Sweet and Sour Taste Preferences of Children

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Sweet and Sour Taste Preferences of Children

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“I will add that formerly it looked to me as if the sense of taste, at least with my own children when they were still very young, was different from the adult sense of taste; this shows itself by the fact that they did not refuse rhubarb with some sugar and milk which is for us an abominable disgusting mixture and by the fact that they strongly preferred the most sour and tart fruits, as for instance unripe gooseberries and Holz apples.”

Charles Darwin, 1877

Aan mijn ouders

ABSTRACT

Sweet and sour taste preferences of children

Ph.D.-thesis by Djin Gie Liem, Division of Human Nutrition, Wageningen University, Wageningen, The Netherlands and the Monell Chemical Senses Center, Philadelphia, PA, USA, November 2nd 2004

In the industrialized countries children have many foods to choose from, both healthy and unhealthy products, these choices mainly depend on children's taste preferences. The present thesis focused on preferences for sweet and sour taste of young children (4- to 12-years of age) living in the US and the Netherlands. Understanding how sweet and sour taste preferences are formed and modified can help health professionals and those working in industry, to develop strategies to decrease the consumption of sweet tasting foods and to increase the variety of children's diet with sour tasting foods.

By using a variety of stimuli that differed in sweet (0.14M - 0.61M sucrose) and sour (0.00M - 0.25M citric acid) taste we investigated 1) how sweet and sour taste preferences of young children can be measured 2) which concentrations of sucrose and citric acid are most preferred 3) how repeated exposure modifies preferences and 4) how these preferences are related to oral physiological processes and food consumption.

From the 7 studies we carried out it can be concluded that sweet and sour taste preferences of young children can consistently be measured with paired comparison and rank-order methods. Children prefer beverages with high concentrations of sucrose (0.61M) and a substantial part of children have a preference for extreme sour foods (0.08-0.25M citric acid). The later is related to intensity and novelty seeking behavior and consumption of fruits rather than oral physiological differences. Preferences for sour taste are related to exposure to sour taste during infancy and can not easily be changed by short repeated exposure during childhood. This is in contrast with preference for sweet taste, which can be heightened by a short repeated exposure during childhood.

The knowledge gained by the present thesis may open the window to low-sweet and high-sour foods. This could be beneficial for fruit consumption and the dietary variety during childhood.

CONTENTS

1

Introduction

In the industrialized countries children have many foods to choose from, both healthy and unhealthy products, these choices mainly depend on children's taste preferences [1;2]. The present thesis focused on preferences for sweet and sour taste of young children who were between the ages of 4-and 11-years. Preferences for sweet taste are of interest because of their association with energy density and therefore its possible role in childhood obesity. Preferences for sour taste are poorly investigated, but may increase dietary variety and interact with the perception of sweet taste. Understanding how sweet and sour taste preferences are formed and modified can help health professionals and those working in industry, to develop strategies to decrease the consumption of sweet tasting foods and to increase the variety of children's diet with sour tasting foods.
In this thesis we investigated 1) how sweet and sour taste preferences of young children can be measured 2) which concentrations of sucrose and citric acid are most preferred 3) how repeated exposure modifies preferences and 4) how these preferences are related to oral physiological processes and food consumption.
In the first chapter, we discuss the relevance and the bases of, sweet and sour taste preferences of children. We then display the current knowledge of sweet and sour taste preferences of young children and discuss how these preferences can be modified by repeated exposure. The last part of this introduction is dedicated to the aim, the research questions and the outline of the thesis.

Relevance of research on sweet and sour taste preferences of young children
One in four children in the US is at risk for overweight (body mass index >85th percentile) [1] and 14% has severe overweight, obesity [3]. European countries tend to follow the same trend [4;5]. Obesity in childhood is associated with risk factors for cardiovascular and metabolic diseases [6]. Research suggested that besides inactivity [7;8], interactions between genetics and environment [9;10], the foods children choose and consume, are of significant importance in the development of childhood obesity. Children's consumption of foods that contain fat [8;10-12] as well as beverages that contain sucrose [12-14] are positively related to children's Body Mass Index (kg/m^2) [13] and childhood obesity [15].
Children in the industrialized countries have a wide variety of products they can choose from, both healthy and unhealthy foods [1-2]. Moreover, they have a large influence on food purchase decisions [16]. The food choices children in the industrialized countries make are influenced by a variety of factors [17-21] including taste preferences.
Previous research suggested that taste preferences are the most important determinants of children's food choice [22-24] and consumption [11;25]. Especially the addition of sucrose to foods, which results in an increase of sweet taste, can drive children's consumption of a wide variety of foods, such as tofu [26], spaghetti [27], applesauce [28] and beverages [22;29-32].

With respect to health it is important that children eat a wide range of products, but adding sugar in order to increase the number of foods children consume will also increase the caloric density. The relationship between sweet preference and body weight in adults is weak (see [33] for review) and it is unknown whether this relationship exists for young children. However, several studies do suggest that high consumption of beverages that are rich in sugar is positively related to children's Body Mass Index [12-14].
Besides the high preference for sweet tasting foods, anecdotal report suggest that some children also have a high preference for sour tasting foods [16;34;35]. Although many of these foods contain sugar they can also be a source of important nutrients. For example, many sour tasting fruits are rich in vitamin C [36]. Sour taste preferences of children have, however, never been thoroughly investigated.
Decreasing preference for sweet taste and increasing preference for sour taste could be an useful approach to improve children's nutrition. That is, a decrease in the consumption of sweet tasting foods and an increase in the consumption of sour tasting foods. The latter supposedly will increase the variety of foods children are willing to consume, without the increase of sugar concentration. Understanding children's sweet and sour taste preferences can also contribute to modification of existing and the development of new products that are highly preferred by children, but that are not high in sugar content. For example, soda could be decreased in sugar content and increased in sourness and still be preferred by children.
In order to decrease preferences for sweet taste and increase the preferences for sour taste, research is needed on how these preferences can be measured and which levels of these tastes are preferred. Furthermore, it is important to investigate how these preferences are related to food consumption, oral physiological determinants and repeated exposure.

Concepts of taste preferences

Before giving insight in the current knowledge and possible gaps in science concerning preferences for sweet-and sour taste of children, we first have to clarify several concepts that are widely used across the present thesis. What in common language is referred to as taste mostly includes a mixture of taste, smell and irritation that is perceived in the oral and nasal cavities [37]. The present thesis will focus only on taste per se. To be more specific, the present thesis will focus on sweet and sour taste preferences of young children. Before a taste quality can be preferred, which takes place in the brain, the taste has to be perceived by the tongue, this analytical tasks is called *taste perception.*

Taste perception

The sensation of taste arises from chemical stimulation of specialized cells, taste receptors, which are grouped in small clusters called taste buds [38]. Each taste bud is innervated by branches of three cranial nerves: facial (VII), glossopharyngeal (IX), and vagal (X) nerves [39]. Taste buds can be found throughout the oral cavity, on the hard and soft palates, the pharynx, the larynx, the tonsils, the esophagus, and the epiglottis. However, they are mainly located in structures on the human tongue, called taste papillae, including fungiform, circumvallate, and foliate papillae [40;41]. Fungiform papillae consist of 20 to 30 taste buds each and are located on the anterior portion of the human tongue. Cicumvallate papillae (8 to 12 in total) contain about 250 taste buds each, and are arranged in a V-shape across the posterior tongue. Foliate papillae consist of around 1280 taste buds per set. In total two sets of foliate papillae are present on the human tongue, one on each lateral border [39;41] (see **Figure 1 panel a**). Chemicals that interact with taste receptors can be grouped into five basic tastes: sweet, sour, salt, bitter and umami. These tastes can be perceived in all areas of the tongue [42]. Binding of sweet tasting molecules (e.g. sucrose) to specific taste receptors (sweet taste receptors) causes the taste receptors to signal to the nerves that innervate the taste buds. In the same line, the perception of bitter taste (i.e. quinine, urea) and umami taste (i.e. MSG) are mediated by binding of bitter or umami tasting molecules respectively, with specific taste receptors [42-45]. The transduction of sour (i.e. citric acid) and salt taste (NaCl) is most likely mediated by specific ion channels located on the apical end of the taste cell rather than binding of molecules to specific receptors [42].

Interaction between different tastes

Although sweet and sour tastes are perceived by their own unique pathway, the perceived intensity of sweet and sour taste is different when both tastes are presented in a mixture [46;47]. This has been described as mixture suppression. Mixture suppression is a phenomenon whereby the perceived intensity of two tastes in a mixture is less than if they were unmixed, at the same concentration [48]. Research in adults suggested that adding high concentrations of citric acid (>0.01M) to a sucrose solution suppresses the perceived sweetness [46;47].

Taste preference

The three cranial nerves that innervate each taste bud, transmit information from the taste buds to specialized area's in the brain (e.g. primary taste cortex, amygdale and orbitofrontal cortex) where the taste quality is decoded and judged on its hedonic value [49] (see **Figure 1 panel b**). Children's food choices and consumption are mainly driven by this hedonic judgment [22;50]. Positive hedonic judgments of tastes are partly determined by nature (e.g. preference for sweet and aversion for sour taste), but can be modified by learned experiences such as caloric conditioning and repeated exposure (see [51] for a review).

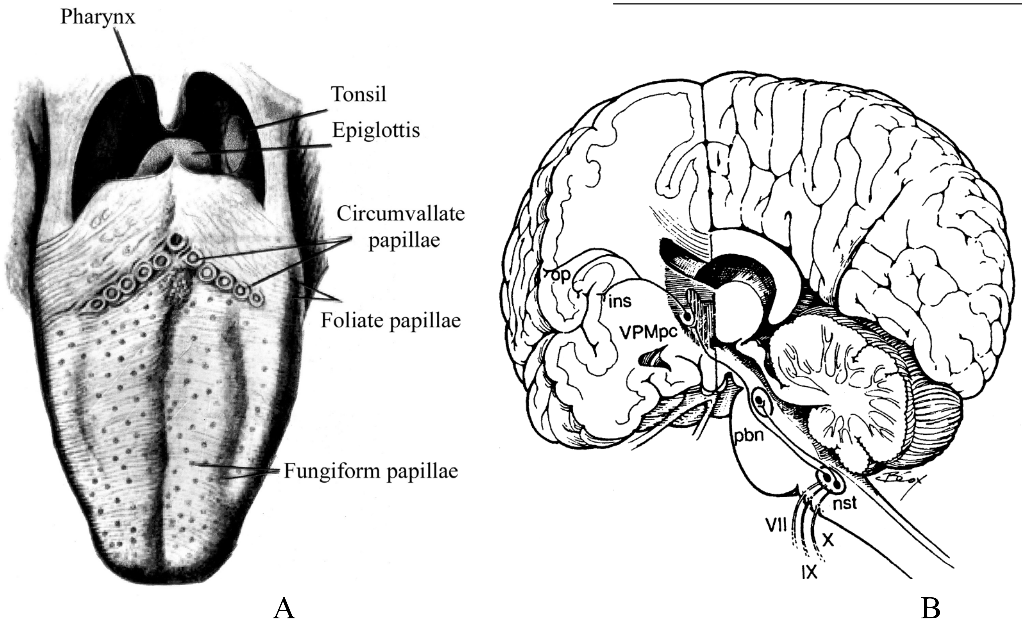

Figure 1 Schematic overview of the human tongue, where taste perception takes place (panel A). Panel B shows the pathway of transportation of signals from the taste receptor cells to specialized areas in the brain where taste perception is judged on its hedonic value. VII, IX, X: cranial nerves; NST: Nucleus of the solitary tract; Pbn: Parabrachial nucleus; VPMpc: Venteroposteromedial nucleus of the thalamus; ins: Insula; op: Opercular cortex (ins and op together are called the primary taste cortex); The taste-sensitive neurons in the orbitofrontal cortex (not indicated in this picture) are called the secondary taste cortex (drawing by Mr. Birck Cox).

Sensory testing with young children

In order to investigate children's sweet and sour taste preferences, the sensory tests need to be validated (e.g. do we indeed measure taste preferences?), reproducible (e.g. can we measure it more than once) and consistent (e.g. the most preferred concentration of sucrose, should be independent of the sensory test that is being used). Sensory tests that are used to measure taste preferences of adults are not always suitable for young children. Complicated sensory tests, such as visual analogue scales and magnitude estimations, may not give valid and precise results, because children are less able to categorize and have less cognitive skills than adults. Even a simple question such as "Do you like this taste" may give unreliable data, because in general children are more likely to answer in the affirmative manner in their reply to such question [52]. In order to obtain valid, reproducible and consistent data concerning taste preferences of children, the sensory tests need to be fairly simple and should not give children the possibility to answer in an affirmative way.

The paired comparison and the rank-order tests are the most widely used tests to measure children's preferences [22;24;26;32;53-57]. Previous studies with different foods as test stimuli, suggested that both methods are able to predict children's food consumption and

are therefore considered to be valid to measure food preferences [24;28;57;58]. Previous research showed a high reproducibility of the paired comparison (88%) [59] and moderate reproducibility of the rank-order method (expressed as tau-correlation, see *chapter 8* for more details, between first and second assessment, mean tau=0.58 [24], mean tau=0.40 [53], tau could range from −1 (total disagreement) to 1 (total agreement)).
It is, however, unclear whether the paired comparison and the rank-order test give consistent data with respect to the most preferred level of a specific taste quality. That is, the most preferred concentration of sucrose could depend on the method that is used. In order to compare studies that used either paired comparisons or the rank-order test, information concerning the consistency of both tests is crucial. Especially when the research is conducted with young children (i.e. 4-and 5-year-olds), the consistency of both methods is not warranted. The attention span of 5-year-old children and their ability to categorize, analyze, and synthesize information rapidly is stronger and more efficient than that of 4-year-old children [60]. Four-year-olds may not be able to carry out simple sensory tests, which are of no problem to 5-year-olds.
The difference between 4-and 5-year-olds becomes even more important when investigating discriminatory ability (e.g. which is sweeter) instead of preference. In order to perform well during the discrimination tests children need to clearly understand what the researcher means by the specific dimension (e.g. sweetness) [48]. Information concerning children's ability to discriminate between different concentrations of sucrose is important in order to judge the consistency of the preference tests. If children are not able to discriminate between different sucrose solutions, it is impossible to identify the most preferred solution in a consistent manner.

Conclusion

Paired comparison and rank-order methods are valid and reproducible methods to measure children's food preferences. However, it needs to be investigated whether they give consistent data when interested in the most preferred concentration of sucrose and children's discriminatory ability. Special attention should be given to young children (4- and 5-year-olds), because their abilities to perform such tasks are rapidly changing.

Preference for sweet and sour taste in young children

In order to understand which levels of sweet-and sour taste children prefer and how this can be modified, it is important to have insight in the development of taste preferences. First we describe which levels of sweet and sour taste newborns and children prefer and whether this is different from adults. We then discuss how these preferences are related to food consumption and what the possible mechanisms are.
In order to protect children from ingesting harmful substances and to promote the consumption of nutritious substances, they already have a sophisticated sense of taste at

birth [61;62]. Based on facial expressions (i.e. facial relaxation) and intake measures, it has been shown that human newborns (i.e. 1-4 days of life) have a preference for intense sweet solutions (0.73M sucrose in water) and that they are able to discriminate between different sugars [63-65]. This preference for high sweet foods continues during childhood and is evident in children around the world [28-31;66-71]. The concentration of sucrose most preferred by children (0.60M sucrose in water) is far higher than which is most preferred by adults (0.075M sucrose in water) [29-31;70]. This is also reflected by large food consumption data, which revealed that 4-to 7-year-old children (n=325) consumed a larger percentage of their total energy intake across the day, by means of mono- and disaccharide's (girls: 33.2 %; boys: 32.1%), than adults (females: 21.3%; males: 23.7%) (n=2799) [2].

It has been hypothesized that preferences for sweet tastes are heightened during periods of maximum growth [71]. This hypothesis has been supported by animal research in which was shown that newborn rats preferred high concentrations of sucrose, this preference decreased with age. This change in preference did not depend on the foods that newborn and older aged rats were fed [72]. An alternative hypothesis is that the preference for sweet taste expands the species' use of available nutritional sources [30]. The addition of sucrose to food can drive children's consumption of a variety of foods, such as tofu [26], spaghetti [27], applesauce [28] and beverages [22;29-32].

Much less is known about the preference for sour taste. Based on facial expressions (i.e. lip pursing combined with negative upper and mid-face components) and intake, it has been suggested that human newborns reject sour tasting substances (0.024M citric acid) [64;65]. Little research, however, has been carried out in children. Anecdotal reports and marketing observations suggest that children prefer higher levels of sour taste than adults. For example, Charles Darwin observed in 1877 that, unlike most adults, his children had a preference for intense sour tasting foods [34]. Nowadays, children's preferences for intense sour foods, are reflected by the clear market for extreme sour tasting candies [16;35]. These candies are highly preferred by some children, but aversive to most adults [35]. Despite these anecdotal and marketing reports no scientific evidence is available to support the assumed preference for intense sour taste of children. It also needs to be determined whether preference for sour taste is evident in all children, or only in a subset of children.

Why would children have a higher preference for sour taste than adults? Hypothetically the rejection of sour taste in newborns is biologically relevant because intense sour tasting foods may cause tissue damage. The possible acceptance of intense sour taste by young children is difficult to explain. Preference for this taste could possibly increase the consumption of sour tasting foods such as certain fruits that can be beneficial for health. For example, sour tasting citrus fruit can be a good source for vitamin C, which

is important for collagen fiber formation in the human body [36]. It has been suggested that preference is the most important determinant of fruit consumption [73]. It, however, needs to be investigated whether preference for sour taste plays a role in the consumption of fruit.
Children's high preference for sour tasting foods can also be explained by the perceived intensity of sour taste. Hypothetically, children who have a preference for extreme high concentrations of citric acid in foods, perceive the sourness as less intense compared to those who do not prefer this taste. In order to come to a similar sensation of perceived taste intensity, those who perceive the sourness less intense need more stimulation with sour taste. Oral physiological differences may play a role in perceived sourness. Research with adults suggested that a high salivary flow [74], high buffering capacity of saliva [75] and low salivary pH [76] are related to a low perceived intensity of sour tasting foods.
An alternative, but not mutually exclusive, hypothesis is that preference for sour taste is driven by or related with sensation seeking behavior of children [16;35]. According to this hypothesis children's preferences for sour tastes are secondary to their generalized preferences for adventures and thrills. To our knowledge, there are no published reports on whether aspects of temperament relate to children's preferences for sour tastes.

Conclusion

Despite the large amount of research dedicated to children's preference for sweet taste, little is know about children's preference for sour taste. Anecdotal reports suggest that children have a preference for intense sour tasting foods, but to our knowledge, this was never scientifically tested. It also remains unknown what drives these preferences for sour taste and whether preference for this taste is related with dietary intake.

Changing preferences for sweet and sour taste in young children

Although children are born with a preference for sweet taste and an aversion for sour taste, these preferences can be changed. There are several mechanisms through which children learn to prefer different tastes, among which classical conditioning (see [51] for review), post-ingestive learning [77], peer pressure [57], interaction between parent and child (see [51] for review) and repeated exposure. The present thesis focused on the role of repeated exposure on the preferences for sweet-and sour taste of children.
The role of exposure starts as early as the third trimester of gestation, when the human fetus is equipped with a functional taste system [78]. While still in the womb, the fetus ingests almost one-liter amniotic fluid per day [79]. It has been shown that amniotic fluid contains flavors that reassemble the flavor of the food previously eaten by the mother [80]. By ingesting the amniotic fluid the fetus is exposed to the flavors of the foods eaten by

the mother. By means of this pre-natal exposure, the fetus learns to accept and prefer the food eaten by the mother [81;82].

Impact of repeated exposure during infancy on sweet and sour taste preferences

The role of exposure continues after birth. By repeatedly exposing infants to the same food, they learn to accept it as familiar and save to consume, if consumption is not followed by negative gastrointestinal consequences [83;84]. Repeated exposure to different tastes during infancy not only affects taste preferences on the short term (i.e. infancy), but is also thought to be important for taste preferences in the long term (i.e. childhood, adulthood) [85]. Similar to the sensitive period for normal vision [86], a sensitive period may exist to learn to prefer different tastes [37]. During this sensitive period it is hypothesized that infants can easily accept new flavors and foods and identify regular eaten flavors as familiar and save [37]. Once this sensitive period comes to an end, the infant is likely to reject all the flavors that are not familiar to him [87]. It is suggested that flavor preferences learned early in life (early flavor experiences), are robust and affect taste preferences later in life [85].

In humans the concept of ‘early flavor experiences’ has been tested for sweet tasting sugar water [88] and sour tasting baby formula [89]. Beauchamp and Moran concluded that infants, who were fed sucrose water, had a higher preference for sucrose water at 2-years-of-age than children of mothers who did not practice this habit. This was also evident for those children who were only fed sucrose water during their first 6 months of life [88]. Along the same line, but now based on sour tasting formula, Mennella and Beauchamp suggested that 4-to 5-year-old children who were fed sour tasting formula during their first year of life, were more likely to prefer juices with a sour taste than those who were fed other formulas (i.e. soy or milk based formulas). This difference in preferences for sour taste was evident years after children’s last exposure to the sour tasting formula [89]. It has been suggested that early taste experience with sour tasting formulas also influences children’s preference for sweet taste [90;91]. However, this has never been thoroughly investigated. Furthermore, it remains unclear whether early flavor experiences with sour tasting formula influences preferences for sweet and sour taste of older aged children.

Impact of repeated exposure during childhood on sweet and sour taste preferences

Repeated exposure remains important during childhood. Previous studies showed that approximately 8 exposures are necessary to increase the liking of a food during childhood [2]. Parents control their children’s degree of exposure to foods [92-95]. Especially sweet food items are often highly controlled by parents because of the assumed negative effects on health [96;97]. By imposing rules that aim to restrict the consumption of foods that contain sugar, parents try to lower their children’s consumption of these foods and preference for sweet taste in general [98]. Two problems arise from this parental approach. First, research on the effect of repeated exposure on subsequent preference has been focused on food products rather than taste per se [99-102]. It remains to be determined whether repeated exposure during childhood to specific tastes such as sweet or sour taste result in an increased liking for sweet and sour taste.

Secondly, it is unclear whether restriction of sweet food items, lowers the preference for sweet taste. On the contrary, restriction may well result in an elevated preference for sweet taste. Several studies suggested that restriction could result in an increased desire to consume the restricted food [103]. This high desire would then increase consumption of the restricted food in the absence of parental monitoring [104]. Whether this mechanism is relevant for sweet taste remains unknown.

Conclusion

It has been suggested that repeated exposure to sour taste early in life (< 1year) influences sweet and sour taste preferences of 4-to-5 year-olds, it remains unknown whether such experience influences the taste preferences of older aged children. Repeated exposure to sweet and sour taste during childhood may also increase the preference for both taste qualities during childhood, but this has never thoroughly been investigated. Despite this lack of evidence many parents try to decrease their children's preference for sweet taste by decreasing exposure. It needs to be investigated whether parental control over the consumption of sweet tasting products is an efficient way to decrease preference for sweet taste.

Overall conclusion

There exists an extensive research on sweet taste preferences of children. However, due to the variety of methods that were used it is difficult to compare the different studies. It needs to be investigated how consistent the results of commonly used methods are, especially when those methods are used to measure taste preferences of young children.

Beside anecdotal and marketing reports, there is a lack of scientific studies concerning preferences for sour taste of children. Research on sour taste preferences of children should be focused on the most preferred intensity, and the physiological and temperamental differences between those who prefer this taste quality and those who do not. Furthermore, it needs to be investigated whether preference for sour taste is related to consumption of sour tasting foods.

It is likely that preferences for sweet and sour taste of children are influenced by a variety of factors among which repeated exposure. The influence of repeated exposure on preference for sweet and sour taste in children starts as early as infancy. It has been suggested that early experience with sour taste influences both sour taste as well as

sweet taste preferences of children. Further research is needed to confirm the possible relationship between early experiences and taste preferences during childhood.
Repeated exposure remains of relevance during childhood as suggested by studies that focused on preference for a specific food. Whether repeated exposure to specific taste qualities is related to an enhanced preference for these taste qualities remains to be determined. Deliberately decreasing the exposure to sweet taste may even have the opposite effect. This, however, needs further investigation.

Outline of the thesis

As has been shown in the overall conclusion research on preferences for sweet and sour taste of children should be focused on the consistency of the commonly used sensory tests, the most preferred level of sour taste and how this is related to oral physiological, temperamental differences and food consumption. Furthermore, it needs to be investigated whether repeated exposure early in life and during childhood are related to preferences for sweet and sour taste, and how preferences for sweet taste are influenced by parental restriction of sweet food items.

Research questions

The research questions were stated as follows (see **Figure 2** for schematic overview)

1. Do paired comparison and rank-order procedures give consistent data concerning the most preferred level of sweet taste and the discriminatory ability of young children? **Chapter 2**

2. Which levels of sour taste are most preferred by children and how is this related to physiological determinants, thrill seeking behavior and fruit consumption? **Chapter 3, 4 and 5**

3. What is the influence of early experience with sour tastes on preferences for sweet and sour taste of young children? **Chapter 6**

4. What is the role of parents on the preference and consumption of sweet foods of their children? **Chapter 7**

5. Can sweet and sour taste preferences of children be changed by a short repeated exposure to sweet and sour tasting stimuli? **Chapter 8**

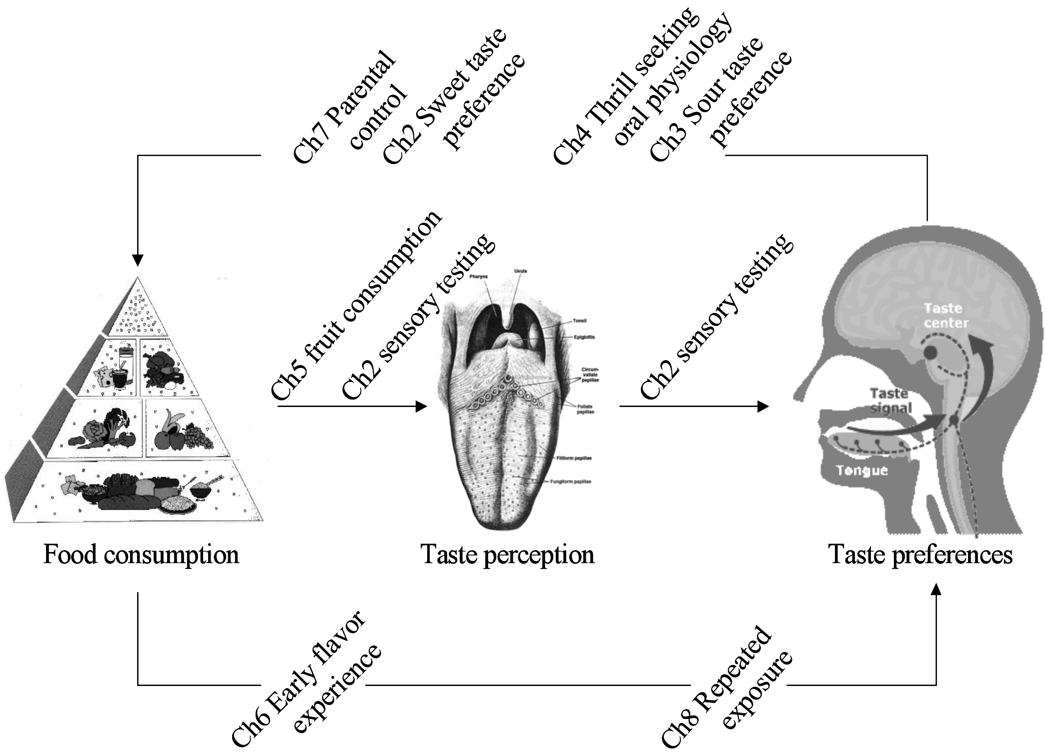

Figure 2 Schematic overview of the chapters of the thesis in relation to food consumption, taste perception and taste preferences.

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Consistency of sensory testing with 4-and 5-year-old children

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ABSTRACT

Background: Rank-order and paired comparison tests are widely used methods to assess sensory perception of young children. Small age differences could, however, influence the ability of children to carry out such tasks.

Objective: This study compared rank-order and paired comparison tests for consistency in 4- and 5-year-old children.

Design: During four sessions, 22 young adults, 21 4-year-old and 47 5-year-old children carried out rank-order and paired comparison tests to measure discriminatory ability (0.22, 0.25, 0.29, 0.34, and 0.39M sucrose in orangeade) and preference (0.14, 0.20, 0.29, 0.42 and 0.61 M sucrose in orangeade).

Results: Young adults and 5-year-old children were able to discriminate between all solutions and showed a high consistency between the rank-order and pair-wise tests for discriminatory ability (>76% consistency) and preference (>71% consistency). In contrast, 4-year-olds detected differences in sweetness during the preference tests, but failed to distinguish sweetness intensities during the discriminatory ability tests.

Conclusion: The dissimilarity between 4-and 5-year-olds in performing sensory tests was due to a difference in their cognitive skills rather than their sensory perceptual differences.

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INTRODUCTION

Children ranging in age from zero to ten years prefer higher concentrations of sucrose in foods than adults [1-5]. Furthermore, it has been suggested that children are less sensitive to sweet taste than adults [4-7]. In order to investigate what children prefer, and which levels of sweet they are able to discriminate, reliable sensory tests are needed. Tests that are reliable for adults do not always give reliable information when used with young children. In general, children tend to answer in the affirmative way, and have less experience with scales than most adults [8].

Rank-order and paired comparison tests are the two most widely used methods to measure sensory perception and preferences in young children [9-19]. Most sensory studies reported in the existing literature, grouped 4- and 5-year-old children together [6;11;14-16]. However, children in this age range are rapidly changing. Their attention spans lengthen and their ability to categorize, analyze, and synthesize information rapidly becomes stronger and more efficient [20]. In the Netherlands, children normally enter kindergarten at the age of four, therefore it is important to asses differences between 4-and 5-year-olds.

Sensory taste tests with young children typically consist of two types: preference tests and discrimination tests. Most studies in children focus on preference rather than discrimination. Stimuli that are commonly used are not only different in taste, but are also different in color and appearance (e.g. different fruits, different sandwiches). It has been suggested that with these stimuli, rank-order and paired comparison tests give consistent data in young children [12].

However, it remains unknown whether rank-order and paired comparison tests give consistent data when the presented stimuli differ only in taste (e.g. orangeade with different concentrations of sucrose). It is also unknown if rank-order and paired comparison tests give consistent data when they are used to measure discriminatory ability in young children (i.e. which stimulus is more sweet) instead of preference.

The present study investigated the consistency between rank-order and paired comparison tests as measures of discriminatory ability and preference, using stimuli that differed only in sweetness. Subjects of the study were young adults, 4-year-olds and 5-year-olds.

METHODS

Subjects

Twenty-one 4-year-old children (4.4 ± 0.3 yrs, 12 girls and 9 boys), 47 5-year-old children (5.4 ± 0.3 yrs, 23 girls and 24 boys) and 22 young adults (22.6 ± 1.8 yrs, 17 females and 5 males) participated in the study (see **Table 1**). The children attended a kindergarten in Bennekom, a village 4 km from Wageningen. Since the research study took place in October, and children in the Netherlands start kindergarten in September, the 5-years-olds (second graders) had almost one year more experience with kindergarten and were present in a greater number than 4-year-olds (first graders). The young adults were students at Wageningen University (see Table 1).

