Study to chew on

Nutritious stimulation and consumption rate on chewing behaviour on non-food items in humans.

MSc thesis report

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Abstract

Background: Chewing and swallowing enable the human to survive. Food and nutrients get into the digesting system. The act of chewing is also observed on non-nutritive non-food items such as on gum, pens or ‘stim toys’.

Aim: The aim of this study was to investigate how nutritious stimulation and consumption rate affect chewing on non-food items in humans. Further, it was of interest how nutritious stimulation and consumption rate affect satiation on a follow-up ad libitum snack. Supportingly, a visual analogue scale (VAS) 100mm rating on appetite was conducted.

Methods: The study had a 2x3 design and twenty-two healthy subjects (24.7±2.8y, BMI 21.8±2.1 kg/m^2) were included. Subjects were randomly assigned to either a fast or a slow consumption rate group. In each group, subjects were exposed to three different fluids (sugar lemonade, sweetener lemonade, water (control)). One fluid was given per session, which consisted of chewing on a chewable tube pumping the fluid in the subjects’ mouth. In total, each subject came in three times. While chewing, the subject played the game ‘Tetris’ to be distracted from the actual chewing activity. After the chewing task an ad libitum snack of M&M’s was offered. The complete session was recorded on tape to determine the chewing activity afterwards.

Results: The stimuli (sugar lemonade, sweetened lemonade, water), of which only the sugar lemonade had nutritious value, did significantly influence the number of chews ($p=0.031$). Sugar and sweetener lemonade exposure resulted in a higher number of chews compared to the control. Yet, the stimuli did not affect the chewing duration ($p=0.260$) nor the follow-up ad libitum snack intake ($p=0.141$). The consumption rate did not influence the number of chews ($p=0.287$), chewing duration ($p=0.357$) nor the follow-up ad libitum snack intake ($p=0.193$). The desire rating to eat sweet ($p=0.001$) and to drink sweet ($p<0.001$) had a greater decline in prior-post chewing task difference in the sugar stimuli compared to water, and sweetener stimuli compared to water.

Discussion: The number of chews was significantly influenced by taste perception (oro-sensory perception) rather than by the energy content of the exposure fluids. Sugar and sweetener lemonade stimuli had a greater number of chews than water (control) ones. No influence on the follow-up ad libitum M&M’s consumption was observed. However, a pre-defined minimum chewing time might have resulted in a different outcome. The difference in sweet desire rating for eating and drinking showed a decline in sweet desire when exposed to taste stimuli. Thus, an sensory specific satiety is the most likely explanation for this finding.

Conclusion: Oro-sensory stimulation rather than nutritional feedback stimulated subjects to an increased number of chews. Thus, this study demonstrates that taste stimuli plays an important role in affectation to chew. Neither nutritional feedback nor consumption rate influenced the follow-up ad libitum snack intake. In addition, the taste exposure suppressed the sweet desire rating for eating and drinking and thereby, indicated a sensory specific satiety. Even though the sweet desire rating outcomes where given the opportunity to be put into actual action in the follow-up ad libitum sweet snacking behaviour, no compliance was found in the results. Thus, it is to suggest that future studies put more attention and emphasis on rating and actual behaviour effect. More research is recommended in order to understand the mechanism and impact of chewing on non-food items, such as on stim-toys.

Contents

1 – Background information

Back in time

The history of chewing and chewing gum is thousands of years old and still a present phenomenon worldwide.
The origin of chewing may be found in bark tar (Aveling & Heron 1999). Nine thousand years ago, as early as in Neolithic Europe, tar was used for chewing. Evidence was found in many populations and also other material besides bark tar, e.g. Pinaceae resin or fir resin (Vilkuna 1964) could be identified. In the past, the reasons for chewing were similar to our reasons today: keeping the teeth clean, freshening up the breath or relieving thirst. Other causes might have been to ease dental problems, to kill time or even to mitigate a sore throat.
Also in pre-Columbian cultures, such as the Maya and Aztec culture, chewing was actively practiced (Mathews, 2009; Redclift, 2004). Chicle latex was used. It is extracted from the sapodilla tree. The latex is excreted by the tree as a whitish fluid as defence against insects and other natural enemies. Not only enjoyed as pleasure, the Maya used the chicle also "for many practical purposes such as adhesive or sealant" according to Mathews (2009). Other reasons for chewing were to stimulate their saliva and to clean teeth in those cultures.
The chewing gum continued its success story from the pre-Columbian cultures further to the Mexican Republic in the nineteenth century (Redclift, 2004). World War I and World War II promoted its popularity as the soldiers took gum in their rations (Mathews, 2009). According to Redclift (2004) "chewing gum was among the first mass-produced products of the twentieth century". This fact impressively shows how chewing gum found its way to everyday life. Soon its success forced the search for synthetic substitution (Mathews, 2009).

Examples of chewing

The Oxford Dictionary defines 'chew' amongst others as "[b]ite and work (food) in the mouth with the teeth, especially to make it easier to swallow." and as "[g]naw at (something) persistently." (Press, 2017a). Gum for example is bitten and gnawed on in the mouth with the teeth for a longer time. However, it is not swallowed. And gum is no exception of being chewed on but not swallowed. In daily life, people use different things to chew on, e.g. nails or pens. This happens mainly unconsciously. The latest trend are so called 'stim toys' used by children and adults (Stimtastic, 2014). In general, stim toys are made for keeping hands and fingers busy by playing with them. They are produced out of chewable material, serve the purpose of being played with and being chewed on and are sold as jewellery or trinkets (Stimtastic, 2014).
As mentioned, those chewy items are not swallowed. By definition 'swallow' is "[c]ause or allow (something, especially food or drink) to pass down the throat." (Press, 2017c). Since gum, nails and stim toys are not swallowed and do not provide any "nutritious substances" (Press, 2017b) the term 'food' is probably not appropriate. These items are not considered as 'real' food (non-food) and the human body does not benefit in terms of nutritional value. Nevertheless, the consumption and usage of them seems popular. Chewing gum is present in everyday life and stim toys are even chewed on by grown-ups (Stimtastic, 2014). This allows the assumption that there might be more complex reasons for chewing rather than the bare desire of having something to do or to chew on.

Speaking in terms of the gum, the reasons why it is chewed on, shifts the focus more to coping with mental issues such as stress or anxiety. At least this seems to be the cause in the Western society with its fast moving and developing life style. Already in the 1930s, people used gum as a mean to cope with inner tensions and restlessness. Hollingworth (1939) was a pioneer in the research field because he made a link between gum chewing and mood and performance.
Smiths' study in 2009 showed that people who chewed gum compared to not chewing ones, had a lower likelihood of depression and "were associated with lower levels of alcohol consumption and with cigarette smoking". The perception of stress exposure at work and life was reported as being lower in gum chewers. His results pointed out that chewing gum might help avoiding stress and could reduce stress caused health issues. These findings could be helpful as a well work-life-balance does not seem to be easy in our fast moving time.
With his results Smith (2009) is not alone. Much research was done on stress related to gum and chewing behaviour. Scholey and his colleagues (2009) outlined that gum chewing subjects had a lower stress level than non-chewing ones. Further, the researcher wrote that alertness was significantly better and the condition of anxiety decreased in chewing subjects compared to non-chewers. Alertness, mood, anxiety and lifestyle factors show a positive influence by chewing a gum and its habit to do so (Hollingworth, 1939; Smith, 2009; Scholey et al., 2009; Allen & Smith, 2011).
However, the study outcomes have to be treated with caution. For example, the duration of which a gum is chewed "may moderate any effect" (Allen & Smith, 2011) and seems to not have been studied yet. Also, the positive association between using a gum and e.g. alertness performances could be stimulated due to an increased cerebral blood flow while chewing (Scholey et al., 2009). Furthermore, as the review by Allen & Smith in 2011 stated "[c]hewing gum seems to be associated with reduced chronic stress, but the research does not suggest an effect on acute stress."
In which way pen, nails biting or chewable stim toys influence the stress perceptions or any other lifestyle factor has not been researched yet.

Chewing and feeding motivation

As outlined previously, chewing gum may influence stress and anxiety related aspects.

However, one may wonder why we put foods and non-foods in our mouth? Feeding motivation and feeding behaviour might be important aspects to consider in this term. Speaking of humans, it is assumed that feeding behaviour partly aims for a specific “goal” and “expectations of needs” (Day, Kyriazakis & Roggers, 1998; Rogers, 1999). When research is often dealing with talking about motivation, it is actually referred to as low or high feeding motivation. Amongst others, Day and colleagues (1996) conducted a study on pigs to clarify the animals feeding motivation with either a high or low level of feeding motivation. Feeding motivation was manipulated by different energy supply (digestible energy DE) of the very same feed sample.

Manipulating feeding motivation intervenes in the two main factors which influence it. Those are metabolic (endogenous) and environmental (exogenous) factors (Toates, 1981; Day, Kyriazakis & Roggers, 1998; Booth, 1992). An example for an environmental factor is the sweetness (Toates, 1981) or the palatability of a food item (Day, Kyriazakis & Roggers, 1997). A lack of a nutrient is an example for an endogenous factor (Day, Kyriazakis & Roggers, 1997). Endogenous and exogenous factors affect the feeding motivation. In this context, satiation plays an important role. In literature it is described as the process leading to the end of eating/consumption (Blundell et al., 2010).

As described previously, the motivation to eat is driven by both endogenous and exogenous factors such as a lack in nutrients and sweetness. It is assumed that nutritional feedback is required to reinforce feeding motivation. Day and colleagues (1996) tested how feeding motivation could be manipulated for non-food items in growing pigs. Feeding motivation was assessed as chewing activity. The pigs showed a tendency towards an increased chewing motivation when exposed to sucrose compared to saccharin or water. Even though that the pig’s behaviour was a phenomenon learned over time since the study was conducted for ten days, their behaviour indicated that a higher nutritional feedback had a greater impact on the chewing activity than a lower one.

However, little is understood about how humans show feeding motivated chewing on non-food items when being exposed to different nutritious stimuli and different consumption rates.

Therefore, the aim of this study was to investigate how different nutritious stimuli and consumption rate affect motivation to chew on non-food items in humans. The motivation to chew was assessed as number chews and as the chewing duration. The second aim was to investigate how nutritious stimulation and consumption rate affect satiation on a follow-up ad libitum snack. Supportingly, a visual analogue scale (VAS) 100mm rating on appetite was conducted.

