# Sugar reduction in flavoured beverages: The robustness of aroma-induced sweetness enhancement

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## Abstract

Aroma-induced sweetness enhancement (AISE) is a cross-modal perceptual interaction repeatedly demonstrated in model foods but rarely in real foods. Previously, we hypothesized that the taste of flavoured foods can be enhanced by aroma components associated with naturally occurring taste-intense versions of that food. This was proven for apple juice sweetness by adding ethyl hexanoate (HEX), an odourant synthesized in apples during ripening. Here, we investigated whether AISE persists after repeated exposure or whether humans eventually learn to discriminate between sugar- and aroma contributions to sweetness. A case series study was performed to assess the effects of sucrose feedback on the perceived sweetness of apple juice. Feedback effects were assessed by pre-test/post-test evaluations by 21 subjects of the sweetness of HEX-containing (0, 1, 2, 5 ppm) apple juices with 0% or 2% sugar added. Juices were evaluated naively and after 4 and 8 intermittent sucrose feedback sessions. Finally, subjects rated sweetness after a 35-day washout period in which no further feedback was given.

Significant enhancement of sweetness by HEX confirmed previous findings that AISE occurs with naïve subjects (HEX effect: $p < 0.001$) and is most profound at low sucrose concentrations (HEX x Sucrose effect: $p < 0.05$). Furthermore, AISE was suppressed to an extent proportional to the amount of feedback received (HEX x Feedback effect: $p < 0.001$), but recovered significantly after washout for all but the highest HEX concentration (HEX x Washout effect: $p < 0.05$). Results contradict that subjects acquired perceptual skills to distinguish between sucrose- and odour-induced sweetness. Instead, we conclude that subjects temporarily adopted the response strategy to reduce sweetness ratings with a factor proportional to the perceived intensity of the HEX odour. Results indicate the long-term applicability of AISE to reduce sugar in naturally flavoured beverages.

## Introduction

Overweight and obesity are increasing health threats in the western society [1]. In part, their incidence is attributed to a global shift in diet towards increased intake of energy-dense foods that are high in fats and sugars [2, 3]. Sweetened beverages appear to play an exceptional role in this dietary shift as drinking beverages results in higher ad-lib calorie intakes than spooning beverages [4, 5]. A recent investigation under Dutch primary school pupils substantiated the effect of liquid calorie intake on weight gain. It showed that the sugar content of fruit-based juices, consumed as single 250-mL servings during daily lunch, significantly contributes to weight gain over a period of one year [6]. Hence, lowering the sugar contents of beverages that are drunk on a daily basis may reduce overweight incidence.

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In order to reduce sugar in beverages without affecting taste, synthesized intense sweeteners are being applied extensively in spite of resistance from consumer organizations against the use of synthesized sweeteners [7, 8]. Yet, alternative sugar reduction strategies are available that do not require substitute sweeteners. First of all, consumers may adapt to a gradual reduction of sugar from all beverages. This could challenge consumer loyalty for beverages and the successful gradual reduction of sugar would therefore require broad commitment in the industry. More elegantly, compensation of sweet taste may be compensated for by aromas, as was shown for simple taste solutions [9, 10].

Reports on such aroma induced sweetness enhancement (AISE) of real foods are few. This may be due to the fact that foods have pronounced aromas already, which makes improvement more challenging than in simple aqueous taste solutions. To deal with the complexity of adjusting existing food aromas for taste enhancement, we introduced a modified approach to the AISE paradigm [11]. This approach was born from the hypothesis that, by mere exposure to many instances of foods, humans learn to associate food aromas with the taste (intensity) that it usually accompanies. If so, mimicking the aroma of sugar-rich versions of a food would raise perceived sweetness by mere suggestion through the aroma. This was confirmed by the demonstration that ethyl hexanoate (HEX), an aroma component that is synthesized simultaneously with sugars in apples during ripening [12], raises the sweetness of apple juice. Although demonstrated repeatedly [11], it is yet unclear whether this effect is robust over long-term repeated presentations to the same subjects.

In the present study, we tested the robustness of the previously demonstrated enhancement of sweetness by mimicking the aroma of sweeter versions of the same food. To that end, we monitored AISE of apple juice by HEX over repeated exposures during a 6-month period. To maximize the opportunity of panellists to learn to distinguish between the contributions of sucrose and aroma to sweet taste, explicit feedback on sucrose-calorie content was provided intermittently in dedicated sessions. To prevent that sweetness differences could only be attributed to aroma differences, two concentrations of sucrose were used in the apple juices. This was expected to aid subjects in distinguishing between AISE-induced and sweetener-induced sweetness. After repeated sucrose-feedback sessions, a wash-out period was observed during which no sucrose feedback was given. AISE is still to be considered robust if it recovers from initial suppression by sucrose feedback during this wash-out period.