Table 1 Characteristics of subjects (mean ± sem) kindergarten and young adults

	Kindergarten		**Young adults**
	4-year-olds	**5-year-olds**	
Sex (female : male)	12:9	23:24	17:5
Age (yrs)	4.4 ± 0.3	5.4 ± 0.3	22.6 ± 1.8
Height (m)	1.1 ± 0.04	1.2 ± 0.04	1.7 ± 0.07
Weight (kg)	18.9 ± 3.5	20.9 ± 2.9	65.1 ± 6.3
BMI (kg/m^2)	15.5 ± 2.2	15.5 ± 1.8	21.6 ± 1.9

Informed consent for the children were obtained from parents prior to testing. The young adults filled out the informed consent forms by themselves. Exclusion criteria for participation were diabetes, sugar restriction in the diet, and presumed allergies for sugar and/or orange beverages. During the actual taste tests, 2 children were excluded because they refused to taste the beverages.

The study protocol was approved by the Medical Ethical Committee of the Division of Human Nutrition of Wageningen University.

Sensory tests

Stimuli

A beverage with orange flavor was used for the discriminatory ability and the preference tests. The beverages were prepared by dissolving 23 g of orange beverage concentrate and additional sugar in water for a total volume of 1.0 liter. The concentrate (Lim 7644, Quest International, Naarden, The Netherlands) was composed of concentrated orange juice, which contained natural orange flavor (80 ppm/l beverage solute), 4.2% w/v citric

acid, 1500 ppm benzoic acid, 4100 ppm ascorbic acid, and 44% w/v sugar (50% sucrose, 25% glucose, and 25% fructose).
Two series of solutions with different concentrations of sucrose (Sundale, Suiker Unie, Breda) were prepared. Series A, which was used during the preference tests, consisted of 5 stimuli ranging from 0.14M to 0.61M sucrose with a middle concentration of 0.29M sucrose, and a difference of 44% between adjacent concentrations: 0.14, 0.20, 0.29, 0.42, and 0.61M sucrose (4.8%, 6.9%, 10.0%, 14.4%, 20.8% w/v sucrose/l solute). Series B, which was used during the discriminatory ability tests, consisted of 5 geometrically spaced concentrations ranging from 0.22M to 0.39M sucrose, with a middle concentration of 0.29M, and a difference of 15% between adjacent concentrations: 0.22, 0.25, 0.29, 0.34, and 0.39M sucrose (7.6%, 8.7%, 10.0%, 11.5%, 13.2% w/v sucrose/l solute). Similar beverages were previously used by de Graaf and Zandstra [5]. About 15 mL of each of the stimuli were offered at room temperature in 25 mL transparent cups. The beverages were prepared the evening before each session and were stored overnight at a temperature of 4°C.

Procedure

Paired comparison and rank-order tests were used to measure preference and discriminatory ability. The children were tested at their kindergarten in a room that was familiar to them. Each child had personal guidance from an adult, who was trained beforehand to become familiar with the procedures. The testing room consisted of 10 to 15 low tables, depending on the number of children that were tested at the same time. The children sat in a circle facing outwards, with an adult in front of them. The adults faced the middle of the circle.
The sensory tests with the children took place on two days, separated by one day. On the first day, both the tests for discriminatory ability were carried out. On the second day both tests for preference were carried out. On both days the paired comparison tests were followed by the rank-order tests, with a 20-minute pause between both tests. Each test lasted for about 10 minutes. The subjects were allowed to taste the stimuli as often as necessary to make a decision.
The young-adults received oral and written instructions. They followed a similar procedure as the children did. Adults performed all tests during one morning, which involved two sessions, one session for discriminatory ability and one session for preference. These two tests took about twenty minutes separated by a 10-minute break. The adult tests were conducted in the tasting booths of the Wageningen University.

Paired comparison tests for discriminatory ability and preference

During the paired comparison tests, subjects judged 5 stimuli in 10 different pairs. For the discriminatory ability test, subjects answered the question: "In which beverage did we put the most sugar?". Before the discrimination tests began, subjects were told that the sweet food items contained more sugar than non-sweet food items. Subsequently the children were asked to name foods with a lot of sugar. All children mentioned sweet food items. For the preference tests, they were asked: "Which one do you like best?". The order of presentation between and within pairs was randomly assigned. Before the actual taste test began, the subjects were offered a pair of stimuli to become familiar with the beverages. This pair consisted of the second and the fourth stimuli from the series that was used during the actual paired comparison tests. The subjects used the sip and swallow procedure, and took a sip of water between each pair of stimuli.

Rank-order tests- for discriminatory ability and preference

During the rank-order test, subjects divided the stimuli into two categories. For the discriminatory ability tests, the categories "most sugar" and "least sugar" were visualized by means of different numbers of sugar cubes. For the preference tests the categories "most preferred" and "least preferred" were visualized by means of a "happy face" and a "sad face". The procedures resulted in a rank-order from most sweet to least sweet for the discriminatory ability test, and a rank-order from most preferred to least preferred for the preference test. For further details see de Graaf and Zandstra [4].

Statistical analyses

Scores for discriminatory ability were calculated based on subjects' performance of the discriminatory tasks. A score of 10 indicated that subjects ranked the sweet solutions from least sweet to most sweet in an errorless fashion. A score of –10 indicated that subjects ranked the sweet solutions from least sweet to most sweet in the opposite way; i.e. the highest concentration of sugar was ranked as least sweet, the lowest concentration of sugar was ranked as most sweet.

Scores for sweet preferences were calculated based on the amount of sugar that was most preferred. A score of 40 indicated that subjects preferred the highest concentration of sugar in orangeade. A score of 20 indicated that subjects preferred the lowest concentration of sugar, see de Graaf and Zandstra [5] for further details).

Pearson correlation coefficients were calculated between the scores of the rank-order and the paired comparison tests. Friedman analyses of ranks were performed in order to determine whether subjects were able to discriminate between the different beverages. For the paired comparisons a rank-order was constructed on the basis of the number of times subjects chose each solution as most preferred (for preference), or as most sweet (for discriminatory ability).

In order to compare the results of the rank-order tests with the results of the paired comparison tests, the data obtained by the rank-order tests were transformed into paired

comparisons. Consistency between both methods was defined as the percentage of pairs that was judged to be identical during both tests.
Unpaired t-test tests were applied to determine whether there were differences in preference and discriminatory ability scores between children and young adults, and between 4- and 5-year old children. *P*-values smaller than 0.05 were considered significant. All summary statistics are expressed as means ± sem.

RESULTS

Discriminatory ability, children and young adults

Figure 1 shows the results of the rank-order and paired comparison tests for discriminatory ability. Children, compared to young adults, were less able to discriminate between the five different sugar concentrations. This was evident during the rank-order tests (t(88df)=-5.2; $P<0.0001$) and during the paired comparison tests (t(88df)=-6.2; $P<0.001$; see **Table 2**). No significant differences were observed between girls and boys during the rank-order tests (t(66df)=-0.48; $P=0.63$), or during the paired comparison tests (t(66df)=1.9; $P=0.07$). Likewise, no significant differences were observed between adult females and males during the rank-order tests (t(20df)=0.63; $P=0.54$), or during the paired comparison tests (t(20df)=-0.25; $P=0.80$).

Table 2 Mean (± sem) discriminatory ability score for rank-order and paired comparison test; young adults, kindergarten, 4- and 5-year-olds

Discriminatory ability test	Young adults	Kindergarten	Kindergarten 4-year-olds	Kindergarten 5-year-olds
	n=22	*n*=68	*n*=21	*n*=47
Rank-order	9.2 ± 0.38[c]	2.3 ± 0.76[a,c]	-1.6 ± 1.32[a,b]	4.11 ± 0.80[a,c]
Paired comparison	8.9 ± 0.36[c]	3.4 ± 0.54[a,c]	1.9 ± 1.0[a]	4.0 ± 0.62[a,c]
Pearson correlation coefficient between rank and paired	0.09 (*P*=0.72)	0.33 (*P*=0.03)	0.02 (*P*=0.95)	0.37 (*P*=0.04)
Mean % consistency	85.9 ± 2.7	76.1 ± 3.1[a]	62.7 ± 5.7[a,b]	82.0 ± 3.1

A discriminatory ability of -10 means a reversed order, +10 means a perfect order.
0 is equivalent to a random order
[a] Significantly different compared to young adults; $P<0.05$
[b] Significantly different compared to 5-year-olds; $P<0.01$
[c] Significantly different from 0, $P<0.01$

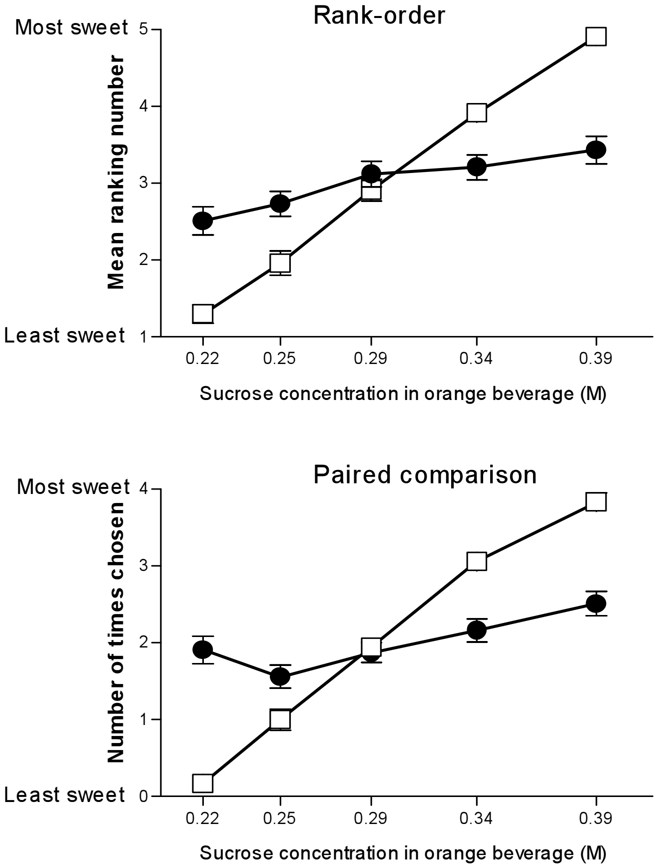

Figure 1 Result of the rank-order test (mean ranking number per solution ± sem) and paired comparison test (mean number of times each solution was chosen as most sweet ± sem) for discriminatory ability, children (●) and young adults (□)

Discriminatory ability, 4- and 5-year-olds, and young adults

Figure 2 shows the results of the rank-order and paired comparison tests for discriminatory ability, grouped by 4-year-olds, 5-year-olds and young adults. Five-year-olds were able to identify differences in sweetness across the five different sugar concentrations. This was the case for both methods (rank-order tests: $F_r(4df)=33.7$, $P<0.0001$; paired comparison tests: $F_r(4df)=63.5$, $P<0.0001$). Both methods showed an 82% consistency with each other and were significantly correlated with each other (Pearson's r=0.37; $P<0.05$).

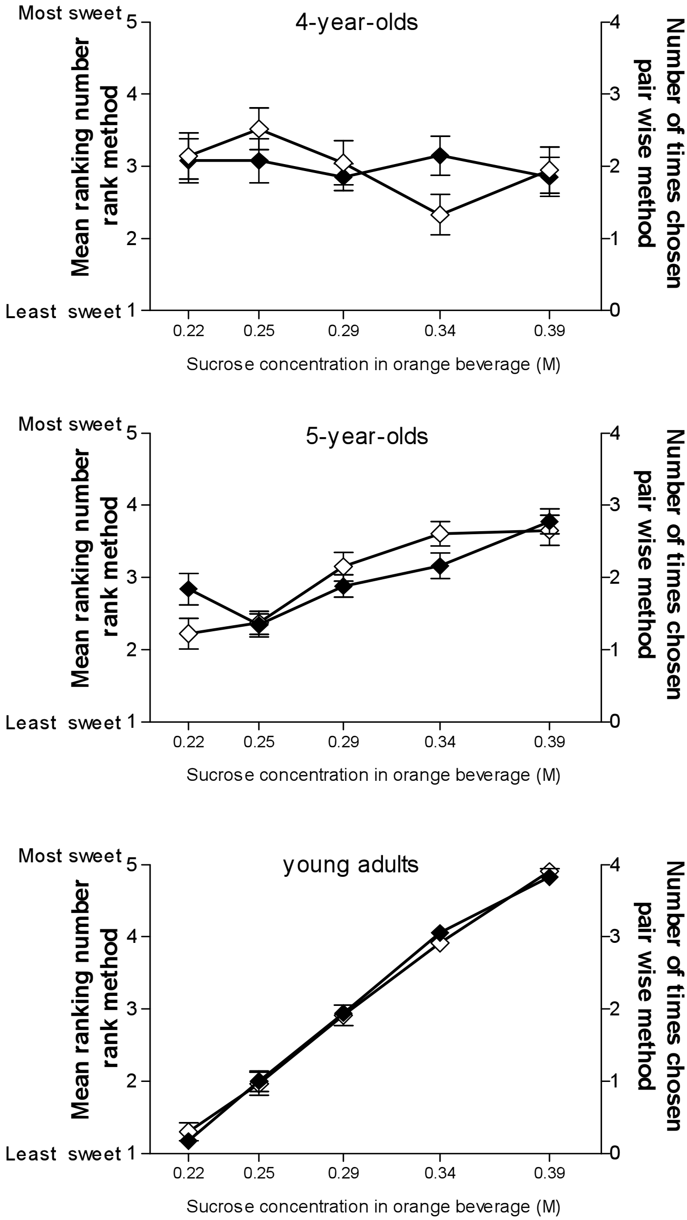

Figure 2 Result of the rank-order test (◊) (mean ranking number per solution ± sem) and paired comparison test (♦) (mean number of times each solution was chosen as most sweet ± sem) for discriminatory ability, 4-year-olds, 5-year-olds and young adults

Four-year-olds were not able to identify differences in sweetness across the five different sugar concentrations. This was the case for both methods (rank-order tests: $F_r(4df)=6.3$, $P=0.18$; paired comparison tests: $F_r(4df)=1.5$, $P=0.82$). Both methods showed a 62.7% consistency with each other and were not significantly correlated with each other (Pearson's r=0.02; *P*=0.95).

Young adults were able to identify differences in sweetness across the five different sugar concentrations (rank-order tests: F_r(4df)=77.9, P<0.0001; paired comparison tests: F_r(4df)=63.5, *P*<0.0001). Both methods showed an 86% consistency with each other. However, there was a low correlation between both tests (Pearson's r=0.09; P=0.72) (see Table 2).

Preference, children and young adults

Figure 3 shows the results of the rank-order and paired comparison tests for preference. Children preferred higher sugar concentrations in orangeade than adults.

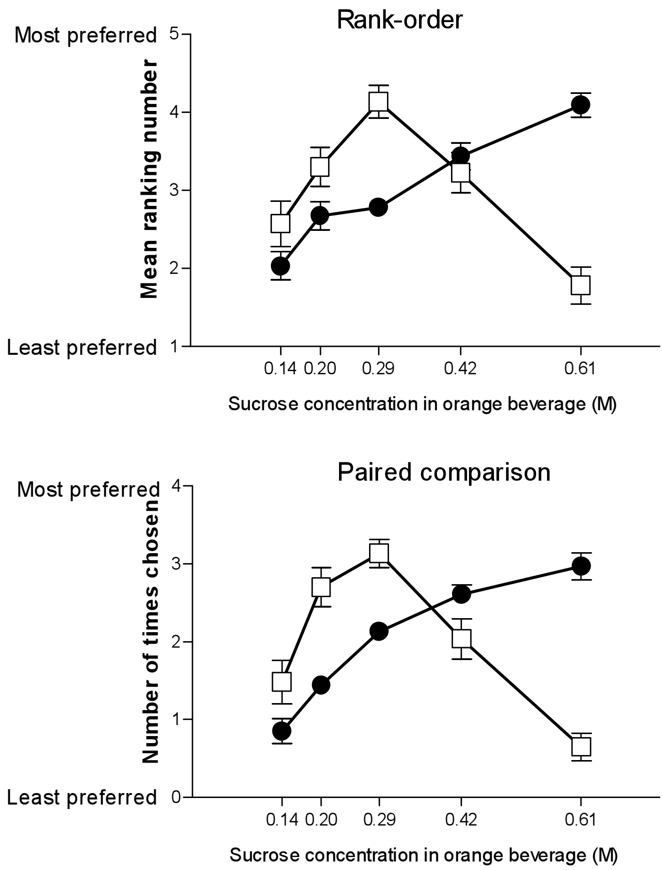

Figure 3 Result of the rank-order test (mean ranking number per solution ± sem) and paired comparison test (mean number of times each solution was chosen as most sweet ± sem) for preference, children (●) and young adults (□)

This was evident in both tests for preference (rank-order tests: (t(88df)=4.2, *P*<0.0001; paired comparison tests: t(88df)=5.6, *P*<0.001). No significant differences were observed between girls and boys during the rank-order tests (t(66df)=0.70; *P*=0.50), or during the paired comparison tests (t(66df)=1.3; *P*=0.18). Likewise, no significant differences were observed between adult females and males during the rank-order tests (t(20df)=2.0; *P*=0.06), or during the paired comparison tests (t(20df)=1.7; *P*=0.10).

Preference, 4-and 5-year-olds, and young adults

All subjects could identify differences in preference across the five stimuli (4-year-olds: rank-order tests: F_r(4df)=12.0, *P*<0.05; paired comparison tests: F_r(4df)=2.2, *P*<0.0001- 5-year-olds: rank-order tests: F_r(4df)=47.7, *P*<0.0001; paired comparison tests: F_r(4df)=67.4, *P*<0.0001- young adults: rank-order tests: F_r(4df)=28.4, *P*<0.0001; paired comparison tests: F_r(4df)=38.9, *P*<0.0001; see **Table 3**). No significant differences in sweet preference were observed between 4- and 5-year-olds (rank-order tests: t(66df)=-0.71, *P*=0.48; paired comparison tests: t(66df)=-0.88, *P*=0.38).

Five-year-olds and adults, showed a high consistency (young adults: 87.1 ± 2.2; 5-year-olds: 76.2 ± 3.4) and a significant correlation (5-year-olds: Pearson's r=0.74, *P*<0.0001; young adults: Pearson's r=0.91, *P*<0.0001) between both tests for preference.

Four-year-olds showed a 61.2% consistency and no significant correlation between preference tests (Pearson's r=0.32; *P*=0.37; see Table 3). However, both tests showed an increased preference with an increased concentration of sugar.

Table 3 Mean (± sem) preference score for rank-order and paired comparison test; young adults, kindergarten, 4- and-5-year-olds.

Preference test			Kindergarten	
	Young adults	**Kindergarten**	**4-year-olds**	**5-year-olds**
	n=22	*n*=68	*n*=21	*n*=47
Rank-order	28.4 ± 1.4	34.9 ± 0.80[a]	33.8 ± 1.4[a]	35.3 ± 1.0[a]
Paired comparison	27.7 ± 5.9	35.4 ± 0.7[a]	34.6 ± 1.2[a]	35.7 ± 0.9[a]
Pearson correlation coefficient between rank and pair-wise	0.91 (*P*=0.0001)	0.71 (*P*=0.0001)	0.32 (*P*=0.37)	0.83 (*P*=0.0001)
Mean consistency	87.1 ± 2.2	71.6 ± 3.1[a]	61.2 ± 6.1[ab]	76.2 ± 3.4[a]

Minimum=20, maximum=40. The higher this score, the higher the sugar concentration of the most preferred solution

[a] Significantly different compared to young adults; *P*<0.01

[b] Significantly difference compared to 5-year-olds, *P*<0.05

DISCUSSION

The present study suggests that 5-year-old children were able to carry out rank-order and paired comparison tests for discriminatory ability in a consistent manner. However, 5-year-olds were less able to discriminate between different sugar concentrations in an orange drink than adults. Four-year-olds failed to carry out both discriminatory ability tests in a consistent way. This was in contrast with the tests for preference. In these tests, young adults and both 4-and-5-year-old children, responded consistently during the rank-order and paired comparison tests for preference. Furthermore, the present study suggests that children preferred higher concentrations of sugar in orangeade than adults.

A possible explanation for the lower discriminatory ability of 5-year-olds compared to adults, is that children analyzed taste mixtures differently [21]. The beverages that were used in the present study, were mixtures of sucrose and citric acid. Oram and colleagues suggested that in a mixture of sucrose and citric acid, children only recognize sweetness, whereas adults have the ability to recognize both tastants [21]. It is possible that children's taste system may not be fully developed for processing taste mixtures. This could result in lower discriminatory ability. In the same line, the use of orange flavor in the present study may have distracted the children. If this were the case, sensory tests with sugar water would result in a similar discriminatory ability of children and adults. But, studies that used sugar water as the test stimulus, have resulted in conflicting results [3;5]. De Graaf and Zandstra [5] suggested that, similar to sweetened orange drinks, children are less able to discriminate between different concentrations of sucrose in water than adults. This is in contrast with Enns and colleagues [3], who found a steeper slope of the psychophysical function of sucrose for 8-10-year-old children compared to adults. The latter study, however, used magnitude estimation. The steeper slope could therefore be a result of a difference in rating behavior. More research is needed to determine whether the lower discriminatory ability of children is specific to taste mixtures.

It is unlikely that the lower discriminatory ability of 5-year-olds was a result of the children's inability to understand the tests. Recall that in the present study, both adults and 5-year-olds showed a high consistency between the rank-order tests and the paired comparison tests. Furthermore, our findings are in line with earlier research that found that young children are less able to discriminate between different sugar solutions [4-6].

The observed difference in discriminatory ability between 4-and 5-year-old children was unlikely due to differences in sensitivity for sweet tastes. Although 4-year-olds could not tell the difference between the highest and lowest concentration of sucrose during the discriminatory ability test, they consistently preferred the sweeter solution within each pair.

Perhaps 4-year-olds were less able to maintain attention than the 5-year-olds [20]. However, the preference tests, which the 4-year-olds were successfully able to perform, took the same amount of time as the discriminatory ability tests. The discriminatory ability tests were supposedly more difficult than the preference tests. In contrast with tests for preference, in order to perform well during discriminatory ability tests, subjects had to clearly understand what the researcher meant by the specific dimension (e.g. sweetness) [22]. The preference tests were fairly intuitive tasks, in which stimuli could be judged holistically [23]. In other words the discriminatory ability tests required more cognitive abilities than the preference tests. It has been suggested that 4-and 5-year-old children are rapidly changing with respect to cognitive abilities [20]. Therefore, four-year-olds may require more intensive training in order to do well during the discriminatory ability tests.

In the present study subjects were made more familiar with the task and stimuli by offering them two solutions from the series that was used. This may not be sufficient for 4-year-olds. Watson and colleagues offered 8-9-year-old children three sweet solutions with different taste qualities (i.e. sweet, salty, water), and trained them to recognize each taste quality [24]. They suggest that this procedure overcame cognitive problems some children had in recognizing tastants in mixtures.

In the present study, the paired comparison and the rank-order tests for preference gave consistent data for both young adults and 5-year-old children. This is in line with previous research that used stimuli that were easy to discriminate [9;12;13;16]. The present study suggests that both methods are also reliable when using stimuli that are difficult to discriminate. For 4-year -olds, consistency between both tests was lower than for older children, which is in agreement with previous research [12]. However, in the present study both tests showed an increase in preference as sweetness increased. This is in line with the published literature concerning sweet preference in children [1;4;25-30]. It is therefore concluded, that rank-order as well as paired comparison tests are useful tools to measure sweet preference in orange beverages for 4-year-olds. The finding that children preferred higher levels of sweet compared to adults is consistent with the published literature [1-5].

The findings in the present study need to be viewed with caution. At least six limitations could have influenced the results of the present study. First, because testing took place at the beginning of the school year, there were more 5-year-old-children than 4-year-old children included. More 4-year-olds were expected to enter school during the following months. It is, however, unlikely that this difference explains our finding. Recall that no differences between the two age groups were observed for sweet preferences.

Secondly, in the present study only rank-order and paired comparison tests were carried out. However, the researchers suggest that the present findings can be generalized to other and more complex indirect scaling methods, such as stair-case method [31;32] and duo-trio [6]. These tests, as well as those investigated in the present study, depend largely on the subjects' cognitive abilities.

Thirdly, in the present study only orange beverages were used as test stimuli. It needs to be determined whether the results of the present study can be replicated by using other sweet stimuli, such as solid food items.

In the present study the paired comparison tests always preceded the rank-order tests. In this way subjects could get used to the testing procedures and environment while performing the paired comparison tests, which were considered to be easier than rank-order tests. A further limitation of this study is that the order of testing could have resulted in an order effect. Subjects might have paid less attention to the rank-order tests, because it was the second test in line. It could be that subjects got less concentrated. This could have resulted in a higher random error in the outcome of the rank-order tests. However, the standard errors of the paired comparison and the rank-order tests were similar.

In the present study the discriminatory ability was measured on day one, whereas preference was measured on day two, to avoid confusion. If the tests were carried out in random order, children and the adults who supervised the children could easily have confused "sweeter" with "more preferred". Hypothetically, subjects performed better on the second day, because they obtained experience with sensory testing during the first day. This could explain why 4-year-olds performed better during the preference tests than during the discrimination tests.

A sixth limitation of the present study is the way the question during the discrimination tests was asked; i.e. "In which beverage did we put the most sugar". Although all children appeared to understand that large amounts of sugar are present in sweet food items, the question could potentially have confused 4-year-old children. On the other hand, visualization of the categories "more sweet" and "less sweet" as done in the present study, could help these children understand both categories. Printed words could not be used, because 4-year-olds lack reading skills. Instead, we used more or less lumps of sugar. In order to match the question with the categories, we asked "In which beverage did we put the most sugar".

In conclusion, rank-order and paired comparison tests are not suitable to measure discriminatory ability in 4-year-old children. These tests are, however, suitable to measure sweet preferences in these children. This study showed large differences between 4-and 5-year olds in performing discriminatory ability tests. More research is needed do develop a reliable tool to measure discriminatory ability in 4-year-old children.

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Heightened sour preferences during childhood

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ABSTRACT

Background: Basic research has revealed that the chemical sensory world of children is different from that of adults, as evidenced by their heightened preferences for sweet and salty tastes. However, little is known about the ontogeny of sour taste preferences, despite the growing market of extreme sour candies.

Objective: The present study investigated the level of sourness most preferred in a food matrix and whether the ability to discriminate differences in sour intensity differed between 5- to 9-year-old children and their mothers.

Design: Preference and discriminatory ability were measured by a rank-by-elimination procedure embedded in the context of a game. Mothers also completed a variety of questionnaires and children were asked several questions to assess whether children's temperament and food preferences and habits related to sour preferences.

Results: The results indicated that, although every mother and all but two of the children (92%) were able to rank the gelatins from most to least sour, more than one-third (35%) of the children, but virtually none of the adults, preferred the high levels of sour taste (0.25 M citric acid) in gelatin. Those children who preferred the extreme sour tastes were significantly less food neophobic ($P<0.05$) and tended to experience a greater variety of fruits when compared with the remaining children ($P=0.11$). Moreover, the children's preference for sour tastes generalized to other foods, such as candies and lemons, as reported by both children and mothers.

Conclusion: These findings are the first experimental evidence to demonstrate that sour taste preferences are heightened during childhood and that such preferences are related to children's food habits and preferences. Further research is needed to unfold the relationship between the level of sour taste preferred and the actual consumption of sour-tasting foods and flavors in children.

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INTRODUCTION

> I will add that formerly it looked to me as if the sense of taste, at least with my own children when they were still very young, was different from the adult sense of taste; this shows itself by the fact that they did not refuse rhubarb with some sugar and milk which is for us an abominable disgusting mixture and by the fact that they strongly preferred the most sour and tart fruits, as for instance unripe gooseberries and Holz apples. (Darwin, 1877)[1]

Charles Darwin keenly observed that children live in different chemical sensory worlds than adults, as evidenced by their heightened preference for sweet and sour tasting foods [1]. Although basic research confirmed his observations on sweet preferences a century later [2-8], little scientific investigation has focused on the ontogeny of sour taste preferences [9;10]. In spite of this paucity of basic research, industry has clearly found a niche in children for extreme sour candies, which are reported to be quite aversive to adults [11].

Some contend that children's preference for extreme sour tastes is secondary to their desire for adventure, thrills and excesses [11;12], of which these products supposedly provide. To our knowledge, there are no published reports on whether aspects of temperament or attitudes toward foods, in general, relate to children's preferences for sour tastes. However, a study on adults revealed that those who experienced greater dietary diversity preferred higher levels of sour intensity when compared with food neophobic adults [13].

The present study aimed to test the hypotheses that children prefer higher levels of sourness in foods when compared with adults, and that such preferences are positively related to their willingness to try new foods and experience with dietary diversity. A rank-by-elimination and randomized order procedure, embedded in the context of a game, was used to assess sour preferences as well as the ability to discriminate differences in sour intensity in 5- to 9-year-old children. The mothers of these children were also tested using identical procedures in order to determine sour preferences in an adult population. Mothers also completed a variety of questionnaires and children were asked whether they had ever tried sour-flavored candies, to determine whether personality factors, such as temperament and attitudes towards or experience with foods in general, contribute to preferences for sour taste and flavors.

METHODS

Subjects

Mothers were recruited from advertisements in local newspapers. The mothers (31 Caucasian, 26 African American, 1 Asian and 3 from other ethnic groups) were, on average, 37.8 ± 0.7 yrs of age, and their children (29 girls, 32 boys) ranged in age from 5 to 9 years (mean=7.4 ± 0.2 years). During a telephone interview, the mother, who was not informed of the hypothesis of the study, was told that she and her child would participate in a 'taste study' in which they would be asked to taste different flavored gelatins. Seven additional children began, but did not complete, testing because they could not understand the task. All children were reported by their mothers to be healthy at the time of testing. The testing procedures were approved by the Office of Regulatory Affairs at the University of Pennsylvania. Informed consent was obtained from each parent and assent was obtained from each child who was eight years of age or older.

General procedures and stimuli

Children and their mothers were individually tested at the Monell Center in a closed room specifically designed for sensory testing, with a high air-turnover ventilation system. After subjects acclimated to the room and personnel, we assessed their preferences for sourness. To this end, four lemon-flavored gelatins were made by either adding no citric acid (0.00 M) or different concentrations of citric acid (0.02, 0.08 and 0.25M; Sigma Chemical Co., St Louis, MO) to 22 g of a stock gelatin dissolved in 473.2 g of water (JELL-O-., Kraft Foods, Inc., Rye Brook, NY). It should be noted that the stock gelatin has a sweet taste because it contains ~0.24 M sucrose and 0.30 M glucose (First Data Bank, Inc, San Bruno, CA). Twenty milliliters of each gelatin was poured into a 30 mL clear medicine cups (Delaware Valley Surgical Supply, Boothwyn, PA) and refrigerated for at least 4 h to obtain firmness. During testing, the gelatins were stored on a tray containing crushed ice.

