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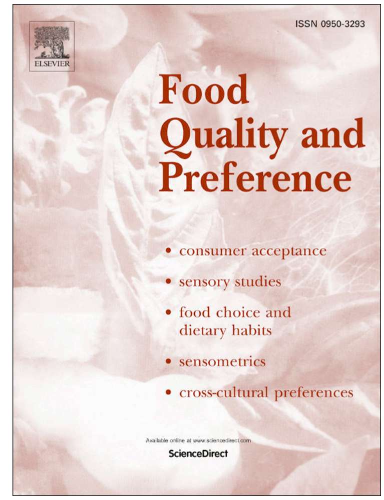

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Modulation of sweetness perception in confectionary applications

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Abstract

The development of sugar-reduced food products is a strategy to reduce the high sugar intake, which is a leading cause of global health concerns. Replacement and/or reduction of sucrose often leads to reduced sweetness perception with the consequence of decreased consumer acceptance. The aim of this work is to implement sensory modulation principles in a model confectionery system with the goal of enhancing sweetness perception. By using 3D-printing, confectionary samples were meso-structured by inhomogenous distribution of sucrose concentrations and assessed, with a trained panel regarding sweetness. All samples were made up of a high and low sucrose phase and compared to a homogeneous reference sample. The overall sugar content was kept constant at 22.8 % in all samples and sweetness perception was compared. A significant increase of sweetness perception by over 30 % could be noted for samples consisting of a sweet outer shell and an inner less sweet core with a high sucrose gradient between the two phases. Whilst texural effects on sweetness perception could not be fully excluded, results can be seen as a strong indication that sweetness modulation by inhomogenious distribution has a potential to be applied directly in solid food products.

Keywords: Sweetness modulation, Pulsatile stimulation, Sugar reduction, Multiphase-food-printing

1. Introduction

The rising consumption of free sugar in the diet is believed to be one of the leading causes for non communitable deseases (NCD) which account for an estimated 68 % of global deaths (Organization et al., 2014). Although often a sugar-reduced reformulation of products is possible, such products are often linked with decreased sensory properties and thus lower consumer acceptance (Markey et al., 2015). To be successful in the combat of sugar consumption, approaches with high consumer acceptance are needed.

By tailoring the spacial and textural properties of products, modulation of sensory perception has been reported in literature. By varying the stimulation intensity of taste receptors over time, an enhancement of tastant perception has been demonstrated for example in liquid systems for the perception of saltiness by Yamamoto and Nakabayashi (1999); Metcalf and Vickers (2002). Holm et al. (2009) applied this concept to gelled solid foods and could demonstrate increased sweetness perception in samples with inhomogeneous sugar distributions. In further experiments Mosca et al. (2010); Mosca, van de Velde, Bult, van Boekel and Stieger (2012), sucrose concentrations were reduced successfully by up to 20 % without decreasing the sweetness intensity. Using this layered gelled system with inhomogeneous distribution has also been shown to increase saltiness perception (Emorine et al., 2015), or to reduce perception of bitterness (Hutchings et al., 2015). In systems with emulsified fat, perception of fat related attributes such as creaminess can also be increased by applying this concepts (Mosca, Rocha, Sala, van de Velde and Stieger, 2012). Similar results were achieved in other solid foods, such as bread, where this concept has been shown to allow a salt reduction by up to 25 % without sacrificing product acceptance (Konitzer et al., 2013; Noort et al., 2010, 2012).

