JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Effects of Pulsation Rate and Viscosity on Pulsation-Induced Taste Enhancement: New Insights into Texture–Taste Interactions

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ABSTRACT: Oral stimulation with high-tastant concentrations that are alternared with low-tastant concentrations or water rinses (pulsatile stimulation) results in taste intensity ratings that are higher than continuous stimulation with the same average tastant concentration. This study tested the combined effects of taste pulsation rate and viscosity on pulsation-induced taste enhancement in apple juice. According to a tastant-kinetics hypothesis, less pulsation-induced taste enhancement is expected at enhanced pulsation rates in the high-viscous proximal stimulus compared to lower viscous stimuli. High-concentration sucrose apple juice pulses and low-concentration sucrose apple juice intervals were alternated at different pulsation periods (pulse + interval in seconds) every 2.5 s (period length = 5 s) or every 1.25 s (period length = 2.5 s). Pulsed stimuli were presented at two viscosity levels by the addition of pectin (0 and 10 g/L). Sweetness intensities of pulsed stimuli were compared to a continuous reference of the same net but nonalternating sucrose concentration. Sweetness ratings were higher for pulsatile stimuli than for continuous stimuli. In low-viscous stimuli, enhancement depended on the pulsation period and peaked at 5 s periods. In high-viscous stimuli, the same enhancement was observed for both pulsation periods. These results contradict a tastant-kinetics hypothesis of viscosity-induced taste suppression because impaired tastant kinetics by viscosity would predict the opposite: lower pulsation-induced taste enhancement for viscous stimuli, especially at higher pulsation rates. Instead, these observations favor an explanation based on perceptual texture–taste interactions, which predict the observed independence between viscosity and pulsation rate.

KEYWORDS: pulsatile stimulation, aroma–taste interaction, texture–taste interaction, sweetness enhancement, pulsation period

INTRODUCTION

For health reasons, regulatory bodies advise a reduction of sugar levels in foods.[1] As this conflicts with the innate preference for sweet taste, compensation strategies for low-sugar products are required. One strategy achieves taste enhancement by tastant concentration contrasts.[2−6] In liquid applications, tastant concentration contrasts are evoked by stimulation with high-intensity tastant pulses that are alternated by tasteless or low-intensity tastant intervals.[4,5,7] This "pulsatile stimulation" enhanced sweet taste intensity of model solutions if compared to stimulation with the same net but not alternating (continuous) sucrose concentration.[4,5,7] Furthermore, the degree of sweet taste enhancement by pulsatile stimulation depended hereby on different factors: the length of the pulsation period (summed length of pulse and interval in seconds),[5] the magnitude of the pulse-interval sucrose concentration contrast,[7] and the presence of additional flavor compounds such as a congruent aroma[4] or a qualitatively contrasting tastant.[7] Viscosity is another factor that might influence the degree of pulsation-induced taste enhancement. An increase in viscosity by the addition of (hydrocolloid) thickeners to tastant solutions commonly results in taste intensity reduction.[8−11] Kinetic explanations of this taste suppression suggest the involvement of reduced in-mouth tastant release and reduced diffusion rates. Tastant release and diffusion may be diminished due to binding of tastants to the thickener, the inhibition of transport of tastants from the bulk phase to the taste receptors, or inefficient tastant mixing in the solution due to entanglement in overlapping hydrocolloid chains.[12−14] As for pulsatile stimulation, if concentration fluctuations in the distal stimulus are dampened as a result of decreased tastant mobility, the pulse-interval tastant concentration contrast in the proximal stimulus will be reduced[7] and decrease the taste intensity gain upon pulsation. Therefore, if increasing viscosity reduces tastant mobility, we hypothesized that increasing the tastant's pulsation rate at high viscosity will reduce taste-contrasts in the proximal stimulus which, in turn, would reduce pulsation-induced taste enhancement in comparison to stimuli with a lower viscosity. In other words, increasing the stimulus viscosity is assumed to reduce tastant mobility, which in turn would suppress overall taste intensity as well as pulsation-induced taste enhancement.

