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Stimulus and recognition thresholds for the basic tastes in deionized water

Are the recommendations for citric acid too high?

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Summary

During the recruitment of sensory panelists (e.g. when screening assessors suitability or training of assessors), the sensitivity for basic tastes is often measured with threshold tests. The present study examined the concentrations of taste stimuli used in the threshold tests in accordance with ISO and DIN [1, 2], with respect to their applicability in deionized water. The taste thresholds for sweet, sour, salty, bitter, umami and metallic were determined for 70 young European women. With eight increasing concentrations, it was found that the individual taste stimuli for sweet, salty, bitter, umami and metallic were well distributed. However, sour was recognized by all study participants within the two lowest concentrations. Clear differences in sensitivity were achieved by reducing the citric acid concentration to a fifth of the original value. Thus, if deionized water is used as solvent, the citric acid concentration must be reduced if the expected differences in perceived sourness are to be found.

Keywords: taste sensitivity, threshold, citric acid, type of water, sensory science

Introduction

The international standard ISO 3972 [1] and the corresponding national DIN standard [2] provide guidelines for examining human taste sensitivity in the context of sensory trials. For example, recommendations are given on the chemicals and reagents to be added, e. g., the type of water used for preparing solutions of taste stimuli. The water should be "neutral, tasteless, still and odourless, preferably of known hardness" [1]. For international taste research, deionized water is mostly used to prepare the solutions (see e.g. [3–6]). This ensures that comparability between different study centres is ensured and that the composition of the water is relatively stable – independent of the site of production. Nevertheless, the evaluation of the results of a large study [7] raised doubts as to whether deionized water is suited for the measurement of taste sensitivity for sour.[1] It is thought that either (a) the citric acid concentration would have to be adjusted or (b) another type of water would have to be used to dissolve the substance.

Moreover, ISO 3972 [1] (with the corrigendum of 2012 [8]), as well as the corresponding national DIN ISO 3972 [2] refer – in contrast to the previous versions [9, 10] – to lower thresholds for bitter and sour in demineralised water, without explaining what is meant by "lower" or giving detailed references to alternative concentrations for the substances.

The present study therefore investigates the thresholds for the five basic tastes (sweet, sour, salty, bitter and umami), as well as the metallic sensation, in deionized water. In addition, various citric acid concentrations in deionized water were examined with respect to taste sensitivity in the threshold test.

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[1] In the original study design [7], it was intended to investigate the influence of sensory training on the perception of sweet, sour and bitter. However, the data for the perception of sourness were not evaluated, as the results were unclear.

Taste substance	Chemical formula	Molecular weight (g/mol)	Taste quality	Stock solution (g/L)	Concentration series*	Unit
Sucrose[a]	$C_{12}H_{22}O_{11}$	342.3	sweet	24.00	0.98–35.06	mmol/L
Caffeine[b]	$C_8H_{10}N_4O_2$	194.19	bitter	0.54	0.29–1.39	mmol/L
Sodium chloride[c]	NaCl	58.44	salty	4.00	2.81–34.22	mol/L
Monosodium glutamate monohydrate[b]	$NaOOCCH_2CH(NH_2)COOH(H_2O)$	187.13	umami	2.00	0.44–5.34	mmol/L
Iron(II) sulfate heptahydrate[b]	$FeSO_4$ (7 H_2O)	278.01	metallic	0.016	0.0024–0.029	mmol/L
Citric acid monohydrate[a]	$C_6H_8O_7$ (H_2O)	210.14	sour			
Group A (ISO)				1.20	0.60–2.86	mmol/L
Group B (ISO/5)				0.24	0.12–0.57	mmol/L
Group C (ISO/10)				0.12	0.06–0.29	mmol/L

Table 1: **Series of taste dilutions for the basic tastes and the metallic sensation, in accordance with [1, 2]**

* Prepared by dilution of the stock solution (eight sequential 10-fold dilutions; D8–D1)
[a] Riedel-de Haen, Sigma-Aldrich Laborchemikalien GmbH, Seelze, Germany; [b] Fluka, Sigma-Aldrich Chemie GmbH, Steinheim, Germany;
[c] Merck KGaA, Darmstadt, Germany

Materials and methods

Study participants

70 female students of European origin (mean age = 22.1 years, standard deviation [σ] = 2.5; mean BMI = 21.5 kg/m², σ = 2.4) took part in the present project. They were part of a larger study in the Hamburg University for Applied Sciences (HAW) [7].