When exposed to a stimulus, taste-receptor cells are triggered to release neural signals, the firing rate of a receptor cell is governed by intensity of a stimulus, thus already translated onto timescale. Under constant exposure to a stimulus, firing rates of receptors decrease causing adaptation leading to a decreased perception over time. Vice versa, a lack of stimuli leads to disadaptation and recovery of these receptors. By alternating phases of high and low stimulation, adaptation is reduced or prevented, explaining the higher overall reception under pulsed stimulation (Kaissling et al., 1987). Furthermore, the intensity of stimulus solutions is judged differently if it is preceded by high- or a low-concentration solution owning to a stronger sensation of contrast between the solutions. (Schifferstein and Oudejans, 1996). However, as shown by Burseg, Brattinga, de Kok and Bult (2010), the sweetness perception does not depend on conscious perception of contrasts. Pulsatile stimulations can lead to enhanced sweetness perceptions even at frequencies below the detection threshold of individual pulses. The key determining factors for the effect of pulsatile stimulation have been identified to be the pulsation period, the concentration gradient, and the presence of additional aromas such as congruent or contrasting flavors. For liquid systems, it has been shown that perceived sweetness intensity is dependent on the viscosity of a solution. Increased solution viscosity leads to a decrease in perceived sweetness(Walker and Prescott, 2000; Pangborn et al., 1978). Generally, this effect is explainable by a kinetically reduced tastant release from the matrix, lower diffusion rates, binding of the tastant to the thickener polymers or poor mixing of the bulk solution. Depending on the thickening agent applied, the magnitude of sweetness reduction has been shown to vary (Baines and Morris, 1987; Ferry et al., 2006).

3D printing techniques allows to arrange food in a 3D space in a targeted manner. Tailored deposition of differently composed masses (e.g. masses with different functional ingredients such as sugar) is suitable for establishing concentration gradients, which may allow product properties such as sensory perception to be adjusted. The resolution of the internal product structure is merely limited by the nozzle diameter(s), the layer height as well as the material properties. Therefore, 3D printing is seen here as an enabling method that allows the investigation of more sophisticated internal gradient structures and their effects on sensory perception further than it has been possible so far. This may lead to new insights into structure design rules with the aim of reducing nutritionally critical or expensive components or to enhance desired perceptions.

In this work, the goal was to investigate (a) how different spacial anisotropic distributions of sucrose as well as the gradient impact sweetness perception and (b) if pulsatile stimulation is the concept to be favored to enhance sweetness perception in solid food items. Model chocolate confectionery products were manufactured with inhomogenuously distributed sucrose quantities to create sucrose gradients in the product with spatially different arrangements. Upon melting in the mouth, sucrose was expected to be released at different concentrations ant varying timepoints, leading to increasing, decreasing or "pulsed" sucrose perception over consumption time and thus altered sweetness perceptions.

2. Materials and Methods

2.1. Materials

For all samples, gelatin from pig skin with a Bloom nr. of 100, manufactured by Gelita AG (Eberbach, Germany), was used. Cocoa butter was obtained from Max Felchlin AG (Schwyz, Switzerland), mono- & diglycerides of fatty acid as emulsifiers were purchased from Danisco (Grindsted, Denmark). Sucrose and cocoa powder were purchased in local grocery stores and used directly. All samples were prepared with tap water.

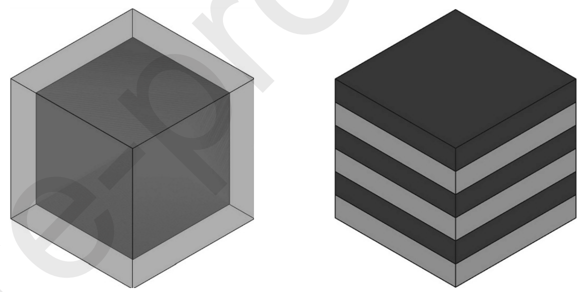
2.2. Sample preparation

Two different types of phase arrangements were tested in this study, illustrations are shown in Fig. 1. Cube in cube samples were arranged with an inner cube consisting of one phase surrounded by an outer cubic shell consisting of the second phase, these samples were named $In_{XX}Out_{YY}$ with XX and YY indicating the sugar concentrations of the inner and outer phase, respectively. The layered structure was named $L_{XX/YY}$. For all samples the overall sugar content was the same as the reference with 22.8 % sugar. All sugar concentrations in this manuscript are indicated as w/w percentages.