Alternatively, viscosity-induced taste suppression may not be caused by changes in tastant kinetics to start with. Possibly, the commonly observed taste suppression is caused by perceptual cross-modal inhibition as appears to be the case for viscosity-induced aroma suppression.[9,15] If tastant kinetics do not play a part in the observed viscosity-induced taste suppression, it is not expected that pulsation-induced taste enhancement is affected by viscosity.

The tastant-kinetics hypothesis is tested in the present study by investigating the combined effects of viscosity and sucrose pulsation rate on the sweet taste of apple juice. This was realized by the alternation of high-concentration sucrose pulses and low-concentration sucrose intervals in apple juice. Apple juice

Received: January 21, 2011
Revised: April 7, 2011
Accepted: April 9, 2011
Published: April 22, 2011

Table 1. Apple Juice Solutions at Different Concentrations of Sucrose ([S]; Low, Average, High) and Pectin ([P]; −, Not Present; +, Present) Used To Produce Gustometer Apple Juice Stimuli

apple juice solution	$[S]_{added}$ in g/L	$[S]_{total}$ in g/L	$[P]_{added}$ in g/L
$S_{low}P^-$	0	54	0
$S_{average}P^-$	54	108	0
$S_{high}P^-$	108	162	0
$S_{low}P^+$	0	54	10
$S_{average}P^+$	54	108	10
$S_{high}P^+$	108	162	10

stimuli were presented at two viscosities. The pulsation rates chosen were (a) period length = 5 s, which was previously found to induce the most sweetness enhancement for sucrose-in-water solutions,[5] (b) period length = 2.5 s, a higher pulsation rate that under the tastant-kinetics hypothesis would hamper taste enhancement most in the higher viscous apple juice, and (c) a continuous stimulus. The following research questions were addressed: (1) Does pulsatile sucrose stimulation enhance sweetness intensity in apple juice? (2) Does an increase in viscosity suppress sweet taste intensity enhancement? (3) Does viscosity-induced taste suppression depend on the pulsation rate? (4) Does pulsatile sucrose stimulation modulate sourness and aroma intensity in apple juice?

MATERIALS AND METHODS

Subjects. Twenty subjects (ages 22−52 years, 9 male) were recruited. They were pretrained on automated tastant delivery by means of a gustometer[15] as well as on the attribute rating procedure using aqueous reference solutions with different levels of sucrose ("sweetness"), malic acid ("sourness"), apple flavor concentrate ("aroma"), and pectin ("viscosity"). Subjects were allowed to drink only water during the last hour prior to testing. The materials and methods used did not require medical ethical approval under Dutch regulations (retail ingredients, oral delivery). Subjects gave written informed consent and were paid for their efforts.

Stimuli. Six apple juice solutions of different sucrose and pectin levels were prepared (Table 1). Apple juice (Appelsientje "Goudappel", FrieslandCampina, The Netherlands) was diluted with bottled water (Evian, Danone, France) at a ratio of 1:1 (v/v). Sucrose (S) and pectin (P; high-methoxyl pectin, CP Kelco GmbH, Germany) were added at the concentrations given in Table 1. Pectin was first dispersed in apple juice at 25 °C using a paddle stirrer. The apple juice was then heated in an open container to 80 °C for 1 h and then left for 2 h at 25 °C for hydration under continuous stirring. Samples without pectin received the same heating and stirring treatment. Solutions were prepared 1 day before presentation and stored at room temperature. For sensory testing, the solutions of Table 1 were used to prepare six stimuli by a computer-controlled gustometer:[15] (1) ScP^-, continuous delivery of $S_{average}P^-$; (2) $Sp_{5.0}P^-$, $S_{high}P^-$ and $S_{low}P^-$ alternated every 2.5 s (= 5 s period); (3) $Sp_{2.5}P^-$, $S_{high}P^-$ and $S_{low}P^-$ alternated every 1.25 s (= 2.5 s period); (4) ScP^+, continuous delivery of $S_{average}P^+$; (5) $Sp_{5.0}P^+$, $S_{high}P^+$ and $S_{low}P^+$ alternated every 2.5 s (= 5 s period); (6) $Sp_{2.5}P^+$, $S_{high}P^+$ and $S_{low}P^+$ alternated every 1.25 s (= 2.5 s period). Stimulus duration was 20 s each (5 s periods were repeated 4 times; 2.5 s periods were repeated 8 times). The net sucrose concentration of all stimuli was 108 g/L. All solutions were delivered at a rate of 15 mL/min in-mouth through a Teflon tube mouthpiece. To verify nominal sucrose concentrations, sucrose contents of stimuli collected over 20 s were analyzed in triplicate by refractometry (PAL-1, ATAGO, USA). Image analysis was used to verify that the pulse shape of stimuli was the same whether pectin was present or not. To this end, an aqueous solution made of 16.2 g/L sucrose and red food colorant and a colorless aqueous solution made of 5.4 g/L sucrose were alternated every 2.5 (= 5 s periods) or 1.25 s (= 2.5 s periods). The same procedure was repeated for an aqueous solution made of 16.2 g/L sucrose, 10 g/L pectin, and red food colorant and a colorless aqueous solution made of 5.4 g/L sucrose and 10 g/L pectin. For each stimulus, the light transmitted at a cross-section of the transparent mouthpiece was recorded (30 frames/s), and recordings were subjected to image analysis (Matlab version 2007a).