In order to study this, a 2x3 study design was performed. Three different stimuli (sugar lemonade, sweetened lemonade and water (control)), of which only the sugar lemonade had nutritious value, and two consumption speed rates (fast and slow rate) were tested.

A greater motivation to chew was assumed when exposed to sugar lemonade compared to the sweetener lemonade and water. The ad libitum snack intake was expected to be smaller in the sugar lemonade group than in the sweetener lemonade group or in the water control group.

2 – Research methods and materials

2.1 - Subjects

The study was conducted at Wageningen University and Research WUR (The Netherlands) with voluntary subjects from the general university population. The sample size calculation resulted in 34 subjects (see 2.3.2.) The recruitment was done via online media postings, flyer distribution and through word-of-mouth recommendations. Subjects had to be healthy, had to have a BMI between 18.5 and 27.0kg/m^2 and had to be between 18 and 35 years of age. According to the World Health Organisation (2000) the average BMI range goes form 18.50 – 24.99 kg/m^2. As the BMI does not consider body composition the range limit was set at 27.0kg/m^2. The age range was chosen as it comes close to the age distribution amongst students enrolled at Universities. Subjects were excluded if they suffered from a food allergy.
Before the first study session, all subjects were informed about the procedure and had to sign an informed consent.
After signing the informed consent, subjects filled in a questionnaire about their age, gender, height and weight. In addition, they filled in a medical and allergy questionnaire aimed for accessing possible health issues.

After the recruitment subjects received an email invitation with their individual time scheduling. Together with the email invitation, subjects were sent an information folder. It included the explanation of study procedure and requirements. Each subject joined three study sessions. It was agreed on that each session had a minimum stay of 30 minutes (min). These information were given in the information folder as well as in the oral form at the beginning of each session. If the test session was finished in less than 30min, they were allowed to play 'Tetris' or wait till the 30min were reached.
At the end of their participation, each subject was handed a present.
Study subjects were not educated on the actual objective of the study. Thus, a so called 'cover-up' story was used. The study focus was pretended to be on mood condition affected by chewing.

2.2 - Design of the study

The study was conducted in different consumption speed exposures of fast and slow rates (compare Figure 1). First, subjects were randomly assigned to either the fast or the slow consumption rate group. In each of the two groups subjects were exposed to three different fluids. The fluids were a sugar containing lemonade, a sweetener containing lemonade and water (control). In the following report the term 'lemonade' is used and represents to a beverage which is made by mixing a flavoured sweet concentrate with water in order to receive a diluted drink. Depending on the region or dialect, it could also be referred to as 'syrup'.
Each subject passed through all three drinks, per test session one.
Subjects got a chewable tube in the mouth and were told to chew on it (compare drawing of Figure 2).
While chewing on the tube subjects played the PC game 'Tetris' on a low and non-engaging level. It was done to distract the subjects and to stimulate a preferable unconscious chewing activity.
The fluid was pumped through the tube in the subject's mouth during chewing. This happened in a slow consumption rate of ca. 6.5 millilitre (ml) per minute or at a fast consumption rate of ca. 12.4ml per minute.
Each study session was video recorded as to determine the number of chews. At the end of each session an ad lib snack of M&M's chocolate was offered as to investigate to what extend a follow-up ad libitum intake was influenced by nutritional stimuli and consumption rate.

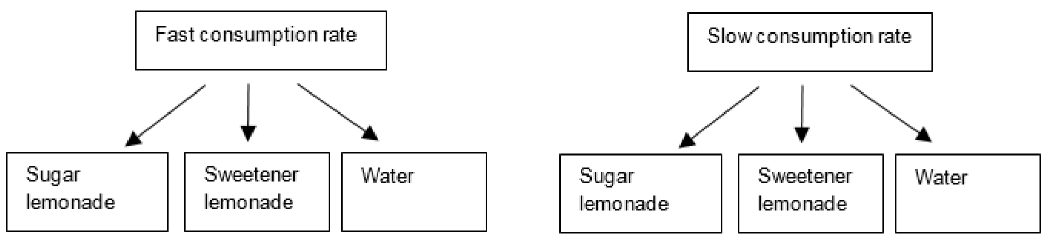

Figure 1: Overview study design

2.3 - Materials and methods

2.3.1 - Fluids

This study used three kinds of fluids given through tubes. Plain tab water with no source of digestible energy was given as control fluid. Further, two lemonade products were chosen which were identical in the appearance and had the same sensory characteristics: *Siroop Bosvruchten 0% toegevoegde suiker ('Forestfruit Lemonade 0% added sugar') and Siroop Bosvruchten 75% fruit ('Forestfruit Lemonade 75% fruit').*

Both lemonades were from the same brand (*Albert Heijn* by Albert Heijn B.V., 2017). The *Forestfruit Lemonade 0% added sugar* was the sweetener and the *Forestfruit Lemonade 75% fruit* was the sugar version.

The actual feeding fluids were made by using the amount of lemonade according to the products label. In order to have one litre of lemonade the following ingredients were used: 80ml *Forestfruit Lemonade 0% added sugar* and 920ml water for the sweetener beverage, and 125ml *Forestfruit Lemonade 75% fruit* and 875ml water for the sugar fluid.

By doing so, the nutritional values per 100ml of the three feeding fluid were as following (based on the nutritional label on the bough lemonade products): 0.0 kilocalories (kcal) energy, 0.0g carbohydrates, 0.0g of sugar for the water fluid, 2.5kcal energy, 0.4g carbohydrates, 0.3g of sugar for the sweetener lemonade, and 38.0kcal energy, 9.5g carbohydrates, 9.5g of sugar for the sugar lemonade.

2.3.2 - Pilot study

A pilot study was conducted prior to the actual study procedure.

Its purpose was to determine the required sample size and which products should be used as consumption fluids. To do so, four products were tested on liking, sweetness, flavour and desire. Compare Appendix 2 Table 6 and Figure 8 for outcome details.

The products tested were 'Ice Tea Zero Peach' with 3kcal and 0.3g sugar per 100ml, 'Ice Tea Perzik' with 27kcal and 6.5g sugar per 100ml, 'Forestfruit *Lemonade* 0% added sugar' with 2.5kcal and 0.4g sugar per 100ml, and 'Forestfruit *Lemonade* 75% fruit' with 38kcal and 9.5g sugar per 100ml.

The two ice tea drinks were of same brand, flavour but different sweet basis (brand *Freeway* by ©Lidl Netherlands GmbH, 2017) as were the two lemonades (brand *Albert Heijn* by Albert Heijn B.V., 2017).

Six people were asked to try a sip of each product and to rate on a visual analogue scale (VAS) of 100mm 'How much do you like it?', 'What do you think about fruit flavour?', 'How sweet it the taste for you?' and 'How much do you desire another sip?'. The testing people did not know what kind of product they were trying. Because the means of rating of liking, sweetness, flavour and desire for the lemonade products showed a tendency towards mutual agreement they were used for the actual study procedure. The ice tea products could not convince (see Appendix 2 Table 6 and Figure 8).

The sample size calculation was done with the program G*Power 3.1.9.2. The "test family" was set as t tests, the statistical test was set to "Means: Differences between two independent means (groups)" with "two tail(s)", an estimated "effect size d" of 1, "α err prob" of 0.05, "power" of 0.8 and the "allocation ratio N2/N1" of 1. With this parameters G*Power calculated a total sample size of 34. An extra of 15% was estimated in case of loss of follow up. Thus, the final desired number was 40 subjects.

2.3.3 - Tube

All fluid products were given to the subject through a chewable tube (tube versitec 4.8x8 mm, Saint – Gobain Performance Plastics 2017, France; Tolérances selon ISO 3302 – E2). One end of the tube (see Figure 2 end A) was placed into the subjects mouth and pumped fluid at a constant rate of either fast or slow rate. The other end (end B) was connected to a small tank which was filled with either water, sugar or sweetener lemonade. The subjects were requested to chew on the tube (end A). From the mouth outwards the tube was fixed with an elastic hair band to the face. This way, a comfortable tube fixation and chewing were provided. The fluid tank was connected to a small machine (323DU/D Pump 400RPM EU, Watson-Marlow Bredel Pumps, England). The whole constellation of the machine, the tank and the tubes are usually used in hospitals for tube feeding people.

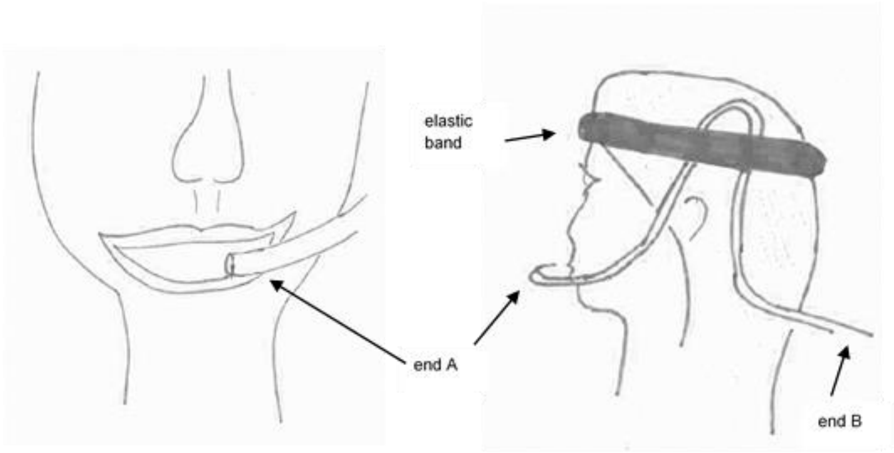

Figure 2: Sketch of tube constellation

2.3.4 - Tetris game

Subjects played the computer game ‘Tetris’ while chewing on the tube. This was done to keep subjects busy, so they did not get bored while tube chewing. The primary goal of ‘Tetris’ is to clear lines by filling up the complete space of one line with the given game pieces. As many lines as feasible should be cleared that way. If one reaches the top of the ‘Tetris’ field the game is lost. ‘Tetris’ was played on a low level to keep the enthusiasm and frustration level low throughout the session duration. It was assumed that subjects were familiar with the game.