The preparation of the basic masses (BM) ($BM_{9.8}$, $BM_{19.5}$, $BM_{22.8}$, $BM_{26.0}$, $BM_{35.8}$) was as follows where all data refer to 100g of the final product: Gelatin (4 g, 3.3 g, 3.0 g, 2.5 g, 1.0 g, respectively) was weighted and mixed into the corresponding amount of tap water (41.5 g, 32.5 g, 29.54 g, 26.7 g, 18.5 g, respectively) and left to swell for a minimum of 5 minutes. The mixture was heated to 55 °C for the gelatin to dissolve. After the addition of sugar (9.8 g, 19.5 g, 22.8 g, 26.0 g, 35.8 g, respectively) and cocoapowder (9.8 g), the mixture was homogenized at 10'000 rpm using a Polytron PT 3100 D (Kinematica AG, Switzerland). Simultaneously cocoa butter (34.3 g) and the mono- & diglycerides of fatty acid (0.7 g) were melted at 75 °C and stirred to dissolve. To produce an o/w emulsion, the oil mixture was slowly added to the aqueous phase under constant mixing. Once the entire oil phase had been added, the sample was left to homogenize for further 10 minutes at 55 °C. To prevent phase separation, the samples were stirred with a Kenwood Major Titanium KMT056 (Kenwood Swiss AG, Switzerland) while cooling to reach an optimal printing temperature of 25 ± 2 °C. Once this target temperature was reached, the mass was transferred into a piping bag and vacuum sealed to 40 mbar in order to remove any air inclusions, followed by its transfer into stainless-steel printing cartridges.

2.3. Printing

Samples with a size of 16x16x16 mm^3 were printed in two distinct structures, a layered and a cube-in-cube, as illustrated in Fig. 1. All masses were printed with a stainless-steel syringe type extrusion setup with 1.7 mm nozzles, the cartridge temperature was kept constant at 25 ± 2 °C by an aluminum heating jacket. The printing stage consisted of a custom built three-axis Cartesian printer shown in Fig. 2 designed by the Institute of Printing-Technology (IDT) of the Bern University of Applied Sciences. To achieve multi-phase printing, the printer was equipped with three separate extruders, of which two were used in this work. To ensure rapid solidification of the masses after exiting the nozzle, the printer was placed in a cooling chamber KK-1000 CHLT (Kambic, Slovenia) set to 5 °C. G-codes were generated using Slic3r Prusa Edition software, while Repetier-Host software was used to control the printer. To prevent any further physical changes during storage, samples were kept at -40 °C for storage.

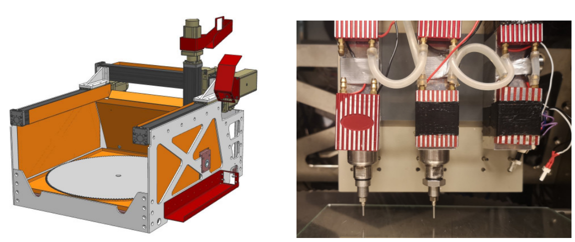


(a) (b)

Figure 1: Schematics of the spacial arrangement of two masses with varying sugar concentration: a) Cube-in-cube and b) layered. The ratio of masses corresponds to 1:1 (w/w) in both cases

2.4. Rheological and penetration tests

Penetration force was recorded using a texture analyzer TA-XTplus (Micro Stable Systems, UK), with



(a) CAD Model of the printing stage used for sample preparation

(b) Closeup of printhead with two nozzles installed

Figure 2: Printing setup

a 5 N load cell and equipped with a cylindrical probe with a diameter of 5 mm. The probe was lowered at a speed of 1 mm/s. At a trigger force of 2.0 g measurements were started and the probe was inserted 8 mm into the sample.

To assess melt viscosity as well as gelling and melting temperatures, oscillatory measurements were performed with a Physica MC302 (Anton Paar, Austria), equipped with a CC27 geometry. Experiments were performed with a strain of 0.5 % and a frequency of 1 Hz at a temperature of 55 °C. The sample was first cooled to 5 °C using a linear temperature ramp with a gradient of 1.25 °C/min, hold for one hour and reheating to 55 °C using the same linear temperature ramp.