## Experimental

### *Subjects*

Forty-five naïve subjects enrolled in the experiment. Of these, 26 passed tests for normogeusia and normosmia. In line with ISO guidelines on the selection of panellists (ISO 8586-1:1993) normogeusia entailed the correct identification of 9 out of 12 duplicate presentations of water and aqueous tastant solutions of sucrose, sodium chloride, citric acid, caffeine and mono-sodium glutamate. Smell acuity was assessed by the Dutch odour identification test GITU; [13], an odour recognition test using 36 common odours varying in familiarity. Test results of 18 or more correct identifications were considered normosmic. Of the 26 selected subjects, 21 completed the study (mean age 40.7 years, 7 male). Their acuity scores were 10.0 and 22.6, respectively. This panel size was considered sufficient to replicate the AISE studies by Knoop [11], involving 17 or 18 subjects.

Under the Dutch regulations, a medical-ethical evaluation was not indicated at the time of execution of this behavioural study (2009-2010). However, the application of stimulus materials in naïve panellists was medically-ethically approved by the Wageningen University medical ethical board (NL25364.081.08). The study was conducted in compliance with the Declaration of Helsinki on Medical Research Involving Human Subjects. Subjects gave written informed consent and were paid for their participation.

*Methods*

Stimuli: Stimuli were apple juices with varying contents of sucrose and additional aromas. Apple juices were prepared by diluting a commercial apple juice concentrate, low in aroma content (‘medium acid’, FrieslandCampina, the Netherlands) in water (Evian, Danone, France) at a concentration of 130 g/L. To this dilute, 10 ppm (vol/vol) of a commercial food-grade apple aroma (Aroma Type ‘apple’; IFF, Hilversum, the Netherlands) was added. Crystalline sucrose (0 or 2 % w/w) obtained from the local supermarket and food-grade ethyl hexanoate (0, 1, 2 or 5 ppm (vol/vol); Sigma-Aldrich, Zwijndrecht, the Netherlands) were added to the apple juice in a full-factorial manner to obtain eight different apple juices.

Procedure: Thirty-ml medicups (King, Tiel, the Netherlands) were filled with 25-ml aliquots of apple juice and closed by a lid with a straw (8.0 mm o.d.) stuck through a hole of the same diameter. In this way, retronasal aroma stimulus presentation was favoured as it is assumed that AISE is optimal for retronasal aroma presentation [14]. The apple juice without added HEX or sucrose was presented as reference stimulus in transparent plastic cups of 100 ml (King, the Netherlands). These cups were sealed with Parafilm® (Pechiney Plastic Packaging, US) through which a straw was inserted. Stimuli were labelled with 3-digit codes for identification without revealing their composition.

Subjects started each session by first evaluating the reference stimulus. They could taste this reference stimulus at will throughout the experiment. Subjects then rated the sweetness of the apple juices on visual-analogue scales anchored 0 at the left extreme (‘not sweet at all’) and 100 at the right extreme (‘very sweet’). To prevent that perceived stimulus differences due to aroma manipulation would, for lack of response alternatives, be dumped into the sweetness ratings [15], three additional attributes were evaluated in parallel to sweetness on identical scales. These attributes, ‘sourness’, ‘apple-like aroma’ and ‘flowery-like aroma’, were previously identified as important quality descriptors of the apple juices in the Knoop studies [11]. Reference scores for these attributes were also derived from the Knoop studies. The stimulus presentation order was individually randomized. Attribute ratings were collected with EyeQuestion software (Logic8, Elst, the Netherlands).

*Experimental design*

Subjects evaluated apple juices that varied systematically in HEX content (0, 1, 2, 5 ppm) and added sucrose content (0, 2% w/w), combined in a full-factorial manner. In addition, the amount of sucrose-content feedback given was manipulated systematically. For this, the experiment consisted of 7 phases (Figure 1) during each of which all 8 apple juices were evaluated. The first, naïve, evaluation phase (I) was in essence a replication of the study by Knoop [11] performed in two consecutive sessions. During the subsequent sucrose feedback phase (4 sessions), sucrose content information was presented to subjects during stimulus evaluation. In a subsequent informed evaluation phase (II; 2 sessions), subjects evaluated stimuli in an identical fashion as during the first stimulus evaluation phase. Because subjects received feedback prior to this evaluation phase, they were not naïve regarding possible discrepancies between the perceived sweetness and the actual sucrose contents. This procedure was repeated in a second sucrose feedback phase (4 sessions) and a further informed evaluation phase (III; 2 sessions). After a subsequent wash-out period of 3 weeks, subjects performed another evaluation (IV; 2 sessions), which took place 5 weeks after they received the last sucrose feedback.