Preference rankings methods

An age appropriate, game-like task that was fun for children and minimized the impact of language development was used to examine sour preferences. Using a rank-by-elimination and randomized ordered procedure [14], subjects tasted each of the four gelatins and were asked to point to the one that they liked best. This gelatin was then removed after which subjects were asked to taste the remaining three gelatins again and then indicate which of the three was most preferred. This procedure continued until a rank order preference was established. To determine reliability, subjects were presented, in counterbalanced order, the gelatins ranked as their two most preferred and were asked to point to which of the pair they liked best. Subjects rinsed their mouth twice with bottled water after tasting each gelatin.

Intensity rankings methods

A second test session was conducted ~7.0 ± 0.5 weeks later to determine whether subjects could distinguish the different intensities of sour taste in the gelatins. We randomly selected 24 children (10 boys, 14 girls) and their mothers from those who participated in the first session. Before testing, each subject was trained to distinguish three of the basic tastes: sweet (0.30M glucose; Sigma), salty (0.30M Na gluconate; Sigma) and sour (0.01M citric acid; Sigma). Subjects were then given three pairs of solutions that differed in sour intensity and were asked to focus only on sour taste and to point to which of the pair tasted more sour, rinsing their mouths twice after tasting each sample. All subjects were able to perform this task.

After a five-minute break during which subjects were offered an unsalted cracker and a cup of water to cleanse their palate, they were asked to rank the four gelatins used during the first session (0.00, 0.02, 0.08 and 0.25M added citric acid) from most to least sour. The rank-by-elimination procedure was identical to that described above for the preference test, except here subjects indicated which one tasted most sour. At the end of the test session, subjects were asked to taste, in counterbalanced order, the least (0.00 M added citric acid) and the most sour (0.25M added citric acid; hereafter referred to as 'extreme sour') gelatin. Immediately after tasting each gelatin, subjects were asked to respond in the affirmative or negative to the questions: Does this gelatin taste sweet? Does it taste sour? Subjects rinsed their mouths with water twice between tastings. One child did not understand the task and therefore was excluded.

Food habits, food neophobia and child temperament measures

Without communicating to the child, mothers completed a series of questionnaires which included a 10-item scale that measured food neophobia [15] and a eight-item scale that measured general neophobia in adults [15], and a 25-item scale that measured five temperament dimensions (i.e. emotionality, shyness, activity, sociability and negative reaction to foods in general) in their children [16]. In a few cases, mothers did not answer all questions and therefore some measures could not be calculated (see **Table 1**). Because we were also interested in assessing the children's reaction to new foods, mothers were asked to indicate on a 5-point scale (1= not at all characteristic of the child; 5= very characteristic of the child) their agreement with the statements 'My child is afraid to try new foods' and 'My child does not trust new foods' [15], as well as to indicate whether they regarded their child as a picky eater and whether they were picky eaters as children themselves. We also asked each mother whether she thought her child went 'through a sour phase' exhibiting strong preferences for sour candies or raw lemons. As a first step in investigating whether experience with a variety of sour foods (e.g. fruits) impacts upon children's sour preferences, all but six of the mothers indicated which of the following fruits their child had experienced at home during the past week (i.e. grapes, bananas, oranges, pineapple, melon, apples, apple sauce, pears, apricots, raisins and berries). Children were asked directly whether they had ever eaten and whether they liked extreme sour candies such as Warheads™. (Foreign Candy Company, Hull, IA); all but three of the children responded to these questions. In addition, all but one of the children were weighed and measured for height.

Statistical analyses

Sour taste preference and intensity rankings

The null hypothesis tested was that there were no systematic differences in children's or mothers' preference ranking of the four gelatins that differed in citric acid content (i.e. 0.0, 0.02, 0.08 and 0.25M added citric acid). To test this, each of the four gelatins was ranked according to subject's preferences (1= most preferred; 4= least preferred). Data obtained from mothers were analyzed separately from children. Separate Friedman two-way analyses of ranks were then conducted on these preference ranking scores. Similar analyses were conducted on the sour intensity ranking scores. When significant, multiple comparisons were performed to determine which differences among the gelatins were significant [17]. To test the reliability, identical responses between test and retest were defined as reflecting guessing if the proportion was below the upper limit of the 95% confidence interval for 50% correct responses, the latter being the predicted proportion if subjects were guessing [18]. All summary statistics are expressed as means ± sem and levels of significance were $P<0.05$.

To investigate whether there were differences in sour taste preferences between children and mothers, the frequencies of subjects who classified the extreme sour tasting gelatin (0.25M added citric acid) as either their most preferred (ranked 1 or 2; hereafter referred to as High-Sour group) or least preferred (ranked 3 or 4; hereafter referred to as Low-Sour group) were also determined. Chi-square statistics were then performed to determine whether children's preferences differed from adults; the Yates correction for continuity was applied to all chi-square analyses. To determine whether the children's sour preferences were related to their mothers' sour preferences, Kendall tau correlations were calculated for each child–mother pair.

Subject demographics, child temperament and food habits

One-way analyses of variance (ANOVA) were conducted to determine whether there were significant differences between the groups (High-Sour versus Low-Sour) on a variety of measures such as the ages, body mass index (BMI, in kg/m^2) and temperament scores of the children, and the age, and food and general neophobia scores of the mother. Chi-square analyses with Yates correction for continuity were performed to determine whether there were group differences in sex ratio, ethnicity and the proportion of children who were perceived as picky eaters.

RESULTS

Sour taste preferences

A striking difference emerged between children and their mothers in their preferences for the extreme sour gelatin. That is, more than one-third (35%) of the children (High-Sour group), but virtually none of the mothers, ranked the highest concentration of citric acid (0.25M added citric acid in gelatin) as one of their most preferred gelatins (χ^2(1df)=24.46; *P*<0.0001). Friedman analyses indicated significant differences in preference ranking of the four gelatins in both groups of children (Low-Sour group: F_r(3df)=81.73; *P*<0.0001; High-Sour group: F_r(3df)=23.07; *P*<0.001) as well as the mothers (F_r(3df)=127.20; *P*<0.00001). Post-hoc analyses revealed that children in the High-Sour group ranked the extreme sour gelatin as their most preferred and the least sour gelatin as their least preferred whereas the exact opposite was true for the remaining children (Low-Sour group) and mothers (all *P*<0.05) (see **Figure 1**). Furthermore, children in the Low-Sour group, as well as adults, preferred the gelatin containing 0.02M citric acid more than gelatins containing 0.08 and 0.25 M citric acid, although there were no significant differences in preference between the gelatin with no added citric acid and that with 0.02M citric acid.

That both groups of children and mothers understood the task is suggested by the strong agreements between their first and second preference rank ordering of the gelatins (High-Sour group: 80% (95% confidence limits=50 ± 23%); Low-Sour group: 92% (95% confidence limits=50 ± 17%); mothers: 98% (95% confidence limits=50 ± 13). No significant difference in sour preference was observed between children in the Low-Sour group and mothers (χ^2(1df)=0.04; *P*=0.84). Nor was a significant relationship observed between mother-child pairs in their preferences for sour flavors in gelatin (Kendall tau correlation, all *P*>0.30).

Sour intensity rankings

There were significant differences in the intensity ranking scores of the four gelatins in both groups of children (Low-Sour group: F_r(3df) = 45.00; *P*<0.0001; High-Sour group: F_r(3df)=24.87; *P*<0.0001) as well as in mothers (F_r(3df)=72.00; *P*<0.00001). That is, every mother and all but two of the children (92%) were able to rank the gelatins from most to least sour in an errorless fashion thus suggesting that they perceived the different sour intensities in the gelatins. Furthermore, every child and adult reported that the 0.25M citric acid gelatin tasted sour but the 0.0M citric acid gelatin did not. Likewise, the majority of subjects (High-Sour group: 66.7%, n = 6, Low-Sour group: 80%, n = 12, Adults: 95.8%, n = 23) reported that the 0.0M citric acid gelatin tasted sweet. There were no significant differences between the groups (High-Sour versus Low-Sour group, χ^2(1df) = 0.06; P=0.81; High-Sour group versus adults, χ^2(1df)=2.85; P=0.10; Low-Sour group versus adults, χ^2(1df)=1.09; P=0.30).

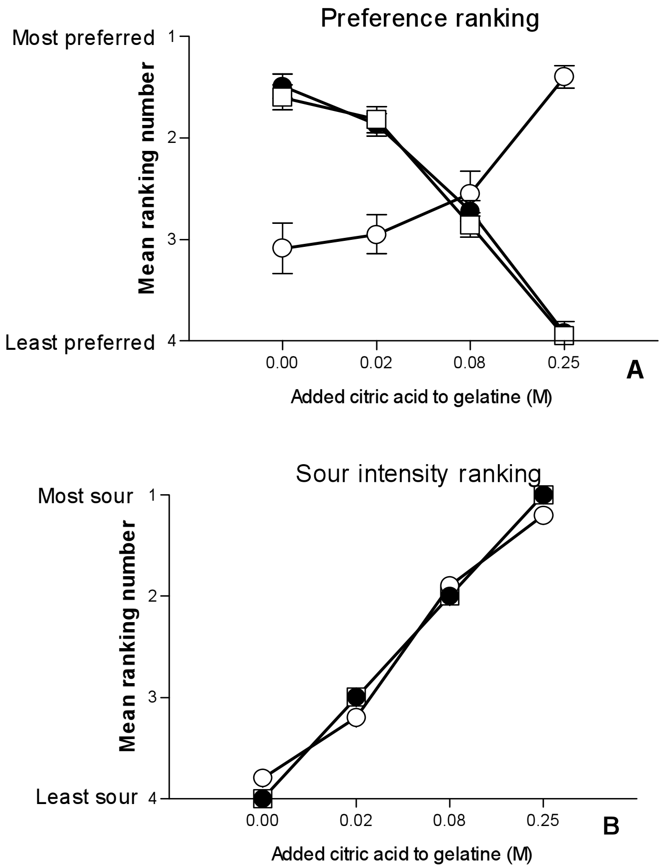

Figure 1 Mean (± sem) preference ranking (Top Panel A) and sour intensity ranking (Bottom Panel B) for 0.00, 0.03, 0.08 and 0.25M of added citric acid to gelatin in children (○,●) and their mothers (□). Children were divided into groups based on the level of sourness preferred in gelatin; those in the High-Sour Group (○, n=22) ranked the 0.25M gelatin as one of their most preferred gelatins whereas those in the Low-Sour Group (●, n=39) did not

Subject characteristics, child temperament and food experience

Further analyses were then conducted to determine whether there were significant differences on any of the measures studied between these High-Sour and Low-Sour groups of children. Salient characteristics of these two groups of children and their mothers are listed in the Table. No significant differences between the groups were observed for the children's ages ($F(1,59df)=0.99$; $P=0.32$), BMI ($F(1,58df)=1.28$; $P=0.26$), proportion of girls to boys ($\chi^2(1df)=0.31$; $P=0.58$) and ethnicity ($\chi^2(3df)=6.61$; $P=0.09$); nor was there any difference between the groups in the mothers' age ($F(1,58df)=0.89$; $P=0.35$), or the mothers' food neophobia ($F(1,59df)=0.74$; $P=0.39$) and general neophobia ($F(1,59df)=1.94$; $P=0.17$) scores.

In addition, there were no significant differences in the children's temperament dimensions of shyness ($F(1,59df) = 0.25$; $P=0.162$), emotionality ($F(1,58df) =0.10$; $P=0.75$), sociability ($F(1,59df)=0.53$; $P=0.38$), activity ($F(1,59df)=1.14$; $P=0.29$) or overall negative reactions to foods ($F(1,58df)=0.32$; $P=0.57$). However, mothers of children in the Low-Sour group were significantly more likely to perceive their child as a picky eater ($\chi^2(1df)=4.47$; $P<0.05$), and were more likely to agree with the statements 'My child is afraid to try new foods' ($F(1,56df)=5.11$; $P<0.05$) and 'My child does not trust new foods' ($F(1,56df)=6.02$; $P<0.05$) when compared with those in the High-Sour group.

In addition, mothers who reported that their child was a picky eater tended to be more likely to report that they themselves were considered picky eaters when they were children ($\chi^2(1df)=3.41$; $P<0.06$). Furthermore, mothers who were considered picky eaters when they were children were more likely to be food neophobic as adults ($F(1,57df)=10.68$; $P<0.001$) when compared with mothers who were not considered picky eaters as children.

Those children who preferred the extreme sour flavor in gelatins, as assessed in the laboratory, were significantly more likely to report not only that they had tried sour candies ($\chi^2(1df)=4.65$; $P=0.03$), but they liked their taste as well ($\chi^2(1df)=7.02$; $P<0.008$). That such sour preferences generalized to other foods is suggested by the findings that mothers of children in the High-Sour group were significantly more likely to report that their child had gone, or was going through, a phase of preferring sour foods such as lemons or candies ($\chi^2(1df)=3.76$; $P<0.05$), and tended to report that, at least in the home, their child experienced a larger variety of fruits during the last week when compared with the Low-Sour group ($F(1,53df)=2.59$; $P<0.11$).

Table 1 Subject characteristics of children who preferred 0.25 M citric acid in gelatin (High-Sour group) and those who did not (Low-Sour group)

	High-Sour group	**Low-Sour group**
Children's characteristics		
Age (yrs)	7.6 ± 0.3	7.3 ± 0.2
Sex (girls:boys)	12:10	17:22
BMI (kg/m^2)	18.2 ± 1.3	16.9 ± 0.5
Temperament measures[a]		
Shyness	2.2 ± 0.2	2.3 ± 0.1
Emotionality	2.8 ± 0.2	2.9 ± 0.2
Sociability	3.7 ± 0.1	3.6 ± 0.1
Negative reactions to foods	3.0 ± 0.2	3.2 ± 0.1
Activity	3.5 ± 0.2	3.7 ± 0.1
Percentage who tried sour candies	100.0	73.7*
Percentage who preferred sour candies	95.0	57.9*
Mothers' characteristics		
Age (yrs)	36.9 ± 1.2	38.4 ± 1.0
Food neophobia score[b]	37.1 ± 2.6	34.3 ± 2.0
General neophobia score[c]	21.5 ± 1.4	24.4 ± 1.4
Percentage of mothers who reported child went through a 'sour phase'	60.0	29.7*

[a] Child temperament measures could range from 1 to 5 (1= not at all characteristic of the child and 5= very characteristic of the child 16)
[b] The food neophobia score could range from 10 to 70
[c] The general neophobia score could range from 8 to 56
*Statistically significant differences between groups at P <0.05
All summary statistics are expressed as mean ± sem

DISCUSSION

The present study demonstrated, as Darwin [1] observed 125 years ago, that some children have heightened sour preferences when compared with adults. One-third of the 5- to 9-year-old children, but virtually none of the mothers, preferred extremely sour tastes in a novel context, that is, sour-flavored gelatin. Their preferences increased with increasing levels of citric acid. The remaining children exhibited the adult pattern such that preferences decreased with increasing levels of citric acid, a finding that is consistent with previous research on both young adults [19-21] and the elderly [21;22]. Those children who preferred the extreme sour tastes were less food neophobic and tended to experience a greater variety of fruits, as reported by their mothers, when compared with the remaining children. Moreover, the children's preferences for sour tastes generalized to other foods, such as candies and lemons, as reported by both children and mothers, and were not related to their mothers' sour taste preferences.

Three hypotheses, not mutually exclusive, could account for the differences in sour preferences within children and between children and adults. First, perhaps children who preferred the extreme sour taste could not discriminate between the different sour gelatins when compared with the other children and adults [23]. However, this seems highly unlikely for several reasons. First, the vast majority of the children were able to rank the gelatins from most to least sour in an errorless fashion. Secondly, children's ranking of the gelatins from most to least preferred was found to be a reliable measure, regardless of the level of sour most preferred. Thirdly, children, like adults, recognized the sour taste components in a complex food matrix such as gelatin. Nevertheless, additional research is needed to determine whether the two groups of children perceived sour tastes differently [6;24].

A second hypothesis, and one that is popular in the marketing field, is that children's preferences for sour tastes are secondary to their generalized preferences for adventure and thrills [11;12]. To our knowledge, there is no scientific basis for such claims. It is unknown whether children who have heightened preferences for sour tastes generalize this preference to other senses, such as vision (e.g. bright colors) and hearing (e.g. loud noises), or other tastes (e.g. sweet). However, previous research in our laboratory revealed that preference for extreme sour taste in children was not related to heightened sweet preferences [25]. Although the present study did not measure thrill-seeking behavior or sensory reactivity *per se*, children who preferred extreme sour tastes did not differ significantly from the other children in a variety of temperament dimensions, such as shyness, emotionality or sociability. What appears to be significant is the degree of adventure as it relates to new foods, however. That is, like adults [13], children who preferred extreme sour tastes were less food neophobic and less likely to be perceived as picky eaters by their mothers.

Perhaps individuals who are less food neophobic were more likely to experience extremely sour foods and, after repeated exposure, developed preferences for such flavors [26-28]. Consistent with this suggestion is the finding that children who preferred the extreme sour tastes in the gelatin (High-Sour group) were significantly more likely to try extreme sour candy such as Warheads™., and to report that they liked the flavor of these candies when compared with the Low-Sour group. Therefore, we hypothesize that children who preferred extreme sour tastes are not only more likely to *try* extreme sour foods but they continue to eat such foods and subsequently develop a preference for extremely sour flavors.

This hypothesis is consistent with previous research on adults [19] and children [25;29] that revealed that repeated exposure to sour flavors may lead to subsequent preferences. Children who were fed a formula that has a sour and bitter flavor component (i.e. protein hydrolysate formulas) during their infancy preferred sour-flavored juices significantly more than did children who were not exposed to such formulas [29]. Because no differences were observed in their sweet preferences, the effect of early experience appeared to be specific to sour tastes. Of interest is the finding that Indian laborers, whose diet consists of many sour foods, such as tamarind fruits, preferred higher levels of citric acid in water when compared with those living in Western populations whose diet had less of an emphasis on sour foods [19].

The present study revealed that children who preferred extreme sour candies such as Warheads™ were significantly more likely to prefer the extreme sour tastes in gelatins. It should be noted that the methods used to assess preference for gelatins (i.e. rank-by-elimination procedures) are limited because the determined level of preferences is relative to the other stimuli presented. However, we emphasize that children in the High-Sour group also reported that they preferred a variety of other sour-flavored food items thus suggesting that these children indeed preferred sour taste.

Whether the heightened sour preference for these sour tasting candies and food exhibited in one-third of the children decreases with age is unknown. Nor do we know whether the preference for sour tastes is due to repeated exposure to these extreme sour candies, which were introduced into the American market during the past decade [11]. However, Darwin's description of his children's preference for tart apples [1] and the report by mothers in the present study that their children went through a sour phase (e.g. preferred lemons) suggest that the heightened sour preferences during childhood can be expressed via a variety of sour-tasting foods, not just candies.

An alternative explanation is that the effects of experience with sour foods may be secondary to effects of experience with dietary diversity since the present study also revealed that children who preferred extreme sour tastes tended to experience a larger variety of fruits. Of interest is the recent finding that children who are food neophobic consume a diet consisting of less dietary variety when compared with children who do not exhibit such behavior [30;31]. That dietary diversity enhances acceptance of new foods has been demonstrated in human infants [32] as well as animal models [33]. In young children, dietary diversity is determined, in part, by the availability of foods provided by their caretakers and dietary patterns and attitudes towards foods are largely influenced by mothers [34;35]. In particular, fruit and vegetable intake by young children is positively related to parental fruit and vegetable intake [35]. The present study revealed that mothers, who reported being picky eaters as children, tended to view their own children as picky eaters and food neophobic. Whether such mothers are providing less dietary diversity, which, in turn, is related to their children's sour preferences and consumption of sour tasting foods, is unknown.

A third, and not mutually exclusive, hypothesis is that there are ontogenic changes in taste perception, independent of experience, that underlie the heightened sour preferences in some children. Responsiveness to salt and sweet tastes provides perhaps the clearest example of a developmental change to taste stimuli that occurs postnatally [4;36;37]. Although human newborns are indifferent to salt taste, preference for salt emerges at ~4–6 months of age, remains heightened throughout childhood and adolescence, and then decreases to levels resembling that of the adult during late adolescence [4]. The shift from indifference to preference is thought to be largely unlearned and due to postnatal maturation of central and/or peripheral mechanisms underlying salt taste perception, as suggested in animal model studies [38]. Like salt taste, preferences for sweet tastes remain heightened during infancy and childhood and decrease to levels resembling that of the adult during late adolescence [36]. That heightened sweet preferences during development has been observed in animal model studies [39] suggested that experience with sweets during ontogeny cannot exclusively account for this decline in sweet preference [36].

Although the mechanisms underlying these age-related changes in sweet and salt preferences during late adolescence remain unknown, we suggest that similar age-related changes may be occurring for sour taste in some children. In adults, the perception of sour is related, but not exclusively, to pH and salivary flow [40-42]. That is, adults with high salivary flow rates and pH rated sour stimuli consistently more intense when compared with those with lower salivary flow rates and pH [42]. This elevation in perceived intensity is presumably due to the greater contrast between the pH of the stimulus and individual's salivary pH. Whether the enhanced sour preferences observed in some children is related to differences in these physiological measures remains to be determined. Moreover, longitudinal studies on sour preferences, like those conducted on the ontogeny of sweet and salt taste preferences, are needed.

The findings of the present study further support the contention that children are living in different chemical sensory worlds when compared with each other as well as to adults. Such differences in sensory preferences may play a role in acceptance of and preference for certain foods and flavors. Previous research suggests that sensory preferences and experiences with foods are better predictors of fruit and vegetable consumption in children than the foods' nutritional content or social value [43]. Consideration of the relationship between the level of sour taste preferred and actual consumption of sour-tasting foods and flavors is an important area for future research.

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4

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Sour taste preferences of children relates to preferences for novel and intense stimuli

Chemical senses, in press

ABSTRACT

Background: Previous research suggested that some children have a preference for sour tastes. The origin of this preference remains unclear.
Objective: We investigated whether preference for sour tastes is related to a difference in rated sour intensity due to physiological properties of saliva, or to an overall preference for extreme (i.e. bright colors) and new stimuli.
Design: Eighty-nine 7-to-12-year old children carried out a rank-order procedure for preference and category scale for perceived intensity for 4 gelatines (i.e. 0.0, 0.02, 0.08, and 0.25M added citric acid) and 4 yellow cards that differed in brightness. In addition we measured their willingness to try a candy with an unknown flavor, their salivary flow and the buffering capacity of their saliva.
Results: Fifty-eight of the children tested preferred one of the two most sour gelatines. These children had a higher preference for the brightest color ($P<0.05$) and were more likely to try the candy with the unknown flavor ($P<0.001$) than children who did not prefer the extreme sour gelatines. Preference for sour taste was not related with differences in rated sour intensity, however those who preferred sour taste had a higher salivary flow ($P<0.05$).
Conclusion: A substantial part of young children have a preference for extreme sour taste. This appears to be related to the willingness to try unknown foods and preference for intense visual stimuli. Further research is needed to investigate how these findings can be implemented in the promotion of sour tasting foods such as fruit

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INTRODUCTION

The food choices of children in industrialized countries are an important determinant of the development of obesity during childhood [1;2]. Children's food choices are for the most part determined by their taste preferences [3-8].

Since the late sixties researchers try to understand the sensory-taste world of young children [9]. In the past four decades the investigations were mainly focused on sweet- , salt- and more recently bitter taste (see [10] for a review). However, little research focused on preferences for sour taste. Darwin (1877) already noted that his children had a preference for this taste quality [11]. A systematic scientific investigation of sour taste preferences of children, however, has not been carried out until recently. To our knowledge Liem and Mennella [8] were the first to show that a substantial part of the children (5-to 9-year-olds) they tested had a preference for high concentrations of citric acid in gelatin, which were perceived as extremely sour by their parents.

The basis of these sour taste preferences remains unknown. One hypothesis is that children who have a preference for high concentrations of citric acid in foods, rate this as less sour compared to those who do not prefer these concentrations of citric acid. In order to come to a similar sensation, those who rate a lower intensity need more stimulation of sour taste. In adults it has been suggested that a high salivary flow [12], high buffering capacity of saliva [13] and low salivary pH [14] are related to a low rated intensity of sour tasting foods.

An alternative, but not mutually exclusive hypothesis, is that preference for sour taste is secondary to their desire for adventures, thrills and excesses [15;16]. In this view, preferences for sour taste might be related to preferences for unfamiliar foods and intense stimuli perceived by other senses such as vision (e.g. bright colors). More general, preference for sour taste might be related to an overall thrill seeking behavior. Research suggests that this behavior is reflected by the rise in cortisol after encountering a challenging or stressful situation. That is, the increase in cortisol concentration in saliva, shortly after encountering a challenging or stressful situation, is larger for thrill-seekers compared with non-trill-seekers [17-19].

The present study had two main objectives. First, we investigated whether children who preferred sour taste, rated sourness as less intense. This might be due to a high salivary flow, a high buffering capacity of saliva and/or a low salivary pH. Secondly, we investigated whether children who preferred sour taste were more likely to prefer intense colors, are more likely to try new foods and/or are more thrill seekers in general, than those who do not prefer this taste.

METHODS

Subjects

Parents of 116 children were invited to participate in the study. They received a brochure with information that explained the procedures of the research. Parents of 92 children signed the informed consent. All children attended one of the two primary schools where the study was carried out. Exclusion criteria for participation were diabetes, sugar restriction in the diet, presumed allergies for one of the test stimuli and color blindness. In addition subjects with non-removable braces (*n*=3) where excluded from participation, because of the use of chewing gum during the test procedure. The final sample of subjects who participated in the study existed of 89 children (40 females and 49 males) ranging in age from 7-to 12- years (mean=10.3 ± 1.0 yrs) (see **Table 1**). The study protocol was approved by the Medical Ethical Committee of Human Nutrition of the Wageningen University.

Table 1 Children's characteristics of children who preferred 0.08M and 0.25M citric acid in gelatin (High-Sour group) and those who did not (Low-Sour group) (mean ± sd)

	Preference group based on level of sourness preferred in gelatine	
	Low-Sour group	**High-Sour group**
Children's characteristics		
Age (yrs)	10.2 ± 1	10.5 ± 1
Sex (girls: boys)	19:18	21:31
Weight (kg)	41.0 ± 9.1	41.0 ± 2.9
Height (m)	1.5 ± 0.1	1.5 ± 0.1
BMI (kg/m^2)	18.5 ± 2.9	17.9 ± 2.9

General overview

The present study involved a training session and two days of testing. During the training session, children were trained in recognizing sweet, sour, salt and bitter taste. During the two days of testing, separated by two days, the children carried out a variety of sensory tests. These tests were conducted at the children's primary school in a room that was familiar to them. This room existed of 10 low tables each, separated by a screen that prevented the children of seeing each other during testing. Children had personal guidance from a trained adult who sat in front of them.

The first day of testing aimed to determine children's preference (± 5 min) and rated sourness (± 5 min) for 4 gelatins that varied in the amount of added citric acid. After a 5-minute-break, in which we measured children's weight and height, we determined

children's willingness to try a novel food. We subsequently took a salivary sample, in order to determine the pH and cortisol concentrations of children's saliva.
The second day of testing started with a second salivary sample. After which we tested children's preference and perceived brightness for 4 yellow colored squares that varied in brightness. Furthermore, we measured the buffering capacity and flow rate of children's saliva. The order of testing (i.e. preference-intensity vs. intensity-preference) was balanced across subjects.

Stimuli

Gelatins

The gelatins were sweet lemon flavored (Rowntrees Wobbly Fruity Fun, Nestlé, UK) with different amounts of added citric acid (0.00, 0.02, 0.08 and 0.25M, Sigma Chemical Co., St Louis, MO, USA). Similar stimuli were previously used by Liem and Mennella [8]. Twenty milliliters of each gelatin were poured into a 30 mL clear medicine cup and refrigerated at 4^0C for at least 4 h to obtain firmness. They were transported in boxes were the temperature was kept constant at 4^0C. Several minutes before the actual test began, the gelatins were removed from the boxes and presented to the subjects.

Colors

The 4 colors were printed on small squares of 5 cm x 5 cm and were placed on larger white colored squares of 7 cm x 7 cm. They were different in intensities of the color yellow (soft yellow (SOY), lemon yellow (LY), canary yellow (CY) and sulphur yellow(SY) (Modo van Gelderen, Amsterdam, the Netherlands) and were used to measure children's preference and perceived brightness of colors. The colors were all different in brightness according the judgments of 6 adults (30 ± 8.2 yrs, 3 female and 3 males). From least intense to most intense, all adults, but one placed the colors in the following order: SOY, LY, CY, SY.

Training session

In order to assure that children were able to recognize, sweet, sour, salt and bitter taste, they were presented with 10 mL of a sweet solution (20% w/v sucrose in water) a sour solution (30% w/v natural lemon juice in water), a salt solution (20%w/v NaCl in water) and a bitter solution (Tonic-water, Schweppes International Ltd, Amstelveen, the Netherlands). After tasting each solution, the researcher asked the children whether it was sweet, sour, salty or bitter. The majority of the children were able to correctly identify the different solutions.

Preference test

The preferences for the different gelatins and colors were measured with a rank-by-elimination procedure [8]. Subjects tasted the four gelatins in a randomized order after which the researcher asked: “Which one do you like most?”. The children could either tell or point at the gelatin that was most preferred. This was then removed after which subjects were asked to taste the remaining three gelatins again. Subsequently the researcher asked: “Which one of these three do you like most?”. This procedure continued until a rank-order of preference was established. In order to determine reliability children were asked to rank the four gelatins again according to their preference. After tasting each gelatin, subjects drank a sip of water. The same procedure was followed for the four different colors, with the difference that subjects did not drink a sip of water after each stimulus.

The results of the rank-order test gave insight in how the different gelatins were preferred relative to each other. In order to have a direct measurement of preference, subjects were presented with two pictures, a smiley face and sad face. The researcher told the children the following: “I am going to give you one gelatin. If you like the taste I want you to give it to “smiley face”. If you do not like the taste, I want you to give it to “sad face””. Subsequently the researcher gave the children the gelatin with no added citric acid. After tasting each gelatin the subjects drank a sip of water

Rated sourness/ brightness test

During a child friendly game children were presented with each of the four gelatins in a randomized order. Children rated each gelatin on perceived sourness by using a 5-point-category scale. The 5 categories were labeled with, “not sour at all”, “a little bit sour”, “sour”, “very sour”, and “extremely sour”. Before the actual test began, the researcher explained the game by explaining each category of the 5-point scale. After subjects rated the four gelatins on perceived sourness, the gelatin with 0.08 M added citric acid was presented again in order to determine consistency of the test. A similar procedure was followed for the four different colors. The 5-point category scale was now labeled, “not bright at all”, “a little bit bright”, “bright”, “very bright”, “extremely bright”. In order to test consistency the color CY was presented twice.

Willingness to try a novel food

In order to test children’s willingness to try a novel food, children were presented with three identical white opaque cups in a randomized order. The cups were labeled with ‘z’, ‘y’ and ‘x’ and placed up side down. The researcher explained the test by saying: “Under each cup a candy is hidden. Each candy had its own taste. Under cup ‘z’ a candy with a strawberry flavor is hidden, under cup ‘y’ a candy with a raspberry flavor is hidden and under cup ‘x’ a candy with a mysterious flavor is hidden.” The subjects could not see the actual candies that were hidden inside the different cups. After the researcher clarified the content of each cup, the subjects were told that they were allowed to pick one candy to try. They could either point to the cup or tell the research which one they wanted to try. A similar procedure was used by Raudenbush and colleagues [20].