Study design and groups

The study participants were requested to come to the HAW Sensory Science Laboratory (designed in accordance with the guidelines in [11]) on one of three weekdays and at one of four possible times. They were not informed of the study design or objectives. A session lasted about 1.5 hours and included various tests on taste sensitivity: [a] a matching test, to make the study participants familiar with the five basic tastes, and the metallic sensation; [b] an intensity test with one sweet and one bitter sample and [c] two times three threshold tests with a break to allow regeneration of the taste buds. The sessions on the three weekdays only differed in the concentrations used to prepare the sour solutions (citric acid); the other tastes (sweet, salty, bitter, umami and metallic) were tested in all sessions in the concentrations recommended in [1]:

- Day 1 (Group A): Original concentration as given in [1]: citric acid stock solution = 1.2 g/L; n = 15;
- Day 2 (Group B): A fifth of the original concentration: citric acid stock solution = 0.24 g/L; n = 30;
- Day 3 (Group C): A tenth of the original concentration: citric acid stock solution = 0.12 g/L; n = 25.

Taste substances and type of water

The substances (◆ Table 1) were dissolved in deionized water – provided by Sensient Food Colors GmbH, Geesthacht, Germany. ◆ Table 2 gives the details of the taste solution for citric acid, as prepared by dilution of the three stock solutions (Group A: ISO concentration as given in [1], Group B: ISO/5 and Group C: ISO/10).

Determination of the stimulus and recognition thresholds

The stimulus and recognition thresholds for the six taste qualities were measured consecutively (two trays with three series each). For each tray, the study participants were presented with three taste series of nine samples each (in 40 mL plastic cups, each containing 20 mL solution); the first sample always consisted of deionized water. The following eight solutions (D8–D1, ◆ Tables 1 and 2) were tasted in the order of increasing concentrations (method

		Group A (ISO concentration)	Group B (ISO/5)	Group C (ISO/10)
Stock solution g/L		1.20	0.24	0.12
Dilution	D1	0.60	0.12	0.06
	D2	0.48	0.096	0.048
	D3	0.384	0.0768	0.0348
	D4	0.3072	0.06144	0.03072
	D5	0.246	0.0492	0.0246
	D6	0.1968	0.04936	0.01968
	D7	0.1572	0.03144	0.01572
	D8	0.126	0.0252	0.0126

Table 2: **Series of taste dilutions for sour taste perception (citric acid) in three study groups**

	sweet (log [μmol/L])		salty (log [μmol/L])		bitter (log [μmol/L])		umami (log [μmol/L])		metallic (log [μmol/L])	
	μ	σ	μ	σ	μ	σ	μ	σ	μ	σ
Group A (n = 15)	**3.2**	0.2	**6.5**	0.1	**2.5**	0.03	**2.7**	0.1	**0.4**	0.04
Group B (n = 30)	**3.1**	0.3	**6.6**	0.2	**2.5**	0.1	**2.8**	0.2	**0.5**	0.2
Group C (n = 25)	**3.2**	0.3	**6.5**	0.1	**2.5**	0.1	**2.7**	0.2	**0.5**	0.2
overall (n = 70)	**3.2**	0.3	**6.5**	0.2	**2.5**	0.1	**2.7**	0.2	**0.5**	0.2

Table 3: **Means (μ) and standard deviation (σ) for the stimulus thresholds (sweet, salty, bitter, umami and metallic) in (log [μmol/L]) as in the series of taste dilutions recommended in [1] (see ◆ Table 1)**

	sweet (log [μmol/L])			salty (log [μmol/L])				bitter (log [μmol/L])			umami (log [μmol/L])			metallic (log [μmol/L])			
	μ	σ	n	μ		σ	n	μ	σ	n	μ	σ	n	μ		σ	n
Group A (n = 15)	**3.7**	0.5	14	**6.8**	*	0.4	13	**2.7**	0.2	14	**3.3**	0.5	11	**0.6**	**	0.4	14
Group B (n = 30)	**3.8**	0.5	30	**7.2**		0.5	18	**2.7**	0.3	26	**3.1**	0.3	29	**1.0**		0.3	29
Group C (n = 25)	**3.7**	0.4	24	**7.0**		0.4	23	**2.7**	0.2	25	**3.1**	0.4	23	**0.8**		0.5	21
overall (n = 70)	**3.7**	0.5	68	**7.1**		0.4	54	**2.7**	0.2	65	**3.2**	0.4	63	**0.8**		0.4	64
				F = 4.23; P ≤ 0.019										F = 5.14; P ≤ 0.008			