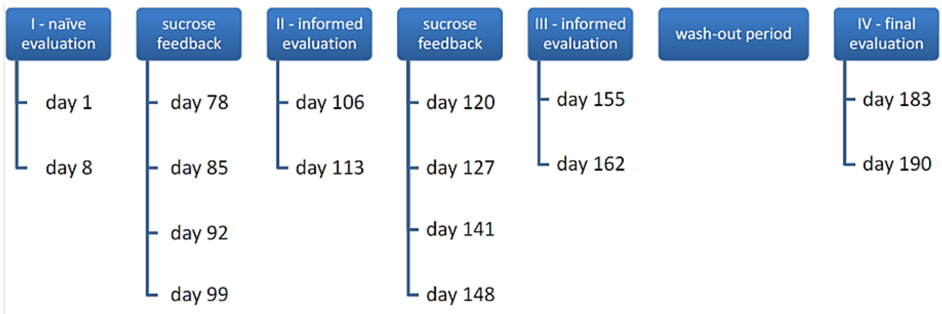


**Figure 1**: Scheduled experimental sessions in days after start. Subjects rated the sweetness of apple juices in duplicates on fixed week days under four information conditions: (I) 'naïve' regarding the actual sucrose contents, (II) after 4 evaluation sessions in which sucrose content feedback was provided, (III) after further 4 sessions in which sucrose content feedback was provided, and (IV) 5 weeks after the last exposure to sucrose content feedback.

*Data analysis*

Only sweetness ratings are evaluated since aroma and sourness attributes were merely included in the experiment to prevent dumping. The statistical evaluation of sweetness results is divided in three relevant sub-tests:

Naïve evaluation. First, to test whether the AISE results of Knoop [11] were reproduced, sweetness ratings from the (naïve) evaluation phase I were subjected to repeated-measures ANOVA, testing for main effects of HEX concentration (HEX; 0, 1, 2, 5 ppm; within-subject), added sucrose (Sucrose; 0, 2% w/w; within-subject) and Replicates (within-subject), and for the respective 2-way and 3-way interactions.

Sucrose content feedback. Second, the effect of sucrose content feedback on sweetness ratings was tested by comparing sweetness ratings for the three conditions of increasing sucrose content feedback (Feedback; 'naïve evaluation I', 'informed evaluation II' and 'informed evaluation III'). Between these evaluation conditions, the total exposure of subjects to sucrose content information was the distinguishing variable. Hence, the factors tested were Feedback (naïve, 4x feedback, 8x feedback), HEX (0, 1, 2, 5 ppm), Sucrose (0, 2% w/w) and Replicates and their mutual 2-way and 3-way interactions (all within-subject).

Recovery. Third, to test whether recovery of AISE occurred after the wash-out period, sweetness ratings collected after the wash-out period in evaluation phase IV were compared to ratings collected during evaluation phase III. The factors thus tested by ANOVA were Recovery (most-informed [III] vs. after washout [IV]), HEX, Sucrose and Replicates, along with their mutual 2-way and 3-way interactions (all within-subject).

All statistical tests were performed with Statistica (version 10, 2011; Statsoft, Inc, Tulsa, OK).

## Results and discussion

### *Naïve evaluations*

For the naïve evaluations of apple juice, significant effects were observed for Sucrose [$F(1,20) = 49.1$; $p < 0.001$], HEX [$F(3,60) = 8.91$, $p < 0.001$] and the Sucrose x HEX interaction [$F(3,60) = 3.06$, $p < 0.05$]. Observed sweetness ratings (Figure 2) reflect the observation by Knoop [11] that both sucrose and HEX enhance sweetness, and that the contribution of HEX to sweetness is more pronounced at lower sucrose concentrations. No significant effects were observed for replicates or for any of the interactions involving replicates.