2.3.5 - Ad libitum snack

M&M’s (M&M chocolate candies regular; Mars UK Ltd) were used as ad libitum snack. This snack was provided at the end of each of the three sessions. Subjects were told that if the first provided M&M serving was not enough they could have an additional bowl. Both portion were each of 100g and contained 468 kilocalories.

2.4 - Study procedure

2.4.1 - General description

The study sessions took place in a small, windowless room at the Helix building at Wageningen University.
Test sessions were scheduled in the morning between 09.00 – 12.00 a.m. or in the afternoon between 01.00 – 04.00 p.m. Each subject was asked to come in three times. One break day was required to be in between the test sessions. The exposure order for each volunteer was randomised.

Prior to the first test session, volunteers received an official email invitation including a file with all important subject information. It included an explanation about the study procedure subjects were asked not eat or drink anything except water three hours prior to the session.

In the following study procedure visual analogue scale VAS 100mm ratings were applied. According to Blundell et al. (2010) it is the most frequent approach to assess self-reported perceptions and it is not difficult to apply. On a horizontal line subjects were asked to make a vertical line at the point being most appropriate to them. The scope of the horizontal line goes from e.g. ‘Not at all’ from one end to ‘None’ to the other end. Depending on the rating the titling of the endings varies.

After the very first arrival subjects received an explanation of the study procedure. They were required to sign the consent form as well as the information file and were asked if they had any remaining questions (compare Figure 3). Then, subjects were placed in front of a computer screen, a keyboard and a mouse. The software ‘EyeQuestion’ (Version 4.6.6. (Build 562)) guided the subjects through the whole study procedure on the screen. A Dutch Eating Behaviour Questionnaire (DEBQ) and general information questionnaire had to be answered (see Appendix 1 and Figure 3 T0). A VAS 100mm rating on thirst, appetite, emotions, attention, mood and liking was done (T1). Next, a glass of 150ml water had to be drunk (T2). This was done to minimise the thirst and to prevent subjects chewing on the tube for bare thirst desire. The chewing tube was then placed on the subject allowing a comfortable testing execution. One rehearsal round was conducted to familiarise subjects with procedure of chewing on a tube while liquid is pumped in. After rehearsal they played 'Tetris' on the PC while chewing on the tube simultaneously (T4).

Subjects were independent on deciding when they wish to stop playing or chewing. By indicating this the actual study procedure of chewing ended. Tube was removed from their mouth and face. Next, a VAS 100mm rating on thirst, appetite, emotions, attention, mood and liking of the tube content was requested (T5). Afterwards, an ad lib snack of M&M's chocolate candies was offered (T6).

The chocolate candies were served as a 100g portion. It contained 468kcal. According to the food label a portion size of 40g is suggested (M&M chocolate candies regular; Mars UK Ltd). If the provided portion had not been satisfied, another 100g bowl would have been handed out. The amount of M&M's eaten was defined as the difference in weight between the amount handed out and the amount of given back weight. These chocolate candies were chosen because previous studies already applied that snack in their studies (Swoboda & Temple, 2013; Miras et al., 2012). Each subject was offered M&M's once at each session and three times in total.

Independent of how much time the subjects require for each session, a minimum stay of 30 minutes was compulsory. This was done to avoid people coming over just for enjoying free food. To fulfil the time request subjects were offered to play 'Tetris' after their active participation (T7).

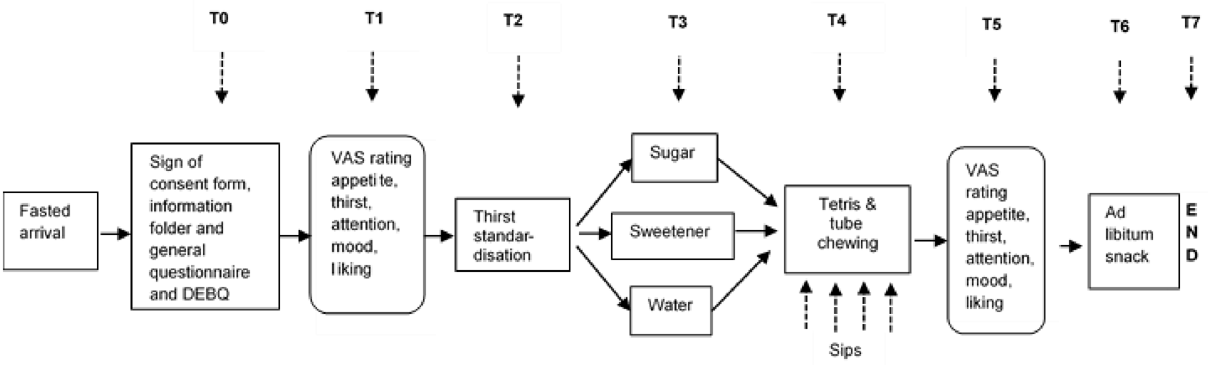

Figure 3: Overview study procedure

2.4.2 – Chewing activity

All subjects were filmed throughout each session. It was done with the software 'VideoPad Video Editor 5.20' from © NCH Software. This method was used by other studies previously (Allison et al., 2004; Hennequin et al., 2005; Nicolas et al., 2007). Both, the tube chewing and the ad libitum follow-up snack were recorded.

In total, each subject was filmed three times. To allow an efficient analysis of the chewing activity on the recorded material, nose and chin stickers were used. One sticker was place on the tip of the nose and another one on the chin. A chew can be identified by the movement of the chin (case) compared to the non-movement of the nose (control).

The video material of interest were the masticatory cycles (chew), the total of chewing time and the chewing rate in minutes (chews per minute divided by the total chewing time).

As defined by Allison et al. (2004) a masticatory cycle is "the number of 'closing' masticatory actions" and is the actual chew. The chewing time was the time span beginning with the first chew on the tube when starting 'Tetris' and ending with the last chew before announcement of ending the task. Of interest was the difference in chewing activity when exposed to different nutritional stimuli and to different consumption rates.

2.4.3 – DEQB

The Dutch Eating Behaviour Questionnaire (DEBQ) assessed the level of emotional eating, external eating and restrained eating behaviour (Van Strien, 2005). Each subjects filled it in once for her/his study participation right before the first test session (Figure 3 T0).

2.4.4 – Liking rating

Onetime, at the very beginning (T1 of Figure 3) subjects rated the game 'Tetris' and the study products. This rating was based on a visual analogue scale VAS with line scales of 100mm. Questions were to rate from 'weak/not at all' to 'strong/extremely' and were 'How much do you enjoy playing Tetris?', 'How much do you like drinking tap water?', 'How

much do you like drinking lemonade?' and 'How much do you like M&M's?'. The aim of this rating was to determine, if any subjects had be excluded. Exclusion criteria applied when two or more questions were rated either higher than 90mm or lower than 25mm on the 100mm VAS. A comparative higher or lower rating could indicate an increased or decreased enthusiasm influencing the study procedure. By not including those ratings of volunteers, a possible 'dislike error' could have been prevented.
At T5 (see Figure 3) after the chewing task, the subjects liking and sensory awareness of the study products were asked. It was asked on a 100mm VAS. Every subjects answered this liking rating once per session and three times in total. The scope of rating followed 'weak/not at all' to 'strong/extremely'. Questions asked were 'How much did you like the tube content?', 'How sweet was the content for you?', 'How much do you desire to consume the tube content?' and 'How likely are you to buy the tube content if possible?'.

2.4.5 - Appetite rating

The appetite rating was done in the scope of 'weak/not at all' to 'strong/extremely'. It was asked after each arrival of the volunteer before the actual task (Figure 3 T1) and after each ending of the task before the ad lib snack offer (T5). Each subject answered two appetite ratings per sessions and six in total. Questions asked were 'How hungry do you feel?', 'How full do you feel?', 'How much do you desire to eat something?', 'How much do you desire to eat something savoury?' and 'How much do you desire to eat something sweet?' It was assessed how nutritional stimuli and consumption rate affect the appetite rating.

2.4.6 - Thirst rating

On a 100mm VAS the thirst rating was evaluated. Timing of the rating was after each arrival before the actual task (Figure 3 T1) and after each ending of the task before the ad lib snack offer (T5). This way each subject answered the thirst rating twice per session and six times in total. The scope of rating was 'weak/not at all' to 'strong/extremely'. Questions asked were 'How thirsty are you at the moment?', 'How dry feels your mouth at the moment?', 'How much do you desire a glass of water?' and 'How much do you desire a glass of flavoured beverage?'. It was assessed how nutritional stimuli and consumption rate affect the thirst rating.

2.4.7 - Emotion and attention rating

The emotion and attention rating was asked on a 100mm VAS as was the appetite one. As subjects were not educated on the actual study reason the emotion rating was the cover-up story. Subjects were questioned after each arrival before the actual task (Figure 3 T1) and after each ending of the task before the ad lib snack offer (T6). The rating was to answer two times per session and six times in total. The scope of rating was 'weak/not at all' to 'strong/extremely'. Question asked were 'How happy are you at the moment?', 'How stressed are you at the moment?', 'How joyful are you at the moment?' and 'How focused do you feel at the moment?'. It was assessed how nutritional stimuli and consumption rate affect the emotion and attention rating.

2.5 – Data analysis

The data analysis was done by using SPSS (version 23; IBM SPSS Statistics 23.0. © IBM Corporation, 1989 2015. Armonk, New York). Following results are reported as mean ± SEM except when stated otherwise. Significance was set at a level below 5% ($p<0.05$).

A mixed ANOVA model was applied in order to compare number of chews, the chewing duration and the chewing rate response between-subject and within-subject conditions when exposed to different nutritious stimuli and consumption rate. Fixed effects were the nutritious stimuli, the consumption rate and their interaction. Random effects were the subjects and their interaction with the consumption rate.
The comparison was made for sugar lemonade, sweetener lemonade and water exposure, and for fast and slow consumption rate. The between-subjects respond was present between the consumption rate groups. The within-subject respond was present within the sugar lemonade, sweetener lemonade and water exposure.
The variables of the nutritional exposure were nested within the consumption rate groups.

Subsequently, the follow-up snack consumption was evaluated. Again, a mixed model ANOVA was used following the same model assumption as of the chewing behaviour. For all analysis the logarithmical transformed output variable of 'M&M's consumption' in grams was used as intake was not normally distributed.

Also, for testing the VAS 100mm ratings the mixed ANOVA model was applied. The influence of the nutritious stimuli and the consumption rate on appetite, thirst, attention, mood and liking rating was analysed using the same model approach as with analysing the chewing behaviour.