Shear Rate Determination. At high shear rates, viscous media may exhibit shear thinning behavior.[16] Therefore, the theoretical shear rates applied on the apple juice solutions (Table 1) by pumping them through the gustometer tubing and mouthpiece (length = 4 cm; inner diameter = 0.32 cm) were calculated to predict a possible change in viscosity

$$\text{shear rate } (s^{-1}) = \frac{\text{linear speed}}{\text{mouthpiece diameter}} \quad (1)$$

$$\text{linear speed } (\text{cm s}^{-1}) = \frac{\text{flow rate}}{\text{cross-sectional area (tubing)}} \quad (2)$$

with mouthpiece diameter = 0.32 cm, flow rate = 0.25 mL/s, and area (tubing) = 0.079 cm^2.

Viscosity Determination. The viscosity of the solutions in Table 1 was determined by rotational viscometry using an ARG2 rheometer (TA Instruments, USA) equipped with a double-concentric cylinder (stator inner radius = 20.00 mm, rotor outer radius = 21.96 mm). Samples (30 mL; 2 replicates) were analyzed at 25 °C at a shear rate range from 0 to 100 s^{-1} over 30 min that was preceded by a 15 min equilibration step.

Procedure. Subjects kept the mouthpiece between their central incisors while the gustometer delivered the apple juice stimuli on the extreme section of the anterior-dorsal tongue. First, the continuous reference ScP^- was delivered. Then, after a 3 s pause, a stimulus was delivered. After stimulus delivery, subjects rated the stimulus' "sweetness intensity", "sourness intensity", and "aroma intensity" relative to the reference on separate 15 cm vertical line scales (end points indicating "not sweet at all" and "very sweet", etc.). The reference intensity of each attribute was placed at 7.5 cm. Subjects swallowed at will, and tongue movements were not restricted. Each subject evaluated all six stimuli four times in a randomized order including the reference (ScP^-) as blind. Stimuli were given in blocks of four with a 5 min break between blocks (total session time = 1 h). Between stimuli, at least 1 min was given to rinse the mouth with water and to eat crackers. At the beginning of each session, subjects received two warm-up stimuli.

Data Analysis. Line scale ratings were converted into numbers by manually measuring the position of each mark with a ruler. Main effects of the fixed factors [replicate, pectin (two categories: present/absent), pulsation period (three categories: continuous stimulation, 5 s pulsation period, and 2.5 s pulsation period)] on averaged intensity ratings for sweetness, sourness, and aroma were analyzed by full-factorial multifactor repeated measures ANOVA (SPSS, Chicago, IL, version 17). All tests were at $\alpha = 0.05$.