Collection of saliva

Salivary production was stimulated by having the child chew on sugarless gum (Freedent™ Wrigley’s Menthol without sugar, France). Before the collection started, children were asked to rinse their mouth with water and to swallow the saliva left in their mouth. Subsequently children were instructed to chew for 30 seconds on a piece of sugarless gum, without swallowing any saliva. After these 30 seconds, they expectorated their saliva directly in plastic tubes [21]. This procedure continued until at least 4 mL saliva was collected. If after 5 minutes the collected saliva did not reach a total of at least 4 mL, the collection was terminated. A similar procedure was previously used by Schwartz and colleagues [21]. Saliva was stored on dry ice and transported to the lab for the measurement of pH and cortisol concentration.

pH was measured by using pH indicator strips non-bleeding (pH 6.5-10.0) (Merck, Darmstadt, Germany). Salivary cortisol concentrations were measured by the LDN Cortisol saliva test (DSL Diagnostic Laboratory Systems, Houston, TX, USA)

Overall thrill seeking behavior

In order to measure general thrill seeking behavior in children, salivary cortisol was measured in a stress situation (hereafter referred to as stress-cortisol) and a non-stress situation (hereafter referred to as baseline-cortisol). We expected the children to be in a stress/challenge situation during the first day of testing due to the novelty of the sensory tests. The baseline cortisol was measured at the beginning of the second day of testing. We expected the cortisol in saliva to be at baseline, because the measurement took place at the start of the stressor/challenge (sensory tests).

Salivary flow and buffering capacity

Before the measurement of salivary flow and buffering capacity, children were asked to rinse their mouth with water and to swallow the saliva left in their mouth. Salivary flow and buffering capacity was determined by having children rinse their mouth for 30 seconds with 10 mL citric acid solution (0.03M citric acid, Sigma Chemical Co., St Louis, MO, USA , pH=2.5). After which they expectorated the citric acid solution in a plastic cup. Subsequently, the pH (Piccolo II, Hanna instruments, Bedfordshire, UK) and weight (Sartorius GMBH, Göttingen, Germany) of the expectorated solution was measured. A similar procedure was previously used by Dawes and colleagues [22]. The buffering capacity of saliva was defined as:

$$\frac{\text{pH after rinsing - pH before rinsing}}{\text{volume solution after rinsing - volume solution before rinsing}}$$

Statistical analyses

Sour taste preference and rated intensity

Subjects were divided into two groups based on their sour preferences. Subjects who classified at least one of the two most sour gelatins as their most preferred or second most preferred were grouped in the High-Sour group. The remaining subjects were grouped in the Low-Sour group. Reliability of the preference test for gelatins was defined as: the percentage of subjects that were grouped in the High-or Low-Sour group based on the first and the second preference ranking. Chi-squares were conducted to determine differences between the two group in the distribution of boys and girls, and differences in preference during the direct measurement of preference. Student t-tests were used to determine differences in age, height, weight, bmi. Wilcoxon analyses (Z) were conducted to determine differences between the first and the second intensity ratings for sour taste. Separate Friedman two-way analyses of ranks were performed to determine differences between the High- and Low-Sour group in intensity rank-order. When significant, multiple comparisons were carried out to determine which differences were significant [23]. Student t-tests determined differences in salivary pH, salivary flow rate and buffering capacity of saliva.

Sour taste preference, color preferences and thrill seeking behavior

Reliability for the preference test for colors was defined as: the percentage who preferred the brightest color the same during the first and the second ranking. Wilcoxon analyses (Z) were conducted to determine differences between the first and the second intensity ratings for color. Mann-Whithney U tests were performed to determine differences in preference for the 4 colors, between the High-and Low-Sour group. Chi-squares were conducted to determine differences in children's willingness to try a novel food. Log transformation were applied to the cortisol concentration in order to normalize the data. Student t-tests were carried out to determine differences in cortisol concentrations. All summary statistics are expressed as means ± sd.

RESULTS

Sour taste preferences and subject characteristics

Fifty-two children (58%) preferred one of the two most sour gelatins as either their most or second most preferred gelatin (hereafter referred to as High-Sour group). The consistency between the first and the second rank-order was 91%. Subjects in the High-Sour group showed an inverse U curve for preference with an increasing concentration of added citric acid. The most preferred stimulus was the gelatin with 0.08M added citric acid. Subjects in the Low-Sour group showed a decrease in preference with an increase in concentration of added citric acid (see **Figure 1 panel a**).

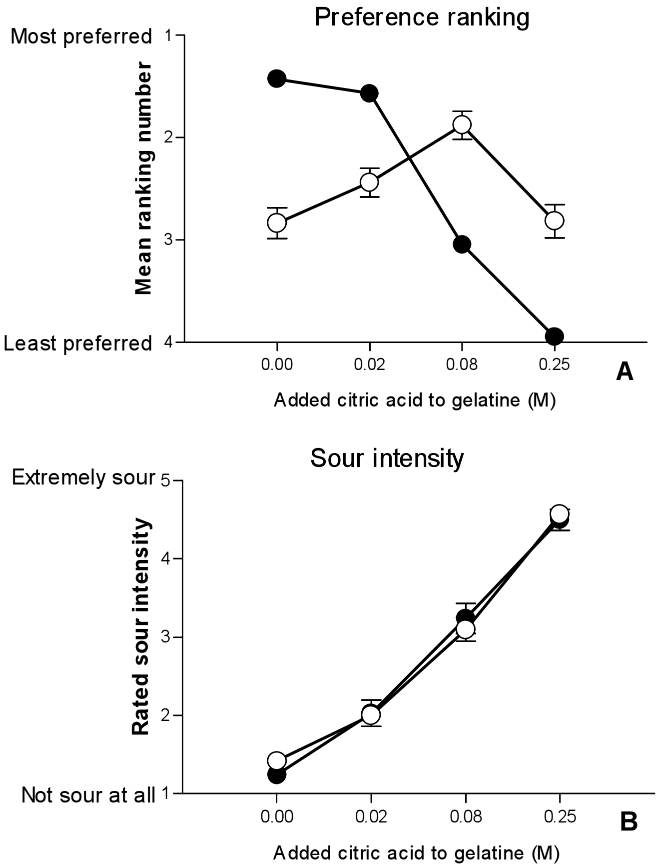

Figure 1 Mean (± sem) preference ranking (A) and sour intensity score (B) for 0.00, 0.03, 0.08 and 0.25M added citric acid to gelatin. Shown for children who preferred 0.08 and/or 0.25M added citric acid in gelatin (○, *n*=52) and those who did not (●, *n*=37)

Ninety-five percent of the children in the Low-Sour group and 65% of the children in the High-Sour group gave the gelatin with no added citric acid to “happy face”. This was significantly different (χ^2(1df)=10.6, *P*<0.01). Children in the High-and Low-Sour group were not significantly different in age (t(84df)=1.3, P=0.18), BMI (t(87df)=1.0, *P*=0.30) or proportion boys to girls (χ^2(1df)=1.1, *P*=0.31) (see Table 1)

Rated sour intensity

Children did not rate the perceived sourness of the gelatin with 0.08M added citric acid significantly different during the two times this gelatin was presented (Z=-3.0, *P*=0.76). No differences between the High- and Low-Sour group were observed in the rated sourness of any of the gelatins (no added citric acid: U=868.0, *P*=0.32; 0.02M added citric acid: U=960.0, *P*=0.99; 0.08M added citric acid: U=899.0, *P*=0.59; 0.25M added citric acid: U=918.5, *P*=0.80; see **Figure 1 panel b**). Children in both groups recognized differences in sourness across the 4 gelatins (Low-Sour: F_r=128.0, *P*<0.001; High-Sour: F_r=181.8, *P*<0.001).). Post-hoc analyses revealed that the gelatins with 0.08M and 0.25M added citric acid were rated as the most intense gelatins by children in the Low-Sour group and High-Sour group (*P*-value’s<0.05) (see Figure 1, panel b).

Salivary Flow, pH and buffering capacity

Children in the High-Sour group produced significantly more saliva, after stimulation with water with added citric acid, than children in the Low-Sour group (High-Sour: 1.8 g ± 1.0, Low-Sour: 2.3 g ± 0.8; t(79df)=-2.4, *P*<0.05). No differences, between the Low- and High-Sour group were observed between the salivary pH (High-Sour: 7.2 ± 0.12, Low-Sour: 7.2 ± 0.12; t(85df)=0.01, *P*=0.99) and the pH of the sour solution after subjects rinsed their mouth with this solution (t(79df)=-1.23, *P*=0.22). Children in the High-Sour group, however, had a significantly lower buffer capacity of their saliva than children in the Low-Sour group (High-Sour: 0.18 ± 0.07 (ΔpH/ml produced saliva), Low-Sour: 0.33 ± 0.44 (ΔpH/ml produced saliva); t(79df)=2.3, *P*<0.05).

Color preferences and rated brightness

Children in the High-Sour group were more likely to judge the SY color as their most favorite than children in the Low-Sour group (High-Sour: 75%; Low-Sour: 51%, χ^2(1df)=5.1, *P*<0.05, see **Figure 2 panel a**). Both children in the Low-and High-Sour group recognized differences in brightness across the 4 colors (Low-Sour: F_r=103.1, *P*<0.001; High-Sour: F_r=56.8, *P*<0.001). Post-hoc analyses revealed that the CY and SY colors were rated as the most intense by children in the Low-Sour group and High-Sour group (*P*-value’s<0.05) (see **Figure 2, panel b**). A significant difference was found between the first (3.1 ± 0.9) and the second time (3.4 ± 1.1) children rated the CY color on perceived brightness (Z=2.8, *P*<0.01).

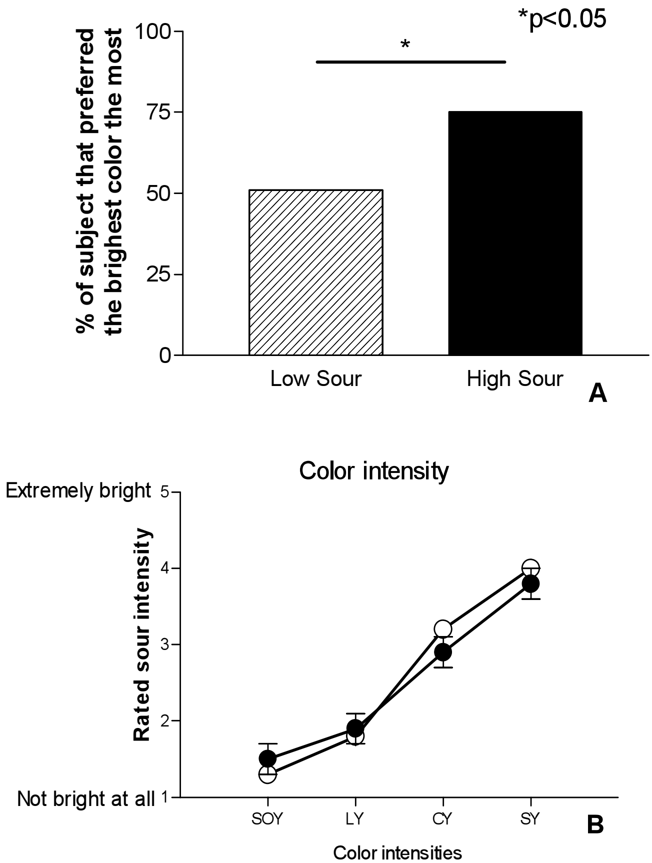

Figure 2 Percentage of subjects who preferred the brightest color the most (A) and intensity scores for brightness of the soft yellow (SOY), lemon yellow (LY), canary yellow (CY) and sulphur yellow (CY). Shown for children who preferred 0.08 and/or 0.25M added citric acid in gelatin (○, n=52) and those who did not (●, n=37)

Willingness to try a novel food and overall thrill seeking behavior

The candy with the unknown flavor was significantly more often chosen by children in the High-Sour group (67%) than by children in the Low-Sour group (36% (χ^2(1df)=7.6, P<0.001).

Children aged 7 through 10 yrs did not show a significant difference between their stress-cortisol and baseline-cortisol concentration (Low-Sour: t(27df)=-.80, P=0.43; High-Sour: t(39df)=-1.4, P=0.16). Children aged 11 through 12 yrs, however, did show a significant lower baseline-cortisol concentration than stress-cortisol concentration (Low-Sour: baseline 0.52 ± 0.04, stress 0.73 ± 0.06, t(13df)=3.2, P<0.01; High-Sour: baseline 0.67 ± 0.06 ng/mL, stress 0.94 ± 0.09 ng/mL, t(22df)=2.9, P<0.01).

Children in the Low- and High-Sour group were not statistically different with respect to baseline-cortisol (0.69 ± 0.38 ng/mL vs. 0.74 ± 0.46 ng/mL; t(85df)=-1.5, P=0.60) or stress-cortisol (0.69 ± 0.35 ng/mL vs. 0.81 ± 0.39 ng/mL; t(87df)=-1.5, P=0.14). The difference between baseline-cortisol and stress-cortisol concentration was also not different between the Low-Sour group and the High-Sour group (t(35df)=0.15, P=0.88).

DISCUSSION

The results of the present study suggest that young children's preference for sour taste is more likely to be related to behavioral determinants than differences in rated sourness as measured with a 5-point category scale. Salivary flow may play a role in the preference for sour taste, but a high salivary flow is not related to large differences in rated sourness in children who prefer this taste.

In the present study preferences for sour taste were measured by a rank-by-elimination procedure. Previous research showed that this is a reliable method to measure taste preferences of young children [24;25]. Moreover, children in the present study showed a high consistency between the two times they rank-ordered the gelatins according to their preference. The finding that a substantial part of the children tested (58%) had a preference for high concentrations of citric acid in gelatin, is in line with a previous study [8]. In the present study sour taste was defined as gelatins with added citric acid concentrations of 0.08 or 0.25M. In general children rated these gelatins between sour and extremely sour. It is likely that those who preferred sour taste during our sensory tests also preferred this taste outside the testing environment, as suggested by previous research [8;26]. This could be beneficial for the consumption of citrus fruit, which are in general high in vitamin C content. In the present study children, who preferred sourness in gelatins, appeared not to prefer the gelatins with no added citric acid. Supposedly, these gelatins were too bland in taste for those who preferred sour taste.

As shown in the present study, preference for sour taste was not related to a difference in rated sour intensity as measured with a 5-point category scale. The consistency between the first and second time subjects rated the gelatin with 0.08 added citric acid on perceived sourness, suggests the consistency of the testing procedure. Moreover, in general children rated the gelatins as more sour as citric acid content increased. We hypothesize that children in the High- and Low-Sour group not only rated the sour intensity similar, but also perceived the sour intensity similar. This is supported by the similarity of the pH of children's expectorated sour solutions. However, due to the methodology used in the present study to measure sour intensity, such hypothesis can not be confirmed. We can not assure that the adjectives used in our 5-point scale mean the same to all the children. Children in the High-sour group (preference for extreme sour taste) may judge a stimulus as extremely sour at lower perceived intensities than children in the Low-Sour group. Children in the High-sour group may have been limited in their responses by the scale and anchors that were used. Differences might have occurred when we used the label magnitude estimation scale with the anchors "not sour at all" and "most extreme sour ever tasted", but it needs to be determined whether such procedure is reliable when testing young children. Future research is needed to determine whether children who prefer sour taste, perceived it as less intense compared with children who do not prefer this taste.

Despite the similarity in rated sourness, as measured with a 5-point category scale, children in the High-Sour group had a higher salivary flow than children in the Low-Sour group. Initially, we hypothesized that high salivary flow would be related to a lower rated sourness, due to the dilution of the citric acid and the large amount of buffering agents [12]. However, as shown by the present study, the high salivary flow of children who preferred sour taste was not related to large differences in rated sourness. Previous investigations on the relationship between salivary flow rate and perceived taste intensity gave conflicting results. Some suggest that high salivary flow rates are related to a lower perceived intensity [12], whereas Norris and colleagues suggested the opposite [14]. Others, however, did not observe any relationship [13;27-29]. Hypothetically, the saliva of children in the High-Sour group had a lower buffering capacity per mL saliva compared with saliva of children in the Low-Sour group. This could explain why no differences were found in the rated sourness between the High- and Low-Sour group despite the differences in salivary flow. This hypothesis can, however, not be fully confirmed by the present study, because buffering capacity was not measured by means of titration.

It is more likely that preference for sour taste is related to children's general preference for intense and new stimuli. In the present study children who preferred sour taste were more willing to try a novel food than children who did not prefer this taste. A previous study suggests that parents of children who preferred sour taste were less likely to report that their child was afraid to try new foods [8]. Pliner and Loewen showed that children who were willing to try a novel food were less shy and emotional compared with those who were reluctant to try a novel food [30].

Children in the High-Sour group were also more likely to prefer a bright color as shown in the present study. Previous studies demonstrated that bright colors are more preferred by people with extrovert temperament than by people with an introvert temperament [31]. It needs to be noted that a significant difference was observed between the first time and the second time children rated the CY color on perceived brightness. It is unlikely that children were not able to carry out the test. Recall that children rated the colors in the same order of intensity as adults did. The difference between the first and the second rating of the color CY, could be a result of an order effect. The duplo was always presented last.

We suggest that children in the High-Sour group were more likely to be sensation seekers than children in the Low-Sour group. But, children in the High-Sour group and the Low-Sour group did not differ in overall sensation seeking behavior as measured by salivary cortisol. Studies by Gunnar suggest that surgent temperament (e.g. extrovert, sensation seeking) in children was related to a high reactivity of cortisol in reaction to a stressor [17-19]. The lack of difference between the High- and Low-Sour group in the reactivity of cortisol in the present study can be explained by at least three hypotheses.

First, especially in young children may experienced stress at the baseline measurement. Children were aware when testing was supposed to take place, this could have resulted in a stress response even before the actual testing began. This could explain why no differences in the reactivity of cortisol were measured in the young children. However, previous research indicated that baseline salivary cortisol concentrations of young children are most likely between 0.7ng/mL- 3.7 ng/mL [17-19;32;34]. In the present study, salivary cortisol concentrations in response to our 'stress' situation were on average between 0.73 ng/mL and 0.9 ng mL. A second hypothesis is therefore that the 'stress' situation in our study did not initiate real stress. In order for the "cortisol" system to respond, the situation must be perceived as potentially threatening [19]. A third hypothesis is that the cortisol concentration was not correctly measured. Cortisol, as well as other hormones in the body, follow a circadian rhythm. In general cortisol levels peak during the early morning and decreases during the afternoon [17;35;33]. In the present study stress-cortisol was measured during the late afternoon, whereas baseline-cortisol was measured during early afternoon. The circadian rhythm of cortisol concentration could potentially have diminished the difference between stress-and baseline cortisol levels.

Although the results of the present study are in favor of the behavioral determinants in the development of sour taste preferences, it cannot be excluded that the biological development of sense of taste play a role as well. Previous research suggested that the development of preferences for sweet and salt taste, are influenced by biological determinants such as energy requirement (sweet taste) [34] and postnatal maturation of central and/or peripheral mechanisms (salt taste) [35]. It remains unknown whether similar mechanisms are important in the development of preference for sour taste.

It has been suggested that children who prefer sour taste, experienced a large variety of fruit [8]. Preference for sour taste could therefore play an important role in the consumption of sour tasting fruits. Increasing preference for sour taste of children who initially do not like sour taste, is most likely to succeed if the sour food is not presented as novel and exciting. Children who do not prefer sour taste, should carefully be introduced to this taste quality. Subsequent repeated exposure could than slowly increase the preference for sour taste and hypothetically increase the consumption of sour fruits. This, however, needs to be investigated.

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Fruit consumption of young children is related to preferences for sour taste

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ABSTRACT

Background: It has been suggested that fruit consumption of children is mostly determined by children's preference for fruit. It remains unclear whether these preferences entail specific taste preferences.

Objective: The present study investigated whether preferences for sweet and sour taste of young children (n=50, mean age 9.2 ± 0.9 yrs) are related to their consumption of fruit.

Design: Preference for sour taste was measured with a rank-by-elimination procedure with 7 orangeades that differed in sourness (i.e. 0.009, 0.013, 0.020, 0.029, 0.043 and 0.065M added citric acid). Fruit consumption was assessed with a validated food-frequency questionnaire that was completed by the children's parents.

Results: Preference for sour taste of boys, but not girls was positively correlated with their consumption of fruit ($P<0.05$).

Conclusion: Preference for sour taste may be associated with the consumption of fruit in boys. In girls, preference for sour taste seems to be of less importance. Hypothetically, their consumption of fruit is more influenced by their parents, availability and health related motives.

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INTRODUCTION

An adequate consumption of fruit lowers the risk for cancer [1] and cardiovascular diseases [2]. Public health authorities in many industrialized countries have therefore defined a recommended daily consumption of fruit [3]. However, many children fail to meet these recommendations [4]. It has been suggested that the consumption of fruit of young children is determined by a variety of factors, among which parenting styles [5;6], fruit consumption of parents [7], availability and accessibility of fruit [8] and, most importantly, taste preferences [9-12].

In order to increase the consumption of fruit by means of children's preference for fruit, we first have to investigate what drives these preferences. Previous research suggests that children's food preferences can be established by a variety of factors, such as the energy density of the food [13], peer modeling [14] and parent-child interaction (see [15] for review). Most likely also *taste* preferences (e.g. sweet and sour taste) are related to children's preference for fruit. It has been suggested that preference for sweet taste is correlated with the consumption of sweet tasting products [16;17]. Preference for sour taste has been related with the consumption of sour tasting food [18;19]. Sweet and sour taste are major taste components of fruit (e.g. citrus fruit) generally consumed in the Netherlands [20]. It remains, however, unknown whether preferences for sweet and sour taste are important predictors of children's consumption of fruit. We therefore investigated whether sweet and sour preferences are related to children's total consumption of fruit.

METHODS

Subjects

Fifty healthy children (9.2 ± 0.9 yrs) participated in the study (see **Table 1**). Exclusion criteria for participation were: sucrose restriction in the diet on medical indication, and presumed allergies to sucrose and/or orangeades. The study protocol was approved by the Ethics committee of Wageningen University. Written informed consent was obtained prior to testing from all parents of the participating children.

Table 1 Characteristics of the study population (mean ± sd)

	Girls (n=25)	Boys (n=25)	P difference[a]
Age (yrs)	9.1 ± 0.9	9.3 ± 1.0	0.40
BMI (kg/m²)	15.9 ± 1.9	17.5 ± 3.1	0.06
Height (cm)	142 ± 7	143 ± 8	0.59
Weight (kg)	32.2 ± 6.1	36.1 ± 8.9	0.07
Fruit consumption[b]	1.3 ± 0.7	1.2 ± 0.6	0.77
SS-preference score[c]	127 ± 14	122 ± 15	0.16

[a] *P*-values for differences between girls and boys (Mann-Whitney U test).
[b] pieces per day
[c] ss-preference score represents the most preferred balance between sweet and sour taste. This score could vary from 84 (sour preference) to 140 (sweet preference)

Questionnaire for consumption of fruit

Fruit consumption during the past month was assessed by using an short fruit frequency questionnaire, which was validated in adult women by using plasma vitamin C and total and specific carotenoids as biomarkers of intake [21;21]. Parents were asked to indicate how many days a week their child consumed 5 of the following fruits: tangerines; oranges, grapefruits, lemons; apples, pears; bananas; or other fruits. Subsequently parents were asked to fill out how many pieces children consumed of each fruit each time they consumed it. The fruits in the questionnaire were selected to represent the most frequently eaten fruits in the Netherlands [22].

Sensory tests

Subjects rank-ordered a series of sweet orangeades (0.42M sucrose) with 7 different concentrations of added citric acid (i.e. 0.0, 0.009, 0.013, 0.020, 0.029, 0.043 and 0.065M added citric acid, according to their preference. A subset of children (*n*= 20, 10.1 ± 0.5 yrs) were retested in order to check reliability. Previous research showed that the addition of citric acid to a sucrose solution suppressed the perceived sweetness intensity [17;23;24]. The orangeade with no added citric acid (hereafter referred to as Sweet-orangeade), is therefore perceived as sweeter than those with added citric acid. The orangeade with added citric acid is perceived as more sour than the orangeade with no added citric acid [17] (see [25] for more details).

Statistical analyses

A sweet-sour preference score (hereafter referred to as SS-preference score) for each individual was calculated by multiplying the solution number (1 through 7) with the preference rank number (1 through 7). By carrying out this calculation, each subject received a SS-preference score, from 84 to 140 for the series of orangeades. This SS-preference score represented the most preferred balance between sweet and sour taste in the orangeade. The lower the SS-preference score the more subjects preferred sour taste over sweet taste and vice versa (see [25;26], for further details). In order to determine the agreement between the first and second preference ranking, for those who were retested, Kendall's tau correlation coefficient (T) was calculated between the most preferred orangeade during the first and second preference rank-order (see [25;26;], for further details). In order to investigate the relationship between fruit consumption and sweet-sour preference, fruit consumption, expressed in pieces per day, was regressed on the sweet-sour preference score using linear regression analysis. Regression models were checked for normality, linearity and homogeneity of variance. Models were adjusted for the possible influences of sex and BMI on fruit consumption. One child was considered an outlier and excluded from the analysis because she reported a consumption of 8.6 pieces of fruit per day. Nine children were excluded from analysis because of missing values on fruit consumption or SS-preference score, leaving a total number of 25 girls and 25 boys.

RESULTS

Table 1 summarizes characteristics of the study population. Boys and girls did not differ significantly for any of the variables studied, although boys tended to have a higher BMI. As reported by the parents, girls consumed on average 1.3 ± 0.7 pieces of fruit per day, and boys 1.2 ± 0.6 pieces of fruit per day. A significant agreement was observed between the first and the second time children ranked the orangeades on preference (T=0.54, $P<0.0001$).

Figure 1 plots children's SS-preference scores against their fruit consumption. Since there was an indication of interaction between SS-preference score and sex (p-value of interaction term = 0.079), the regression models were run separately for boys and girls. In boys a decrease of one in the preference score, indicating a higher preference for sour taste, was associated with a increase in fruit consumption of 0.029 pieces a day (95% CI 0.043 - 0.014; standardized regression coefficient= 0.67, P=0.001). Among girls this association was absent (standardized regression coefficient= 0.06, P=0.796). The adjusted R^2 of the models was 0.39 for boys and –0.08 for girls.

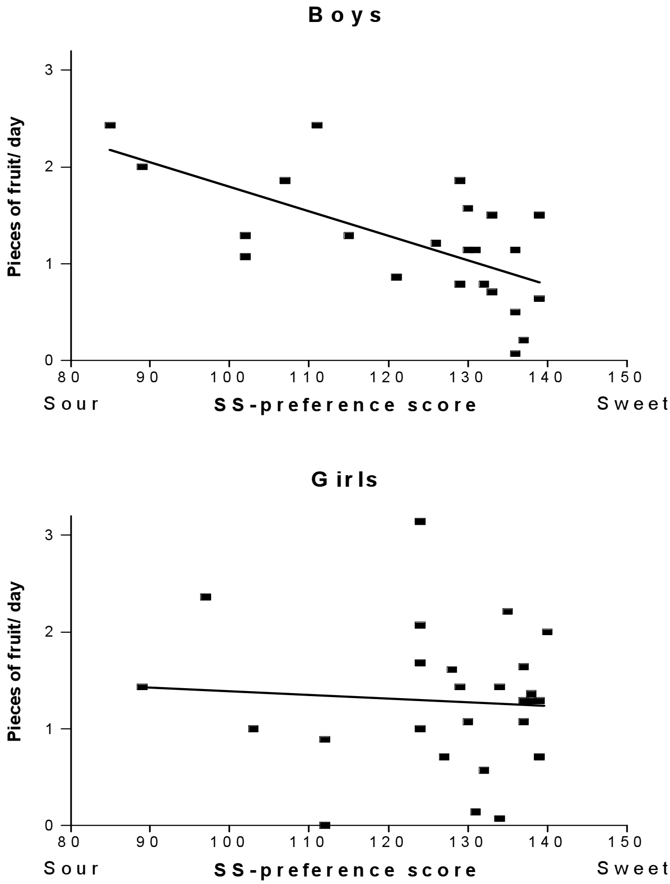

Figure 1 Relationship between sweet-sour preference score and fruit consumption in girls and boys. SS-preference score represents the most preferred balance between sweet and sour taste. This score could vary from 84 (sour preference) to 140 (sweet preference)

DISCUSSION

The present explorative study gives the first evidence that sour taste preferences are positively related to the consumption of fruit in boys but not girls. Previous studies already suggested that preference for fruit is the most important determinant of fruit consumption. However, our data seems to suggest that the main driver of these preferences might be different for boys and girls.

It has been shown that boys are better than girls in adjusting their food consumption in response to internal cues (e.g. satiety [27]). Hypothetically, girls' preferences for fruit are more determined by external cue's such as parental control, health related motives and availability. In a study among 5- to 8-years-old children, Tiggemann suggested that mothers' dietary restraint predicted the degree of monitoring of daughters', but not sons', eating behavior [28]. Hypothetically mothers are more likely to monitor their daughters' fruit consumption. Klesges and colleagues showed that children are more likely to make healthy food choices when parental control is high [29]. Besides a difference in parental control, it has been speculated that girls compared to boys are more likely to adopt their mother's eating behavior such as dieting [30]. Since according to girls as young as five years of age, dieting includes "eat more fruits" [31], dieting behavior of young girls could be related to their preference for fruit. Another external cue that could explain the difference between boys and girls is the availability and accessibility to fruit. Cullen and colleagues showed that availability and accessibility accounted for only 1% of the variance in the fruit consumption of boys, but accounted for 35% of the variance of fruit consumption of similar aged girls [8].

The questionnaire that was used in the present study was design to measure total fruit consumption rather than to discriminate between different fruits with different sensory profiles. The relationship between the consumption of fruit and preference for sour taste, is not necessarily due to the sour taste of fruit. Moreover, we cannot conclude from our results whether preferences for sour taste was a result of repeated exposure to fruit or vise versa. It is important to note that we did not assess the contribution of taste preferences relative to other potential determinants of fruit consumption. Furthermore, the variation in sweet-sour taste preferences of girls is smaller than in boys, which could explain the difference between boys and girls with respect to the relationship between sour taste preferences and fruit consumption. Further research should focus on the relationship between *sour* fruit and preferences for sour taste in a larger population of subjects.

In conclusion, our results suggest that preference for sour taste may be associated with the consumption of fruit in boys. In girls, preference for sour taste seems to be of less importance. Hypothetically, their consumption of fruit is more influenced by their parents, availability and health related motives. Future research is needed to confirm this hypothesis.

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Sweet and sour preferences during childhood: role of early experiences

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ABSTRACT:

Background: It has been suggested that early flavor experiences might influence taste preferences of children years after the last exposure during infancy.

Objective: We investigated the effects of early experience on sweet and sour preferences in children.

Design: Eighty-three children were divided into four groups based on the type of formula fed during infancy and age. By using a forced-choice, sip-and-swallow procedure, we determined the level of sweetness and sourness preferred in juice.