DEBQ rating for restrained eaters was tested with an independent sample t-test. Differences between restrained and non-restrained eaters were tested for significance on chewing and follow-up ad libitum consumption behaviour.

3 – Results

3.1 – Subjects characteristics

22 study subjects participated in this study. 20 female and two male subjects were recruited. Subjects were on average 24.7±2.8 years of age (mean ± SE) and their BMI was 21.6±2.0[kg/m²] for females and 23.8±2.5[kg/m²] for males. DEBQ restrained eating scores were 2.7±0.2 for females and 2.2±0.4 for males. All study subjects completed the study conduction.

The general questionnaire (Figure 3 T0) asked how often subjects consumed low sugar or calorie restricted drinks/lemonade. 45.5% claimed of consuming those products not at all. 22.7% responded of consuming them two to three times a month, 18.2% indicated a consumption of two to three times a week, 13.6% claimed of drinking those products two to three times a year and none of the subjects consumes low sugar or calorie restricted drinks every day.

Onetime, subjects were asked to rate their liking of the products used for the study procedure on a VAS 100mm scale at the first session. M&M's candies were rated as 69.2±3.1 (VAS 100mm mean ±SE), playing 'Tetris' as 54.5±3.4, liking of tab water as 71.0±2.8 and liking of lemonade in general as 54.3±3.4.

An overview on how much energy in kilocalories were consumed per minute of tube chewing when exposed to different consumption rates and nutritious stimuli is given in Table 1.

Table 1: Manipulation descriptive: Energy consumption per minute [min] of tube chewing per stimuli and consumption rate

Rate	Stimuli	Sugar lemonade	Sweetener lemonade	Water
Fast rate = 12.4 ml/min		ca. 4.73 kcal/min	ca. 0.31 kcal/min	0.00 kcal/min
Slow rate = 6.5 ml/min		ca. 2.48 kcal/min	ca. 0.16 kcal/min	0.00 kcal/min

In total four out of 22 subjects were restrained eaters (DEBQ score >3.39 for female; Van Strien, 2005) of whom all were female. Restrained eaters ate on average 22g M&M's which equals 14% more than the non-restrained eaters ate. They consumed on average 110ml of study fluids which equals 35% more than the non-restrained eaters did. No significant influence on number of chews (p=0.338), chewing time (p=0.405), ad libitum follow-up M&M's consumed (p=0.720) or on study fluids drunken (p=0.720) could be observed between restrained and non-restrained eaters.

3.2 – Chewing behaviour

The number of chews (Figure 4) were significantly influenced by nutritional feedback ($F_{(2,44)}$=3.767; p=0.031), whereas the number of chews were not influenced by the consumption speed ($F_{(1,22)}$=1.189; p=0.287). The pairwise comparison between the stimuli indicated that the sugar exposure had 62 more chews compared to water (p=0.022), as had the sweetener exposure 62 more chews compared to water (p=0.022). There was no significance between the two lemonade stimuli (p=0.994).
The chewing duration resulted in a non-significance towards nutritious stimuli ($F_{(2,44)}$=1.4; p=0.260), consumption rate ($F_{(2,44)}$=0.9; p=0.357) and their interaction ($F_{(2,44)}$=1.2; p=0.306). Compare Figure 5 for details.
Neither differed the chewing rate [chews/min] (Figure 6) significantly between nutritional feedback ($F_{(2,44)}$=1.2; p=0.306) nor between the consumption rates ($F_{(2,44)}$=2.4; p=0.134). See Figure 6 for details and Table 2 for the overview.

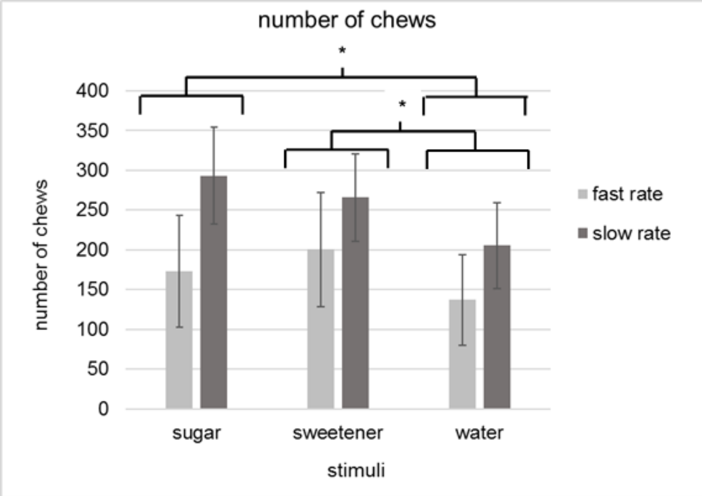

Figure 4: Mean ± SE Stimuli and consumption rate on number of chews
* Indicates a statistical significant difference ($p<0.05$)

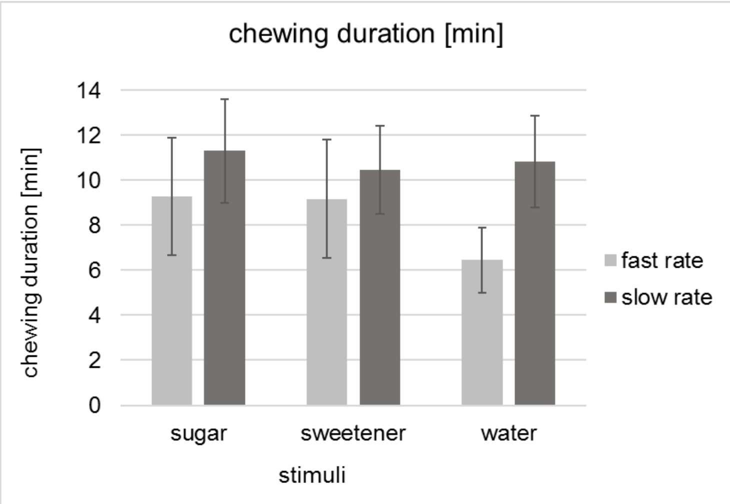

Figure 5: Mean ± SE Stimuli and consumption rate on chewing duration [min]

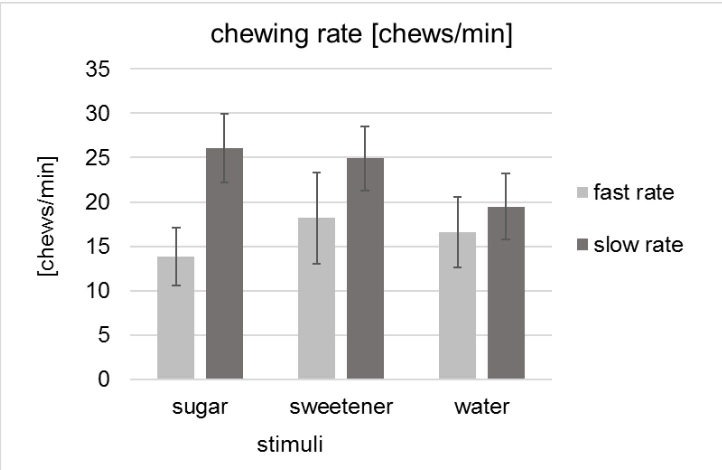

Figure 6: Mean ± SE Stimuli and consumption rate on chewing rate [chews/min]

Table 2: Mean ± SE Stimuli and consumption rate on chews and chewing rate (‘H_2O’ represents water)

Consumption rate	**Fast**			**Slow**			**Mixed model** (p-values)		
Stimuli	Sugar	Swee-tener	H_2O	Sugar	Swee-tener	H_2O	stimuli	Speed	Speed* stimuli
# of chews	173±70[a]	200±72[a]	137±57[b]	293±61[a]	266±55[a]	205±54[b]	0.031*	0.287	0.504
Chewing duration [min]	9±3	9±3	7±1	11±2	11±2	11±2	0.260	0.357	0.306
Chewing rate [# chews/min]	14±3	18±5	17±4	26±4	25±4	20±4	0.306	0.134	0.132

* Indicates a statistical significant difference
Means with different letters differ significantly in the same line

3.3 – Follow-up ad libitum consumption

Nutritional stimuli and the consumption rate did not seem to have a significant impact on the follow-up ad libitum M&M snack consumption ($F_{(2,2)}$=2.048; p=0.141 and $F_{(1,2)}$=1.804; p=0.193). See Figure 7 and Table 3.
A tendency towards significance was assumed, thus a post-hoc test comparing water to sweetener lemonade was done (p=0.060). On average water exposed subjects snacked 6.8% more than sugar exposed and 2.9% more than sweetener exposed subjects.

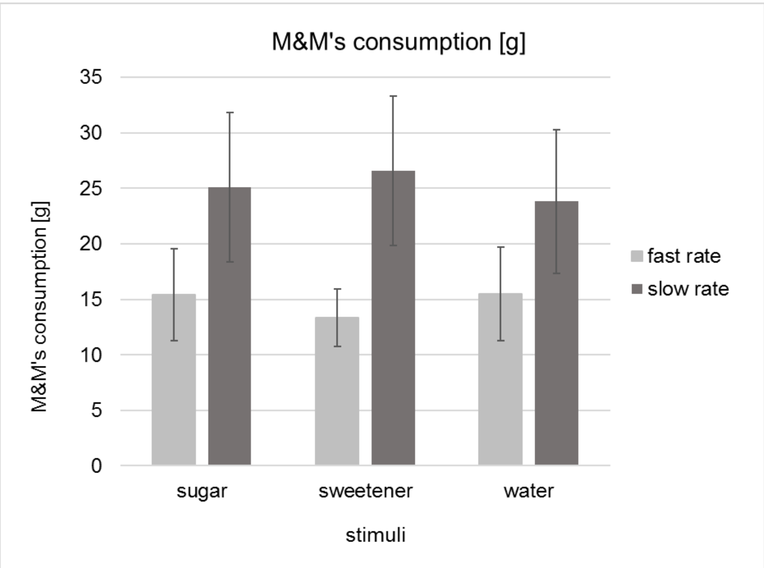

Figure 7: Mean ± SE Stimuli and consumption rate on M&M snack consumption [g]

Table 3: Mean ± SE Stimuli and consumption rate on M&M snack consumption [g] (‘H_2O’ represents water)

Consumption rate	Fast			Slow			Mixed model (p-values)		
Stimuli	Sugar	Sweetener	H_2O	Sugar	Sweetener	H_2O	Stimuli	Speed	Speed * stimuli
M&M consumption	15±3	15±5	15±3	23±7	26±7	27±6	0.141	0.193	0.220
* Indicates a statistical significant difference									

3.4 - VAS ratings

3.4.1 – Appetite and thirst rating

By comparing the difference Δ of prior (Figure 3 T1) and post chewing (T5), the VAS rating had a significant effect on the desire to eat sweet in nutritious stimuli ($F_{(2,44)}$=9.0;p=0.001). Sweetened lemonade stimuli had a 9.4 higher Δ desire to eat sweet rating than water (p=0.027), and sugar lemonade had a 17.5 higher Δ rating than water (p=0.000). Thirst prior-post ratings showed a significance for the interaction ($F_{(2,44)}$=5.3; p=0.008) of slow rate feeding with water (p=0.006). The desire rating for drinking sweet drinks indicated a significance Δ for the nutritious stimuli with $F_{(2,66)}$=10.1 and p<0.001. Whereby the sweetened stimuli had a 15.0 higher Δ desire to drink sweet rating than water (p=0.001) and the sugar stimuli showed a 17.4 higher Δ rating than water (p<0.001). The VAS rating of desire to drink water had a significant interaction ($F_{(2,44)}$=6.0; p=0.005) of slow consumption rate with water of p=0.019 (see Table 4 and Appendix 2 Figure 9).