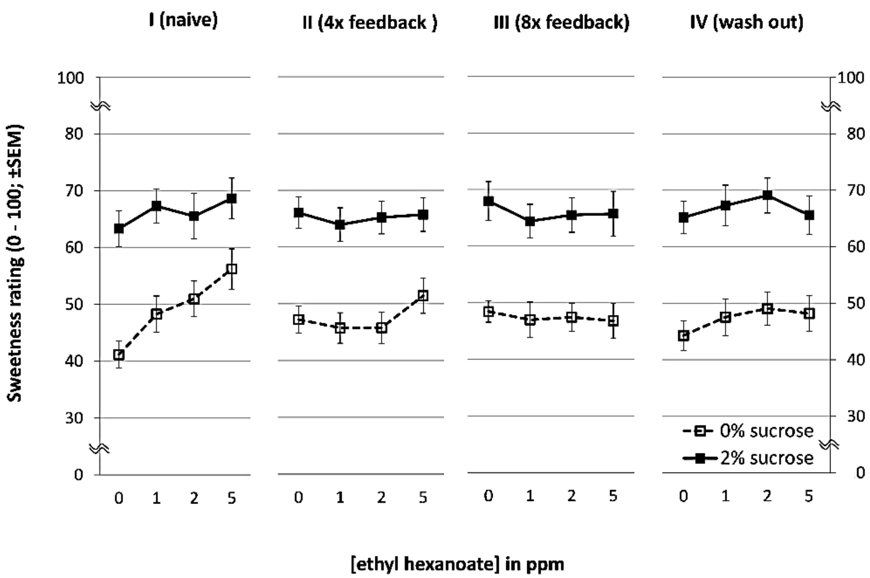


**Figure 2:** Rated sweetness as a function of ethyl hexanoate- and sucrose concentrations in apple juice under different sucrose content feedback conditions: I = no information provided, II = after 4 sucrose content feedback sessions, III = after 8 sucrose content feedback sessions and IV = after a subsequent 'wash-out' period of 5 weeks during which no sucrose content information was communicated.

### *Sucrose content feedback*

Sweetness ratings were affected by Sucrose [$F(1,20) = 85.4$, $p < 0.001$], HEX [$F(3,60) = 3.81$, $p < 0.05$] and the HEX x Feedback interaction [$F(6,120) = 5.85$, $p < 0.001$]. No main effects of Feedback or Replicate or other interactions were observed. Inspection of sweetness ratings for the first three evaluation phases in Figure 2 shows that the main effect of Sucrose is due to an overall sweetness enhancement upon addition of 2% w/w sucrose. The addition of increasing concentrations HEX results in increasing sweetness ratings in the naïve evaluation setting, and to a lesser extend also in the evaluation after the first sucrose-content feedback phase, explaining the main HEX effect. The observed HEX x Feedback interaction is reflected in a gradual decrease of HEX-induced sweetness enhancement for increasing amounts of feedback on sucrose content. In fact, after two feedback blocks, no HEX-induced sweetness enhancement is observed at all (Figure 2, plot III).

*AISE recovery effects after wash-out*

Analysis of the effects of a wash-out period on AISE (evaluation IV versus evaluation III) resulted in significant effects of Sucrose [$F(1,20) = 70.7$, $p < 0.001$] and a non-significant trend for the HEX x Recovery interaction [$F(3,60) = 2.56$, $p = 0.063$]. Inspection of the interaction effect of HEX x Recovery on sweetness learns that AISE appears to be restored for the lower HEX concentrations (0, 1 and 2 ppm) but not for the highest HEX concentration (5 ppm). For the 3 lowest HEX concentrations, this comparison results in a significant HEX x Recovery interaction [$F(2,40) = 3.62$, $p = 0.036$]. No main effects of Replicate or HEX were observed, nor for any of the remaining interactions.

*Discussion*

Aroma-induced taste enhancement is a cross-modal perceptual interaction that received a fair amount of attention in the scientific literature. This interest could originate from the prospect of exploiting cross-modal effects to reduce sugars and sodium in foods. Nonetheless, only few reported on the successful application of single odourants to enhance the taste of model systems reminiscent of real foods [11, 16, 17], possibly because of the challenge to modify the existing aromas of these foods without ill effects on their quality. The aroma-induced taste enhancement paradigm proposed by Knoop [11] differs from classical cross-modal interaction approaches because it entails the balancing of selected aroma components in line with their natural occurrence in sweeter versions of the food, rather than the classical combination of singular aroma components or entire aroma mixtures with a mere taste solution. Consequently, literature reports on AISE in simple taste solutions may still be a poor indicator of the reliability and robustness of the few observations of aroma-induced taste enhancement by Knoop. Therefore, the repeated observation of AISE in apple juice by an odourant that is synthesized in sugar-rich apples provides further support for the hypothesis that AISE exists in real foods and that it relies on previously learned aroma-taste associations.