Results: Children who were fed protein hydrolysate formulas, which have a distinctive sour and bitter taste and unpleasant odor, preferred higher levels of citric acid in juice when compared to older children who were fed similar formulas. No such difference was observed between the groups for sweet preference. However, the level of sweetness preferred in juice was related to the sugar content of the child's favorite cereal and whether the mother routinely added sugar to their foods.

Conclusion: These data illustrate the wide variety of experiential factors that can influence flavor preferences during childhood.

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INTRODUCTION

In the biography on the life of his children, Charles Darwin (1877) [1] astutely noted that children are living in different sensory worlds than adults. One century after these keen observations were made, the scientific investigation of taste preferences during human ontogeny began. This body of research has focused primarily, but not exclusively, on sweet tastes and has repeatedly demonstrated, as Darwin noted, the strong acceptance of sweet-tasting sugars during infancy and childhood [2-7], with preference levels decreasing to resemble that of the adult during late adolescence [8].

The heightened preference for sweet taste during early development is universal and evident in children around the world (e.g., Brazil [9]; France [10]; Iraq [11]; Israel [7]; Mexico [12]; Netherlands [13]; North America [2;14]). However, individual variations do exists [3;15;16]. Genetic variation [17-19], as well as early experience [15;20], play a role in establishing such individual differences. For example, children who were fed sweetened water during the first several months of life exhibited a greater preference for sweetened water at two years of age when compared to those who had little of no experience with sweetened water [15]. Through dietary experiences, children develop a sense of what foods should or should not taste sweet [21;22].

In comparison to sweet taste, much less is known about the ontogeny of sour taste preferences. Unlike the innate preference for sweet tastes [23], newborns reject the sour taste of citric acid as evidenced by facial grimacing [7] and reduced intake [24]. That experiences during infancy impact upon sour preferences during childhood is suggested by our recent study [25]. In that study, we exploited the substantial flavor variation inherent in commercially available infant formulas, traditional milk-based formulas and those based on hydrolyzed proteins. Although each formula brand has its own characteristic flavor profile, milk-based formulas often are described as being slightly sweet and having ‘‘sour and cereal-type’’ aromas whereas protein hydrolysate formulas (e.g., Nutramigen, Alimentum) have an extremely unpalatable, offensive taste and ‘‘off’’ odor due primarily to its sourness and bitterness, perhaps because many amino acids taste sour or bitter [26]. When compared to children who had been fed milk-based formulas during their infancy, 4- to 5-year-old children who were fed protein hydrolysate formulas during the first year of life were significantly more likely to prefer sour-flavored (0.04 M citric acid) apple juices and less likely to make negative facial responses during the taste tests. We suggest that the inherent variation in these two classes of formula provides a particularly apt model system to study the role of preweaning flavor experiences on later preference behaviors because these formulas differ profoundly in flavor and because exposure is frequent and repeated [27;28].

The present study was built upon these previous findings and was designed to determine whether early experience with hydrolysate formulas influences preferences for a wider range of sour-flavored (0-0.070M citric acid) apple juices in 4- to 5-year-old as well as older aged (6- to 7-year-old) children. Preference for a range of sweetened apple juices also was assessed for two reasons. First, the addition of citric acid to apple juice not only enhances its sour taste but also suppress its sweetness [29]. Therefore, heightened sour preferences may be secondary to diminished sweet preferences. Second, there have been reports in the literature that infants who are feeding, or have been fed, protein hydrolysate formulas have a reduced preference for sweet foods [30;31]. As a first step in evaluating the role of maternal factors on children's flavor preferences, we also queried children and mothers individually about their habits and preferences.

METHODS

Subjects

Mothers of 4- to 7-year-old children were recruited from advertisements in local newspapers. Four groups of children (*n*=83; 42 girls, 41 boys) were formed based on their age (age groups: 4–5 yrs vs. 6–7 yrs) and early formula history (formula groups: milk formula vs. hydrolysate formula). There were no significant differences among the groups in the number of months that these children were fed formula during infancy, $F(1, 79df) = 2.19$, $P=0.14$. However, as expected, children in the hydrolysate group fed a milk- or soy-based formula during their first months (1.9 ± 0.4) of life and then, usually following their pediatrician's recommendation, were switched to hydrolysate until, on average, 11.8 (± 0.9) months of age. The vast majority of children in the hydrolysate group (83%) began this type of formula during the first 3 months of life. None of the children who were fed milk-based formulas were ever fed hydrolysate formulas.

All children were reported by their mothers to be healthy at the time of testing. Thirteen children were excluded because they could not understand the task. The procedures used in this study were approved by the Office of Regulatory Affairs at the University of Pennsylvania, and informed consent was obtained from each mother prior to testing.

Procedures

Children and their mothers were tested at the Monell Center on 2 days separated by 3.0 ± 0.5 days. Testing took place in a closed room specifically designed for sensory testing with a high air-turnover ventilation system. Each child sat at a small table designed for children. The mother, who was unaware of the hypothesis being tested, completed questionnaires about her child's feeding habits and preferences (discussed later) and sat approximately 2 ft behind the child, out of the child's view (see [32]). Mothers were asked to refrain from talking during the testing session, and replays of videotapes verified that they indeed did not talk during testing.

After the child acclimated to the room and personnel, we assessed the child's preference for sourness (0–0.070M citric acid) during one test session and their preference for sweetness (0.16–0.93M sucrose) during another. To this end, six solutions with different concentrations of citric acid (0, 0.007, 0.012, 0.022, 0.039, and 0.070M) were made by dissolving citric acid (Sigma Chemical) in apple juice and six solutions which differed in sugar concentrations were made by either diluting the stock 0.34M solution of apple juice (Mott's Inc., Stamford, CT) with water (0.16 and 0.22M) or by adding sucrose to the stock solution (0.47, 0.66, and 0.93M).

An age-appropriate, game like task that was fun for children and minimized the impact of language development were used to examine flavor preferences. Using a forced-choice procedure, each child was presented with all possible pairs of solutions (six solutions yield 15 possible pairs), one pair at a time, on each testing day. Ten mL of each stimuli were presented in identically colored opaque tumbler cups, containing a slit through which the child could sip its contents without seeing the color of the solution presented. The child was asked to taste each solution of the pair and to point to which of the pair he or she liked better. The order of presentation of the solutions was randomized within and between each pair. A 10-sec interval separated each pair of solutions, and a 5-min interval separated the tasting of the first eight pairs from the last seven pairs. During these intervals, children were offered a sip cup containing water and a small unsalted cracker to cleanse their palate.

Mothers completed a 10-item scale that measured their food neophobia, an 8-item scale that measured general neophobia [33], and a 25-item scale that measured temperament and food neophobia in their children [34]. All but four of the mothers completed the temperament scales. They also were asked to indicate how often they added sugar to their child's diet and whether they perceived their child as a picky eater. Without communicating to the child, each mother was asked to write down their child's favorite cereals and candies. It should be noted that mothers completed this task before their child answered similar questions: ''What is your favorite cereal (or candy) in the whole world?'' and ''Which cereal (or candy) do you ask your mom to buy the most?''. All but four of the children were able to answer these questions. Mother–child agreement was 65% for the child's most favorite cereal and 54% for their favorite candy. The sugar content (g/100 g) of these cereals then was determined.

Statistical analyses

Separate ANOVAs were conducted to determine whether there were significant differences between the groups on the age, body mass index (BMI: mass (kg)/height

(m^2), and temperament scores of the children as well as the age and variety seeking (with regards to foods), and food and general neophobia scores of the mother. Least significant difference analyses were performed to investigate significant effects. Pearson chi squares were performed to determine whether there were group differences in ethnicity, proportion of girls to boys, and the mothers' habit of adding sugar to their children's diet (e.g., those who never vs. those who frequently added sugar to their child's diet).

To determine whether early experience was related to sour preferences, we determined the number of times (of 10) each child preferred the two most sour juices (0.04 and 0.07 M citric acid) as ''tasting better.'' Please recall that our previous findings revealed that children who were fed hydrolysates were more likely to prefer sour apple juice (0.04 M). Similarly, we also determined the number of times (of 15) that each child preferred the apple juices with added sugar (0.47, 0.66, and 0.93 M sucrose). To determine whether there were significant differences in sweet and sour preferences between the groups, separate ANOVAs were conducted with age group (4–5 yrs vs. 6–7 yrs) and formula history (milk vs. hydrolysate) as the between-subject variables. Finally, to determine whether preference for sweetness in juices was related to preference for sweetness in cereal, a multiple regression was performed between the average sugar concentration of the child's most favorite cereals and the number of times children preferred the apple juices with the added sugar. All summary statistics are expressed as means ± sem.

RESULTS

Subject Characteristics

Salient characteristics of the four groups of children and their mothers are listed in **Table 1**. For those children who were 4- to 5-years of age, there was no significant difference between the formula groups (milk vs. hydrolysate) in their age: $F(1,37df)=1.26$, $P=0.27$, BMI (kg/m^2): $F(1,37df)=0.51$, $P=0.48$, the proportion of girls to boys: $\chi^2(1df)=0.02$, $P=0.88$, or the age of the mothers: $F(1,37df)=1.13$, $P=0.30$.

Likewise, there were no significant differences between the formula groups for those who were 6 to 7 years of age on any of these measures, children's age: $F(1,41df)=0.07$, $P=0.80$, BMI: $F(1,41df)=0.18$, $P=0.68$, sex ratio: χ^2 $(1df)=0.10$, $P=0.76$, or mothers' age: $F(1,42df)=3.77$, $P=0.06$. However, the two formula groups did differ in their ethnicity, χ^2 $(2df)=6.53$, $P<0.04$. That is, significantly more subjects in the hydrolysate group were Caucasian and less were African American (77% Caucasian, 17% African-American, 6% Other) when compared to those who were fed milk formulas (44%

Caucasian, 52% African- American, 4% Other). However, there was no main effect of ethnicity on any of the outcome variables examined (*P*'s>0.05).

Table 1 Subject characteristics and type of formula children were fed during infancy

	Group 1		Group 2	
Characteristics	**Milk-based formula**		**Hydrolysate formula**	
Age range (yrs)	**4-5**	**6-7**	**4-5**	**6-7**
Age (yrs)	5.5 ± 0.1	6.8 ± 0.1	5.4 ± 0.1	6.9 ± 0.1
Sex (girls:boys)	11:10	14:13	9:9	8:9
BMI (kg/m²)	16.6 ± 0.6	16.1 ± 0.6	16.7 ± 0.5	17.2 ± 0.5
Child temperament measures [a]				
Shyness [b]	2.3 ± 0.2	2.5 ± 0.2 [c]	2.6 ± 0.2	1.9 ± 0.2
Emotionality	2.9 ± 0.2	2.7 ± 0.2	2.9 ± 0.2	3.1 ± 0.2
Sociability [b]	3.8 ± 0.1	3.4 ± 0.1 [c]	3.3 ± 0.2	3.8 ± 0.2[d]
Negative reactions to food	3.2 ± 0.2	3.1 ± 0.2	3.0 ± 0.2	3.5 ± 0.2
Activity [b]	4.2 ± 0.2	4.0 ± 0.1 [c]	3.5 ± 0.2	4.0 ± 0.1
Percentage of mothers reporting that their child is picky eater	47.6	51.9	44.4	64.7
Number of mother-child pairs	21	27	18	17

[a] Child temperament measures could range from 1 to 5 (1= not at all characteristic of the child and 5= very characteristic of the child [33])
[b] Significant age (4–5, 6–7 years) by formula (milk, hydrolysate) group interaction; *P*<0:05
[c] Significantly different from 4–5 hydrolysate group; *P*<0:05; [d] Significantly different from 4–5 milk group; P<0:05. All summary statistics are expressed as mean ± sem

With regards to child temperament measures, there were no significant interactions between age or formula-history groups on the children's negative reactions to foods, F(1,76df) =1.98, *P*=0.16, or emotionality, F(1,79df) =1.02, *P*=0.32, nor was there a significant difference in the percentage of mothers who reported that their child was a picky eater (Fisher's exact *P*=0.36). However, there was a significant interaction between age and formula-history groups on children's shyness, F(1,78df) =4.62, *P*<0.05, sociability, F(1,78df) =11.42, *P*<0.01, and activity levels, F(1,79df) =4.70, *P*<0.05. Post hoc analyses revealed that 4- to 5-year-old children who were fed hydrolysate formulas were perceived by their mothers as being less social (*P*<0.05) and less active (*P*<0.05), and tended to be perceived as more shy (*P*=0.05) when compared to 6- to 7-year-old children who were fed similar formulas. In contrast, younger aged children who were fed milk-based formulas were perceived by their mothers as being more social (*P*<0.05) when compared to older aged children who were fed similar formulas. Further analyses of the children's temperament revealed no significant main effect of child's temperament on any of the outcome variables examined (P's>0.05).

For mothers' eating habits, there was no significant interaction between age and formula-history groups on the mothers' variety-seeking behavior to foods: $F(1,78df) = 0.02$, $P=0.90$, food neophobia: $F(1,76df) = 0.03$, $P=0.90$, or general neophobia: $F(1,76df) = 1.90$, $P=0.17$, as determined by questionnaires nor were there differences between the groups in the mothers' habit of adding sugar (Fisher's exact $P>0.05$) to their children's foods.

Effect of early experience and age on sour preferences

As seen in **Figure 1**, there was a significant interaction between age group (4–5 yrs vs. 6–7 yrs) and formula group (milk vs. hydrolysate) in the children's preference for the extreme sour juices, $F(1,79df) = 4.91$, $P<0.05$.

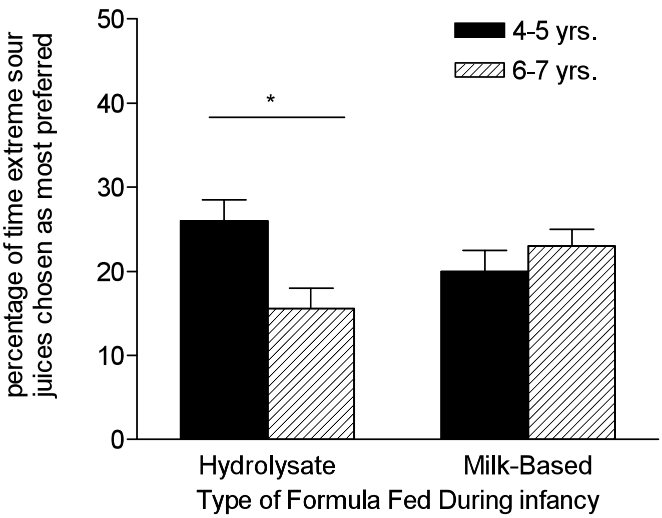

Figure 1 The percentage of time that 4-to 5-year-old children (solid bars) and 6-to 7-year old children (hatched bars) preferred the two most sour juices (0.04 and 0.07M citric acid) as "tasting better". The groups differed in the type of formula (i.e., milk, hydrolysate) that the children were fed during infancy. There was a significant interaction between age group (4-5 yrs. vs. 6-7 yrs) and formula group (milk vs. hydrolysate) in the children's preference for the extreme sour juices. * children who were 4- to 5-years of age and fed hydrolysate formulas during their infancy preferred these sour juices significantly more than did older children who were fed similar formulas ($P< 0.05$)

That is, children who were 4-to 5-years of age and fed hydrolysate formulas during their infancy preferred these sour juices significantly more than did older children who were fed similar formulas, $F(1,33df) = 5.81$, $P<0.05$. No such age-related difference was observed in children who were fed milk-based formulas during infancy, $F(1,46df) = 0.54$, $P = 0.50$.

Effect of early experience and age on sweet preferences

Unlike that observed for sour preference, there was no significant interaction between age group and formula group in their preference for sweetened juices, $F(1,79df) = 0.10$, $P = 0.76$ (**Figure 2**). That is, children of both age groups and early feeding history were significantly more likely to prefer the juices with added sugar (P's<0.001). Moreover, there was no significant interaction between the groups in the sugar content of the children's favorite cereals, $F(1,75df) = 0.63$, $P = 0.43$, which was, on average, 30.6 ± 1.5 g/100 g.

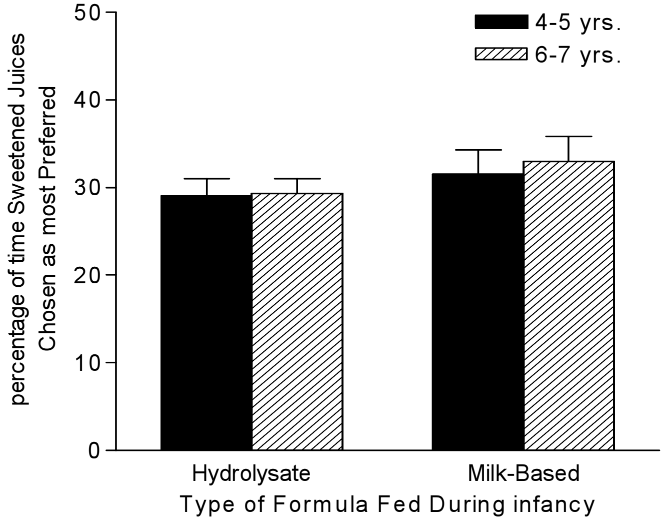

Figure 2 The percentage of time that 4-to 5-year-old children (solid bars) and 6-to 7-year old children (hatched bars) preferred the apple juice with added sugar (0.47, 0.66, and 0.93 M sucrose) as "tasting better". The groups differed in the type of formula (i.e. , milk, hydrolysate) that the children were fed during infancy. There was no significant interaction between age group and formula group in the children's preference for sweetened juices.

Because there were no significant differences of age or formula-history groups on the mothers' habit of adding sugar to their child's foods, children were divided into two groups: those whose mothers reported that they never added sugar to their foods (n=29) versus those whose mother did so on a routine basis (n=51) to further investigate whether maternal behaviors are related to children's preferences. **Table 2** reveals that children whose mothers reported adding sugar to their foods on a routine basis were significantly more likely to prefer apple juices with added sugar, $F(1,78df) = 4.68$, $P<0.05$, and reported that they preferred a cereal with a significantly higher sugar content, $F(1,74df) = 6.02$, $P<0.05$, when compared to children whose parents reported that they never added sugar to their children's foods. The mothers who added sugar also were more likely to be African Americans, χ^2 (2df) = 17.06, $P<.0001$, and were older in age, $F(1,78df) = 11.03$, $P<0.001$, when compared to mothers who never added sugar to their child's diet.

Table 2 Mother's habit of adding sugar to child's food: subject characteristics and preference

	Does mother add sugar to child's food?	
	Frequently	**Never**
Children's age (yrs)	6.2 ± 0.1	6.2 ± 0.2
Ethnicity (%) [a]		
African American	47%	3.5%
Caucasian	47%	93%
Other	6%	3.5%
Mother's age (yrs)	34.1 ± 0.8	38.3 ± 1.0 [b]
Children's BMI (kg/m^2)	16.3 ± 0.4	17.0 ± 0.4
No. times (of 15) that child preferred apple juices with added sugar	9.6 ± 0.32	8.4 ± 0.47 [a]
Sugar content of favorite cereals (g/100g)	32.9 ± 1.7	25.2 ± 2.8 [a]
Number of mother-child pairs	51	29

[a] $P < 0.05$; [b] $P < 0.001$

DISCUSSION

This study expands upon our previous findings and demonstrates that the type of formula fed during infancy influences sour, but not sweet, preferences several years after the last exposure to the formula. However, these effects were only observed when children were 4-to 5-years of age. In other words, children who were fed protein hydrolysate formulas, which have distinctive sour and bitter tastes and unpleasant odors, preferred higher levels of citric acid in juice when compared to 6- to 7-year-old children who were fed hydrolysate formulas. Because there was no significant difference in sweet preference between the groups, the preference for the more sour juices (which also were less sweet) in the younger children does not appear to be due to a reduced sweet preference.

Why were the effects of early experience not seen in the older children? It is unlikely that the enhanced sour preference in the younger children was due to a poorer ability to perform the sensory tests. The pair-wise comparison test has been frequently used to determine preference and discriminatory ability in young children [35-37]. Moreover, there were no age-related differences in the level of sweetness preferred, which was determined by procedures identical to those used for determining sour preferences.

We hypothesize that the observed shift in sour preference in those children who were fed hydrolysate during infancy was due to the older children's more expanded experience with foods and flavors [38] which, in turn, leads to learning the appropriate level and context of sour taste in different foods. On the other hand, we hypothesize that the heightened preference for sour tastes would persist with continued exposure to hydrolysate formula. Consistent with this hypothesis is the report that adolescent patients with phenylketonuria who were fed a type of protein hydrolysate formula (which is specifically treated with charcoal to remove most of the phenylalanine) throughout childhood and adolescence reported that one of their most preferred flavors, which was often added to the formulas, was lemon [39].

Because we did not randomly assign children to groups (parents decided which formulas they would feed their infants.), this was not a strict experimental study. Nonetheless, we attempted to match the groups as closely as possible to maximize the probability that any group differences were due only to differences in early formula-feeding experiences. Because there were no significant differences among the groups in the mothers' variety-seeking scores or food-neophobia scores, the differences observed in the children's behavioral responses to the sour flavor were unlikely to be due to the mothers' eating habits or attitudes toward foods.

There were significant age-related differences with temperament. Four- to five-year-old children who were fed hydrolysate formulas were perceived by their mothers as being less social and less active when compared to 6- to 7-year-old children who were fed similar formulas. Despite the paucity of research on sour taste preference, a recent study on sweet taste preference suggested that young adults who scored high on arousability and pleasure gave higher intensity ratings to a sweet solution under mild stress conditions when compared to those who scored low on these temperament scores [40]. However, despite the significant age-related differences in temperament observed in the present study, no significant main effect of child temperament was observed for any of the outcome variables.

Are mothers of young children who were on hydrolysate more likely to perceive their children differently? Of interest here is the finding that mothers who switched their children's formula during infancy because of feeding problems were more likely to perceive their 3.5-year-old children as more vulnerable several years after last exposure to the formula, despite no observed difference in the children's personalities or incidence of asthma or eczema [41]. Recall that every mother in the hydrolysate group switched her child's formula during infancy. Forsyth and Canny [41] suggested that the mere experience of changing formula during early infancy has long-term effects on mother–infant interaction and perceptions. Nevertheless, controlled experimental studies in which these formulas are introduced at differing ages and in which later preferences and temperament are determined by examining the child directly and not by maternal reports are needed for confirmation. Such studies are ongoing in our laboratories.

The findings from the present study also indicate that, at least for cereals, it is possible to relate sweet preference as measured in a laboratory setting to preferences for sweets in everyday foods such as cereals, as reported by the child. That experience influences sweet preferences is suggested by the finding that children whose mothers routinely added sugar to their diet preferred higher levels of sugar in apple juice and cereals when compared to children whose mothers reported never adding sugar. Such findings are consistent with previous research demonstrating that experience with sweetened water during infancy resulted in higher preferences for sweetened water at 2 years of age [15;20] and that preference for sweet taste, as assessed by psychophysical methods, is related to carbohydrate intake in healthy adults [42].

To be sure, mothers have an important influence on their children's diet [43], such as the types of foods eaten [44] and frequency of exposure to sweet foods [45]. However, these data underscore the importance of obtaining such information from the child directly because parents are not always reliable sources of this type of information. For example, mothers may be more accurate in identifying their child's favorite cereal when compared to candy because parents do most of the cereal purchasing. In conclusion, the findings of the present study illustrate the wide variety of experiential factors that can influence flavor preferences during childhood and highlight the importance of assessing children's preferences directly. The goal of our research program is to identify the early experiences that influence why we like the foods we do.

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Sweet preferences and sugar consumption of 4-and 5-year-old children: role of parents

Appetite in press

ABSTRACT

Background: Although the relationship between sugar consumption and health is unclear, many parents in the industrialized counties try to modify the consumption and preference for sugar of their children by imposing rules that restrict sugar consumption.

Objective: We investigated the relationships in children between rules that restrict consumption of mono and disaccharides (MDS), consumption of MDS and preferences for sucrose-containing orangeade. The background ideas of restriction rules we also investigated.

Design: To this end, 44 children (5.1 ± 0.5 yrs) performed a rank-order and paired-comparison test of preference for five orangeades, which differed in sucrose concentration (0.14, 0.20, 0.29, 0.42 and 0.61M sucrose). Parents filled out a questionnaire concerning restriction rules and their children's consumption of MDS-containing foods.

Results: Stronger restriction rules were related to a lower consumption of beverages that contained MDS and to a lower consumption of MDS-containing foods during breakfast and lunch. The most freedom to choose foods that contain MDS was given during the afternoon. Fifty-five percent of the children who were highly restricted showed a preference for the highest concentration of sucrose in orangeade. None of these children preferred the orangeade with the lowest concentration of sucrose. While 19% of the children who were little restricted preferred the beverage with the lowest concentration of sucrose, 33% preferred the beverage with the highest concentration. These parents generally believed that sugar has a bad effect on health and had similar background ideas concerning restriction rules.

Conclusions: Rules that restrict sugar consumption of children may lead to a lower consumption of sweet tasting foods. These rules may have the unintended effect of increase children's preference for sweet foods.

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INTRODUCTION

Human infants are born with a preference for sweet taste [1-4]. Also infants at 1-3-days-old can discriminate between different kinds of sugars (e.g. fructose, glucose) and different concentrations of the same sugar [3]. Preference for sweet taste remains during childhood and decreases during late adolescence, until the adult preference for moderate sweet taste has been established [5;6]. Preference for high levels of sweetness is evident in children around the world (for review see [7]), and is positively related with children's consumption of sweet foods [8;9]. Along the same lines, Dutch survey of food consumption has shown that 4-to 7-year-old children consumed a larger percentage of their daily energy intake as means of mono and disaccharides (girls: 33.2 %; boys: 32.1%) than adults (females: 21.3%; males: 23.7%) [10].

The effects of high consumption mono and disaccharides (hereafter referred to as MDS) on health are not clear. Excessive consumption of MDS is positively related to dental caries [11-13], but there is no conclusive evidence that consumption of MDS causes harm to health. Several studies suggested that a high intake of beverages that contain MDS is positively related to obesity [14;15;16]. However, other studies failed to detect such a relationship [17;18]. Also, behavioral and cognitive changes caused by high consumption of MDS have not been clearly demonstrated in healthy subjects [19;20].

Despite this inconclusive scientific evidence, many parents in the industrialized world try to lower their children's consumption of MDS and preference for sweet foods by applying rules that aim to restrict the consumption of MDS (hereafter referred to as 'MDS restriction rules'). It is unclear which thoughts and attitudes of parents are related to these rules [21]. Three different attitudes may play an important role in the formation of MDS restriction rules.

First, it has been suggested that parents who used foods that contains MDS to comfort their children or used these foods as a reward (the instrumental functions of MDS), were more likely to give their children these food items [22]. Secondly, the well established attitude, "sugar is bad for your health", may also be of importance in the formation of rules that aimed to restrict consumption of MDS [18;23]. Thirdly, parents may think that sweet preferences can be modified (e.g. lowering sweet preference) by imposing MDS restriction rules [24].

Besides the unclear attitudes behind MDS restriction rules, it is also unclear whether these rules result in a lower consumption of MDS, or lower preference for sweet taste as aimed by the parents. Several studies suggested that restriction could result in an increased desire to consume the restricted food [25]. This high desire would then increase consumption of the restricted food in the absence of parental monitoring [26]. It is important to note that the latter studies focused on particular food items and not on one particular taste dimension per se. From these studies it is therefore unclear whether MDS restriction would change subjects' preferences for MDS.

The present study investigated the background ideas of MDS restriction rules. Subsequently, this study investigated whether MDS restriction rules were related to consumption of MDS and preference for high concentration of MDS in orangeade.

METHOD

General overview

The present study involved two parts. The first part consisted of a survey questionnaire concerning three main topics: a) the child's consumption of a variety of foods that contain MDS, b) restriction rules concerning consumption of those foods, and c) the background ideas of the use of MDS and MDS restriction rules. The parents of the children, who participated in the sensory tests, filled out this questionnaire. The second part consisted of sensory tests with orangeade that contained different amounts of sucrose. These tests were completed by children who attended kindergarten. In the present study "lunch" refers to the second cold meal and "MDS" refers to MDS present in the foods that were listed in the questionnaire. "Restriction" refers to "the degree children were restricted to freely choose a food that contained MDS". "MDS restriction rules" refers to "rules that restrict children to freely choose a product that contained MDS".

Subjects

The subjects who completed the questionnaires were the parents of the children who participated in the sensory tests. Forty-five of the sixty questionnaires were returned to the researchers. Sixty children (5.0 ± 0.6 yrs, 33 girls and 27 boys) were involved in the sensory tests; all attended a kindergarten in a nearby village. Two children refused to taste the beverages and were therefore excluded. Exclusion criteria for participation of the children were, restriction of MDS in the diet on medical indication, and presumed allergies to MDS and/or orange beverages. This resulted in the inclusion of 43 children (5.1 ± 0.01 yrs, 22 girls and 21 boys) who completed the sensory tests and whose parents returned the questionnaires. The study protocol was approved by the Medical Ethical Committee of the Department of Human Nutrition of Wageningen University. Informed consent was obtained prior to testing from all parents of the children who participated.

Survey questionnaire

The survey questionnaire consisted of 42 questions divided into 4 sections: 1) a food frequency section, organized to assess consumption of a variety of foods that contain

MDS during specific meals and times of day (i.e. breakfast, during the morning, lunch, during the afternoon and dinner) 2) a section with MDS restriction rules that referred to the same food items as in the food frequency list 3) Statements about the background of MDS restriction rules 4) General questions about each parent (gender, age, and education).
The 13 food items in the questionnaire were the MDS-containing food items most frequently eaten by children (4-7 yrs) in the Netherlands 10 (see Table 1).

Table 1 Average mono and disaccharide (MDS) contents and standard portion size of the food items mentioned in the food frequency questionnaire [31].

Food Items	Average MDS content (g/100g product)	Standard portion (g)
Sweet beverages		
Sweetened beverages	10	150
Juices	9	150
Candies		
Small candy	70	5
Large candy	70	15
Candy bars	50	15
Cake	30	50
Biscuits	30	10
Others		
Sweet yogurt	75	12
Apple sauce	17	10
Sweet dessert	12	150
Sweet spreads for bread	65	15
Sweet breakfast cereals	20	150
Sweeteners	100	5

Parents filled out how many times a day, a week, or a month their children consumed each specific food item, during each specific time of the day (i.e. breakfast, during the morning, lunch, during the afternoon, during the dinner) during the past month. During breakfast, Dutch children normally eat bread or breakfast cereals. During the morning and afternoon snacks are eaten. Lunch normally consists of bread and dinner normally consists of a warm meal. The Dutch food composition table was used to calculate the MDS content of each food item (see Table 1). The questionnaire included foods that represented about 75% of the regular MDS intake of children 4-7 years of age [10]. Fruit and milk were not included in the questionnaire, because these foods do not contain

added MDS. For the statistical analyses the food items were categorized into three main food groups: 1) sweet beverages, 2) candy, 3) others (see Table 1).
The second section contained the same food items as the first section. In this section parents filled out whether their children were "always", "often", "sometimes", "rarely" or "never" free to choose specified food items. The food items were organized according to the time of day they are normally eaten in the Netherlands. When children did not eat particular food items during a specific meal, parents could fill out the option, "does not apply". Similarly to the section concerning MDS consumption, the food items were categories into three main food groups.
The third section contained 23 statements concerning the background of the MDS restriction rules. The agreement on these statements was measured with 5-point Likert-scales. The statements, which were quoted at random, involved 3 main issues: 1) "Preference for sweetness is modifiable" (based on [24]), 2) "Sugar consumption has a negative effect on health" (based on [18;23]) and 3) "Sweetness can be used as an instrument" (based on [22]). **Table 3** shows the different statements ordered by their main issue (see Table 3).
The fourth section contained general questions. Parents reported their gender, age, weight and height and the highest level of education they participated in (Low: primary school, lower level of secondary school, lower vocational training; Medium: higher level of secondary school, intermediate vocational training; High: higher vocational training, university degree).