Table 4: Mean ± SE VAS 100mm prior-post chewing difference Δ rating of appetite and thirst rating on stimuli and consumption rate (‘H_2O’ represents water)

Consumption rate	Fast			Slow			Mixed model (p-values)		
Stimuli	Sugar	Sweetener	H_2O	Sugar	Sweetener	H_2O	Stimuli	Speed	Speed * stimuli
Δ hunger	10.8±4.4	7.0±3.7	10.2±5.4	10.0±3.1	8.8±4.4	2.2±5.3	0.468	0.608	0.347
Δ fullness	-17.1±6.1	-20.3±5.2	-16.6±5.3	-22.5±5.4	-6.8±6.0	-8.6±3.6	0.257	0.272	0.124
Δ desire to eat	9.4±3.0	1.9±4.4	8.5±3.4	9.6±3.2	4.7±5.0	2.0±4.1	0.181	0.748	0.372
Δ desire to eat sweet	21.4±6.6[a]	13.0±4.0[a]	3.9±2.5[b]	15.0±4.7[a]	7.3±4.8[a]	-2.5±4.9[b]	0.001*	0.156	0.996
Δ desire to eat savoury	3.3±2.2	2.1±3.5	8.5±3.3	-0.9±6.2	-3.3±4.9	-1.3±2.0	0.492	0.075	0.711
Δ thirst	21.5±6.8	22.8±6.0	44.0±5.8	28.3±4.9	28.1±7.0	19.6±5.2	0.369	0.437	0.008*
Δ desire to drink sweet	24.6±3.9[a]	20.2±3.6[a]	11.6±4.3[b]	32.8±4.0[a]	32.4±5.0[a]	11.1±5.4[b]	0.000*	0.057	0.313
Δ desire to drink water	23.5±6.2	23.4±6.0	43.8±5.6	23.2±6.2	31.1±4.9	23.0±4.0	0.069	0.436	0.005*
* Indicates a statistical significant difference Means with different letters differ significantly in the same line Δ represents the rating difference of prior-post chewing task									

3.4.2 – Post liking and desire rating

Post liking and desire rating indicated a significant influence for the nutritious stimuli, yet not for the consumption speed rate (Table 5 and Appendix 2 Figure 10). The liking of the tube content was rated significantly for the nutritious stimuli ($F_{(2,44)}$=5.4;p=0.008) and the interaction term ($F_{(2,44)}$=5.2;p=0.009). Sugar lemonade had 12.5 higher rating points than the water stimuli (p=0.004), and sweetened lemonade had 10.7 more rating points then the water stimuli (p=0.004). The interaction was found when slow consumed with water (p=0.004). Sweetness perception of the tube content was significantly different rated for the nutritious stimuli with $F_{(2,44)}$=66.7;p<0.001. Sugar lemonade had 38.6 higher rating points than the control group (p<0.001), and the sweetener one had 35.4 more rating points than water (p<0.001). The desire rating for the tube content indicated a significant difference for the stimuli ($F_{(2,44)}$=9.0; p=0.001). Water stimuli was rated 14.2 points lower than the sugar lemonade (p<0.001) and 9.9 points lower than the sweetener stimuli (p=0.006). The desire rating of buying the tube content indicated a significance in the stimuli ($F_{(2,44)}$=7.9; p=0.001). Whereby the sugar stimuli showed 14.5 more rating points than the water one (p=0.001), and the sweetener stimuli had 13.4 more rating points compared to the control (p=0.002).

Table 5: Mean ± SE VAS 100mm post liking and desire rating ('H_2O' represents water)

Consumption rate	**Fast**			**Slow**			**Mixed model** (p-values)		
Stimuli	Sugar	Sweetener	H_2O	Sugar	Sweetener	H_2O	Stimuli	Speed	Speed * stimuli
Liking of tube content	45.4±5.5[a]	45.9±5.8[a]	45.4±4.9[b]	56.1±4.2[a]	52.2±5.5[a]	31.2±3.6[b]	0.008*	0.846	0.009*
Sweetness perception	63.4±3.6[a]	62.5±5.8[a]	24.1±3.8[b]	61.4±3.6[a]	55.9±5.4[a]	23.4±5.4[b]	0.000*	0.513	0.706
Desire of tube content	43.0±5.0[a]	41.1±6.2[a]	36.0±3.4[b]	44.8±5.0[a]	38.2±4.9[a]	23.4±2.5[b]	0.001*	0.357	0.114
Desire to buy tube content	39.0±6.6[a]	38.2±6.8[a]	29.5±4.3[b]	39.3±6.1[a]	37.9±5.6[a]	19.9±2.2[b]	0.001*	0.582	0.403

* Indicates a statistical significant difference
Means with different letters differ significantly in the same line

3.4.3 – Emotion and attention rating

None of the emotion or attention ratings indicated a significant difference by comparing the prior-post chewing difference with nutritious stimuli, consumption speed rate or their interaction. See Appendix 2 Table 7 for details.

3.4.4 - Reasons for stopping chewing

The reasons to stop chewing were evaluated after the chewing task (Appendix 2 Figure 11). The top three reasons mentioned were 32% being bored of playing 'Tetris', 19% stated of having enough of the taste, and 18% of the subjects had enough of chewing. See Appendix 2 for more information.

4 – Discussion

This study focused on the question how nutritional stimulation and consumption rate affect chewing on non-food items in humans, and how it affects satiation on a follow-up ad libitum snack.

A significant difference in the number of chews was found when subjects were exposed to different stimuli. Sugar lemonade had a significant greater amount of chews than the water-control group, and sweetener lemonade had a significant greater amount of chews than the water-control group. No significant difference was found between the two lemonade groups. Hence, an oro-sensory stimulation of sweetened or sugared lemonade affected our subjects to significantly chew more on the non-food item than when stimulated with bare water. Since the sweetened lemonade compared to the sugar lemonade did not provide any energy but only taste, we argue that the taste and not the energy (sugar) content was the stimuli of the greater chewing behaviour. Meaning, an oro-sensory stimuli rather than a nutritive one is the reason for this result. Similar results were found by Neyraud and colleagues (2005) who investigated how taste bitterness can affect mastication. They found that with increased bitterness in their study products the number of chews decreased. In their paper the researcher conclude that compositions of taste might have the power to impact mastication and number of chew, respectively. It could be assumed that if bitterness decreases the number of chews, sweetness, on the other hand, may increase it. Another paper by Day and colleagues (1996) researched chewing behaviour of growing pigs. Amongst others, they found out that an exposure to a “sweet taste did slightly enhance chewing behaviour” of growing pigs. It needs to be mentioned that their outcome was associated to a learning effect over time since the chewing behaviour was observed over the range of ten days. In this context the learning effect describes the increase of familiarisation with a product over time. In our study, 54.5% of the subjects reported that they consume low sugar or calorie restricted drinks on a regular basis. Those drinks are usually artificially sweetened with sweetener, as was the sweetened lemonade exposure of our study. This could be regarded as a learning effect on artificial sweetened drinks over time. Thus, the learning effect in growing pigs and in our study subjects might support the significant difference in number of chews between the oro-sensory stimulation compared to the water control-group. A possible limitation may have been that no sufficient learning effect could have been developed by our subjects because they were only exposed to each stimuli once.

Further, it could be argued that the brain associates chewing on taste-providing material with food consumption and consequently, with energy uptake. Energy uptake is essential for life sustainment. Thus, independent of nutritous value, a sweet taste is ‘rewarded’ and might lead to a greater stimulation of chewing. Day et al. (1996) also touched on this approach. The writers point out that “preference for sweeteners per se could reflect a strategy to obtain dietary energy”. As “obtaining dietary energy” entails the act of chewing, sugar (sugar lemonade) as well as sweetener (sweetener lemonade) may have acted as stimuli on the number of chews.

The number of chews was independent of the consumption rate. The slow consumption rate was approximately 6.5ml/min and the fast consumption rate was approximately 12.4ml/min. This research might have been the first one that studied affectation to chew on non-food items depending on consumption rates in humans, yet no significant outcome was observed. Thus, according to these results we conclude that in our study subjects the number of chews on a non-food item was unaffected by the consumption rate.