Table 4: **Means (μ) and standard deviation (σ) for the recognition thresholds (sweet, salty, bitter, umami and metallic) in (log [μmol/L]) as in the series of taste dilutions recommended in [1] (see ◆ Table 1)**

* P ≤ 0,05; ** P ≤ 0,005

as given in [1]). Between the series and between the trays, there was an interval that was long enough to allow regeneration of the taste buds. For this purpose, the study participants rinsed with deionized water and were allowed to eat unleavened bread (matzo) if they wanted. The presentation of the series of taste dilutions was randomised between the study participants.
The stimulus threshold is defined as the minimum concentration of a sensory stimulus needed to give rise to a sensation (different to the sensation from the initial water sample). However, the sensory sensation must not be identifiable quantitatively. The recognition threshold is the minimum concentration of a sensory stimulus that permits the assessor to make a qualitative recognition of the sensory impression.

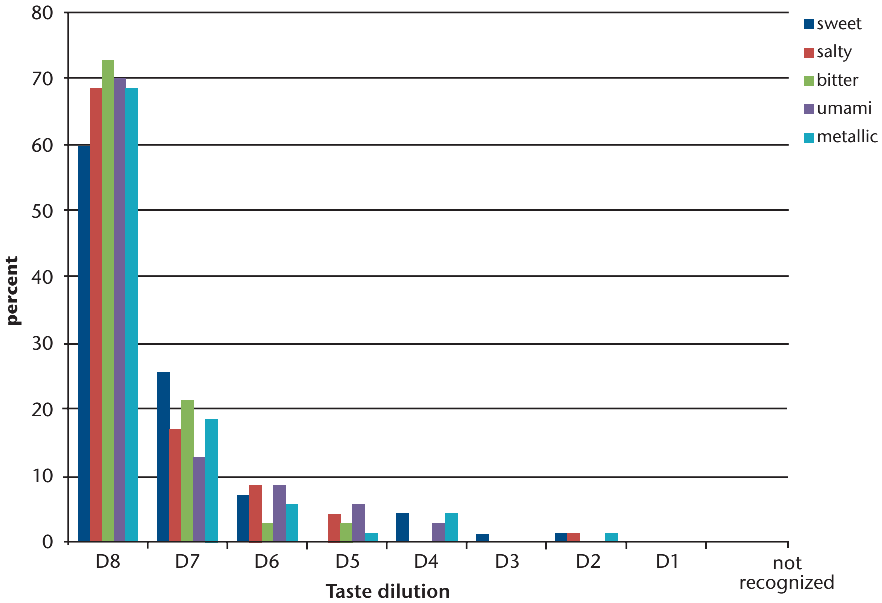

Figure 1: **Stimulus thresholds for the five tastes (sweet, salty, bitter, umami and metallic) in deionized water (n = 70) as in the series of taste dilutions recommended in [1] (see ◆ Table 1)**

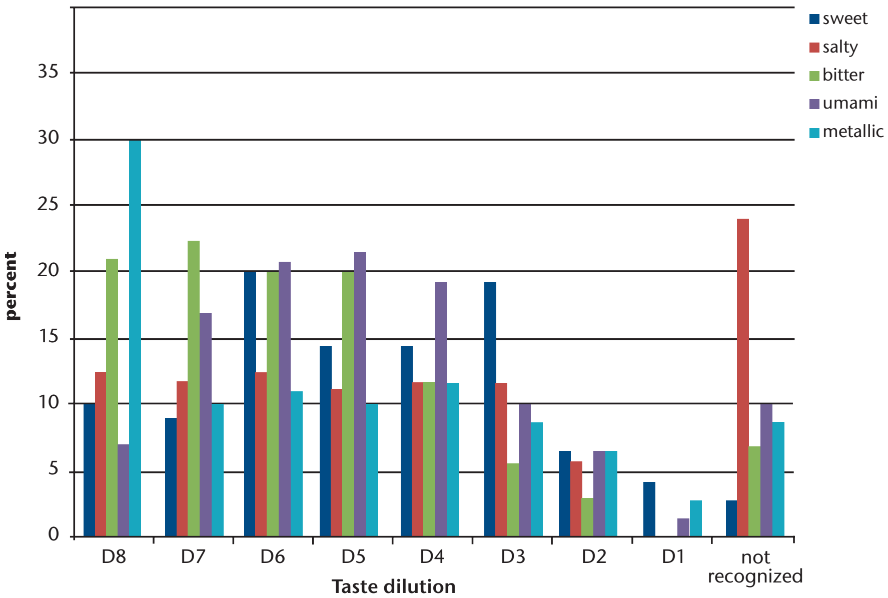

Figure 2: **Recognition thresholds for the five tastes (sweet, salty, bitter, umami and metallic) in deionized water (n = 70) as in the series of taste dilutions recommended in [1] (see ◆ Table 1)**