2.5. Sensory evaluation

Sensory assessments were performed in two stages: A first simple descriptive test (DIN 10964:2014-11) followed by rating of sweetness intensity on a categorical scale were performed with a selected group of 5 to 7 employees of the institute to narrow down the number of samples to those considered most promising and relevant. For the consecutive static and dynamic sensory profiling, the external trained panel of the institute was invited to for six sessions. The panel was composed of 8 women, six of the panelists remained the same for all sessions, two panelists were replaced in between due to availability reasons. All panelists took part in two evaluations per session with a break in between. The establishment of the sensory profiling was carried out following the general guidance of the ISO 13299 norm. Training consisted of three sessions prior to the static evaluation and one additional session prior to the dynamic evaluation. As summarized in the table 1, the training ensured an alignment of the panelist on the attribute list and definition as well as on the oral processing protocol and the scale usage.

The training sessions were conducted in a training room allowing exchanges between panelists and panel leaders. The evaluation sessions were conducted in a sensory laboratory with panelists sitting at individual booths equipped with red light and laptops for data entry. Samples were served to panelists on plastic trays with random three-digit codes. The oral processing protocol for all evaluation sessions was: "Place the sample upright in your mouth, cut it in halves with your molar teeth and let it melt by tongue movements.". No instructions were given concerning swallowing. Taste was neutralized between each sample evaluation with water and plain crackers. All panelists tested each of the five samples within one session but in varying order according to a William square design and the product sequences were randomly assigned to the panelists.

Static evaluation was performed by handing over trained panelists a sample and the homogenous reference simultaneously and asking them to rate the sweetness perception of the sample compared to the reference on a unipolar linear scale (0 – 100, 0 = much weaker, 50 = reference, 100 = much stronger). For each new test sample, panelists received an additional reference sample.

Dynamic evaluation consisted of four test samples and only one homogeneous reference which was considered like an individual sample (.lind reference).

Table 1: Overview of training and evaluation sessions

Session Nr.	Training axes
1	Attribute list generation & Oral processing protocol
2	Training on sweetness perception & Attribute intensity training
3	Further training on oral processing protocol & Evaluation training
4	Static evaluations
5	Training on the dynamic evaluation
7	Dynamic evaluation

Table 2: Experimental design indicating samples which were analyzed in (t) technical, (s) static and (d) dynamic sensory trials

Gradient [%]	Sweet outside	Layered	Sweet inside
9.8/35.8	t/s/d	t/s/d	t/s/d
16.3/29.3	t	t	t
19.5/26.0	t/s/d	t	t

The samples were presented in monadic sequence. Panelists were asked to rate the sweetness perception on a predefined scale (0 – 100, 0 = not sweet, 100 = extremely sweet) at three distinct timepoints defined as: **T1**: Sweetness intensity after the first bite and two tongue movements (first impression), **T2**: Maximum sweetness intensity and **T3**: Sweetness intensity before swallowing (last impression).

2.6. Statistical analysis

Data collection in the sensory laboratory was performed with the EyeQuestion software (EyeQuestion, Netherlands, v 4.11.20). Statistical analysis was performed with R packages *nlme* and *emmeans* (Pinheiro et al., 2018; Lenth, 2019). Continuous sweetness intensity ratings were analyzed by two-way ANOVA with sweetness intensity as the dependent variable, samples and time points were treated as fixed factors whilst panelists and replicates were treated as random factors. For significant results with $p < 0.05$ a pairwise comparison was performed with a Tukey test.

3. Results & Discussion

3.1. Characterization of basic masses

A physical characterization of the basic masses $BM_{9.8}$, $BM_{19.5}$, $BM_{22.8}$, $BM_{26.0}$, $BM_{35.8}$ showed firmness values of: 2.70±0.50 N, 2.82±0.74 N, 2.94±0.76 N, 4.13±0.80 N, 7.5±1.9 N, respectively. Rheological measurements of viscosities at various temperatures indicated that all masses are molten and liquid at temperatures above 32 °C, whereas the viscosity in the molten state increased with increasing sugar concentration.