Sensory tests

In order to track small differences in preferences for different concentrations of sucrose in orangeade, rather than investigate whether subjects preferred sucrose in orangeade or not, orangeades with different concentrations of sucrose were used. Five beverages were prepared by dissolving different concentrations of sucrose (Sundale, Suiker Unie, Breda, the Netherlands; 0.14, 0.20, 0.29, 0.42 and 0.61M sucrose) in 23 gram concentrated orangeade with water for a total volume of 1.0 liter. The concentrate (Lim 7644, Quest International, Naarden, The Netherlands) was composed of concentrated orange juice, which contained natural orange flavor (80 ppm/l beverage solute), 4.2% w/v citric acid, 1500 ppm benzoic acid, 4100 ppm ascorbic acid, and 44% w/v sugar (50% sucrose, 25% glucose, and 25% fructose). Similar concentrations were previously used in our lab [6;27]. About 15 mL of each stimulus was offered at room temperature in 25 mL transparent cups. The beverages were prepared one evening before each session and were stored overnight at a temperature of 4°C.
A paired comparison test and a rank-order test were used to measure preference for different concentrations of sucrose in orangeade. Previous research suggested that both

paired comparisons [27;28] and the rank-order method [29;30] are reliable methods when conducted with young children. Children were tested at their kindergarten in a room that was familiar to them. The testing room existed of ten or fifteen low tables. This depended on the number of children (ranging from 10 to 15 children) that was tested at the same time. In order to minimize the influence of peers during the sensory tests, the tables were set one meter apart from each other. Each child had personal guidance from an adult, who was trained beforehand to become familiar with the procedures. The children sat in a circle facing outwards, so they could not see the other children who were tested at the same time. First the paired comparison test and subsequently the rank-order test were performed, with a 20-minute pause between the two tests. Each test lasted for about 10 minutes. The subjects were allowed to taste the stimuli as often as was necessary to make a decision.

To determine preference with the paired comparison test, the subjects had to judge five stimuli in ten different pairs. The pairs were presented one at the time in a random order. Children were asked to taste both stimuli, after which the researcher asked the child: "Which one do you like most?". The child could either tell or point to the one he liked the best. Before the actual test began, the subjects were offered a pair of stimuli to become familiar with the beverages. This pair consisted of the second and the fourth stimuli of the series, which were used during the actual paired comparison test. The subjects used the sip and swallow procedure and took a sip of water between each pair of stimuli, but not within.

During the rank-order test subjects divided the stimuli into two categories, "most preferred' and "least preferred", which were made visual by a "happy face" and a "sad face". First, children were presented with all five stimuli in a random order. Secondly, the researcher asked the child: "I want you to taste all the beverages. If you like the taste of the beverage, you give it to the "happy face", if you do not like the taste I want you to give it to the unhappy face". After all stimuli were grouped in either the "happy face" group, or "unhappy face" group, the researcher asked the child: "Now I want you to rank the beverages from most preferred to least preferred, starting with the beverages in the "happy face" group. After the stimuli in the "happy face" group were rank-ordered, the same procedure was followed for the stimuli in the "unhappy face" group.

The procedure resulted in rank-orders from most preferred to least preferred. This method was a modification of a method previously used by Birch [30]. In her research, Birch used three categories (happy face, neutral face and sad face). Because of the small number of stimuli in the present study we decided to use only two categories.

Statistical analyses

The consumption of MDS was estimated by multiplying the frequency of consumption of each food item by its standard serving size and the MDS content of each serving size [31]. This resulted in the estimated consumption of mono and disaccharide in g/day per child. Differences in the consumption of mono and disaccharide within a group, between different eating moments, or between different food groups, were tested for significance by using Friedman analyses for ranks (F_r) and post hoc analyses [32]. Mann Whitney U tests were performed to identify significant differences in MDS consumption, children's age, children's BMI (weight/height^2), parent's age and parents' BMI between different groups of children

In the analyses concerning restriction, we only analyzed the number of times parents answered "never" to give their children freedom to choose a specific product. It appeared that the options "sometimes, rarely and often" were ambiguous terms for some of the parents according to the remarks parents wrote on the questionnaires, whereas "never" was not ambiguous. In order to calculate an individual score for MDS restriction, the number of times parents reported "never" to give their children freedom to choose a specific product was summed.

Subsequently, children were divided into two groups based on the frequency parents reported "never" to give their child freedom of choice. Children of parents who reported to be stricter than half of all the subjects (upper median) were grouped together (High-Restriction group). All children of parents who were less strict than half of all the subjects (lower median) were grouped together in another group (Low-Restrictive group). Children, whose parents were on the median concerning strictness, were grouped in the High-Restriction group.

The different statements concerning parents' background ideas about sugar received a score and were categorized into three main issues: 1) "The liking of sweetness is modifiable", 2) "Sugar has an instrumental function (i.e. Reward, comfort) " and 3) "Sugar has a negative effect on health". For each main issue the mean score ± sd was calculated. The internal consistency of the three main issues was assessed with Crohnbach's alpha. Because of the exploratory character of this part of the questionnaire, the lower limit for Crohnbach 's alpha was set at 0.60 [33].

In order to have individual scores that indicate the subjects' preference for sucrose in orangeade, preference scores were calculated as follows. The preference score for the paired comparison method was calculated by assigning a score of "5" to the solution with the highest concentration of sucrose. The solution with the second highest concentration of sucrose received a score of "4" etc. The total score was obtained by summing the numbers of the most preferred solution within each pair. This gave each person a preference score between 20 (1+1+1+1+2+2+2+3+3+4) and 40 (5+5+5+5+4+4+4+3+3+2). In which a score of 20 indicated that the subject always picked the lowest concentration of sucrose in orangeade as most preferred. A score of 40 indicated that the subject always picked the highest concentration of sucrose in orangeade as most preferred.

The preference score for the rank-ordering method was calculated by assigning a score of "5" to the solution with the highest concentration of sucrose. The solution with the second highest concentration of sucrose received a score of "4" etc. For the rank-order test for preference, a preference score was estimated by the following formula:

Preference score=4 * P1 + 3 * P2 + 2 * P3 + 1 * P4,

Where P1 is the 'score' of the concentration with that was most preferred; P2 is the 'score' of the concentration that was second-most preferred; P3 is the 'score' of the concentration that was third-most preferred; and P4 is the 'score' of the concentration that was fourth-most preferred. This resulted in a second preference score between 20 and 40 ([34] for further details).

In order to determine significant correlations between the paired and the rank-order tests, Spearman's rho correlation coefficients were calculated between the measures of the paired comparison tests and rank-order tests. The consistency between the paired and rank-order test for preference was calculated by transforming the rank-order tests into 10 pairs, with the following assumption: if solution "a" was preferred over solution "b", and solution "b" was preferred over solution "c", then solution "a" was preferred over solution "c". Next, these 10 pairs were compared to the 10 pairs of the paired comparison test. The consistency was then calculated by determining the percentage of pairs that was judged in the same order in both the rank-order and the paired comparison test. Mann-Whithney U tests were applied to determine whether there were significant differences in preference between High- and Low-Restriction children.

All data were analyzed by using SPSS (SPSS version 11.01. LEAD technologies, US) for the main frame at a critical value of $P<0.05$. All summary statistics are expressed as means ± sd.

RESULTS

MDS restriction rules and social-demographics

Parents in the High-Restriction group, answered "never" on more than 42% (median) of the questions concerning freedom of choice (mean=51 ± 7%). Parents in the Low-

Restriction group were less strict. These parents reported "never", on less than 42% of the questions (mean=21 ± 12%, hereafter referred to as Low-Restriction group). Children in the High- and Low-Restriction groups were not significantly different in age (U=197.5, *P*=0.42) or BMI (kg/m^2) (U=199.0, *P*=0.44) (see **Table 2**). Children's BMI fell within the average range of a normal population (mean=15.5 ± 1.6 kg/m^2) [35]. Parents of children in the High-Restriction group were significantly, older than parents of low restricted children (mothers: 39.7 ± 4.1 yrs vs 37.2 ± 3.3 yrs, U=99.0, *P*<0.01; fathers: 37.7±3.8 yrs vs. 35.0±3.1 yrs, U=126.0, *P*<0.05). Mothers of children in the High-Restriction group had a significantly lower BMI than mothers of children in the Low-Restriction group (21.5 ± 2.0 kg/m^2 vs. 23.8 ± 2.9 kg/m^2, U= 116.0, *P*<0.01). A similar trend was observed for fathers (High-Restriction: 24.2 ± 2.8 kg/m^2, Low-Restriction: 26.1 ± 3.6 kg/m^2, U=136.0, *P*=0.09). More than half of the parents in the High- and Low-Restriction groups were highly educated (High-Restriction: 62%, Low-Restriction: 51%). The remaining parents had either a medium level of education (High-Restriction: 38%, Low-Restrictive: 42%) or low level of education (High-Restriction: 0%, Low-Restriction: 7%).

MDS restriction and consumption during the day

Parents were not equally strict across the five eating moments during the day (F_r(4df)=63.7, *P*<0.001). In general they were the least strict during the afternoon and the strictest during breakfast (all *P*-value's<0.05). A similar pattern was observed after dividing children in High-Restriction and Low-Restriction groups (see **Figure 1**).

Table 2 Characteristics and sweet preferences of 4-and 5-year-old children (mean ± sd), one group whose parents have High sugar restriction rules, and one group whose parents have Low sugar restriction rules

	Low-Restrictive[a]	High-Restrictive[b]
Children		
Age (yrs)	5.1 ± 0.6	5.0 ± 0.5
BMI (kg/m^2)	15.8 ± 1.0	15.2 ± 2.0
Child's sweet preference [c]		
Sweet preference, rank-order	31.7 ± 6.7	36.5 ± 5.8*
Sweet preference, paired	31.9 ± 6.9	37.4 ± 3.5*

a. Low restrictive: parents who reported "never to give their child freedom to choose a product that contained sugar" in 42% or less of the cases (11 girls, 10 boys). *.Signifies difference between High- and Low-Restrictive, *P*<0.05
b. High restrictive: parents who reported "never to give their child freedom to choose a product that contained sugar" in more than 43% of the cases (11 each of boys and girls).
c Ranging from preference for low sweet orangeade=20, to preference for high sweet orangeade=40.

Children in the High-Restriction group were subject to significantly more MDS restriction than children in the Low-Restriction group during breakfast (U=47.5, *P*<0.001), the morning (U=111.5, *P*<0.01), lunch (U=82.5, *P*<0.001) and dinner (U=102.5, *P*<0.001). No such differences were observed for the afternoon (U=210.0, *P*=0.16) (see **Figure 1, panel A**).

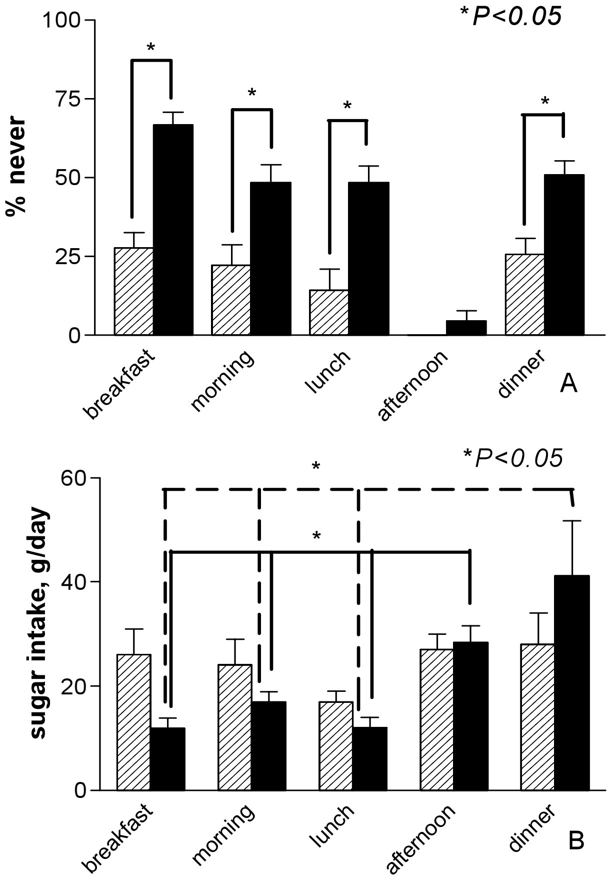

Figure 1 Percentage of times (out of 19 questions) parents reported "Never" to give their child freedom to choose a mono-and disaccharide containing product (panel A) and mono-and disaccharide consumption during different eating moments (breakfast, during the morning, lunch, afternoon, dinner) (panel B). Divided by High-Restriction (solid bars) and Low-Restriction (hatched bars) (mean ± sem).

The average total MDS consumption per day was 116 ± 54 g/day. Children in the High- and Low-Restriction groups did not significantly differ from each other with respect to the total MDS consumption during the day (High-Restriction: 110 ± 54 g, Low-Restriction: 122 ± 54 g, U=188, *P*=0.41). However, children in the High-Restriction group compared with children in the Low-Restriction group tended to consume less

MDS during breakfast (U=153.0, P=0.09) and lunch (U=149.0, P<0.07). Furthermore, children in the High-Restriction group consumed more MDS during the afternoon (29% from the total daily sugar intake) and during dinner (31% from the total daily sugar intake) than during the other eating moments (see **Figure 1, panel B**). However, this was not the case for children in the Low-Restriction group. They consumed about the same amount of MDS across the 5 different eating moments.
When MDS consumption was expressed as 'gram food that contained MDS', significant differences between the High-and Low-Restriction groups were observed. That is, children in the High-Restriction group compared with children in the Low-Restriction group consumed less food that contained MDS during breakfast (U=117.0, P<0.009) and during lunch (U=121.5, P<0.05). High-Restricted children also consumed less MDS-containing foods during the day (U=127.0, P<0.05), than Low-Restricted children.

MDS restriction for and consumption of different food groups
Children in the High-Restriction group received different degrees of restriction for sweet beverages, candies and other sweet food items (F_r(2df)=21.8, P<0.001). That is, they were significantly more restricted to choose a sweet beverage, than to choose candies or other sweet food items (both P's<0.05) (see **Figure 2, panel A**). Children in the Low-Restriction group were not restricted significantly different across the three different food groups (F_r(2df)=0.09, P=0.96) (see Figure 2, panel A).
Children in the High-Restriction group were more restricted to choose sweet beverages (U=33.5, P<0.001) and other sweet food items (U=121.0, P<0.01), than children in the Low-Restriction group. No significant difference was observed for candies in this respect (U=189, P=0.24; see Figure 2, panel A).
Children in the Low-Restriction group consumed significantly more MDS from sweet beverages (63 ± 37 g/day) than children in the High-Restriction group (40 ± 23 g/day) (U=114.0, P<0.01). Not only did they consume more MDS derived from sweet beverages, they also consumed more MDS from sweet beverages as percentage of their total MDS intake (52% ± 18 vs. 40% ± 23 from total MDS intake per day; U= 135.0, P<0.05). No significant differences between the High- and Low-Restriction groups were observed for candies (U=187.0, P<0.40) and other sweet food items (U=189.0, P=0.43).
When MDS consumption was expressed as 'gram MDS-containing foods', significant differences between the High-and Low-Restriction groups were observed. That is, children in the High-Restriction group compared with children in the Low-Restriction group consumed less sweet beverages (U=120.5, P<0.05). Such difference was not observed for "candies" (U=203.0, P=0.66), or "other sweet food items" (U=188.5, P=0.42) (see **Figure 2, panel B**).

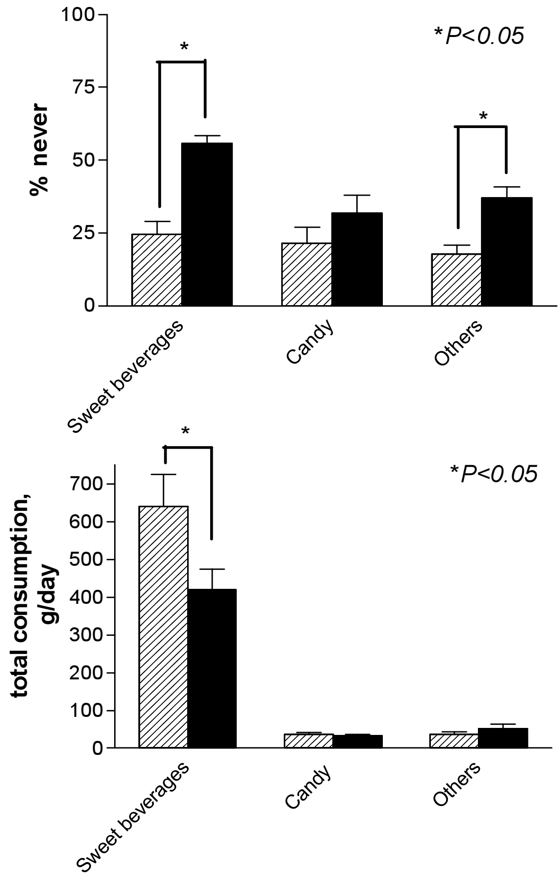

Figure 2 Percentage of times (out of 19 questions) parents reported "Never" to give their child freedom to choose a mono-and disaccharide containing product (panel A) and consumption of mono- and disaccharide containing foods (i.e. Sweet beverages, Candy an Other sweet food items) (panel B). Divided by High-Restriction (solid bars) and Low-Restriction (hatched bars) (mean ± sem).

Background of MDS restriction rules

Table 3 shows parents' response on the different statements. The Cronbach's alpha's of the three main issues reached at least 0.60. In general parents agreed on the statement "Eating much sugar is bad for children" (see Table 3). Parents of the High- and Low-Restriction groups did not show an equal agreement across the different main issues (High-Restriction: $F_r(2df)=10.2$, $P<0.01$; Low-Restriction: $F_r(2df)=16.4$, $P<0.001$). Parents of children in the High-Restriction group agreed the least on statements within the main issue "sweetness can be used as an instrument" (both *P*-value's <0.05). No significant difference in agreement was observed between the statements within the other two main issues (i.e. “sugar has a negative effect on health” and “preference for sweetness is modifiable”). Parents of children in the Low-Restriction group agreed more on the statements concerning “sugar has an negative effect on health” than on the statements concerning “eating much sugar is bad for children” and “preference for sweetness is modifiable” (both *P*-value’s<0.05). No significant difference in agreement was observed between the statements concerning “eating much sugar is bad for children” and “preference for sweetness is modifiable”. No significant difference in agreement that concerned the statements was observed between the High- and Low-Restriction groups.

Preference for sucrose in orangeade

The paired comparison and the rank-order test for preference showed a 70.1 ± 3.6% consistency with each other and a correlation of r=0.63 (see [27] for more details). Children in the High-Restriction group preferred higher concentrations of sucrose in orangeade than children in the Low-Restriction group. This was significant during the rank-order test (U=103.0, *P*<0.01), as well as during the paired comparison test (U=102.0, *P*<0.01) (see Table 2 and **Figure 3**).

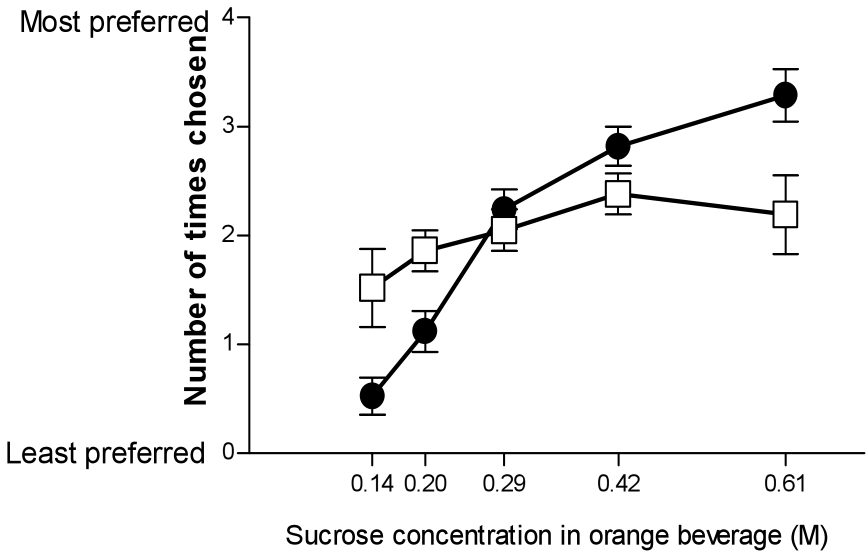

Figure 3 Number of times each solution was chosen as most preferred during the paired comparison test for preference. Shown for High-Restriction (●) and Low-Restriction (□) children (mean ± sem)

Table 3 Parents responses on statements concerning: "Negative health effects of sugar", "Modifiability of preference for sweetness" and "The instrumental function of sweetness" (mean ± sd)

Restriction	Low	High
Negative health effects of sugar	**3.9 ± 0.6**	**3.6 ± 0.6**
When children eat too much sweet foods, they will eat less healthy foods	3.8 ± 1.4	3.8 ± 1.2
Children will grow fat after eating many sweet foods	4.1 ± 1.0	3.7 ± 1.2
Sweet foods are usually unhealthy for children	3.8 ± 0.9	3.4 ± 1.2
Eating too much sugar is bad for children	4.6 ± 0.5	4.3 ± 0.9
*As long as my child brushes his/her teeth, I don't have to limit his/her sugar consumption	4.2 ± 1.1	4.3 ± 1.2
Sweet foods will make kids more active	3.3 ± 1.0	2.9 ± 1.0
Children will become hyperactive from eating many sweet foods	3.4 ± 0.9	2.9 ± 0.9
Modifiability of preference for sweetness	**2.7 ± 0.6**	**2.5 ± 0.4**
*Sweet tooth is caused by predisposition, rather than upbringing.	2.5 ± 1.1	2.4 ± 1.3
If I do not lay down rules, my child will only eat sweet foods	2.7 ± 1.3	2.7 ± 1.3
*Children just like sweet foods, and there is nothing you can do about it	3.0 ± 1.2	2.8 ± 1.2
The fewer sweet foods your child eats, the lower his or her appetite for sweet foods will be.	3.2 ± 1.8	2.6 ± 1.0
When your child has a likes many sweet foods, you gave him or her too many sweet foods	2.7 ± 1.3	2.1 ± 0.6
By prohibiting sweet foods, they will become more attractive	3.4 ± 1.0	3.6 ± 1.3
Eating sweet foods causes children to become less sensitive to other flavors	3.0 ± 0.9	2.7 ± 0.8
Children simply need a lot energy, which they mainly get from sugar	1.9 ± 0.9	1.6 ± 0.9
You can teach children to like sweets less	3.3 ± 1.1	3.5 ± 1.7
The less candy you give your child, the les he/she will like it	2.4 ± 1.0	2.5 ± 1.1
The instrumental function of sweetness	**2.8 ± 0.6**	**2.9 ± 0.8**
Sweetness is one of the most pleasant things in life for children	2.2 ± 1.3	2.6 ± 1.8
Withholding sweet foods from children is unkind	2.6 ± 1.1	2.8 ± 1.3
Sweet foods can sometimes soften pain	2.8 ± 1.3	2.8 ± 1.4
Sweet foods can sometimes easily be used as a reward	2.9 ± 1.2	2.8 ± 1.1
Sweet foods can sometimes easily be used as comfort	2.9 ± 1.1	3.1 ± 1.1
Sweet foods can sometimes easily be used as a treat	3.4 ± 1.1	3.3 ± 1.3

1=totally disagree**------**5=totally agree; *= reversed items totally agree**-----**5=totally disagree

Children in the Low-Restriction group did not significantly differentiate between the different solutions during the paired comparison (F_r(4df)=5.1, *P*=0.28) or during the rank-order test (F_r(4df)=5.5, *P*=0.24). This was in contrast with children in the High-Restriction group. They significantly differentiated between the different solutions during the paired comparison (F_r(4df)=52.9, *P*<0.001) and during the rank-order test (F_r(4df)=35.7, *P*<0.001) (see Figure 3). However, a closer investigation of each subject in the Low-Restriction group showed that the majority of children (71%) had a clear preference for one of the five beverages. That was, 19% preferred the orangeade with the lowest concentration of sucrose, 33% preferred the orangeade with the highest concentration of sucrose and 19% preferred one of the intermediate orangeade. This was in contrast with children in the High-Restriction group. None of those children preferred the orangeade with the lowest concentration of sucrose, 55% preferred the orangeade with the highest concentration of sucrose, and 27% preferred one of the intermediate beverages.

DISCUSSION

The results of the present study suggest that children are the least MDS restricted during the afternoon. Children who were highly restricted, consumed significantly less MDS derived from sweetened beverages, and consumed less foods that contained MDS during breakfast and lunch. Also the total consumption of foods that contained MDS was lower for the highly restricted children, compared with the low restricted children. However, the total daily consumption of MDS per se was not different for high- and low restricted children. Furthermore, the present study suggests that the strength of MDS restriction rules, enforced by parents, was positively related with children's preferences for sucrose in orangeade. With respect to the background ideas of MDS restriction rules, parents agreed in general that sugar has a bad effect on health. In general low and high restrictive parents had similar background ideas concerning MDS restriction rules.

Previous studies have repeatedly shown that parents can significantly influence their children's food consumption. This influence can be established by means of the parents' own attitude and behavior towards food [22], parental monitoring and control [25;26;36-38], and rewarding [39-41]. The present study suggests that parents are able to lower the consumption of sweet beverages, and MDS-containing foods during breakfast and lunch, by means of MDS restriction rules. But these rules appeared to have little influence on MDS consumption per se. This observation can be explained by at least two hypotheses.

First, MDS restriction rules were effective during meals in which bread is generally consumed. The food items that contained the most MDS (i.e. candies and sweet yoghurt) are normally not consumed during these meals, but rather during the afternoon and dinner. During the afternoon and dinner MDS restriction rules did not appear to affect the consumption of foods that contained MDS. Along the same line, foods that were rich in MDS, such as candies, were not affected by MDS restriction rules. Foods that were affected by MDS restriction rules (i.e. sweet beverages) contained the least percentage of MDS. This could explain that children in the High-Restriction group consumed less grams of food that contained MDS, but not less MDS per se.

We speculate that the high level of MDS restriction decreased the consumption of all foods that contained MDS, not only sweet beverages. This decrease in consumption may have resulted in a lower consumption of sweet beverages and equal consumption of the other food items that contained MDS. In an unrestricted setting, with no parental control, children in the High-Restriction group might have consumed more MDS rich foods, than children in the Low-Restriction group. This hypothesis is supported by the finding that children in the High-Restriction group preferred higher concentrations of sucrose in orangeade than children in the Low-Restriction group. Conner and colleagues suggested that preference for high concentrations of MDS in an experimental beverage as measured in a sensory experiment, is related to preference for MDS in a variety of foods in real life [42;43]. Preference for high concentrations of MDS could lead to high consumption of food that contain MDS, if children are or not limited in their access to these foods [8;9]. In order to come to a similar level of consumption of these foods other than sweet beverages, children in the High-Restriction group needed perhaps more restriction rules than children in the Low-Restriction group. In that case one could hypothesize that MDS restriction rules are effective in lowering children's consumption of all foods that contain MDS.

A second hypothesis is that parents are successful in lowering their children's consumption of MDS, but only when the rules are very tight. As soon as parents loosen their tightness, MDS restriction rules do not lower consumption of foods that contain MDS. This hypothesis is supported by the finding that parents in the High-Restriction group were the strictest with respect to sweet beverages. Moreover, it is this food group in which the MDS consumption of children in the High-Restriction group was significantly lower. For the remaining two food groups (i.e. candies and other sweet food items), no significant differences in the consumption of MDS was observed. In general, restriction rules might not always have been strict enough to result in a difference in consumption of MDS between the High-and Low-Restriction groups.

Although MDS restriction rules were related to a lower consumption of foods that contained MDS, restriction rules may result in a preference for high concentrations of MDS in food. This preference could then lead to a high consumption of foods that contain MDS in the absence of parental control. However, this effect may be unintended. This hypothesis is in line with several experimental studies, which suggested that food restriction could lead to an increased desire and preference for the restricted foods [25;26]. Similarly, Spruijt-Metz and colleagues demonstrated that mothers' concern for her children's weight was positively related to higher total fat mass of their children [44]. Furthermore, they suggested that mothers' pressure to eat was related to lower total fat mass of their children. Johnson and Birch suggested that mothers who highly controlled their children's food intake had children who showed less ability to self regulate food intake [37]. Along the same line, Carper and colleagues suggested that high parental control over feeding, has the potential to promote the development of dietary restraint and disinhibition among girls at a very early age [36]. In other words, at least in the case of rules that are determined to restrict or control children's food consumption, in the absence of parental control children may do the opposite of what parents want them to do.

The present study, however, did not investigate the causal relationship between preference for sucrose in orangeade and restriction. We speculate that children who had a preference for high sweet foods were more likely to be subject to restriction. This could explain why no difference in attitude of parents concerning sugar consumption was found between the Low-and High-Restriction groups.

In general, parents in the present study did agree that sugar is not a healthy food choice ("eating much sugar is bad for children"). This agreement might partly be based on the assumed negative role of sugar consumption in child obesity and dental caries. In the present study this idea is supported by the large agreement on the statements "Children will grow fat when eating many sweet foods", and "As long as my child brushes his/her teeth, I don't have to limit his/her sugar consumption".

The results of the present study cannot be generalized to all parents because most parents were highly educated. Previous studies suggested that the educational level of parents is positively related with the tendency to impose rules that aim to restrict the consumption of certain foods [45].

It also needs to be noted that mothers of children in the High-Restriction group had a lower BMI than mothers of children in the Low-Restriction group. This is in contrast with previous research who did not find a relationship between maternal BMI and the degree they controlled their children's food consumption [25;38;46], however, Fisher and Birch suggested that a lower BMI of parents was associated with a larger degree of food restriction they enforced. In the study of Fisher and Birch [25], similar to the present study, parents reported their own height and weight. It has been suggested that restrained eaters [47] and those who were dissatisfied with their own body image [48] were more likely to underestimate their weight. Hypothetically, parents who enforced many restrictive rules for their children, were more concerned about their own weight than parents who enforced less restrictive rules. Previous research suggested that those mothers for whom weight is an issue for themselves, exert most control over their daughter's eating habits [46].

Furthermore, the number of subjects in each group was rather small, this could have resulted in non significant findings. Such as the lack of differences in consumption of MDS between the High-and Low-Restriction groups. It is, however, unlikely that a statistically significant difference in total consumption of MDS can easily be obtained by increasing the number of subjects. In the present study the difference in consumption of MDS between both groups was only 12 gram/day on an average total of 116 gram/day. Also, the lack of increasing preference with an increasing concentration of sucrose in orangeade within the Low-Restriction group could be a result of the low number of subjects. It is, however, more likely that this is a result of the diversity in preferences within this group. Recall that the majority of these children showed a clear preference for a specific concentration of sucrose in the orangeade. Moreover, the preference data obtained from the paired comparison tests and the rank-order tests showed a high consistency with each other. This suggests that preference was reliably measured in the present study (see [27]).