Statistics

The evaluations were performed with SPSS Statistics 21.0. For the calculation of the means (μ) and standard deviation (σ), the concentrations (μmol/L) were transformed to logarithmic values (log [μmol/L]). To calculate the group differences, one-way analysis of variance (ANOVA) was performed. The level of significance was taken as $\alpha \leq 0.05$.

Results

The mean values for the stimulus and recognition thresholds (sweet, salty, bitter, umami and metallic) of the three study groups (A, B and C) are shown in ◆ Tables 3 and 4. If the three groups give the same mean threshold values (all three groups tasted identical concentrations according to [1]), the text gives the group mean values. ◆ Figures 1 and 2 show the distribution of the individual results over the eight threshold concentrations (D8–D1) in ISO concentration for the five tastes. ◆ Table 5 shows the mean stimulus and recognition threshold for the sour taste in three different concentrations (Group A: ISO concentration as given in [1]; Group B: ISO/5; Group C: ISO/10).
◆ Figures 3 and 4 show the distribution of the individual results of the threshold tests for the sour sensation for the three concentration series.

Stimulus and recognition thresholds for sweet, salty, bitter, umami and metallic

As expected, the three study groups

	Stimulus threshold (log [µmol])	σ	n	Recognition threshold (log [µmol])	σ	n
Group A (ISO-concentration as given in [1])	2.8	0.00	15	2.8	0.03	15
Group B (ISO/5-concentration)	2.1	0.1	30	2.2	0.1	30
Group C (ISO/10-concentration)	1.8	0.1	25	1.9	0.1	19

Table 5: **Means (µ) and standard deviations (σ) of the stimulus and recognition thresholds for sour (citric acid) for the three study groups**

(A, B and C; n = 70) do not differ in their stimulus thresholds with respect to sweet (mean for the whole group [µ] = 3.2 log [µmol/L]; ~D7), salty (µ = 6.5 log [µmol/L]; ~D7), bitter (µ = 2.5 log [µmol/L]; ~D8), umami (µ = 2.7 log [µmol/L]; ~D7) or metallic (µ = 0.5 log [µmol/L]; ~D8/D7; ◆ Table 3), as there were no concentration differences in the taste dilutions between the three study groups. As expected, the recognition thresholds for sweet (µ [n = 68] = 3.7 log [µmol/L]; ~D5/D4), bitter (µ [n = 65] = 2.7 log [µmol/L]; ~D6/D5) and umami (µ [n = 63] = 3.2 log [µmol/L]; ~D5/D4) did not differ significantly between the three study groups. Nevertheless, there were significant differences between Groups A and B with respect to the means for the salty and metallic recognition threshold (◆ Table 4).

For all five tastes, the individual results exhibit satisfactory distributions over the eight dilutions (D8–D1; ◆ Figures 1 and 2). At the lowest concentration (D8), about 60–70 % of the study participants perceived a sensation that differed from that of water. Nevertheless, there were also study participants who only perceived a sensation at much higher concentrations (e.g. at D2 for sweet, salty and metallic; • Figure 1).

The individual recognition thresholds were even more clearly distributed over the whole range of the dilutions. Moreover, for each taste, there were study participants who were unable to assign the test quality correctly (e.g. ~23 % for the salty taste; ◆ Figure 2).

Stimulus and recognition thresholds for sour with ISO concentrations

At the lowest concentration (D8), all study participants in Group A (ISO concentration) already perceived a sensation that differed from the water sample (stimulus threshold; ◆ Figure 3). The mean stimulus threshold in this group was 2.8 log (µmol/L; ◆ Table 5). In addition, they recognized the sour taste within the two lowest concentrations (D8 and D7; recognition threshold; ◆ Figure 4). Thus, the mean recognition threshold for Group A was just as low, at 2.8 log (µmol/L; ◆ Table 5). This result confirms the finding in [7], that the citric acid concentrations recommended in [1, 2] give rise to little or no differences in the study participants' taste sensitivity, when the substance is dissolved in deionized water.