RESULTS

Sucrose Concentration. The net average sucrose concentrations delivered over 20 s by the gustometer, as measured by refractometry, were 105 ± 9 and 108 ± 7 g/L (M ± SD) for profiles with continuous sucrose delivery (ScP^-; ScP^+) and 109 ± 12 and 106 ± 16 g/L (M ± SD) for profiles with pulsatile sucrose delivery ($Sp_{5.0}P^-$; $Sp_{5.0}P^+$).

Image Analysis. The delivered pulse shapes were similar for stimuli with and without pectin and consistent with theoretical profiles (i.e., pulsation periods; Figure 1).

Shear Rate Determination. At a flow rate of 15 mL/min, the shear rate applied by the gustometer pumping action was 10^{-1} s.

Viscosity Determination. Solutions were shear thinning at a shear rate ranging from 0.01 to 1 s^{-1} but Newtonian from 1 s^{-1} onward (Figure 2). Hence, viscosity did not change in the shear rate range from 10 s^{-1} (theoretical shear rate calculated for gustometer pumping action) to 50 s^{-1} (estimated in-mouth shear rate[17,18]).

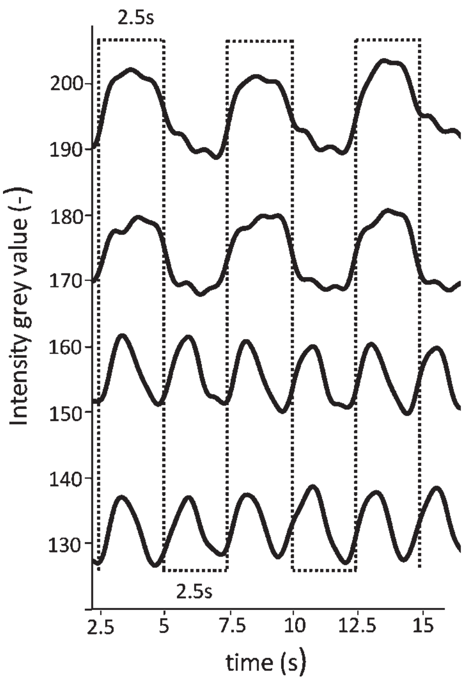

Figure 1. Pulse-interval stimulation profile delivered by gustometer in-mouth of subjects as depicted by image analysis. Pulse-interval combinations consist of high-concentration sucrose apple juice pulses that are alternated with low-concentration sucrose apple juice intervals every 2.5 s (5 s periods; $Sp_{5.0}$) or every 1.25 s (2.5 s periods; $Sp_{2.5}$). Apple juice stimuli contain either no pectin (P^-) or pectin at 10 g/L (P^+). Square-dots represent the superimposed 5 s pulsation period profile as programmed by the gustometer; profiles are shifted on the *y*-axis for clarity.

The in-mouth viscosity of stimuli then correlates with the instrumental values depicted in Figure 2: At a shear rate of around 50 s^{-1} the viscosity increase due to added pectin and sucrose was approximately 100 mPa·s ($S_{high}P^+$).

Psychophysical Results. Multifactor repeated-measures ANOVA revealed the main effects of pectin [$F(1, 431.0) = 20.1$; $p < 0.001$] and pulsation period [$F(2, 31.3) = 4.17$; $p < 0.05$] on sweetness intensity. Sweetness intensity decreased on average by 15% upon the addition of pectin (Figure 3a). Pulsatile stimulation increased sweetness intensity in both nonthickened and thickened apple juice. In nonthickened apple juice, enhancement was more pronounced for 5 s pulsation periods than for 2.5 s pulsation periods. There was a main effect of replicate on sweetness intensity [$F(3, 22.9) = 3.67$, $p < 0.05$]. Closer inspection of the data revealed that the first stimulus of a kind was always scored less sweet. Furthermore, main effects were found for pulsation period on sourness intensity [$F(2, 16.9) = 4.24$; $p < 0.05$], which increased on average by 8% in pulsed stimuli (Figure 3b). Similar to sweetness intensity, there was a main effect of pectin on aroma intensity [$F(1, 452.5) = 23.73$; $p < 0.05$], which decreased by 20% upon the addition of pectin. Aroma intensity did not increase in pulsed stimuli (Figure 3c). No two-way or three-way interactions were observed.

