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Neohesperidin Dihydrochalcone is Not a Taste Enhancer in Aqueous Sucrose Solutions

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Abstract

Neohesperidin dihydrochalcone (NHDC) is an intensive sweetener, obtained by alkaline hydrogenation of neohesperidin. In this investigation a supposed taste enhancing effect of this substance was tested. A three-step procedure was used. In the first experiment, using a pool of 31 subjects, NHDC and sucrose detection thresholds were measured. In the second experiment, psychophysical functions for both tastants were determined. Then, 15 participants closest to the group threshold who, in addition, had produced monotonic psychophysical taste functions were selected to participate in the next two experiments. In the third experiment, taste enhancement was tested. Three psychophysical sucrose functions were constructed, one with a near-threshold amount of NHDC added to each of seven sucrose concentrations, one with a near-threshold amount of sucrose added (control 1) and one without any addition (control 2). No difference was found between the NHDC-enriched sucrose function and the sucrose-enriched sucrose function. Finally, in experiment 4, differential threshold functions were constructed with either NHDC or sucrose added. Neither the overall shape of the functions nor a comparison of the points of subjective equality showed enhancement. It was concluded that weak NHDC does not enhance the taste of aqueous sucrose solutions.

Introduction

Neohesperidin dihydrochalcone (NHDC) is a sweet substance of molecular weight 612.6 produced by alkaline hydrogenation of neohesperidin, which in its turn can be isolated from the peel of oranges. Aqueous solutions of 0.0045% NHDC and 5% sucrose are approximately equisweet (Inglett *et al.*, 1969). Often the relative sweetness of a sweetener is indicated in terms of sucrose sweetness. This relationship may be expressed as the ratio between the weights of both sweeteners at equisweet points. Due to differences in the shapes of the psychophysical functions, the obtained ratios are by no means similar for different concentrations of sucrose. So, values mentioned in the literature may vary between 1000 (Horowitz and Gentili, 1986) and 2000 (Schwarzenbach, 1976). At the sweetness threshold even more extreme ratios may be found. With decreasing sucrose concentration the relative sweetness of NHDC increases exponentially (Guadagni *et al.*, 1974; Bär *et al.*, 1990). This means that with sucrose-sensitive subjects, who are able to carry out low concentration comparisons, the ratio may be even more extreme than 2000.

The sweetness of a substance must be carefully distinguished from its sweetness enhancing potential or, more generally stated, its taste enhancing property. As indicated, the sweetening property may be defined as the relative weight required to make an aqueous solution equisweet to a sucrose solution. Because the addition of a tiny amount of an intense sweetener often has such spectacular effects, it is tempting to call it a taste enhancer. However, a taste enhancer does more then just adding its own taste. We may call a substance a taste enhancer if, irrespective of whether or not it has a taste of its own, its addition to a taste substance increases the taste of that substance. The definition of taste enhancement is very close to that of synergism. Synergism means that both components in a mixture contribute to the enhancement, although this is often difficult to assess if both substances share the same taste quality, like NHDC and sucrose. It may be argued that taste enhancement is just a special case of synergism: the case in which only one of the two components contributes to the enhancement effect. Definition problems like these evaporate when an insight is gained into the underlying mechanisms. However, taste enhancement is little understood in terms of underlying mechanisms. A rule that satisfies both concepts is that either taste enhancement or synergism exists if the total perceived taste intensity of a mixture is beyond the level predicted by the *psychophysical* combination of the two substances. In such a combination the slopes of both psychophysical functions are taken into account (Bartoshuk and Cleveland, 1977; Rifkin and Bartoshuk, 1980; Lawless, 1998).

Besides its properties as an intensive sweetener, it is maintained that NHDC is a taste enhancer in combination with other sweeteners, such as saccharin (Kiyofumi *et al.*, 1972), cyclamate (Inglet *et al.*, 1969) and acesulfame-K (Von Rymon Lipinski and Lück, 1976, 1979). Such effects have also been described for combinations of NHDC with sucrose (Beerens, 1981) and with sugar alcohols (Dwivedi and Sampathkumar, 1981). The aim of the present investigation was to test the taste enhancing effect of a weak NHDC concentration on the taste of sucrose. This was accomplished by comparing the effects of near-threshold NHDC with a control condition in which only near-threshold sucrose was added.