The analysis of the chewing duration [min] showed no significance when exposed to either different nutritious stimuli nor different consumption rates. The chewing duration seems to be independent of our studies exposures. Prior the study conduction, we assumed that the fast rate group (intake ca. 12.4ml/min) had a significant lower chewing duration since a greater intensity of oro-sensory stimulation might have been experienced compared to the slow rate group (intake ca. 6.5ml/min). A greater intensity experience due to the greater quantity of fluid intake may have resulted in subjects ending the chewing task earlier. Our original assumption shares similarities with findings by Rolls and colleagues (1981) who found that as soon as food was not experienced as pleasant anymore the consumption of it was ended. The researchers concluded that a sensation of satiety was sensed. Rolls (1986) described the sensory specific satiety as “the changing [of] hedonic response to the sensory properties of a particular food”. In humans, according to her, this holds for products at the very moment of consumption. In the present study, applying Rolls (1986) approach may have been that in the fast rate group the greater quantity of fluid compared to the slow rate one might have caused an earlier sensory specific related satiety. The fluid intake in the fast group was approximately double as much as in the slow group. Fast rate subjects may have stopped chewing earlier. Eventually, it might have led to a shorter duration of chewing activity in the fast rate group compared to the slow rate one. Surprisingly, even though almost one fifth (19%) of our subjects reported that they stopped chewing as they ‘had enough of the taste’ (see Appendix 2 Figure 11), no significant outcome of consumption rate was observed on the chewing duration. Further, the average fluid consumption in the fast rate group was approximately 103ml and approximately 71ml in the slow rate group. It is to question if those observed amounts of consumption actually lead to a sensory specific satiety as those were quite small amounts. Thus, our findings do not go in line with existing literature. Possible limitations interfering with the study outcome could have been the laboratory settings of the study: ‘discomfort of tube chewing’ (8%, see Appendix 2 Figure 11), ‘discomfort of the tube fixation’ (3%), ‘had enough of chewing’ (18%) or being ‘bored of playing’ ‘Tetris’ (32%).

The follow-up ad libitum M&M's consumption did not show any significant influence by nutritious stimuli or by consumption rate in our study. Prior the study procedure we assumed an appetiser enhancement when exposed to sweetener lemonade or water which eventually did not apply.

Our findings were underlined by other papers. Julis & Mattes (2007) found that fruity sweetened gum was not associated with an increase or decrease of a "post-chewing eating occasion" pattern. Also, Swoboda & Temple (2013) concluded in their report that no evidence can be stated that chewing on a (spearmint and juicy) gum reduces energy intake of a post-chewing meal. Both papers provided evidence suggesting that chewing on flavoured non-food items did not influence the follow-up snack/meal intake. However, other research found a reduction in snack intake after pre-chewing activity. Hetherington & Regan (2011) reported a decrease in snacking of approximately 10% after extended gum chewing. Similar, Higgs & Jones (2013) stated a decline in follow-up snack intake when the pre-meal was consumed with an extended chewing time of 30 seconds per mouth bite. Related articles indicated similar observations at which an extended chewing and a greater amount of chews had decreasing impact on the meal intake itself (Smit et al., 2011; Zhu & Hollis, 2014). In her paper, Hetherington (2016) noted that "masticatory effort will enhance satiation". It appears likely that the actual chewing time and chewing effort, respectively, play an essential role when it comes to a (follow-up) snack/meal intake. The longer and the more extensive, respectively, chewing on the food item is experienced, the more the perception seems to be satisfied. It could be argued that sensory specific satiety (Rolls, 1986) may have been experienced. Further, more extensive chewing is associated with a decrease in hunger feelings (Zhu et al., 2013). As a consequence, the (follow-up) snack/meal intake declines. Experiencing satiation might be the explanation. In this present study, the outcomes of nutritious stimuli and consumption rate on satiation in follow-up ad libitum snack intake are supported by existing research on sweet flavoured gum. Further, our subjects experienced a non-significant decrease in hunger and increase in fullness ratings post the chewing task. Despite this result and the fact that no pre-defined minimum of chewing time on the tube itself was set, the study design was not able to indicate a significant effect on satiation.

As the follow-up ad libitum snack intake did not indicate an influence by the nutritious stimuli, this finding suggests that the ad libitum snack intake was independent of the pre calorie consumption and of the source of sweetness. In his paper, Ludwig (2009) suggested that when "[i]ndiviudals who habitually consume artificial sweeteners (...) reduce[ing] overall diet quality in ways that might contribute to excessive weight gain". Moreover, Fowler and colleagues (2008) support this approach, as their paper looked at weight gain amongst their study population which used artificial sweetened beverages in the long-term. Both papers indicate weight gain which comes along with artificial sweetener consumption. However, the review by Rogers et al. (2016) reported similarities to our study findings. They concluded a declining effect of "low-energy sweeteners in place of sugar" when it comes to "relative energy intake and body weight". Eventually, the "conventional wisdom" (Fowler et al, 2008) of 'low(er)' calorie products stimulating energy intake and eventually the risk of weight gain could not be proven with the present study outcomes. Thus, nutritous stimulation and consumption rate did not affect satiation on a follow-up ad libitum snack.

Also, twenty out of twenty two subjects were female and largely recruited from the University population which did not represent the normal target population. The subjects' higher education level as well as their awareness of the snacks' caloric content might have influenced their snacking behaviour in restrained manners. Further, M&M candies are considered as a snack and not as a full-valued meal from which one consumes when being hungry until fullness. For future studies the choice of the follow-up meal is to choose with greater consideration.

The average intake of M&M's was surprisingly low with 15g in the fast rate group. It is to question to which extend this amount of intake did resemble an ad libitum behaviour. According to the product packing the 'suggested' portion size (M&M chocolate candies regular; Mars UK Ltd) is stated with 40g.

The VAS 100mm on sweet desire ratings prior-post the chewing task indicated a significant difference (Δ) for the desire for eating something sweet and for the desire for drinking something sweet when exposed to different stimuli. Being exposed to either the sugar lemonade (0.38kcal/ml) or the sweetened lemonade (0.025kcal/ml) resulted in a higher Δ in sweet desire rating than being exposed to water (0.0kcal/ml control). The post chewing desire rating declined compared to prior the chewing task. The more the post chewing rating declined, the more the sweet desire rating decreased. That finding is supported by many papers. Rolls et al. (1981) and Rolls (1987) describe this phenomenon as sensory specific satiety. So observed Guinard & Brun (1998) sensory specific satiety as their study outcome indicated a decline in cravings to eat sweet after being exposed to a sweet pre meal. The pure activity of chewing itself seems to have a reductive effect on the sweet desire rating according to Hetherington & Boyland (2007). They compared gum conditions to gum-less condition and found out that chewing on a gum not only blanket the feeling of appetite but, further, reduced the sweet urge and snack intake. The findings of Hetherington & Boyland (2007) suggest a link in our results between follow-up ad libitum snack intake and the sweet desire ratings. The decreased desire rating to eat sweet (see Figure 3 T5) was followed by a not increased ad libitum snack consumption (T6) as originally assumed. An indication towards a sensory specific satiety can be made for both of our study findings. Further, the present outcomes do suggest that chewing on a sweet taste exposure does not encourage the sweet desire rating in our subjects.

By considering the study's outcomes of the post chewing sweet desire ratings (see Figure 3 T5) and the follow-up ad libitum M&M snack intake (T6), an interesting finding could be made. The sweet desire rating indicated a significant decrease in sweet desire to eat comparing sugar lemonade to water, and sweetener lemonade to water. Based on these ratings one might conclude that the follow-up ad libitum (sweet) snack intake consequently shows similar behaviour. However, no significant difference in snack intake was overserved comparing sugar lemonade to water, and sweetener lemonade to water. Even though the significant sweet desire rating outcomes were given the opportunity to be put into actual action/behaviour, no significant compliance was found in the results. Thus, it is to recommend that future studies put more attention and emphasis on rating outcomes and actual behaviour effects.

Hunger and fullness VAS 100mm ratings prior-post the chewing task were independent of nutritious feedback and consumption rate. Neither did any other appetite rating indicate a significant difference. Nutritious stimulation as well as consumption rate seemed to have no significant influence on prior-post appetite ratings. Our findings agree with Hetherington and Boyland (2007). They compared sugar free to regular gums and found no significant association in appetite ratings between those two gum conditions. However, other papers reported different results. So indicated Tordoff and Alleva (1990) an increase in hunger ratings when subjects were exposed to chewing on aspartame-sweetened gum. Steinert and colleagues (2011) indicated that the fullness rating for energy providing drinks (sugar and fructose) lasted longer than for artificial sweetened drinks. Also, the hunger ratings showed similar patterns. Yet, both findings of Steiner and colleagues were not significant. Further, Swoboda & Temple (2013) reported a decrease in subjects hunger ratings when chewing on a gum itself was done right before a food reward task. Thus, present literature does not indicate consistent findings supporting our study outcomes. To which extend nutritious stimuli or consumption rate might contribute to a beneficial appetite rating may still require further investigation. Probably a weak point of our study conduction could have been that the time slot between the end of the chewing task (Figure 3 T4) and the post VAS 100mm rating (straight after the tube was removed from the subject, see T5) was relatively short. This little time frame might have impacted the post rating as appetite and fullness perception which are sensed differently over time. At a later time point of VAS 100mm rating could have resulted in a different outcome.

Our study was conducted as a lab based study. This condition and the fact that chewing on a tube may have caused discomfort for our subjects as it is not a habitual behaviour, might have affected the results. However, we chose this study procedure because it was the best way to investigate nutritious stimuli and consumption rate on chewing behaviour on non-food items. The sample size of only 22 subjects was a great limitation as the desired sample size calculation amounted in 40 subjects. Also, the study population was not balanced in terms of gender characteristics because the majority were female subjects. Thus, it is to recommend that future studies should have a larger and a more balanced design with a study population reflecting more the general target population especially in terms of students and non-students.

5 – Conclusion

The greater motivation to chew, speaking of the number of chews, was observed in sweet flavoured drinks only. An oro-sensory stimulation could be an explanatory reason. It seems that the brain needs a taste stimuli in order to increase the number of chews which, eventually, might constitute a strategy to gain required dietary energy. The consumption rate did not show any influence on neither the chewing motivation nor the follow-up ad libitum snack intake. It may indicate that independent of the rate/speed one consumes, the chewing and snacking behaviour remain unaffected. This conclusion could aid the knowledge of consumption and weight affairs. The follow-up ad libitum snack consumption was not impacted by stimuli. Thus, the present outcomes disconfirm our assumption in a lesser snack intake in the sugar lemonade group than in the sweetener lemonade group or in the water control group. Together with the decrease in sweet desire ratings to eat and to drink, an indication towards sensory specific satiety may be provided.

With ours study findings we were able to identify oro-sensory stimulation affecting chewing on non-food items. Yet more research is recommended in order to understand the mechanism and impact of chewing on non-food items, such as on stim-toys.