Separate contrasts comparing the sweetness ratings between categories "continuous" and "5 s period" or "2.5 s period" revealed significant differences between "continuous" and "5 s period" [$F(1, 19) = 7.9$; $p < 0.05$]. For sourness comparisons, significant differences between "continuous" and "5 s period" [$F(1, 19) = 6.5$; $p < 0.05$] and "continuous" and "2.5 s period" [$F(1, 19) = 5.6$; $p < 0.05$] were found.

DISCUSSION

Image analysis of gustometer stimuli revealed consistent light absorption patterns over time between low-viscous non-pectin-containing and high-viscous pectin-containing stimuli for both pulsation periods. This suggests that at least the distal stimulus was in line with the programmed stimulus profiles. Any pulsation and pectin effects observed are then related to effects of kinetics on the composition of the proximal stimulus or to perceptual interactions.

In line with the literature, the sweetness intensity of apple juice was attenuated in our study by increasing its viscosity upon the

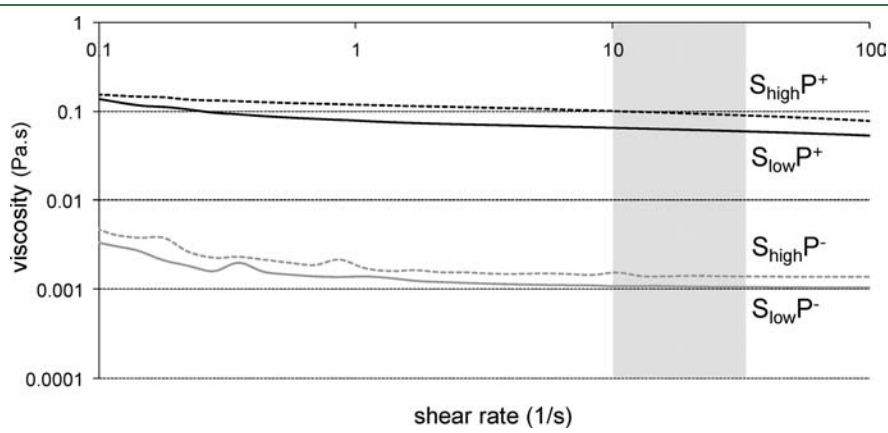

Figure 2. Representation of change in viscosity (Pa·s) with shear rate (s^{-1}) for apple juice solutions (Table1) as measured by rotational viscometry. Solutions contained different sucrose levels (S_{low}, 54 g/L; S_{high}, 162 g/L); pectin was absent (P^-) or added at 10 g/L (P^+). The gray area indicates that the viscosity remained unchanged in a shear rate range from 10 s^{-1} (shear rate applied by pumping solutions through gustometer tubing at 15 mL/min) to 50 s^{-1} (estimated in-mouth shear rate).