Materials and methods

Experiment 1: determining thresholds

In the first experiment the absolute threshold for sucrose and NHDC will be assessed, using a two alternative forced choice method.

Subjects

Thirty-one healthy, non-smoking students, 16 male and 15 female, served as subjects. Their mean age was 22 years (range 18–34 years).

Stimuli

Commercially available refined crystal sugar was used. NHDC was from Zoster SA. Before using NHDC in the main experiments, the substance was used with three subjects (not included as experimental subjects) to find out the approximate threshold concentrations. Seven sucrose concentrations were used, the strongest 27.40 g/l and each next lower concentration half that of the preceding one. Thus the weakest sucrose concentration was 0.43 g/l. The same dillution factor was used for NHDC, the strongest concentration being 0.008000 and the weakest 0.000125 g/l. The tastants were dissolved in distilled water 24 h before the experimental session. All solutions were offered to the subjects at room temperature (21°C) in 25 ml polystyrene cups, each containing 5 ml of stimulus substance. The cups were filled by automatic standardized pipetting immediately before the session in order to minimize evaporation of water.

Each concentration was paired with a blank, resulting in 14 pairs, which were replicated six times, leading to a total of 84 pairs. In half the presentations the blank was the left member of a pair and in the other half it was the right member. The left–right positions as well as the concentrations were randomly distributed. Each subject received a different random order. Between pairs a constant interval of 1 min was observed. Within pairs, the time interval was not controlled. Tasting both members of a pair was obligatory, even when subjects had the impression that the first member contained the tastant. Between pairs, subjects rinsed thoroughly twice with distilled water. One rinse was immediately after a stimulus pair and the second rinse just before each stimulus pair.

Procedure

Subjects were seated at a table in front of a rectangular Plexiglas tray with holes contaning the cups. Next to the tray a drinking glass and a bottle of rinsing water were available. Assistants refilled the bottles whenever necessary and registered the amount of rinsing water used by each subject. Furthermore, a pencil and a response sheet were available. The response sheets were printed replicas of the trays, which made responding very easy. Next to each chair a bucket was placed. Only eight subjects at a time took part in order to make sure that every single subject could be monitored by an assistant. The session was subdivided into two sub-sessions separated by a 7 min break. In the first sub-session 50 pairs were tasted and in the second sub-session the remaining 34 pairs were tasted. The session was opened by extensive instruction and a demonstration of the mouth rinsing procedure. After instruction, three training trials were carried out in order to get used to the procedure. The start of each trial was signalled by a 1000 Hz, 50 dB tone. Immediately after the tone the subjects rinsed in the prescribed way and then sipped the entire contents of the first cup of a pair. After assessing its taste the subjects spat the contents into the bucket. The cup was also thrown into the bucket. Then the second cup of the pair was tasted in the same way. After having tasted both members of a pair, the subjects indicated which of the two cups contained the tastant by putting a cross on either the left or right 'hole' at the corresponding position on the response sheet. All subjects started in the left lower corner and worked through the pairs of all rows from left to right. After having finished the top row of the first tray (sheet) the mid-session break followed.

Experiment 2: determining psychophysical functions

In the second experiment the psychophysical functions for sucrose and NHDC were determined. The same group of subjects took part in this second investigation.

Stimuli

Five concentrations of both substances were used. The solutions were prepared in the same way as in the first experiment. The strongest sucrose solution was 146.3 g/l and each next lower concentration was 0.75 times the preceding one, resulting in a lowest concentration of 46.3 g/l. For NHDC the strongest concentration was 0.16 g/l and each next lower step was 0.50 times the preceding one. Thus here the lowest concentration was 0.01 g/l. The five concentrations were replicated six times. For sucrose and NHDC this made a total of 60 stimuli. These were randomized for each of the subjects and placed in the Plexiglas trays. The inter-stimulus intervals were 1 min. Between stimuli, two rinses were carried out in the same way as in the first experiment.

Responses

The method of magnitude estimation (Stevens, 1975) was used. In this method subjects assign numbers to the perceived intensities of the stimuli. Subjects were free to use any number they felt appropriate, as long as they adhered to requirements of magnitude estimation, i.e. assigning numbers proportionally to the strength of their sensations. The method was clearly explained to the subjects and numerical examples were given to demonstrate it. The subjects wrote the numbers on a response sheet displaying, as in the first experiment, a print of the tray.

