value for metallic sensation when the meat had an internal cooking temperature of 65°C. This can be attributed to the characteristic of blood taste, which can be perceived by subject, when meat was treated at 65°C. According to Mottran (1998) uncooked meat has little or no aroma and only a blood-like taste.

The American Meat Science Association (AMSA, 1995) recommends cooking to an internal temperature of 71°C for sensory evaluation of meat because this temperature should basically guarantee microbiological safety. However, the preference of the consumer can be different and variable with regard to factors such as origin of the meat, age and the degree of doneness (Osornio *et al.*, 2008). The research of Osornio *et al.* (2008) was conducted in different U.S. cities. Using a temperature interval of 63°C to 80°C for the meat, they found that approximately 50% of consumers cooked their beef to "medium and less" and 50% to "medium and more." This finding shows a clear segmentation on consumers' preferences for degree of doneness. Moreover, consumers as a whole can be an influencing factor in choosing how meat will be cooked. The internal cooking temperature is an important factor to be considered when thinking about sensory evaluation in meat. Several papers reported an internal cooking temperature of 72°C for consumers in a study about meat quality (Caine *et al.*, 2003; Oliver *et al.*, 2006). In this manner, the result obtained (2.77 in an unstructured line scale of 9 cm) for an internal cooking temperature of 72°C is equivalent with 0.013 g/L $FeSO_4.7H_2O$ for the *semimembranosus* muscle of the bovine Crioulo Lageano, this value characterizes the meat in question as having a low metallic sensation.

Conclusion

The procedure proposed for selecting and training the panel to evaluate metallic sensations was successful. The results demonstrated that an internal cooking temperature at 65°C can be an adequate temperature to use as a standard reference for evaluating metallic sensations in meat. The *semimembranosus* muscle of the bovine Crioulo Lageano is characterized as having low metallic sensations.

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