To assess whether these firmness/viscosity differences caused effects in sweetness perception, a sweetness assessment of the basic masses was performed by the trained sensory panel. The perception of sweetness intensity for the basic masses is shown in Fig. 3. The masses could successfully be placed in order, all masses except for $BM_{19.5}$ and $BM_{22.8}$ could be significantly distinguished. Due to the correct ranking of the masses as well as the melting at similar temperatures, differences in firmness were concluded to be low enough not to influence further experiments.

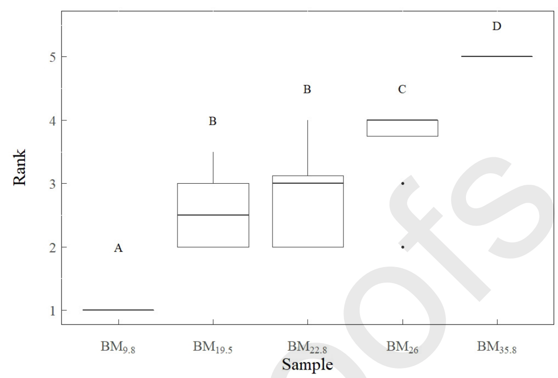


Figure 3: Sweetness intensity ranking of basic masses with varying sugar content. Numerical values in sample names represent sugar concentration in wt%.

3.2. Multiphase Samples

Samples $In_{19.5}Out_{26.0}$, $In_{35.8}Out_{9.8}$, as well as $L_{9.8/35.8}$ did not show sweetness intensities significantly higher than the homogeneous reference. $In_{9.8}Out_{35.8}$ however showed a mean sweetness intensity 33% higher than the reference sample, indicating an overall effect caused by the first contact surface. As seen in Fig. 1, the first contact surface of the layered sample, is comprised of both phases in a 1:1 ratio. This causes an averaged first impression, as the sweetness intensity difference of the sample is ranked between significance group A and B. A contrasting negative first layer effect due to a low sucrose first contact layer for sample $In_{35.8}Out_{9.8}$ was not observed. We assume that the sweet core of the sample was able to compensate a low inital sweetness impression for the overall sample perception. The increased sweetness perception of sample $L_{9.8/35.8}$ could also be explained by the varying viscosities of the two basic masses. As $BM_{35.8}$ shows a higher viscosity than $BM_{9.8}$, it could have remained in the mouth for a longer period and thus influenced the overall perception recorded at the end of consumption. In sample $In_{35.8}Out_{9.8}$, no such effect could be observed, indicating that the effect of the first contact layer could be more dominant for the overall sweetness perception.

Similar sweetness increases for cubes of gelled sucrose ($20 \times 20 \times 20$ mm^3) were shown by Mosca et al. (2010) where a sweetness increase of 20% was achieved in cubes with inhomogenously distributed sucrose content. While Mosca used layered structures which did not show the reported effects in this study, a similar correlation between the sweetness gradient and the sweetness enhancement was also demonstrated. The variation in structure dependency and maximum sweetness enhancement from 15 to 20 % could be related to the different oral processing protocols applied. Samples were completely chewed in the trials performed by Mosca, in this study panelists were asked to bite the sample once into two halves and then let it melt. This protocol was chosen in order to reduce variance resulting from heterogeneous chewing processes, although it does not entirely reflect realistic consumption situations. This kind of oral processing also gives less effect to different gel breaking properties upon chewing as this has also been shown potentially be a significant effect to cause altered sweetness perception Mosca et al. (2015).