Experiment 3: testing NHDC enhancement with scaling

The subjects participating in experiments 3 and 4 were selected on the basis of their results in experiments 1 and 2. Two selection criteria were used. First, subjects with non-monotonies in their scaling results did not pass. Secondly, only subjects close to the group sensitivity mean were included: from those subjects who passed the first criterion, the 15 closest to the group mean were selected, thus constituting a homogeneous group. These were seven males and eight females, coincidentally about an equal number of males and females. The mean absolute thresholds of the selected subjects were 0.01 M (3.47 g/l) and 0.67 M (0.000412 g/l) for sucrose and NHDC, respectively. In this experiment the effect of adding a near-threshold amount of NHDC to sucrose solutions was investigated. There were three conditions in the experiment: (i) a control condition with seven different sucrose solutions, the strongest being 195.1 g/l, each next lower one being 0.75 the preceding one and the resulting weakest concentration being 34.7 g/l; (ii) a control condition with a near-threshold amount of sucrose (15 mM, 5.14 g/l) added to each of the seven sucrose stimuli of control condition (i); (iii) the experimental condition with a near-threshold amount of NHDC (1.63 M, 0.001 g/l) added.

From the threshold functions obtained previously it could be seen that the added amounts were equivalent and of about threshold value for the most insensitive subject of the 15 participants. The subjects were offered nine blocks of 21 randomly ordered stimuli, each block containing seven concentrations × three conditions. Subjects came for three session, one session a day on consecutive days. Each session contained three blocks (63 stimuli). The scaling procedure was carried out in the same way as in experiment 2.

Experiment 4: testing NHDC enhancement by paired comparison

The same subjects as in experiment 3 participated. In this experiment the subjects carried out paired comparisons. The fourth concentration (82.3 g/l) with either NHDC (N) or sucrose (S) added served as the standard. At this concentration S+N was judged slightly more intense than S+S in the previous experiment. Although this difference was far from significant, it appeared at least to be in the direction of the enhancement claim.

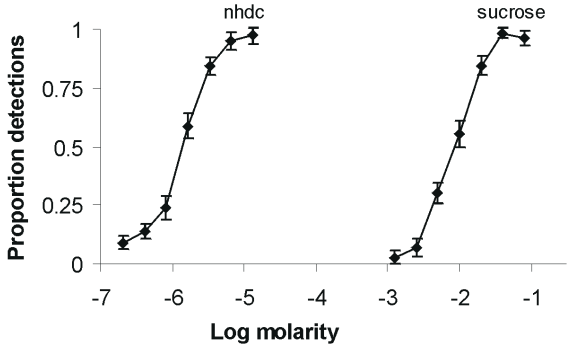

Figure 1 Absolute threshold functions of NHDC and sucrose with standard errors of measurement. NHDC and sucrose have 50% points of 1.349 M (0.000826 g/l) and 8.767 mM (3.001 g/l), respectively. On a weight basis the subjects are 3600 times as sensitive to NDHC as to sucrose.

Sucrose solutions of 5, 10 and 15% below the standard and 5, 10 and 15% above the standard were prepared. Then, by addition of either 5.14 g/l sucrose or 0.001 g/l NHDC to each of the seven sucrose solutions, two types of stimuli were prepared: sucrose-enriched sucrose stimuli (SS) and NHDC-enriched sucrose stimuli (SN).

There were three conditions: (i) each of the SS stimuli, SS-1–SS-7, was compared with the standard SS-4; (ii) each of the SN stimuli, SN-1–SN-7, was compared with the standard SS-4; (iii) each of the SS stimuli, SS-1–SS-7, was compared with the standard SN-4. As each pair was offered twice per subject in each of three sessions, once with the standard at the left and once at the right position, the total number of presentations over three sessions was 126. The subjects' task was to indicate on a printed replica of the tray whether the right-hand member of a stimulus pair tasted less, equally or more intense than the first (left) stimulus.

Results and discussion

Experiment 1

The proportion of detections was calculated for each subject and both substances as well as for the group. The chance-corrected proportions of the group are plotted as a function of log molarity in Figure 1.