Stimulus and recognition thresholds for sour with reduced concentrations

As the starting concentrations of the solutions for Group B (ISO/5) and Group C (ISO/10) were much lower than for Group A, the mean thresholds for these two groups were also much lower: The mean ISO/5 recognition threshold for Group B was 2.2 log (µmol/L), which is somewhat above D7; the mean ISO/10 recognition threshold for Group C was 1.9 log [µmol/L], which lies between D7 and D6 (◆ Table 5).

Moreover, reducing the concentrations to one fifth or one tenth of the original enhanced the distribution of the individual taste sensitivities over the different dilutions. • Figure 3 shows the individual results for the stimulus threshold. The stimulus thresholds for ISO/5 and ISO/10 (Groups B and C) were within the first three concentrations (D8–D6). It is evident from ◆ Figure 4 (recognition thresholds), that only 37 % of Group B (ISO/5) recognized the sour taste at the lowest concentration (D8). Four study participants (16 %) in Group C (ISO/10) recognized the sour taste at the lowest concentration (D8). Moreover, when the concentration was reduced to one tenth, six study participants (24 %) did not recognize the sour taste at all.

Discussion

Comparison of the individual results (◆ Figure 1 and 2) gave the expected picture regarding both stimulus and recognition threshold of all five tastes – sweet, salty, bitter, umami and metallic. There were study participants with sensitive taste abilities, who perceived and recognized a taste at very low concentrations. At the same time, there was a distribution over the whole concentration range (D8–D1) and study participants who failed to assign the taste properly (not recognized).

The situation was different for the sour taste in the ISO concentration (Group A): In this case, 100 % of the study participants detected a sensation at the lowest concentration (D8) and recognized this as sour within the lowest two concentrations (D8 and D7). It can therefore be inferred that the citric acid concentrations recommended in [1, 2] to measure thresholds for taste sensitivity are too high when the substance is dissolved in deionized water. For the other substances, the concentrations in deionized water appear to be more appropriate.
This conclusion is confirmed by the results of another study [12], in which the type of water used to dissolve the substances has a greater influence on sour perception than on the perception of other tastes.
If the concentration was reduced to one fifth, the distribution of taste sensitivity was improved (Group B). Dilution to a tenth appeard to be too low, as then 24 % of the study participants in Group C failed to recognize the taste as sour.
The concentration range of ~120 to ~571 µmol/L (dilution series of one-fifth of ISO [1]) used in our study was comparable to the range of 48–720 µmol/L employed in [13], although the authors did not fully explain its origin. They prepared a series of 15 citric acid dilutions in deionized water (D15–D1; 48–720 µmol/L). The highest concentration in the present study (D1) roughly corresponded to their twelfth highest concentration [13].

In a follow-up project [14] to the present study, it could be shown that the recommended citric acid concentration (original concentrations as given in [1, 2]) is more suitable when the substance is dissolved in spring water or tap water. Both the stimulus and recognition thresholds for sour differ significantly between the three water types. The highest threshold values for sour were determined in tap water, with intermediate values in spring water and the lowest values in deionized water. As explained above, the very low mean threshold values in deionized water were due to the lack of differences between the study participants with respect to taste sensitivity. This homogeneity of the individual taste sensitivity was unexpected and is due to the excessively high initial concentration of citric acid when this is dissolved in deionized water.
After evaluation of the present study results, it is unclear why there are differences between the study groups with respect to their mean recognition thresholds for salty and metallic, although there were no concentration differences in the tasted solutions. In Group B, there was a high proportion of study participants who totally failed to recognize the salty taste (n = 12; 40 %). This might be due to study participants exhaustion or to errors in sample preparation or presentation (e.g. if the NaCl stock solution was too low or in the false sequence in the sample series). Study participants frequently had problems in recognizing the metallic sensation [14]. In future studies, it must be checked whether the concentrations should be increased or whether specific training could lead to improved sensitivity for this unfamiliar taste.
For the sour taste sensation, it must be concluded that special care is required for the selection of the type of water used to dissolve the citric acid. Spring water with low total mineral content can be appropriately used for all basic tastes aside from metallic [14]. As it is so readily available, it is a suitable solvent for practical work – such as screening candidates for sensory testing or for training sensory abilities. In further studies, it should be examined to what extent filtered tap water (e.g. with an ion exchanger or active carbon) gives comparable results for taste sensitivity. With this procedure, it would be even simpler and cheaper to prepare the taste solutions than with spring water. On the other hand, if deionized water has to be used (e.g. to guarantee international comparability), it should be considered reducing the citric acid concentration for the stock solution to prepare the