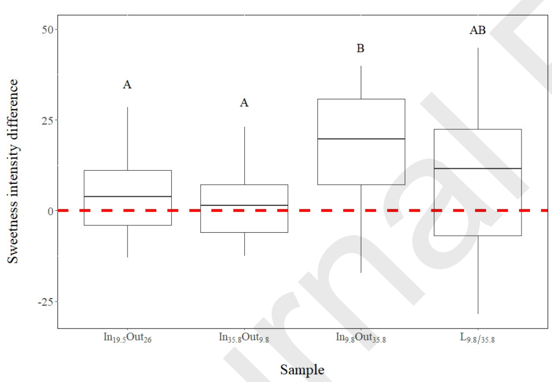


Figure 4: Sweetness enhancement of multiphase samples, all samples were compared to a homogeneous reference which was anchored at a sweetness value of 50 (red, dashed line); data in the graph represents the deviation from this value. Numerical values in sample names represent sugar concentration in wt%.

By comparing the sweetness intensity between $\mathrm{In}_{19.5}\mathrm{Out}_{26.0}$ and $\mathrm{In}_{9.8}\mathrm{Out}_{35.8}$, the importance of the gradient is demonstrated. Samples with the same phase allocation regarding high and low sweetness phases do not show altered sugar perceptions when small gradients are applied whereas larger gradients show a significant effect. The impact of size of the gradient has already been shown for liquid systems by Burseg, Camacho, Knoop and Bult (2010), where larger sweetness gradients are linked with increased sweetness perception under pulsatile stimulation conditions. Obtained results further confirmed the influence of the gradient on the sweetness enhancement. $\mathrm{In}_{19.5}\mathrm{Out}_{26.0}$ was not perceived significantly sweeter than the homogenous reference, while $\mathrm{In}_{9.8}\mathrm{Out}_{35.8}$ was. Burseg has also shown that the pulsation period in sugary liquid systems has a strong effect on the sweetness perception. The pulsation period in solid foods cannot be properly defined, however it can be argued that the spacial arrangement together with melting, breakup and mastication behavior are the most determining factors that account for a pulsation behavior in foods with inhomogeneous sucrose distribution. To achieve this pulsatile stimulation, the approach was to produce layered samples such as $\mathrm{L}_{9.8/35.8}$. However, the first contact layer was a mix of both phases, such mixed impression does not occur for all $\mathrm{In}_{\mathrm{XX}}\mathrm{Out}_{\mathrm{YY}}$ samples, which can thus be viewed as samples consisting of a single pulse. Consequently, samples with multiple pulses (alternating shells of high/low concentrated masses) could be produced to simulate real pulsatile stimulation in future.

3.3. Dynamic evaluation

To compare the sweetness intensity over consumption time, progressive profiles with three time points (initial impression, maximum, final impression) were recorded. Figure 5 shows the resulting profiles for all 5 samples. The structure was not expected to be destroyed entirely after the first bite, therefore an effect from the first contact layer was expected, as discussed in the static evaluation. At T1, the first impression, no significant difference between the samples was recorded. As melting and subsequent sucrose diffusion are required to allow the sucrose to reach the receptors and induce a sweetness perception, some time is required to sense the full sweetness. It is probable that in the period up to T1 (first bite and two tongue movements) not enough melting/diffusion occurred for a significant amount of sucrose to reach receptors, and therefore results remain insignificant. Similarly, the maximum sweetness impression at time-point T2 also showed no significant difference between samples, in contrast to time-point T3 with significant differences. The sample with a low sweetness core and the layered sample were perceived less sweet. We explain this by the fact that last bolus will contain mostly the inner phase and therefore consists of a low sugar mass. In a similar study performed by Holm et al. (2009), significant differences between different samples were found at the beginning of consumption which evened out over time, this strongly contrasts current results, showing differences appearing at the end of consumption time. These differences are likely caused by differing oral processing (chewing versus no chewing). T3 is the only time point at which significant differences were recorded. However, the ranking order of the samples does not reflect the ranking of the samples of the static evaluation. This could indicate that the final perception is less decisive for the overall sweetness perception compared to other factors such as the first impression and pulsatile effects. The static evaluations were performed by comparing each sample to a reference, while the dynamic evaluation contained the reference as a sample and no reference for the scale, such differences have also been show to impact the evaluation in sensory studies by Larson-Powers and Pangborn (1978). Additionally, is worth mentioning that the progressive profiling task was very difficult to perform for the panel, which was also noted by several panelists during trials. To deepen the understanding of the relationship between static and dynamic results, data points from T2 of dynamic sensory experiments were compared to those of static experiments. In Fig.6, all samples show a lower value, with the exception of $In_{19.5}Out_{26.0}$. Along with the added complexity and time requirements, this raises the question if dynamic studies of this type are required to assess the overall sweetness perception in further product development. For screening purposes the static evaluation seems to be faster, easier and sufficient to gain insight into the sweetness perception. To gain a more detailed insight into sweetness development, dynamic methods can be very interesting, however the increased requirement of resources needs to be considered. It would also be beneficial to increase the amount of measuring points to potentially lead to more significant results.