In the present study, explicit feedback on sucrose contents of stimuli elicited a profound suppression of AISE. After four sucrose feedback sessions, HEX contributions to sweetness ratings nearly disappeared and after eight feedback sessions HEX did not contribute to sweetness ratings at all. As the contributions of sucrose to sweetness remain unchanged over feedback conditions, it is expected that the feedback-induced sweetness reductions are entirely due to a changed processing of aroma information, and not to a changed processing of sweetness in general. We therefore conclude that subjects have learned to use aroma information to adjust their sweetness ratings on basis of feedback regarding the stimulus sucrose contents. The conscious exposure to calorie feedback therefore appears to mimic acceleration of the effects of repeated exposure to stimuli on the reduction of sweetness ratings.

Central to the interpretation of these results is the question whether the observed suppression of AISE after sucrose content feedback reflects a limited robustness of AISE for repeated exposure. We think that the answer lies in the mechanism involved in the observed AISE suppression. Either, subjects became unresponsive to HEX after sucrose feedback because (i) they successfully acquired the skill to perceptually isolate the aroma contribution to sweetness from the sucrose contribution, or (ii) they learned to apply the response strategy that if HEX is perceived, the perceived sweetness should be diminished with a corresponding amount. The former explanation reflects a genuine refinement of perceptual skills whereas the latter implies that only response behaviour is affected by feedback. In the latter case, a reduction of AISE after sucrose content feedback does not unequivocally demonstrate a poor robustness of AISE because perception is overruled by the application of a response rule. Given the general long-term persistence of acquired perceptual skills and perceptual odour associations [18, 19] and the more transient nature of non-reinforced cognitive strategies in perceptual tasks [20], it is expected that response strategies will decay after discontinued feedback, whereas perceptual associations will not. If a response strategy invokes AISE suppression, AISE is expected to recover after a wash-out period during which no further feedback is given on sucrose content. Therefore, the observed AISE recovery after the final wash-out period is indicative of robust sweetness enhancement by HEX and of a response rule in decay. Further support for this interpretation is that, after the wash-out period, sweetness enhancement was restored for the two intermediate HEX concentrations (1ppm and 2ppm), but not for the highest (5ppm) HEX concentration. This suggest that AISE recovered, although partially compensated for by a remainder of the response strategy that only kicks in when the enhancing aroma becomes too apparent, i.e. at its highest concentration.

The partial recovery of AISE after a wash-out period favours an explanation in terms of robust AISE and a decaying application of a response strategy. Would AISE have recovered even more after longer wash-out periods? As the present results do not rule out a persistent partial suppression of AISE, we invited the panel for a repeated evaluation of the used stimuli, one year after the start of the experiment, to verify whether further recovery of AISE occurred. Unfortunately, panellist drop out had increased by then and the sweetness ratings collected for the remaining subgroup of panellists showed a decrease of general task performance (higher intrinsic response variation). This may reflect a general problem in longitudinal studies of multimodal perception in which a panel should be kept naïve and untrained during wash-out periods.

In general, studies on taste enhancement by factors other than the tastant concentration show that enhancement is most pronounced at lowered tastant concentrations. This is, for instance, observed for aroma-induced taste enhancement of sweet tasting stimuli [11, 21], salty stimuli [22] and the effects of salt distribution on the salty taste of bread [23]. The present study confirms this dependency, which further supports the applicability of AISE for enhancing the sweetness of beverages with reduced sugar contents.

This study is premised on the idea that by informing panellists on the amounts of sucrose in the apple juice, the worst case scenario is created for the extinction of AISE. If explicit sucrose feedback cannot forestall AISE in the long run, does this therefore really imply that AISE is robust for long-term repeated exposure? Or may, alternatively, the chosen feedback regime in the present study not have challenged AISE robustness enough? For instance, one may argue that after repeated exposure to low-sugar juices with the enriched aroma, subjects may cease to associate the aroma with sweetness and the sweetness-enhancing effect of the aroma would disappear. This scenario is improbable if perceived sweetness were the main driver for the learned aroma-taste association because a full compensation of sugar reduction by AISE would not reduce the perceived sweetness. However, sugar is not only a sweetener but also an important energy source. Pairing a particular aroma with low-energy foods may then, in the long run, invoke consumers to associate aromas with caloric content as was previously shown for rodents [24]. Attempts to replicate such controlled intake experiments in humans failed, as foods in which aromas signalled different calorie contents [25, 26] invoked no behavioural changes due to metabolic impact. With this in mind, we consider the present study a greater challenge to the robustness of AISE than mere repeated consumption. Regarding the comparison with explicit sucrose feedback in real-life, we argue that even if consumers would spell out ingredient lists, the discrepancy between sucrose contents and the perceived taste intensity would not be as explicit as it was in this study. Nonetheless, only a longitudinal consumer study that monitors daily intake under natural conditions without revealing the focus on taste-aroma interactions, taste intensity and preference may provide a conclusive answer.

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