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Appendix 1

Participant folder

Study to chew on

Screening number

Inclusion Questionnaire I – confidential

The aim of this questionnaire is to determine in you are eligible as a participant in the Study to chew on supervised by the Human Nutrition department of Wageningen University and Research WUR. It takes places in September and October 2017.

In case you would like to participant in the study as mentioned above, we kindly ask you to fill in this questionnaire according to the truth with a pen or ballpoint (not with pencil). The data we collect with this questionnaire are solely used for the purpose of this study and will be handled confidentially. Meaning that only the researchers involved in this study have access to the data collected, and those data will not be shared with any third parties.

Personal information

Date: ___/___/______ (day/month/year)

Participant details

First name: ____

Last name: ____

Street and house number: ____________________________________

Postal code: __ __ __ __   __ __

City: ____

Place of birth: ____

Nationality: ____

Phone number: ____

Mail address: ____

01 – General Questionnaire

01.1 What is your gender?

- ☐ Female
- ☐ Male

01.2 Date of birth

___/___/______ (day/month/year)

01.3 What is your current body weight?

_______________ [kg]

01.4 What is your current body height? [cm]

__________________ [cm]

01.5 Do you understand and speak English without any difficulties?

- ☐ Yes
- ☐ No

01.6 Do you smoke?

- ☐ Yes
- ☐ No

01.7 Do you consume more than 28 glasses of alcohol per week?

- ☐ Yes
- ☐ No

01.8 Do you already take part in another study or are you interested in another one?

- ☐ Yes, namely: ____
- ☐ No

01.9 Are you employed at the Human Nutrition department of Wageningen University?

- ☐ Yes
- ☐ No

01.10 Are you an intern or are you writing your thesis at the Sensory and Eating behaviour chair group of the Human Nutrition department?

- ☐ Yes
- ☐ No

02 – Medical history

02.1 Are you currently doing a weight loss diet, or did you do a weight loss diet during the last two month?

- ☐ Yes.
- ☐ No

02.2 Do you experience difficulties in chewing, swallowing or eating?

- ☐ Yes, namely: ____
- ☐ No

02.3 Do you have any smell or taste problems?

- ☐ Yes, namely: ____
- ☐ No

02.4 Do you use medication? Also consider medication you use occasionally.

- ☐ Yes, namely: ____
- ☐ No

02.5 Would you like to mention anything else considering your health before participating in this study?

- ☐ Yes, namely: ____
- ☐ No

03 – Allergies and eating pattern

In this study you are going to consume fruit treacle (brand *Albert Heijn* by Albert Heijn B.V., 2017) and M&M's chocolate filling (M&M chocolate candies; Mars UK Ltd).

The treacle's ingredients are juice concentration (apple, elderberry, rosehip, raspberry, blueberry, blackberry), glucose-fructose treacle, sugar, aroma, natural aroma, acid (citric acid), vitamins (B_3, B_6, C, E), preservative (E202), water, malt dextrin, sweetener (acesulphame -K, sucralose), thickening agent (xanthan gum).

The M&M's ingredients are sugar, Cocoa Mass, Skimmed Milk Powder, Lactose and Milk Proteins, Cocoa Butter, Milk Fat, Palm Fat, Glucose Syrup, Shea Fat, Starch, Emulsifier (Soya Lecithin), Colours (E100, E120, E133, E160a, E160e, E171), Dextrin, Glazing Agent (Carnauba Wax), Flavourings, Salt, Coconut Oil, Milk Chocolate contains Milk Solids 14% minimum, Milk Chocolate contains Vegetable Fats in addition to Cocoa Butter, May contain: Peanut, Hazelnut, Almond).

03.1 Do you have any allergy? Also consider allergies occurring occasionally like hay fever.

☐ Yes, namely: ____

☐ No

03.2 Do you have any food specific diseases, allergies or intolerances?

☐ Yes, namely: ____

☐ No

03.3 Do you have any allergy towards any ingredient listed in the text above?

☐ Yes, namely: ____

☐ No

03.4 Do you avoid eating any food or food products due to believe or habit?

☐ Yes, namely: ____

☐ No

03.5 Do you have a regular eating pattern / eating at the same time?

☐ Yes

☐ No

03.6 Do you consume low sugar or calorie restricted drinks/lemonade?

☐ Yes, namely: □ 2 to 3 x a year □ 2 to 3 x a month □ 2 to 3 x a week □ every day

☐ No

Inclusion Questionnaire II – confidential

Please reach the following questions and answer them on the scale of 'never' – 'rarely' – 'sometimes' – 'often' – 'very often'.
Tick the answer box which fits you best.
Make sure you answer all questions. Do so in a true manner.
If you want to change your answer, change the tick to a cross and tick the correct box.

		never	rarely	sometimes	often	very often
1	Do you have the desire to eat when you are irritated?					
2	If food tastes good to you, do you eat more than usual?					
3	Do you have desire to eat when you have nothing to do?					
4	If you have put on weight, do you eat less than you usually do?					
5	Do you have a desire to eat when you are depressed or discouraged?					
6	If food smells and looks good, do you eat more than usual?					
7	How often do you refuse food or drink offered because you are concerned about your weight?					
8	Do you have a desire to eat when you are feeling lonely					
9	If you see or smell something delicious, do you have a desire to eat it?					
10	Do you have a desire to eat when somebody lets you down?					
11	Do you try to eat less at mealtimes than you would like to eat?					
12	If you have something delicious to eat, do you eat it straight away?					
13	Do you have a desire to eat when you are cross?					
14	Do you watch exactly when you eat?					
15	If you walk past the baker do you have the desire to buy something delicious?					
16	Do you have a desire to eat when you are approaching something unpleasant to happen?					
17	Do you deliberately eat foods that are slimming?					
18	If you see others eating, do you also have the desire to eat?					
19	When you have eaten too much, do you eat less than usual the following days?					
20	Do you get the desire to eat when you are anxious, worried, or tense?					
21	Do you find it hard to resist eating delicious foods?					
22	Do you deliberately eat less in order not to become heavier?					
23	Do you have a desire to eat when things are going against you or when things have gone wrong?					

		never	rarely	sometimes	often	very often
24	If you walk past a snack bar or café, do you have the desire to buy something delicious?					
25	Do you have the desire to eat when you are emotionally upset?					
26	How often do you try not to eat between meals because you are watching your weight?					
27	Do you eat more than usual, when you see others eating?					
28	Do you have a desire to eat when you are bored or restless					
29	How often in the evening do you try not to eat because you are watching your weight?					
30	Do you have a desire to eat when you are frightened?					
31	Do you take into account your weight with what you eat?					
32	Do you have a desire to eat when you are disappointed?					
33	When you are preparing a meal are you inclined to eat something?					

Appendix 2

Table 6: Mean ± SE Liking and sensory perception sweetener and sugar lemonade on VAS 100mm rating pilot study

	liking	sweetness	flavour	desire
sweetener lemonade	50.2±7.4	64.7±9.4	52.7±6.2	40.0±11.9
sugar lemonade	49.3±6.9	60.8±11.6	53.2±7.6	36.5±11.8
sweetener ice tea	20.5±5.0	35.7±15.9	26.7±9.6	19.3±4.0
sugar ice tea	46.2±7.8	45.2±12.2	29.7±10.4	52.7±9.1

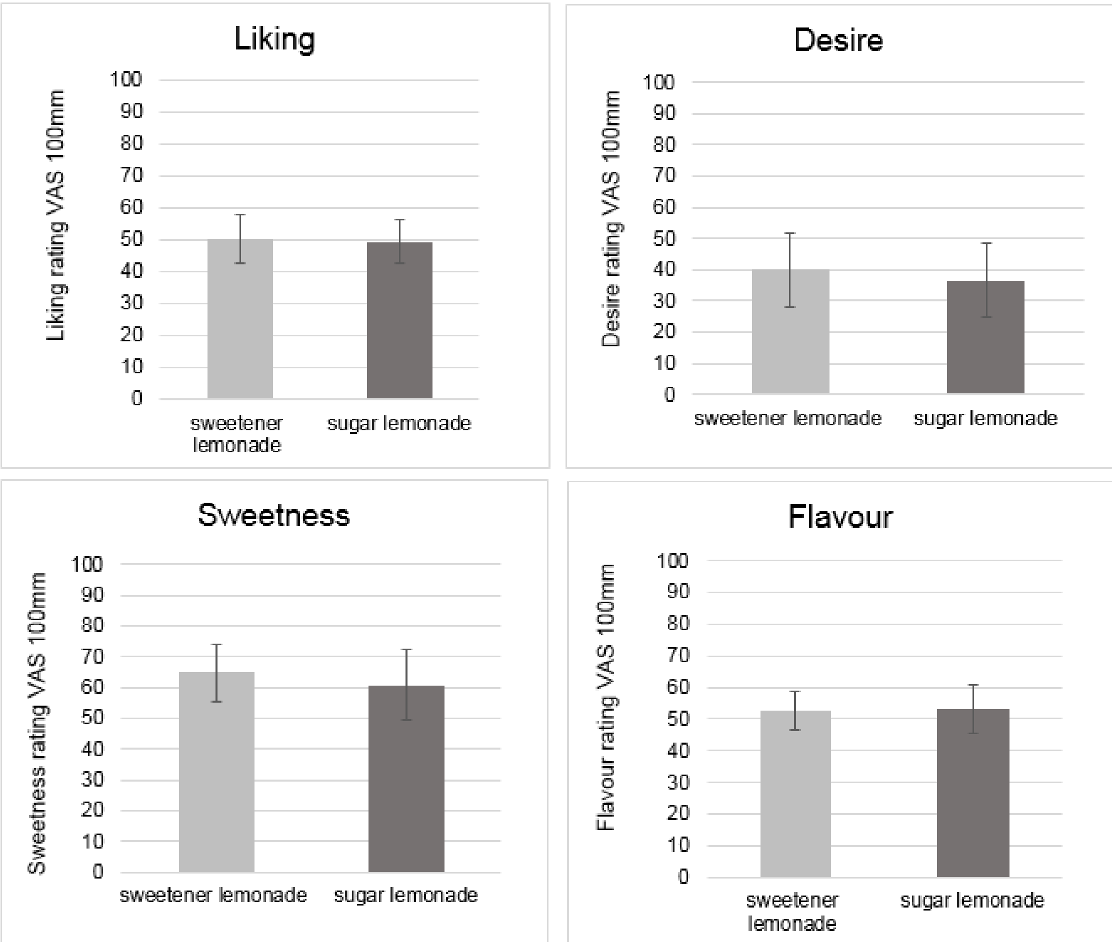

Figure 8: Mean ± SE Liking and sensory perception sweetener and sugar lemonade on VAS 100mm rating pilot study