dx.doi.org/10.1021/jf2002848 | *J. Agric. Food Chem.* 2011, 59, 5548–5553

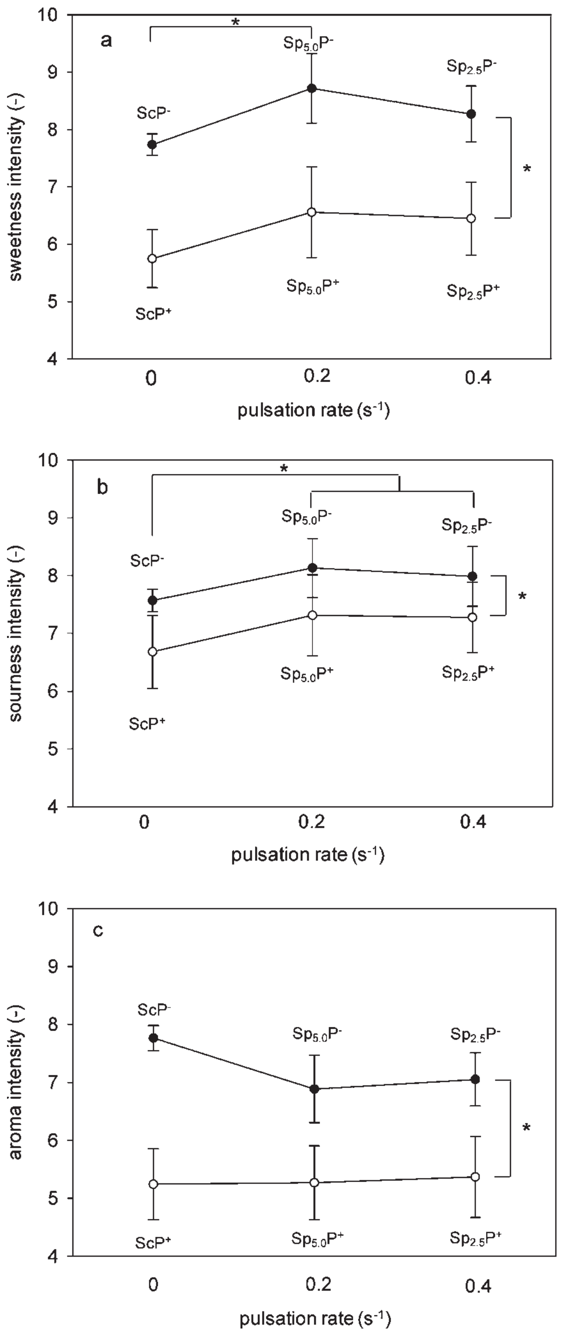

Figure 3. Intensity ratings for sweetness (a), sourness (b), and aroma (c) averaged over 20 subjects (4 replicates) for gustometer apple juice stimuli. Sc, sucrose concentration was kept constant at 108 g/L; Sp, sucrose concentration was pulsed (162 and 54 g/L alternated at 2.5 or 5 s periods); P, pectin was absent (P^{-}) or added at 10 g/L (P^{+}); ∗, stimulus categories are significantly different at $p < 0.05$.

addition of pectin. The significant main effect of pectin indicates that a general thickener-induced attenuation occurred over pulsation conditions. In addition, the sweetness intensities of apple juices were affected by the sucrose pulsation rate. This overall effect was predominantly due to the sweetness enhancement observed at low-frequency pulsation ($t = 5.0$ s) compared to continuous stimulation: Sweetness intensity peaked at intermediate 5 s pulsation periods for nonthickened apple juice. This observed frequency-dependent intensity maximum corresponds with previous observations for sucrose model solutions.[4] The present pulsation-induced sweetness enhancement was observed regardless of the presence of pectin. Surprisingly, and contradicting the tastant-kinetics hypothesis, pulsation-induced sweetness enhancement did not decrease in thickened apple juices when pulsation rates increased. In fact, the attenuation, observed from low- to high-frequency pulsation categories in nonthickened juices, was absent rather than more pronounced in the thickened juices. Therefore, the hypothesized pectin × pulsation period interaction, which can be described as diverging sweetness ratings for the two pectin categories over pulsation frequency, was contradicted by converging sweetness ratings. Although this convergence did not result in a significant pectin × pulsation period interaction, it is a stronger rejection of the tastant-kinetics hypothesis than the mere absence of a statistical pectin × pulsation period interaction with diverging sweetness ratings.

An alternative explanation to the tastant-kinetics hypothesis of viscosity-induced taste suppression is that perceptual interactions govern the effect of texture on taste. This has been described for viscosity-induced aroma intensity reduction: despite unaltered nasal odorant concentration, decreased odorant intensity with increasing viscosities of oral stimuli.[15,19] Neural correlates of such cross-modal interaction between somatosensory tactile and aroma signals were observed at higher (cortical) levels of integration of sensory information.[20−22] Viscosity-induced taste suppression as observed here may equally be the result of such perceptual texture−taste interactions.[14] The observed suppression of taste and aroma intensity in the present study may then be explained by cross-modal texture−taste and texture−aroma interactions, respectively.