It is important to note that the used questionnaire had some important limitations. The amount of MDS consumed was measured by rather rough estimations. It was assumed that a standard portion size was given by the parents each time children consumed a certain food, the standard portion size was, however, not defined in the questionnaire. No actual amount of foods that contained MDS was measured. Furthermore, milk and fruits were not included in the questionnaire, because the researchers were interested in foods with added MDS. Hypothetically, children in the High-Restriction group eat a large amount of milk and fruits, in order to meet their liking for sweet taste. Furthermore, both consumption and restriction of foods that contained MDS were measured in one questionnaire, this could have resulted in bias. Parents could deliberately have underestimated their children's consumption of MDS, just to be in line with their previously given answers concerning MDS restriction. In order to minimize bias, questions concerning consumption and restriction were completely separated in two different parts of the questionnaire.

Not all 13-food items were assessed at each eating occasion, but rather at those occasions they are generally consumed. In case that subjects did not have a 'normal' eating pattern, it is possible that the actual consumption of the 13 food items was underestimated. However, the sugar consumption founded in the present study was in line with large and extended food consumption data [10]. This suggests that the present findings give an accurate estimation of the daily consumption of MDS.

In conclusion, the present study suggests that rules aimed to restrict children to consume foods that contain MDS, are effective during breakfast and lunch. These restriction rules may also be effective in lowering the consumption of beverages that contain MDS. However, these rules may lead to an enhanced preference for high concentrations of MDS. Therefore, restriction rules may have the unintended effect of raising consumption of MDS of children, when parents are not able to control their children's consumption of MDS. Future research is needed to further investigate the background of rules that aimed to restrict the consumption of foods that contain MDS and the long term effect of MDS restriction rules on children's preference for high concentrations of MDS and consumption of MDS.

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Sweet and sour preferences in young children and adults: role of repeated exposure

Physiology & behavior, in press[*]

ABSTRACT

Background: Previous studies suggested that repeated exposure might lead to an enhanced preference of the exposed food.

Objective: We investigated the influence of repeated exposure to orangeades with added sucrose and different concentrations of citric acid, on the taste preferences of 6-to-11-year-old children and young adults.

Design: During an intervention study of eight days, 59 children (9.2 ± 0.9 yrs) and 46 young adults (22 ± 2.0 yrs) received each day either an orangeade with a sweet taste, a sour taste, or no orangeade (control). Before (baseline) and after the intervention, preferences for a series of orangeades and yoghurt that varied in balance of sweet and sour taste, were measured by means of a rank ordering procedure. The variation in balance of sweet and sour taste was established by adding different amounts of citric acid (orangeade: 0.009, 0.013, 0.020, 0.029, 0.043 and 0.065M added citric acid; yoghurt: 0.027, 0.038, 0.056, 0.081, 0.12 and 0.17M added citric acid) to a stock orangeade and yoghurt with 0.42 M sucrose. The sweet and sour tasting orangeade that were consumed during the intervention were equally preferred at baseline.

Results: After an 8-day exposure to the sweet-orangeade, children's preferences for this orangeade (0.42 M sucrose) significantly increased ($P<0.05$). A similar trend was observed for the yogurt with 0.42 M sucrose ($P=0.09$). An 8-day exposure to the sour-orangeade, did not have a significant effect on children's preference for this orangeade. The taste preferences of adults did not change after the intervention. The control group of children and adults did not show any change in preferences for sweet and sour tastes.

Conclusion: Sour taste preferences of children appeared to be more stable than preferences for sweet taste. Adults taste preferences were more stable than children's taste preferences. Future research is needed to investigate whether the changed preferences for sweet taste are stable over time and how these changed taste preferences are related to a change in the consumption of sugar rich foods.

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INTRODUCTION

In recent years obesity in children has been a growing health problem in Western countries [1]. Obesity in childhood is associated with risk factors for cardiovascular and metabolic diseases such as diabetes [2]. Childhood obesity is related with food choice and intake [3;4] . Choice and intake of children are influenced by a variety of factors [5], among which parental behavior [6-10], social environment [11], and sensory preferences [12-14]. The present study will focus on sensory preferences.

Many studies showed that children have a preference for high concentrations of sucrose in foods [15-20]. This preference for high concentrations of sucrose has been positively related to a high consumption of sucrose containing foods [12;21;22]. Besides the preference for sweet taste, a recent study suggested that some children also have a preference for extremely sour tasting foods [23]. In both adults [24] as well as in children [23] it has been suggested that those who preferred high concentrations of citric acid (sour taste), experienced greater dietary diversity when compared to those who did not prefer these high concentrations of citric acid. It remains to be investigated how preferences for sweet and sour taste are developed and how they can be modulated.

It has been suggested that a long term repeated exposure to sweet [25] and sour [22;26] taste during infancy, enhances young children's preference for these tastes. Beauchamp and co-workers suggested that repeated exposure to sweetened water during infancy was positively related to high preferences for sweet taste in water at two years of age [25]. In the same line, recent studies have suggested that repeated exposure to a sour tasting baby formula during infancy is related to a high preference for sour taste in young children [22;26].

Causality could, however, not been drawn from these studies. Studies that did investigate the effect of repeated exposure of a food on subsequent preference in an experimental design, mostly used whole food products rather than being focused on one taste dimension [27-29]. Moreover, most studies used novel foods [30-32]. An increase in preference for novel foods after repeated exposure could also be due to reduction in food neophobia rather than an increase in preference for the specific taste of the food [33].

We investigated whether the preference for sweet and sour taste in two different foods can be changed, in children and adults, after a short repeated exposure to sweet or sour taste in orangeade.

METHODS

General overview

Children and adults were divided into three groups. One group consumed for eight days an orangeade with a sweet taste (hereafter referred to as Sweet-Group). Another group consumed for eight days an orangeade with a sour taste (hereafter referred to as Sour-Group). A third group did not consume any orangeade during these eight days (hereafter referred to as Control-Group). Before (baseline) and after the intervention preference for a series of Sweet-Sour-orangeade and yoghurt was determined. The sweet and sour-orangeade were at baseline equally preferred (see determination of the exposure concentration).

Subjects

Children

Sixty-three children who attended a primary school in Bennekom participated in the study (see **Table 1**). The Sweet-Group and Sour-Group consisted of children from two different classes who were randomly assigned to one of the two groups. Children in the Control-Group were all classmates and shared the same classroom during school time. This was done to prevent possible interaction between the Control-Group, and the experimental groups (i.e. Sweet-Group and Sour-Group) during the time of testing. Four children were excluded from the final analyses because they were not present during one of the testing days. All children had a BMI that fell within the normal range for children their age [34] and were healthy according to their parents. The characteristics of the children are shown in Table 1.

Adults

Forty-six young adults who were students at the Wageningen University were randomly assigned to either the Sweet-Group, Sour-Group or Control-Group. Characteristics of the adults are shown in Table 1. All adults reported to be healthy and had a BMI that fell in the normal range for adults [35].

Exclusion criteria for participation of the children and adults were: sucrose restriction in the diet on medical indication, and presumed allergies to sucrose and/or orangeades. The study protocol was approved by the Ethics committee of the Wageningen University. Written informed consent was obtained prior to testing from all participating adults and parents of the children.

Stimuli

Seven orangeades with similar concentrations of sucrose (0.42M of sucrose) and different concentrations of added citric acid, i.e. 0.0, 0.009, 0.013, 0.020, 0.029, 0.043 and 0.065M added citric acid, were prepared by dissolving the citric acid (BUFA B.V. pharmaceutical products, Uitgeest, The Netherlands) and sucrose (Sundale, Suiker Unie, Breda, the Netherlands) in 23 grams concentrated orangeade and water for a total volume of 1.0 liter. The concentrate (Lim 7644, Quest International, Naarden, The Netherlands) was composed of concentrated orange juice, which contained natural orange flavor (80 ppm/l beverage solute), 4.2% w/v citric acid, 1500 ppm benzoic acid, 4100 ppm ascorbic acid, and 44% w/v sugar (50% sucrose, 25% glucose, and 25% fructose). In a similar manner seven lemon flavored sweet yoghurts (Biogarde™, Almhof, Uniekaas Holding B.V., Veenendaal, the Netherlands; 0.42M sucrose, Sundale, Suiker Unie, Breda, the Netherlands) with different concentrations of citric acid (0.0, 0.027, 0.038, 0.056, 0.081, 0.12 and 0.17M added citric acid) were prepared by dissolving sucrose and citric acid in the lemon flavored yoghurt.

The orangeades and yoghurt were prepared one day in advance and were stored overnight at 4^0C. The orangeades were presented in opaque cups in quantities of 200 gram each. The cups were covered with a lid, so subjects could not see the content of the cup. Subjects drank the orangeade out of a straw. The yoghurts were presented in similar opaque cups in quantities of 50 gram each. Subjects used a plastic spoon to eat the yoghurt. All stimuli were presented at room temperature.

Procedure

Preference test

Children were tested at their primary school in a room which was familiar to them. Each child had personal guidance from an adult, who was trained beforehand to become familiar with the procedure. The testing room existed of 9 low tables, which were situated in a circle. Children sat in front of their personal guide and faced outwards. Preferences for the series of orangeades and yoghurt were both measured by means of a rank-ordering procedure [36]. The preference tests existed of two sessions, one session for the orangeades, one session for the yoghurt, separated by 20 min. The order of the sessions was balanced across subjects. During each session, subjects tasted all stimuli presented in the session and divided them into three categories, “most preferred”, “neutral” and “least preferred”. These categories were made visual by a “happy face”, a “neutral face” and a “sad face”. Subsequently, children were asked to taste and rank all stimuli within each category from most preferred to least preferred. The procedure resulted in a rank-order from most preferred to least preferred (see [36]). The order in

which the stimuli were presented was randomized. Children took a sip of water after tasting each stimulus.
Adults were tested in the tasting booths of the Wageningen University and used the same procedure as children in order to determine their preferences for the series of orangeades and yoghurt.

Determination of the exposure concentration

During the exposure period, subject in the Sweet-Group were, for eight days, exposed to the orangeades with no added citric acid (0.42M sucrose). Previous research showed that the addition of citric acid to a sucrose solution suppressed the perceived sweetness intensity [37;38]. The orangeade with no added citric acid (hereafter referred to as Sweet-orangeade), is therefore perceived as sweeter than those with added citric acid.
Subjects in the Sour-Group were exposed to the orangeade with added citric acid that was at baseline equally preferred as the Sweet-orangeade. The orangeade with added citric acid is perceived as more sour than the orangeade with no added citric acid [37;38]. Preferences at baseline are shown in **Figure 1**.
Figure 1 suggests that at baseline children preferred the Sweet-orangeade similar as the orangeade with 0.02M added citric acid, hereafter referred to as Sour-orangeade (**Figure 1, panel A**). Statistical analyses revealed that the Sweet and Sour-orangeades were indeed not preferred significantly differently (P=0.90). Adults preferred the orangeade with 0.043 M added citric acid not different from the orangeade with no added citric acid (P=0.30). (see **Figure 1, panel B**).
This resulted in the following: children in the Sour-Group were, for eight days, exposed to the orangeade that contained 0.42M sucrose and 0.02M added citric acid. Adults in the Sour-Group were, for eight days, exposed to the orangeade that contained 0.42M sucrose and 0.043M added citric acid.

Exposure period

On each morning, for a period of 8 days, children and adults were presented with 200 mL of orangeade as a midmorning snack. Children drank the orangeade while sitting in their classroom. They were instructed to drink as much as they wanted to. As soon as a child did not want to drink any more orangeade, or drank 200 mL, they were asked to return the cup to one of the researchers who were standing in the back of the classroom. Before and after consumption each cup with orangeade was weighed in order to calculate the daily amount consumed.
Adults consumed the orangeade at home. They were instructed to drink as much as they liked. As soon as they were done drinking they were instructed to mark on the cup how much was left of the orangeade.

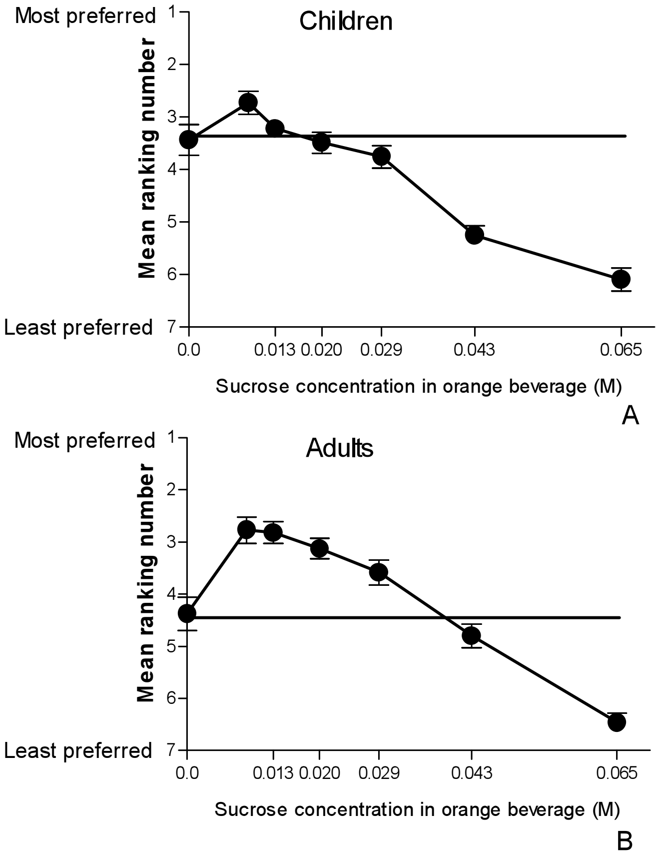

Figure 1 Mean ranking number (± sem) for preference of the series of orangeades at baseline children (panel A) and adults (panel B). Subjects in Sweet-Group were exposed to Sweet-orangeade (0.42M sucrose). Children in the Sour-Group were exposed to Sour-orangeade with 0.42M sucrose and 0.02M added citric acid. Adults in the Sour-Group were exposed to Sour-orangeade with 0.42M sucrose and 0.043M added citric acid

Statistical analyses

Sweet-sour preference score (SS-preference score)

Mann-Whitney U-tests and Chi-squares were conducted to determine differences in subject characteristics. A sweet-sour preference score (hereafter referred to as SS-preference score) for each individual was calculated as follows. Each orangeade/yoghurt was assigned a number according to the amount of added citric acid, i.e. the orangeade/yoghurt with no added citric acid was assigned '1', the orangeade/yoghurt with the highest concentration added citric acid was assigned '7'. Subsequently the number of the solutions was multiplied with the preference ranking (i.e. 1=most preferred, 7=least preferred) and summed across the preference ranking. By carrying out this calculation, each subject received a SS-preference score, from 84 to 140 for the series of orangeades and the series of yoghurt. This SS-preference score represented the most preferred balance between sweet and sour taste in the orangeade. The lower the SS-preference score the more subjects preferred sour taste over sweet taste and vice versa (see [19], for further details).

Spearman correlation coefficients (r) and 95%-confidence intervals (95%CI) were calculated in order to determine 1) whether SS-preference scores for orangeade and yoghurt were correlated and 2) whether baseline preference was significantly correlated with average consumption of orangeade across the eight days of intervention.

To determine the degree of agreement between preference at baseline and after the intervention, tau correlation coefficients (T) were calculated between the most preferred orangeade at baseline and after the intervention. T was defined as [(#agreements - #disagreements)/total number of pairs)]. For example, 10 subjects ranked 7 stimuli according to their preference. After an intervention these 10 subjects ranked the 7 stimuli according to their preference again. If 8 subjects ranked the same stimulus as most preferred before and after the intervention then, T= (8-2/10)=0.5. T could range from –1 (total disagreement) to 1 (total agreement). The significance of T indicated a statistical significant agreement between the most preferred orangeade at baseline and after the intervention [39]. The same calculation was carried out for the most preferred yoghurt.

The changed preference ranking was defined as: [preference ranking at baseline - preference ranking after the intervention]. In order to determine whether subjects changed their preference for the orangeade they were exposed to during the intervention, Mann-Whitney U-tests were calculated between the changed preference ranking of the Sweet-Group and the Control-Group, and between the Sour-Group and the Control group. Similar analyses were carried out for the yoghurt that contained the same amount of sucrose and citric acid as the beverage subjects were exposed to.

Wilcoxon Signed Ranks Test (Z) were performed in order to determine differences in consumption of the orangeade during the first and last day of the intervention. All summary statistics are expressed as means ± sd and levels of significance were $P<0.05$.

RESULTS

The characteristics of subjects in the Sour-Group, Sweet-Group and Control-Group are listed in Table 1. No differences in gender or BMI were observed between the different experimental groups within each age category (i.e. children and adults). Children in the Control-Group were significantly older than children in the Sweet-Group (U=29.0, *P*<0.001) and children in the Sour-Group (U=35.5, *P*<0.001). Children in the Sweet-Group, Sour-Group and Control-Group did not differ in their baseline SS-preference scores.

Children showed a higher preference ranking for the sweetest tasting orangeade with no added citric acid than adults (3.4 ± 2.3 vs 4.4 ± 2.1, were 1= most preferred and 7= least preferred; U=1016, *P*<0.05). Baseline SS-preference scores for orangeades and yoghurt were significantly correlated (children: r=0.40, 95%CI: 0.15 to 0.60, adults: r=0.43, 95%CI: 0.16 to 0.65).

Table 1 Characteristics of subjects, divided by Sweet-Group (Sweet), Sour-Group (Sour) and Control-Group (Control) (mean ± sd)

	Children		
Group	**Sweet**	**Sour**	**Control**
Age (yrs)	8.7 ± 0.8[a]	8.9 ± 0.7[a]	10.1 ± 0.5[b]
Girls:Boys	7:12	12:8	12:8
Height (m)	1.37 ± 0.05	1.40 ± 0.06	1.48 ± 0.07
Weight (kg)	31.7 ± 5.6	32.7 ± 5.8	37.1 ± 9.4
BMI (kg/m^2)	16.7 ± 2.4	16.6 ± 2.2	16.8 ± 3.0
	Adults		
Age (yrs)	22.2 ± 2.0	22.1 ± 2.1	22.2 ± 2.1
Girls:Boys	14:2	13:3	8:6
Height (m)	1.74 ± 0.07	1.74 ± 0.07	1.76 ± 0.09
Weight (kg)	67.6 ± 8.3	66.4 ± 9.4	69.2 ± 7.1
BMI (kg/m^2)	22.4 ± 2.3	21.9 ± 2.8	22.3 ± 1.8

[a-b-] Significant difference *P*<0.05

Repeated exposure to sour, children (Sour-Group)

Children in the Sour-Group showed a significant agreement between the most preferred orangeade at baseline and after the intervention (T=0.30, *P*<0.05). The same was observed for yoghurt (T=0.41, *P*<0.05). They did not change their preference ranking of the orangeade they consumed during the intervention (U=195.5, *P*=0.90) (**Figure 2, panel A**).

During the intervention children in the Sour-Group consumed on average 150 ± 58 mL of the Sour-orangeade per day, this was not correlated with the preference ranking of this Sour-orangeade at baseline (r=0.01, 95%CI: -0.44 to 0.46). No significant differences in consumption were observed between the first day and last day of the intervention (Z=-1.4, P=0.16; **Figure 3, panel A**).

Repeated exposure to sweet, children (Sweet-Group)
Children in the Sweet-Group did not show a significant agreement between the most preferred orangeade at baseline and after the intervention (T=-0.08, P=0.67). The same was observed for yoghurt (T=0.31; P=0.19). Closer investigation suggested that they increased preferences for the Sweet-orangeade (0.42M sucrose and no added citric acid; U=120.0, P<0.05) and tended to have increased preferences for sweet yoghurt (0.42M sucrose and no added citric acid; U=129.5, P=0.09; **Figure 2, panel B**).
During the intervention they consumed on average 184 ± 28 mL Sweet-orangeade per day, this was not significantly correlated with the baseline preference ranking for this Sweet-orangeade (r=0.32, 95%CI: -0.19 to 0.70). Furthermore, they tended to have consumed more of the orangeade during the last day of the intervention than during the first day of the intervention (Z=-1.8, P=0.07) (Figure 3, panel A). Children in the Sweet-Group consumed on average more orangeade during the intervention than children in the Sour-Group (U=374.5, P<0.05).

Control Group, children (Control-Group)
As expected children in the Control-Group showed a significant agreement between the most preferred orangeade at baseline and after the intervention (orangeade: T=0.54, P<0.0001). The same was observed for yoghurt (T=0.45, P<0.05).

Repeated exposure to sour, adults (Sour-Group)
Adults in the Sour-Group showed a significant agreement between the most preferred orangeade at baseline and after the intervention (orangeade: T=0.78, P<0.0001). Such agreement was, however, not statistically significant for the yoghurt (T=0.19, P=0.45). They did not change their preference ranking of the orangeade they consumed during the intervention (U=68.5, P=0.11; **Figure 2, panel C**).
During the intervention, adults in the Sour-Group consumed on average 128 ± 63 mL of the Sour-orangeade per day, this was not significantly correlated with the initial preference ranking of this Sour-orangeade (r=0.38, 95%CI: -0.21 to –0.77). No significant differences in consumption were observed between the first day and last day of the intervention (Z=-0.85, p=0.40; **Figure 3, panel B**).

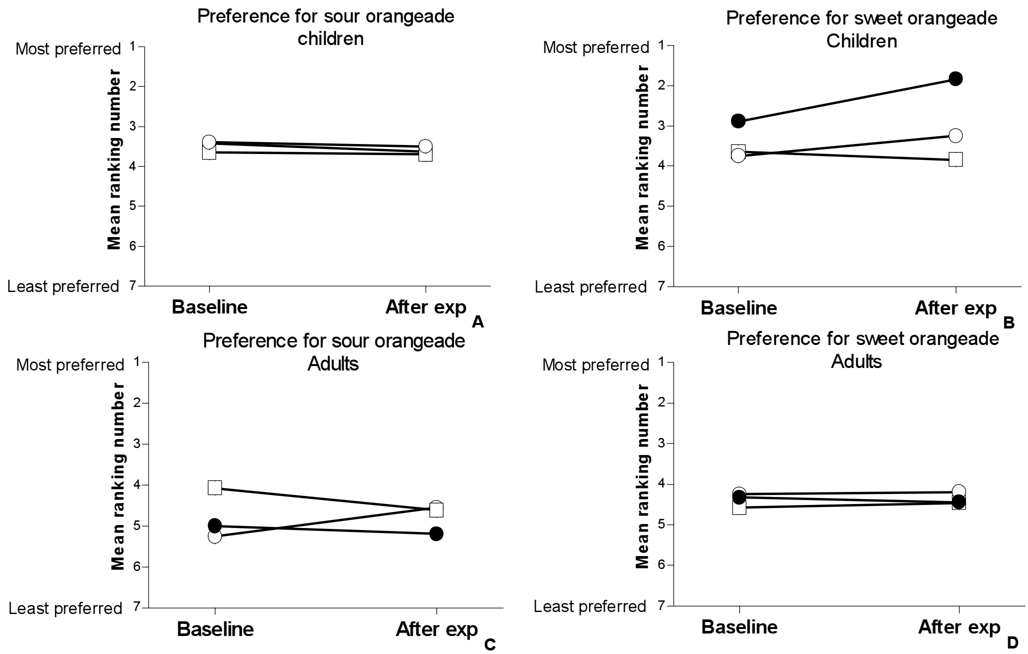

Figure 2 Mean ranking number for preference of Sour-and Sweet-orangeades at baseline and after repeated exposure (after exp) to these specific beverages, shown for children (panel A and B) and adults (panel C and D) (● Sweet-Group; ○ Sour-Group; □ Control-Group)

Repeated exposure to sweet, adults (Sweet group)

Adults in the Sweet-Group showed a significant agreement between the most preferred orangeade at baseline and after the intervention (orangeade: T=0.50, *P*<0.001). A similar trend in agreement was observed for the most preferred yoghurt (T=0.43, *P*=0.09). Nor did they change their preference ranking of the orangeade they consumed during the intervention (U=88.0, *P*=0.68; **Figure 2 panel D**). Adults in the Sweet-Group consumed on average 172 ± 33 mL Sweet-orangeade per day, this was not significantly correlated with the initial preference for the Sweet-orangeade (r=0.14, 95%CI: -0.42 to 0.62).
These subjects consumed significantly less during the last day compared with during the first day of exposure (Z=-2.0, *P*<0.05; Figure 3, panel B). On the first day 94% of the adults consumed all the beverage they were presented with. This percentage decreased to 63% on the last day of exposure.

Control-Group, adults

As expected adults in the Control-Group showed a significant agreement between the most preferred orangeade at baseline and after the intervention (orangeade: T=0.45,

P<0.05). Such agreement was, however, not statistically significant for the yoghurt (T=0.22, P=0.42).

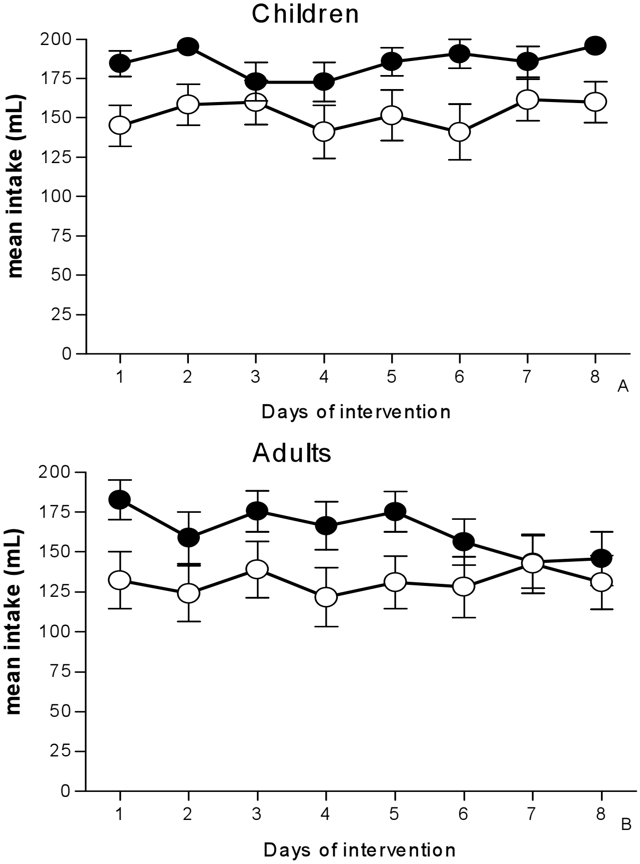

Figure 3 Average consumption (± sem) of orangeade during the 8 days of intervention by the Sweet-Group (●) and Sour-Group (○). Shown for children (panel A) and adults (panel B)

DISCUSSION

The present study showed that after an 8-day repeated exposure to orangeade with a high concentration sucrose during childhood, children's preferences for this orangeade significantly increased. A similar trend was observed for yoghurt with a high concentration of sucrose. This increased preference for a high concentration of sucrose was specific for the exposed high concentration of sucrose and not specific for the food sucrose was exposed in. Adults did not change their taste preferences after an 8-day repeated exposure to a sweet- or sour-orangeade. However, after repeated exposure to a Sweet-orangeade they decreased their consumption of this orangeade. In 8- to 11-year-old children, repeated exposure to the Sweet-orangeade tended to result in an increase of consumption of the sweet tasting orangeade.

Previous research suggested that a short repeated exposure to an initially novel food is likely to result in an increased preference for the exposed food [29-32;40]. From these studies it is unclear whether repeated exposure to a specific taste quality can increase preference for this taste. Beauchamp and Moran suggested that two-year-old children who had been regularly fed sucrose water during infancy (<12 months of age), consumed more sucrose solutions, but not more water than did children who were not fed sucrose water during infancy [25]. In the same line, a recent study suggested that children of parents who added sucrose to their children's diet on a regular basis had a higher preference for apple juice with added sucrose, than children of parents who did not practice this habit [22]. Causality can, however, not been drawn from these studies. The present study is, to our knowledge, the first experimental study with young children that showed that a short repeated exposure to orangeade with a high concentration of sucrose results in an elevated preference for this high concentration of sucrose in both orangeade and yoghurt. Previous research suggested that changed taste preferences, which were initiated by repeated exposure during infancy are relatively stable over time [22;26]. Future studies are needed in order to determine if the changed taste preferences as observed in the present study will be stable over time.

As shown in the present study preference for a high concentration of sucrose in orangeade can be generalized to other foods (i.e. yoghurt). The existence of a sweet tooth has been suggested by several studies in adults [21;41] as well as in children [12]. Sullivan and Birch [32] failed to find support for the suggestion that a sweet tooth might be the result of a generalized preference for sweetened foods. In their research repeated exposure resulted in an increased preference of the exposed sweet food only and not in an increased preference of a novel sweet food. This could, however, be a result of a food neophobic reaction to the novel sweet food, rather than the non-existence of a generalized preference for sweetened foods.

Repeated exposure is thought to reduce food neophobia [42]. Food neophobia, fear of new foods, is commonly seen in young children and supposedly protects children of eating harmful substances [43]. After a repetition of exposures without negative outcomes, children consider the food to be save [30]. Repeated exposure also teaches children which tastes, in which foods, are appropriate [32]. The latter was illustrated by Sullivan and Birch [32]. They showed that repeated exposure to sweet tofu did result in an increased liking for sweet tofu but not for salted or plain tofu. In the same line, as shown in the present study, repeated exposure to sweet orangeade did not necessarily lead to a preference for Sour-orangeade.

Preference for sweet taste has been positively correlated with a high consumption of sugar rich foods [12;21;44]. In the present study, the consumption of orangeade was not related to the preference for orangeade. This is in contrast with previous studies with children which suggested that consumption is positively related to taste preferences [22;23;45-49]. In the present study, however, most children consumed all the Sweet-orangeade they were presented with. This was also the case for adults on the first day of the intervention. Those who had a high preference for the orangeade might have been limited in their consumption by the amount they were given.

An alternative explanation is that children in the same classroom influenced each others drinking behavior. In that case the amount children consumed depended on the amount consumed by peers, rather than their own taste preference. Birch and colleagues suggested that peers can have a large influence on children's eating behavior [11]. We tried to prevent this influence of peers, by using opaque cups that were covered by an opaque lid. Children in the experimental groups could therefore not see how much everyone was drinking.

After the first day of exposure adults decreased their consumption of the Sweet-orangeade which could be caused by boredom [28]. After serving monotonous meals (i.e. identical meals) for several days, researchers showed that intake decreased and acceptance of the meal declined [27] even if the food was initially liked [28]. This phenomenon was not observed in children. Perhaps in the present study children were not offered enough orangeade in order to show a decrease in consumption after repeated exposure.

Unlike to that observed with repeated exposure to sucrose in orangeade, children did not increase their preference for sour taste after repeated exposure to sour taste. It is unlikely that this difference was due to the use of unreliable methodology. The rank-ordering procedures have been shown to be reliable with young children [16;50]. Moreover, in the present study the majority of subjects (except for children in the Sweet-Group) showed a significant agreement between the most preferred stimulus at baseline and after the intervention. The number of subjects in the present study might have been too small to show significant differences in preferences for sour taste. However, the number of subjects in the Sweet-Group and the Sour-Group were equal. Recall that we did observe changes in preference in children in he Sweet-Group.