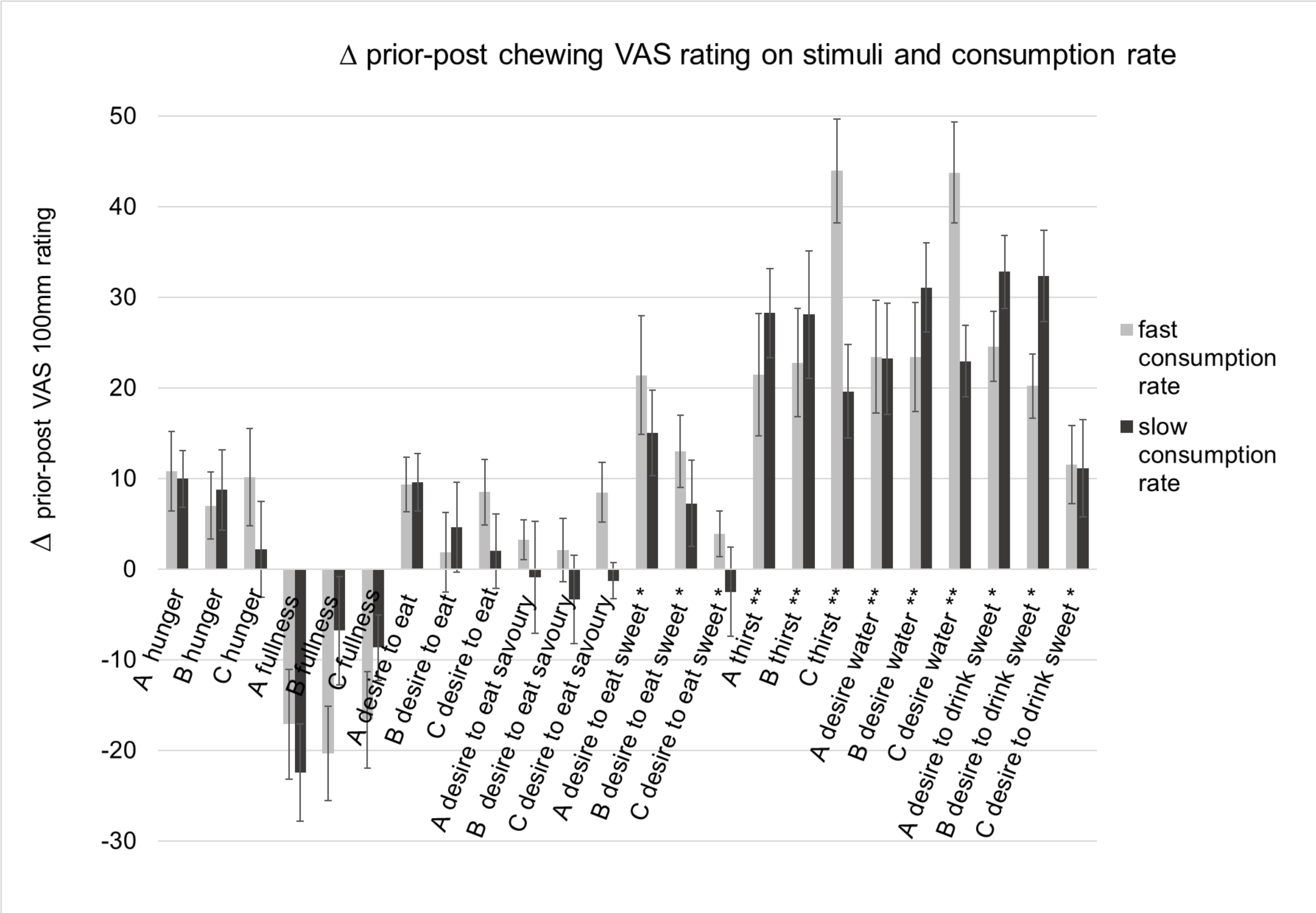

Figure 9: VAS 100mm prior-post chewing rating of appetite and thirst rating on stimuli and consumption rate
A=sugar lemonade stimuli, B=sweetener lemonade stimuli, C=water control stimuli
* indicates a significant difference between the stimuli
** indicates a significant difference between the interaction of stimuli * consumption rate
Δ represents the rating difference of prior-post chewing task

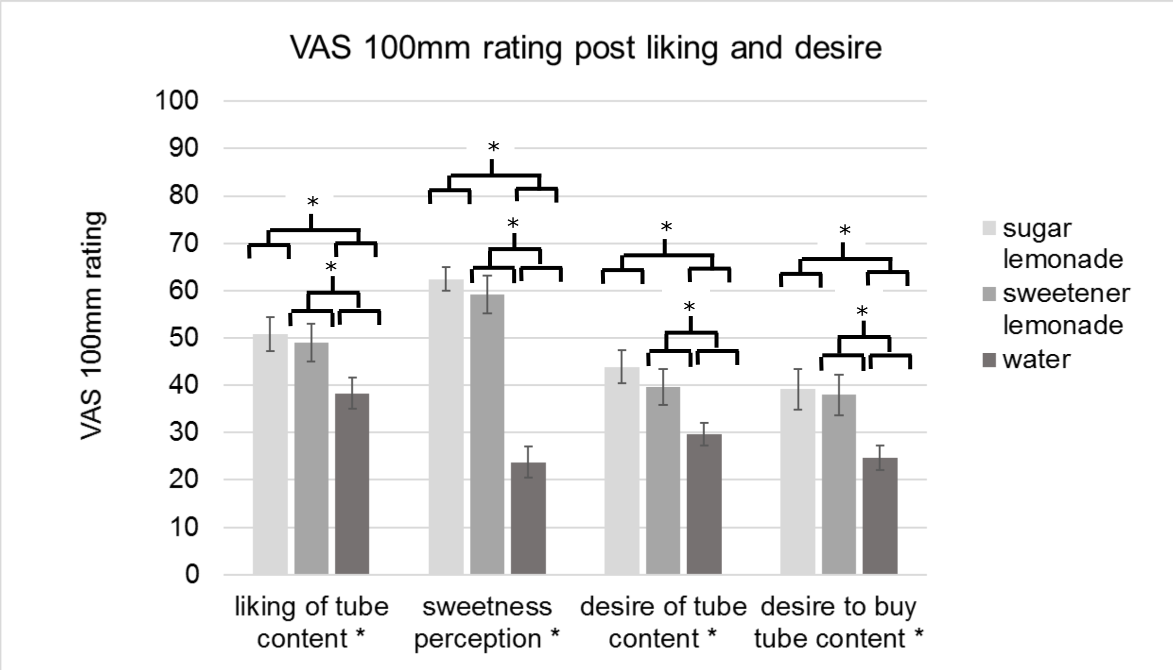

Figure 8: VAS 100mm rating post liking and desire
* indicates a significant difference between the nutritious stimuli

Table 7: Mean ± SE VAS 100mm prior-post chewing difference Δ rating of emotions and attention rating on stimuli and consumption rate (‘H_2O’ represents water)

Consumption rate	**Fast**			**Slow**			**Mixed model** (p-values)		
Stimuli	Sugar	Swee-tener	H_2O	Sugar	Swee-tener	H_2O	Stimuli	Speed	Speed * stimuli
Δ happy	11.0±3.6	8.0±2.9	9.5±3.1	6.5±2.8	4.9±2.8	7.7±3.0	0.494	0.344	0.831
Δ stress	2.1±2.6	3.3±2.4	4.1±1.8	3.6±3.3	5.4±4.0	7.0±2.5	0.612	0.354	0.970
Δ joyful	4.1±2.8	6.1±2.4	3.1±2.2	2.4±2.6	4.9±2.9	9.5±3.2	0.444	0.608	0.188
Δ focused	2.7±3.3	5.2±3.6	2.7±3.5	-3.2±3.7	-1.3±2.8	0.6±3.5	0.738	0.108	0.748

* Indicates a statistical significant difference
Δ represents the rating difference of prior-post chewing task

Reasons for stopping chewing

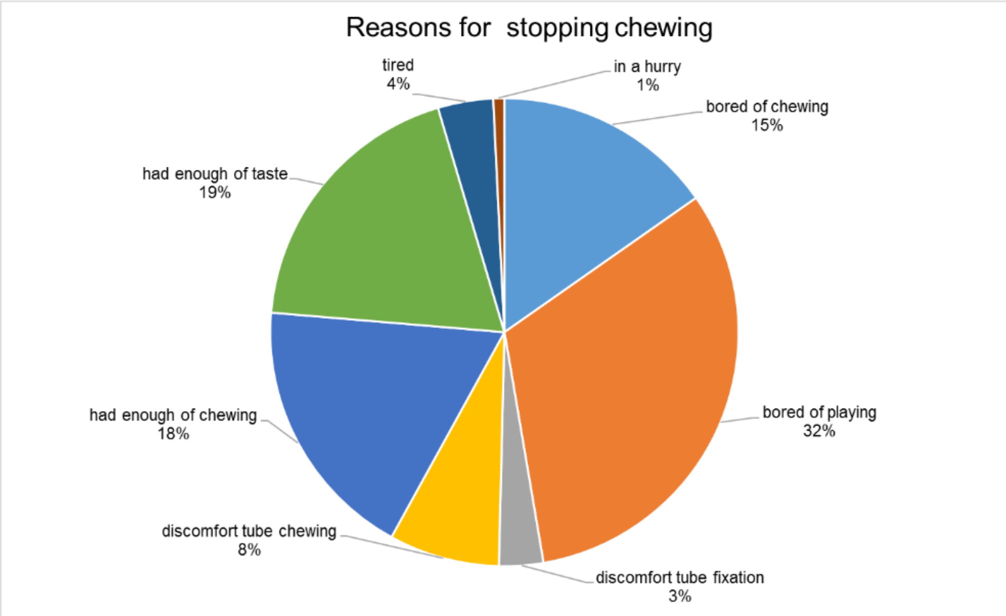

Figure 9: Reasons for stopping chewing [%]