According to the literature,[23−25] we expected an increase in aroma intensity with sweetness intensity. Interestingly, aroma intensity in nonthickened pulsatile stimuli decreased as sweetness intensity increased. This may have been caused by several factors. First, subjects rated both aroma and taste intensities. This is known to reduce the degree of cross-modal aroma−taste interactions invoked by halo-dumping compared to cases when only taste is rated.[26,27] Combined rating of taste and aroma then explains why aroma intensity did not increase with sweetness intensity. In addition, as the aroma concentration was diluted upon sample preparation, the increase in sweetness (and sourness) intensity in pulsed stimuli may have suppressed aroma intensity at a perceptual level. It is also possible that the dilution of apple juice may have created a sensation different from apple juice that subjects consumed in the past. The unfamiliarity of the stimulus may have led to a deconstruction of taste and aroma input and reduced cross-modal aroma−taste integration.

If presented in mixtures, qualitative contrasting taste stimuli suppress each other's intensity with the direction of the suppression depending on the compound of the highest intensity.[28,29] Accordingly, in training sessions, subjects rated low-concentration sucrose apple juice solutions as sour and high-concentration sucrose apple juice solutions as sweet (data not shown). The repeated alternation of high-concentration sucrose apple juice solutions and low-concentration sucrose apple juice solutions in pulsed stimuli then represents the repeated alternation of sweet and sour stimuli. Sequential alternations of contrasting taste qualities may lead to intensity enhancement of the second stimulus as it stands out against the preceding contrasting taste stimulus.[30] The observed sweetness and sourness enhancement in pulsed stimuli in the present study then suggests a mutual enhancement of both taste qualities: High-concentration sucrose apple juice pulses ("sweet") stood out against the low-concentration sucrose apple juice intervals ("sour") and vice versa.

Previously, it was suggested that pulsation-induced taste enhancement is the result of an elevated chorda tympani output when tastants are presented in a discontinuous (pulsatile) fashion.[2] Supporting the involvement of such preconscious stages in gustatory processing, we showed that conscious perception of pulsation is no requirement for pulsation-induced taste enhancement.[5] However, because no peripheral interactions have been observed on concomitant stimulation with sucrose and acids, peripheral mechanisms cannot explain the enhanced sourness ratings upon sucrose pulsation: Unlike sucrose, the concentrations of acidic compounds were the same at all times. Consequently, sourness enhancement must have been induced by sourness intensity alternations, which then are likely to have originated at higher cortical levels of gustatory information processing.

The first of all replicates was always rated the least sweet. This suggests (i) a certain learning effect and (ii) the ability of subjects to distinguish between different stimuli. Hence, this response to repeated stimuli of specific compositions reflects a stimulus sensitization pattern rather than a habituation pattern. In an earlier study, in which pulsatile stimuli were of high-concentration sucrose pulses and low-concentration sucrose intervals, about 50% of subjects could not tell whether stimuli were given in continuous or pulsatile manner at pulsation periods of ≤5.1 s.[5] In the present study, the alternation of high-concentration sucrose apple juice pulses and low-concentration sucrose apple juice intervals represented alternations of sweet and sour stimuli. Such sweet−sour alternations may be easier to detect than sucrose−sucrose concentration alternations and explain why subjects were able to distinguish between stimuli and pulsation periods.

In summary, the alternation of high-concentration sucrose pulses with low-concentration sucrose intervals in apple juice enhanced sweetness and sourness intensity. Sweetness enhancement may have originated at preconscious stages in taste processing that respond differently to continuous and pulsatile tastant presentations. Sour taste enhancement by sucrose pulsation, on the other hand, is likely to have originated at conscious levels of taste processing (e.g., through taste−taste contrasts). This demonstrates that pulsation-induced taste enhancement can be evoked by both concentration and intensity alternations. Sweetness and aroma ratings decreased with increasing viscosity. The absence of pulsation rate × viscosity interactions in combination with the clear presence of a suppression of taste viscosity supports a perceptual origin of texture−taste interactions rather than the involvement of changing tastant kinetics. Overall, the study demonstrates that tastant concentration and intensity contrasts in apple juice enhance sweetness intensity. This concept is interesting for sweetness enhancement in low-sugar beverages at various viscosities.

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