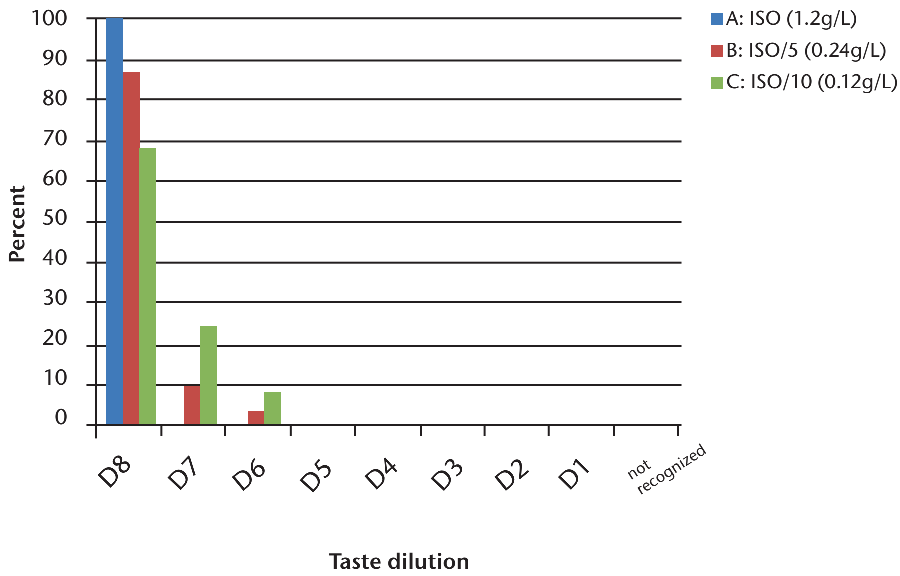

Figure 3: **Stimulus thresholds for sour (citric acid) in deionized water for the three study groups A (n = 15), B (n = 30) and C (n = 25)**

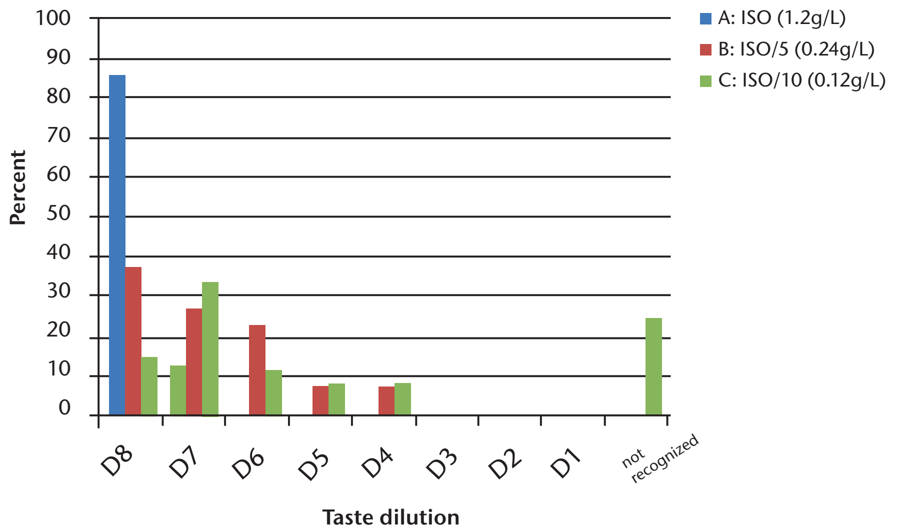

Figure 4: **Recognition threshold for sour (citric acid) in deionized water for the three study groups A (n = 15), B (n = 30) and C (n = 25)**

series of taste dilutions (e.g. to one fifth of the concentration recommended in [1]).

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Conflict of Interest

M. Busch-Stockfisch is charmain of the DIN Standards Comitee for Sensory Sciencie and German ISO Delegate for Sensory Sciencie.
K. Hohl und G.U. Schönberger declare no conflict of interest according to the guidelines of the International Committee of Medical Journal Editors.

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