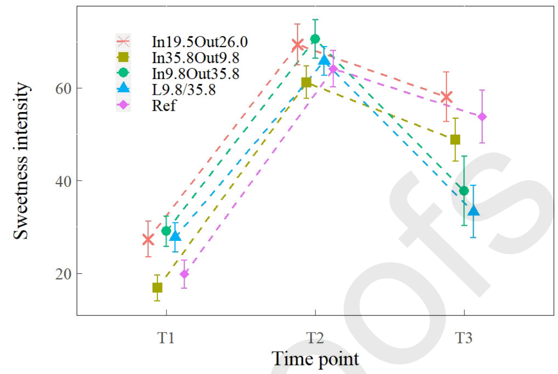


Figure 5: Dynamic evaluation of sweetness intensity on a scale 1-100 for time points T1-3, initial impression, maximum sweetness, and final impression. Dashed lines are there to guide the eye and do not represent measurements. Numerical values in sample names represent sugar concentration in wt%.

4. Conclusions

Results show differing sweetness perceptions in a model confectionery product when inhomogenous sucrose distribution are applied. The sample with a high sucrose shell and a low sucrose core and a high gradient was percieved as significantly sweeter than the homogeneous reference sample, indicating that the first impression of a product influences the overall perception. However this seems to require strong sucrose gradients. A number of effects which can potentially effect sweetness perception are also superimposed on such measurements and have to be taken into account, e.g. the viscosity of basic masses, their melting behavior and how they influence the final impression.

To mimic the pulsatile stimulation as demonstrated in liquid systems, further more intricate designs will be considered. The design with a layered structure does not seem to cause a relevant pulsation of the sweetness sensation. The cube-in-cube

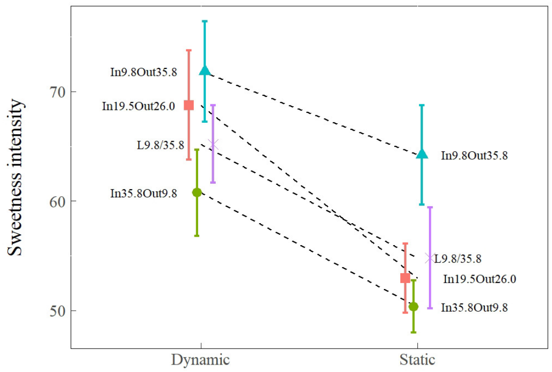


Figure 6: Comparison of the maximum perceived sweetness during the static and dynamic evaluation (time point T2) of the two-phased samples. Dashed lines are there to guide the eye and do not represent measurements. Numerical values in sample names represent sugar concentration in wt%.

design seems to be more suitable to adjust increased sweetness perception. By increasing the number of alternating high/low sugar shells in the cubic sample, it could be possible to increase the number of pulses from one to many and get to a true pulsatile stimulation. If such a 3D-arrangement would further increase the overall sweetness perception to a superior level compared to the cube-in-cube adjustment will be the question of a consecutive study. The 3D-printing technology will enable the production of complex arbitrary structures.

Due to the complex nature of the products and their sensory characterization, a simple protocol for the oral processing was applied. In order to get more generally applicable results, trials have to be conducted using more realistic eating protocols in future, and should include higher time-wise resolution of sweetness perception. Additionally, acceptance trials with real customers need to be performed, to translate results from the lab environment to consumers everyday life.

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