As can be seen, an extremely small amount of NHDC is sufficient at the absolute threshold level. Graphical interpolation at the 50% point in the sigmoidal functions of Figure 2 gives a mean NHDC detection threshold of 1.35 μM (0.000826 g/l) and a mean sucrose detection threshold of 8.767 mM (3.001 g/l). On a weight basis the subjects proved 3600 times as sensitive to NHDC as to sucrose. It can also be observed that the slope of both threshold functions is similar. It looks as if the NHDC function mimics the sucrose function at a lower molar level.

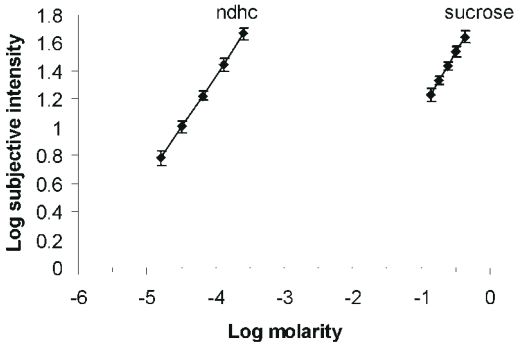

Figure 2 Psychophysical functions of NHDC and sucrose based on five concentrations of each substance with standard errors of measurement. NHDC, log$S$ = 0.73 log$I$ + 4.2980 (fit, r^2 = 0.9871); sucrose, log$S$ = 0.83 log$I$ + 1.9517 (fit, r^2 = 0.9799).

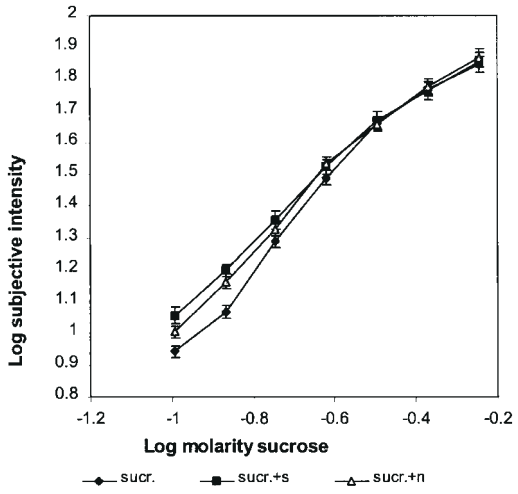

Figure 3 Psychophysical functions with standard errors of measurement of sucrose only (log$S$ = 1.28 log$I$ + 2.2326; fit, r^2 = 0.9801), sucrose + weak sucrose (log$S$ = 1.10 log$I$ + 2.1679; fit, r^2 = 0.9944) and sucrose + weak NHDC (log$S$ = 1.19 log$I$ + 2.2104; fit, r^2 = 0.9934).

Experiment 2

Before constructing a scale, individual differences in scaling ranges, resulting from the free number choice, were eliminated by rescaling all individual responses to a common mean. The common mean was the group mean obtained from all scaling data.

Then geometric means were calculated per subject and concentration for each of the two substances. The arithmetic mean was then calculated over subjects.

The psychophysical functions of sucrose and NHDC plotted on log–log coordinates are displayed in Figure 2. The slopes for sucrose and NHDC are, respectively, 0.83 and 0.73, indicating that perceived NHDC intensity increases slightly less than perceived sucrose intensity with molarity. It can be seen that both plots are perfectly linear on log–log coordinates, meaning that Stevens' power law applies to the sweet sensation associated with both substances.

Experiment 3

The raw data were treated in the same way as in experiment 2. The resulting psychophysical functions can be seen in Figure 3.

If NHDC had acted as a taste enhancer, the perceived intensity of sucrose with NHDC added should have been higher than with sucrose added and thus more different from the control in which neither NHDC nor sucrose was added. It can be seen that in fact the Stevens function of NHDC-enriched sucrose solutions displays a small, although not statistically significant, loss of perceived intensity. An analysis of variance (SPSS, 1998) with repeated measures on concentrations and conditions revealed significant effects for both factors. The concentration effect is always significant in a magnitude estimation experiment and is not of interest here. The main effect of conditions proved significant [$F(2,28) = 11.20$, $P < 0.001$]. Pairwise comparison revealed that this significant effect was entirely caused by the slight but consistent increase in perceived intensity after addition of a constant amount of either NHDC [$F(1,14) = 14.31$, $P = 0.002$] or sucrose [$F(1,14) = 15.67$, $P = 0.001$] to an increasing background concentration. Thus it did not matter whether sucrose or NHDC had been added [$F(1,14) = 0.007$, $P > 0.90$). A linear fit on log–log coordinates showed perfect adherance to Stevens' law. The three psychophysical functions were: sucrose, log$S$ = 1.28 log$I$ + 2.23; sucrose with weak sucrose added, log$S$ = 1.10 log$I$ + 2.17; sucrose with weak NHDC added, log$S$ = 1.19 log$I$ + 2.21. Compared with experiment 2 the slopes in this experiment proved substantially higher. This may be a selection effect caused by discarding subjects with non-monotonies in their psychophysical functions. The slight convergence of the three functions is exactly what one might expect on the basis of Weber's law if a fixed rather than a proportional amount is added to an increasing background concentration. This convergence can be seen in the tendency of both within-factors to interact [$F(12,168) = 1.647$, $P = 0.083$]. It underlines the sensitivity of our procedure. Neither the intercepts nor the slopes indicate any evidence of taste enhancement.

Experiment 4

From the paired comparisons the cumulative distribution 'percent stronger than standard' was calculated for each of the three conditions. These distributions are depicted in Figure 4.

It can be seen that the three functions are virtually identical. By converting the percentages to standard Z scores, linear functions were obtained of which the 0 points represent the points of subjective equality (PSEs). From these linear functions it was calculated that when NHDC-enriched sucrose (82.3 g/l sucrose + 0.001 g/l NHDC) was the standard, a sucrose-enriched sucrose solution of 80.9 + 5.14 g/l sucrose was sufficient to match it. In the case of

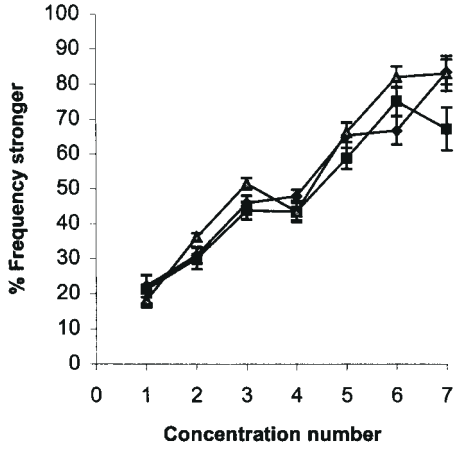

Figure 4 Differential threshold plots of the percentage of cases in which the comparison stimulus was judged stronger than the standard as a function of the concentration of the variable stimulus. Standard errors of measurements are indicated. S+s and S+n mean that either a small amount of sucrose or a small amount of NHDC was added. Diamonds, S+s (comparison) – S+s(standard); squares, S+n(comparison) – S+s(standard); triangles, S+s(comparison) – S+n(standard). Points of subjective equality, represented by the 50% points of the linear least squares plots, are not statistically different.

enhancement it should have been significantly more! With sucrose-enriched sucrose as a standard a NHDC-enriched sucrose solution of 83 g/l sucrose + 0.001 g/l NHDC is required to match it. In this case also enhancement is clearly absent.

When the three PSEs are calculated for each of the subjects in the same way as was done for the group data and then statistically tested with subjects as cases, they are not significantly different for either condition 1 and 2 ($t = 0.884$, df = 14, $P > 0.10$) or for condition 1 and 3 ($t = 0.447$, df = 14, $P > 0.25$).

Conclusion

It may be concluded from the results of the experiments that there is no sign whatsoever of a taste enhancing property of NHDC in sucrose solutions. NHDC does not enhance perceived intensity more than a subjectively equal control solution. The procedure was so designed that had an enhancement effect existed, it should have been found. How sensitive the procedure actually was can be inferred from the significant effect of enriching the sucrose solution either by a small amount of NHDC or a small amount of sucrose. The detectability of NHDC in extremely small amounts is remarkable: we found that at the detection threshold the sugar/NHDC ratio was 3600. Although weak NHDC does not lead to taste enhancement in sucrose solutions, the results do not challenge its possible taste enhancing effect in other media or its quality as a very potent sweetener.

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