An alternative hypothesis is therefore that, during childhood, preferences for sour taste are more stable than preferences for sweet taste. The small number of studies that investigated preferences for sour taste in children, concluded that these preferences can be changed by a long term exposure (approximately eight months) during infancy [22;26]. This could be due to the prolonged exposure or to the timing of the exposure. It has been suggested that early exposures to flavors are more effective than later ones in establishing taste preferences, due to the existence of sensitive periods [51]. The exposure to sour taste in the present study might have been to short or the time of exposure (i.e. during childhood) might have fallen outside the sensitive period in which children easily learn to prefer new tastes.

Adults in the present study did not change their taste preferences for the series of orangeades or yoghurt, as a result of an eight day repeated exposure to the Sweet-orangeade. This could be a result of the difference in preference for sweet taste between children and adults at baseline. In the present study children, compared with adults, had a higher preference for the Sweetest orangeade. It has been hypothesized that repeated exposure to stimuli people already liked, causes them to rate those stimuli even more positively, whereas repeated exposure to initially disliked stimuli leads to even more negative ratings [52]. Due to the difference in preference of the sweetest orangeade children were more likely to increase their preference for the sweetest orangeade than adults.

An alternative hypothesis is that adults are less likely to change their preference for sweet taste, because of their more expanded experience with foods and flavors [53]. As mentioned earlier, repeated exposure of flavors within a specific food context teaches children which flavors are appropriate in which food context. Adults were presumably less influenced by a short repeated exposure of sweet and sour taste in orangeade, because of their expanded experience with this beverage in the past.

In conclusion, the present study suggests that sweet preference of children can be modified by a short repeated exposure during childhood. In contrast with sour taste preferences which appear to be more stable. Adults' preferences for sweet and sour taste are less likely to be influenced by a short repeated exposure. Future studies are needed to determine whether changed preferences for sweet taste are stable over time and whether they are related to a change in the consumption of sugar rich foods.

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9

Discussion

The present thesis focused on sweet and sour taste preferences of children. We investigated how these preferences can be measured, which level of sweet and sour taste children prefer, and how these preferences are related to oral physiological processes, food consumption and repeated exposure. The individual studies are discussed at the end of the previous chapters and will therefore not be discussed in detail.
In the present chapter we give an overview of the main findings, after which we discuss critical design issues. Furthermore, we discuss the new insight in taste preferences of children we gained after 4 years of research. We then continue with the implications and recommendations for future research.

MAIN FINDINGS

The paired comparison and the rank-order tests are widely used methods to assess taste preferences and discriminatory ability of young children. Although 4-and 5-year old children were able to give consistent data for preference, large differences appeared when both tests were used to measure discriminatory ability. That is, 5-year-old children were able to carry out the paired comparison and the rank-order tests for discriminatory ability in a consistent manner, but 4-year-olds failed to carry out both tests in a consistent way (*chapter 2)*.
With respect to children's preferences for sour taste, we discovered that a substantial number of children that we tested had a preference for extreme sour taste in gelatins. This was not related to the rated intensity (*chapter 3 & 4)*, buffering capacity of saliva or salivary pH (*chapter 4*). Preference for sour taste appeared to be positively related to preference for unfamiliar stimuli (*chapter 3 & 4*), intense colors (*chapter 4*), consumption- and variety of fruits (*chapter 3 & 5)*, and early experiences with sour taste (*chapter 6*)
Preference for sweet taste can be modified by a short repeated exposure during childhood (*chapter 8*), however, restricting children's freedom to choose for a sweet tasting product by means of rules is related to a heightened preference for sweet taste (*chapter 7*).
In conclusion, sweet and sour taste preferences of young children can consistently be measured with paired comparison and rank-order methods. Children prefer beverages with high concentrations of sucrose and a substantial part of children have a preference for extreme sour foods. The latter is related to intensity-and novelty-seeking behavior and consumption of fruit. Preferences for sweet and sour taste can be modified by learned experiences (see **Table 1**).

Table 1 Research questions and the main findings, divided by chapter ch

Ch	**Research questions**	Main findings
2	Do paired comparison and rank-order procedures give consistent data concerning the most preferred level of sweet taste and the discriminatory ability of young children?	5-yrs-olds: preference tests, 76%-consistency, r=0.74, $p<0.01$; discrimination tests, 82% consistency, r=0.37, $P<0.05$. 4-yrs-olds: preference test , 61%-consistency, r=0.32, $P=0.37$; discrimination tests, 62% consistency, r=0.02, $P=0.95$.
3, 4 & 5	Which levels of sour taste are most preferred by children and how is this related to physiological determinants, thrill seeking behavior and fruit consumption?	36% (US) to 58% (Neth.) preferred extreme sour taste. This was not related to perceived intensity ($p>0.32$) or salivary pH (7.2 ± 0.12 vs. 7.2 ± 0.12), but was related to a higher salivary flow (1.8g ± 1.0 vs. 2.3 g ± 0.8, $P<0.05$), a lower buffering capacity of saliva (0.18 ± 0.07 vs. 0.33 ± 0.44 Δ pH/ mL saliva produced), preference for unfamiliar foods ($P<0.05$) and fruit consumption of boys (r=0.67, $P<0.001$).
6	What is the influence of early experience with sour tastes on preferences for sweet and sour taste of young children?	Sour formula fed Children (4-to 5-years of age) preferred sour juices significantly more than did older children who were fed similar formulas during infancy ($P<0.05$). No such age-related difference was observed in children who were fed milk-based formulas during infancy ($P=0.50$)
7	What is the role of parents on the preference and consumption of sweet foods of their children?	High sugar-restricted children consumed less sweet foods during breakfast ($P<0.009$) and lunch ($P<0.05$), but showed higher preference for sweet than low-restricted children. ($P<0.01$).
8	Can sweet and sour taste preferences of children be changed by a short repeated exposure to sweet and sour tasting stimuli?	Preference for a 0.42M (14% w/v) sucrose beverage increased after 8-day exposure ($P<0.05$). A similar exposure to beverage with 0.02M (0.4% w/v) added citric acid did not increase the preference for this beverage.

METHODOLOGICAL CONSIDERATIONS

In order to judge the studies that were presented in this thesis on its scientific relevance, we first have to evaluate the methodology that was used during the studies. We made several decisions about the way we selected our subjects, the sensory tests were carried out and how food consumption was measured. In the following paragraphs we discuss each topic and hypothesize how the decisions we made could have influenced the validity of our results

Selection of subjects

Population

The studies described in this thesis were carried out in the United Stated (*chapter 3 & 6*) and the Netherlands (*chapter 2, 4, 5, 7 & 8*). Large differences in preference for sweet taste were not expected and not found. Several studies around the world suggests that children's preference for high concentrations of sucrose is universal (e.g., Brazil [1]; France [2]; Iraq [3]; Israel [4]; Mexico [5]; Netherlands [6], North America [7;8]). Whether this is the case for sour taste is unknown. Preference for extreme sour taste is more common in countries were the general diet contains many sour-tasting foods [9]. The general cuisine in the US and Western Europe (among which the Netherlands) are fairly similar [10]. Furthermore, the environment and situation in which different foods are presented to children are similar in the US and the Netherlands. For example, in both countries the consumption of fruit is encouraged [11;12], sweet foods are seen as unhealthy by parents [13] and over consumption is more likely than foods shortage. We therefore conclude that the results of our studies are valid for both countries. However, differences in recruitment in both countries could have influenced our results.

Recruitment

In the US and the Netherlands the subjects were differently recruited. This could have resulted in restriction and selection bias. Restriction, the admissibility criteria for subjects, is an effective method to prevent confounding, but potentially provides a poor basis for generalization of the study results [14].

In all studies a restriction of age (4-to-12-years of age) was applied. This age range, middle childhood, is situated between the pre-school and adolescence years. During middle childhood children have the skills to perform sensory tests and have a moderate autonomy in the family [15;16].

Selection biases are distortions that result from procedures used to select subjects and from factors that influence study participation [17]. Subjects in the US were recruited from advertisements in Philadelphia newspapers, which were free of charge. The Philadelphia area has a population of approximately 6 million inhabitants from various races (72.5 %

Caucasian, 19.6 % African American, 7.9% others) [18]. Compared to the total population of the US, Philadelphia has a slightly higher percentage of African Americans (12.6% vs. 19.6%) [19]. Our sample (*Chapter 3 & 6*) (56% Caucasian, 39% African American and 4% other), consisted of fewer Caucasian and more African Americans than would be expected from a random sample of the Philadelphia population. Several studies suggested that African Americans have a preference for higher concentration of sucrose than their Caucasian counterparts [8;20-22]. Due to the relatively high number of African Americans the founded preference for sweet taste may not be valid for the total US population. However, as reported in *chapter 6*, no main effect of race on preference for sweet taste was found, therefore the higher number of African Americans did probably not affect our outcome measures (*chapter 3 & 6*). Ethnic differences, within our sample in the US, are also unlikely to explain the observed differences in preference for sour taste after early exposure to sour formula. In *chapter 3*, no ethnic differences were found between children who preferred sour taste and those who did not. Furthermore, in the US as well as in the Netherlands we found a positive relationship between preference for sour taste and novelty seeking behavior, despite the high number of African Americans in the US sample.

In the Netherlands the recruitment of the subjects was organized differently. The study population was restricted to three schools that were located on different sites of the Wageningen area. The studies reported in *chapter 2, 5, 7 & 8* were carried out at a school were the education level of the parents was relatively high compared with the Dutch average [23]. A previous study showed that the education level of parents is positively related with the tendency to impose rules to restrict the consumption of certain foods [24]. Other studies suggested that parental education level [25] and intellect [26] are positively related to children's own intellect (see for further discussion *chapter, 2, 5, 7 & 8*).

The high number of well-educated parents could have resulted in a higher number of parents who were very strict on the consumption of sugar of their children. It is unlikely that this affected our study findings (*chapter 7*) to a large extent, because the aim of this study was to investigate differences between children who were highly restricted and low restricted within our study population rather than investigating how many parents in the Netherlands were strict on sugar. Moreover, the variation of parents' level of education within our sample was not related to any of our outcome measures. Whether our results can be extrapolated to children of parents with a low education level needs to be investigated.

Sensory testing with young children

In order to assess the reliability of our sensory tests, we carried out repeated measurements (see **Table 2**). From these repeated measurement we conclude that young children were able to carry out the tests in a reliable fashion. This is in line with other research on taste preferences of children [27-33].

Table 2 Sensory methods used in thesis

Ch	Age	Sensory test	Outcome	Stimuli	Reliability
2	N=68, 4-5 yrs	Paired comparison Rank-order	Preference Intensity	Orangeade	Repeated measurement
3	N=61 5-9 yrs	Rank-order Rank-order	Preference Intensity	Gelatin	repeated measurement
4	N=89 7-12 yrs	Rank-order 5-category scale	Preference Intensity	Gelatin	Repeated measurement
5	N=59 8-11 yrs	Rank-order	Preference	Gelatin	Repeated measurement
6	N=83 4-7 yrs	Paired comparison	Preference	Apple juice	-
7	N=43 4-5 yrs	Paired comparison Rank-order	Preference	Orangeade	Repeated measurement
8	N=59 6-11 yrs	Rank-order	Preference	Orangeade	Repeated measurement

Caution should, however, be taken when interested in *discriminatory ability* of 4-year-olds. This is an analytical task rather than a hedonic judgment. As reported in *chapter 2*, we demonstrated that commonly used sensory methods to measure discriminatory ability do not give consistent data when used with 4-year-olds (for a further discussion see *chapter 2*).

Although the internal validity and consistency of our sensory tests can be assured by repeated measurements, the external validity is not warranted. Previous studies showed that preferences for high sweet taste as measured in a laboratory setting are related to high consumption of sweet foods in daily life [1;3;34;35]. Our studies reported in *chapters 3 & 5* suggest that also laboratory measurements of preferences for sour taste are related to consumption of sour foods in daily live. This supports the external validity of our studies. It can, however, not be concluded that those who preferred a specific taste during our sensory tests, prefer this taste quality in all foods. Most likely preference for

sour and sweet taste is limited to those foods that, according to culture, are suppose to be sweet and/or sour.
Furthermore, it needs to be noted that, by using traditional sensory methods such as paired comparison and rank-order procedures, bias caused by differences in cognitive skills or lack of attention can never be ruled out. These sources of bias will only be of a larger influence when the research sample exists of young children. Therefore there is a need for an objective measurement of preference and discriminatory ability. In adults it has been suggested that differences in preferences is accompanied with differences in physiological processes in the human body, such as brain activity [36]. Whether this can be used in sensory testing with young children is unknown and worthwhile to investigate.

Setting of the sensory tests

The reliability of the outcome of sensory tests also depends on the setting children are tested in. In the US we tested children individually in a closed room, hereafter referred to as 'single testing'. In the Netherlands we also tested children on a individual bases, but they sat in a classroom with about ten other participants who were all individually tested, hereafter referred to as 'multiple testing'. Single testing gives the researcher the possibility to avoid influences from peers during testing and to highly control the testing environment (i.e. high-air ventilation, no distracting objects present). On the other hand, single testing is time consuming and therefore the sensory tests of a large number of subjects are spread out over several months. This means that seasonal variation, such as the availability of certain foods and outside temperature cannot be controlled for. These variables could be of influence on children's taste preferences. For example, during and shortly after the Halloween festivities children consume large quantities of sweet foods [37], this could result in a higher exposure to sweet foods than normally. During periods of extreme high temperatures children have a large change to become dehydrated [38], this could influence their taste preferences [39].
Multiple testing is less time consuming and the sensory tests of a large number of subjects can be carried out in just two days. On the other hand, one has to be aware of the influence of peers [30]. This influence could result in a more homogenous group with respect to taste preferences than would be the case during individual testing [30]. Furthermore, multiple testing involves more than one researcher that is supervising the subjects.
In the present thesis we used individual testing (*chapter 3 & 6*) as well as multiple testing (*chapter 2, 4, 5, 7 & 8*). The influence of seasonal variation on our results that were obtained with individual testing is likely to be not significant. The founded taste preferences are in line with other research that is carried out during different times of the year in countries with different climates. The variation in preferences for sweet and sour

taste during individual testing are more likely to be related to differences in exposure and children's temperament.

During our multiple testing we minimized the influence of peers by separating the subjects by at least one meter. Furthermore, a trained adult who was instructed to minimize the influence of peers guided each subject. Moreover, all studies, but one, were checked on reliability by repeated measurement.

Food consumption questionnaires

During the studies several questionnaires were used to measure food consumption. The discussion on each specific questionnaire can be found in the previous chapters. The present paragraphs will give a general discussion on the use of food frequency questionnaires in the type of research described in this thesis.

It has been shown in previous research [40] and in the present thesis that young children are able to express their food and taste preferences in a reliable fashion. Children older than approximately 8-years of age are also able to report their food frequency under supervision of an adult [41-43]. Younger aged children are less able to report their own food consumption [44;45], therefore research with these children, relies on parental report when it comes to food frequency [13;41;46-52]. Because our age-range of interest (4-12 years of age) included children below the age of 8, food frequency was measured by means of parental report (*chapter 3, 5, 6, 7 & 8*). It has been suggested that this approach gives reliable data for foods consumed at home, but might not be accurate for foods consumed outside of the home environment [45].

In our studies we did not assess the number of foods consumed out of home. This could have resulted in an underestimation of the sugar (*chapter 7*) and fruit consumption (*chapter 5*). In our studies this is of minor importance because in *chapter 7* we were interested in the sugar consumption that was controlled by parents. In *chapter 5* we were interested in the relative consumption rather than the absolute number of fruits they consumed. When interested in absolute numbers of consumption it is recommended to include both parents and others who take care of the child in the reporting process (see [45] for review)

TASTE PREFERENCES, NEW INSIGHTS

As noted in the introduction of this thesis, there has been extensive research carried out on preferences for sweet taste in children. Research on sour taste preferences of children did, however, not exist. A literature search in Medline on the key words "sour" "taste" "preferences" and "children" resulted in no relevant publications before the initiation of our research in the year 2000. To date a same Medline search resulted in three relevant studies [53-55], two of which are reported in the present thesis. With the submission of three other papers (*chapter 4, 5 & 8*) with relevant research on sour taste preferences of children, we conclude that the results of the present thesis add substantial knowledge to the field of taste preferences of children.

Preference for extreme sour taste

The research reported in *chapter 3* was the first scientific study that investigated children's preferences for extreme sour taste. Marketing-and consumer reports suggested that children have a preference for extreme sour taste [56;57]. Detailed scientific information about this phenomenon was, however, not available. In the past it has already been suggested that children live in a different sensory world. They prefer higher concentrations of sucrose [6;8;58;59] and salt [8] than adults. Based on our research it can be concluded that this heightened preference is also evident for sour taste, in 5- to 12-year-old children (*chapter 3 & 4*). Longitudinal studies with sweet taste, suggested that preference for this taste decreases with age [8]. From our study (*chapter 3*) we can at least conclude that parents of children who preferred extreme sour taste did not prefer this taste themselves. We assume that children's preference for sour is not related to a specific cohort, as suggested by Darwin's observations more than 100 years ago [60]. Longitudinal studies are, however, needed to investigate whether preference for sour taste decreases with age.

There are at least two mechanisms that could explain the decrease of preference for sour taste with age. As suggested for sweet taste, high preference of young children for a specific taste could be explained by their physiological need [7]. However, while the physiological consequences of the consumption of sweet foods are clear (i.e. calorie uptake), this is less evident for the consumption of sour foods. Furthermore, preference for high sweet solutions seems to be innate, while preference for sour taste is not [61-63]. Moreover, preference for sweet taste was seen in almost all children, whereas preference for extreme sour taste was only observed in a subset of the children we tested. It is more likely that some children prefer extreme sour taste as a result of early exposure or as part of sensation seeking behavior. After a more expanded experience with foods mostly eaten by adolescence and adults, children adjust their preference for sour taste when they approach the adult age. Similar to preference for salt taste [64], the most preferred level of sour taste at adult age is likely dictated by dietary experiences. We therefore hypothesize that preference for sour taste decreases with age and that this is due to dietary experiences rather than physiological need.

Preference for sour taste relates to novelty-and intensity seeking

For the first time a scientific investigation related preference for extreme sour taste of children to preferences for unfamiliar (novel) foods (*chapter 3 & 4*) and intense visual stimuli (*chapter 4*). In adults it has previously been speculated that sensation seekers are more likely to prefer sour taste [65] and that those who preferred a wide range of products also liked higher intensities of sour taste [66]. Perhaps sour taste elicits similar sensations as novel foods and intense visual stimuli. This is in contrast with preference for sweet taste, which is associated with comfort and pain relieve [67]. The optimal balance between sweet and sour taste, hypothetically represents the optimal balance between comfort and sensation.

Causality can, however, not been drawn from our studies. Perhaps children develop a preference for sour taste early in life, due to early flavor experiences (*chapter 6*). Subsequently, they not only become to like this intense taste but also the associated intense sensation. During childhood they are more likely to expand this preference for intense sensation to other modalities such as novelty and color intensity. It is, however, unlikely that early exposure to sour tasting hydrolysate formula (*chapter 6*) fully explains the variation in preference for extreme sour taste during childhood. Hydrolysate formulas are only given to a small portion of the infants in the US [68], whereas the prevalence of children's preference for extreme sour taste is close to one third of the children tested. Preference for sour during childhood must then be a result of exposure to other sour tasting foods such as lemons. Unfortunately we have no data on the foods children consumed during their first years of life.

An alternative explanation is suggested in *chapter 3*. We hypothesize that children's novelty and intensity seeking behavior leads to an increased likelihood to try sour foods. After repeated consumption of these foods, which fulfill their need for novelty and intense experiences, they develop a preference for sour taste. Behavioral differences among children can already be observed during early infancy [69] and may precede children's preference for extreme sour taste. New studies are, however, needed to confirm this hypothesis.

Whether our results can be extrapolated to all cultures is questionable. In countries were many extreme sour foods are part of a normal diet, extreme sour taste may not be a novelty. Relationships between preference for sour taste and preference for novel foods may then not exist.

Preference for sour taste does not relate to oral physiology

Preference for sour taste is most likely not due to a low perceived intensity (*chapter 3 & 4*). However, as suggested in *chapter 4*, children who preferred extreme sour taste did have a higher salivary flow. Spielman (1990) [70] suggested that a high salivary flow results in a lower perceived intensity of sourness, due to the dilution of citric acid and the large amount of buffering agents. In their study the used sour stimuli were of a much lower intensity than the stimuli that were used in our studies. A high salivary flow may not be sufficient enough to change the perceived sour intensity of our stimuli. Perhaps in our study the high salivary flow in children who preferred extreme sour taste, affected the aftertaste rather than the sour intensity during and shortly after the stimulus was consumed. Hypothetically, the high salivary flow results in a faster disappearance of the sour stimuli from the oral cavity.

An alternative explanation for the discrepancy between Spielman's study and ours is that saliva of those who preferred extreme sour taste has a low buffering capacity per mL saliva. This hypothesis can, however, not fully be confirmed by our studies, because buffering capacity of saliva was not measured by means of titration (see *chapter 4* for further discussion).

Preference for sour taste relates to fruit consumption

Our explorative study reported in *chapter 5* was the first to suggest that sour taste preferences play an important role in the consumption of fruit in boys but not girls Previous studies already suggested that preference for fruit is an important determinant of fruit consumption [71;72;72-74]. However, our data seems to suggest that preferences for sour taste may play a different role in the fruit consumption of boys than for girls. Previous studies suggested that boys are better than girls in adjusting their food consumption in response to internal cues (e.g. satiety) [75]. Perhaps girls' preferences for fruit are more determined by external cues such as parental control [76], health related motives [77;78] and availability [79]. More studies are needed to investigate the causality if the relationship between preference for sour taste and consumption of fruit.

CHANGING OF TASTE PREFERENCES, NEW INSIGHTS

Before the initiation of our studies in the year 2000 interventions concerning the effect of repeated exposure on subsequent preference only focused on foods, mostly novel foods, rather than *taste per se* [80-85]. Food exists of many variables which influences preferences, such as appearance [86;87] and familiarity. [52]. Enhanced food preferences after repeated exposure is therefore not necessarily a result of an increased preference for the specific taste of the food. Early research of Zajonc showed that people could increase their preference for a meaningless object by repeated exposure [88]. Although several studies already suggested that repeated exposure to sweet taste is positively related with preference for sweet taste, causality could never been drawn from these studies [20;89]. To our knowledge our study reported in *chapter 8*, was the first experimental study in young children that showed that repeated exposure to a specific concentration of sucrose increases preference for this concentration.

Prevent increase rather than promote decrease of sweet preference

It is, however, unlikely that preference for sweet taste during middle childhood can be decreased. Most sweet tasting products are caloric dense and are, due to caloric conditioning, easy to develop a preference for [90]. Sweet tasting products such as candy, ice cream and soda are often associated with celebration, comfort or reward [47]. All these associations will make it difficult to decrease the preference for sweet taste in young children.

An alternative strategy is to prevent the increase of preference for sweet taste. Hypothetically, decreasing the number of exposures to sweet foods would stabilize children's preference for sweet taste and prevent it from increasing. Decreasing children's sugar consumption by means of strict rules, may, however, result in a high preference for sweet taste (*chapter 7*). This is in line with findings of Fisher and Birch who concluded that restricting children's access to palatable foods result in an increase desire and consumption of those foods [91;92].

We hypothesize that decreasing the consumption of sweet foods in order to prevent the increase of preference for sweet taste, can only be effective if children do not perceive themselves as being restricted. Children have to prevent excessive consumption of sugar themselves, rather than obeying rules that are set by their parents. A similar approach was suggested for energy dense foods: "The optimal environment for children's development of self-control of energy intake is that in which parents provide healthy food choices but allow children to assume control of how much they consume." [75].

It needs to be noted that our research population in *chapter 7* were all 4-to 5-year-old Dutch children, whether our findings can be generalized to older aged children is unknown. Supposedly, older aged children are less dependent on their parents with respect to sugar consumption. Rules that parents apply to restrict their children's sugar consumption, have therefore a lower impact on older aged children than on younger aged children. Furthermore, our study was carried out in an industrialized country were over consumption is more likely than foods shortage. In countries were there is a shortage of food, it is questionable if parents would apply rules that restrict sugar consumption of their children.

Changing preference for sour taste, early exposure

As shown in *chapter 8* preferences for sour taste are not easily changed by a short repeated exposure during childhood, but can possibly be changed by repeated exposure during infancy ([53] and *chapter 6*). This suggests that preferences for sour taste are determined early in life. It is unlikely that children were given sour formula because they preferred sour taste. Parent who decided to feed their infants sour-bitter tasting formula, did not base their decision on the infant's ingestion or taste preferences, but rather on the expected reduction of colic (*chapter 6*). The importance of early flavor experiences on food acceptance has previously been shown with these formulas. Infants who were introduced to the formula before 2 months of age continued drinking these formulas during their first year of life. However, infants who are introduced to these formulas at 7 months of age show similar rejections as adults [93;94]. Early flavor experiences do not only affect preferences during infancy and childhood, but possibly also affect preferences during adulthood. Haller and colleagues suggested that adults who were fed a vanilla flavored baby formula during infancy, were more likely to prefer vanilla flavored ketchup than adults who did not experience this formula during infancy [95]. Our study (*chapter 6*) and Haller's study were both observational studies. There is a need for intervention studies to confirm the influence of early flavor experience on taste preferences during childhood and adulthood.

Changing preference for sour taste, prenatal exposure

Besides, taste experiences during infancy, taste preferences are also thought to be influenced by prenatal events. Research on salt taste suggested that preferences for high concentrations of salt in infants [96] and adults [97] are related to reported morning sickness of their mothers. It has been hypothesized that extra cellular dehydration and electrolyte imbalance related with morning sickness influences the development of salt taste preference of the offspring [98]. Mennella and colleagues suggested that prenatal experience with carrot flavor increases infants' preference for this flavor [99]. Along the same line, prenatal experience with anis flavor affected preference for anis after birth [100]. Hypothetically, also preferences for sour taste can be modified before birth by means of the mothers' diet, or other prenatal events. However, as suggested in *chapter 3*, no relationship was found between mothers' and their children's preferences for sour taste. It is, however, unknown whether mother's present diet was a reflection of what she consumed during pregnancy.

Changing preference for sour taste, caloric conditioning

An alternative approach to increase children's preference for sour taste could be caloric conditioning. It has been shown that children learn to prefer flavors that are associated with high energy density (see [101] for review). Exposing children to high-energy dense foods with a predominant sour taste could result in a preference for high sour taste. Studies with rats showed that preference for sour taste can be increased when the oral ingestion of water with added citric acid was paired with the intragastrically infusion of 16% glucose water [102].

Replacing sweet preferences by sour preferences

The prevention of excessive intake of sweet foods could lower children's caloric intake. Increasing children's preference for sour foods could be beneficial for the dietary variety and the fruit consumption of young boys (*chapter 3, 4 & 5*). Replacing sweet preference by preference for sour taste could therefore result in a diet low in calories and high in variety. Increasing preference for sour taste does, however, not entail that sweet preferences are automatically decreased. In the present thesis we have no evidence that children who preferred high sour taste had a lower preference for sweet taste than those who did not prefer sour taste (*chapter 6*). On the contrary, most sour products consumed by young children such as candies are also high in sugar contents. As mentioned earlier, decreasing preference for sweet taste in children is in our opinion not realistic. Children will always prefer sweetness, but by decreasing the exposure to sweet taste a further increase of sweet preference can be tempered. For an important part of children this decrease in exposure can be established by increasing sourness. For example soda could be decreased in sweetness and increased in sourness. It needs to be noted that this approach is not valid for all products. The addition of sugar makes products that are rich in fat more palatable [103]. Whether sour taste can take over this task is questionable. Furthermore, the consumption of large amounts of sour foods can result in teeth erosion. This is mainly due to the low pH. The concentration of H+ ions is, however, not the only determinant of sour taste. It has been shown that sour tasting agents with a similar pH can elicit a different perception of sour taste [104]. It remains to be investigated whether this can be used to produce sour foods that are preferred by children but that do not cause teeth erosion.

MAIN CONCLUSIONS

1. Sweet taste is highly preferred by young children, this can consistently be measured.
2. Repeated exposure to high concentrations of sucrose during childhood can result in heightened preference for this concentration in a variety of foods. However, strict parental control on the consumption of sweet foods hypothetically increases preference for sweet taste.
3. An important part of young children have a preference for extreme sour tasting foods. This preference is most likely driven by a general preference for extreme stimuli and the urge to search for new and exciting events in life. Preferences for sour are likely determined early in life and appear to be related to consumption of fruit in daily life.

IMPLICATIONS AND RECOMMENDATION FOR FURTHER RESEARCH

The knowledge gained by the present thesis can be of great use for health professionals and those working in industry. There is growing evidence that consumption of sugar derived from beverages such as soda and fruit juices is related with childhood obesity. In order to prevent children from developing preference for excessive high concentrations of sucrose, children need to be directed to other foods that are highly preferred but do not elicit a intense sweet taste. The knowledge that an important part of young children have a preference for intense sour foods, may open the window to the promotion of low sweet – high sour foods. Not all children have a preference for sour taste, however, we suggest that preference for sour taste can be modified early in life. This modification of preferences for sour taste could be beneficial for fruit consumption and the dietary variety during childhood.

In our opinion it is unrealistic to assume that children can be taught to get an aversion for sweet taste. However, sweet taste may not be a necessity for products in order to be highly preferred by children. Sour taste is already preferred by a substantial part of children. Moreover, there is now evidence that a preference for sour taste can be created by early exposure to this taste quality.

Although the present thesis gave answers to many prominent questions, it raised even more questions that need to be answered by other investigations. Below are some suggestions.

1. As suggested by the present thesis, conventional methods to measure preference and discriminatory ability of children largely depend on children's skills to perform these tasks. Biological measurements such as functional magnetic resonance imaging (fMRI) may provide more reliable results when children are not able to understand the conventional methods. Although this biological measurement is tested with adults, with mixed results, to our knowledge they were never used during sensory testing with young children. Research with young children would especially benefit from such approach.
2. In order to investigate whether preference for extreme sour taste decreases with age, longitudinal studies are needed. We realize that the influence of dietary experience is hard to record during a longitudinal study with humans. In addition to these longitudinal studies with humans, it is recommended to carry out a longitudinal study with rats. Bertino and colleagues demonstrated that rats showed a similar decrease in preference for sweet taste with age as humans.

During this experiment the researchers were able to keep the diet the same during their study. It was therefore concluded that the decrease in sweet preference is most likely independent from dietary experiences [105].

3. Based on our observational study (*chapter 6*) we suggest that early experiences with sour taste influences preference for sour taste during childhood. Furthermore, it has been suggested that preference for sour taste is related with, novelty and intensity seeking behavior and the consumption of fruit in boys. Experimental studies are, however, needed to confirm our findings. The first step of such study has recently be undertaken by Mennella and co-workers [94]. In this study infants were randomly assigned to different feeding regimens which consisted of sour-bitter hydrolysate formula and/or regular milk based formula. The next step would be to follow up these children and measure their preference for sour taste, fruit consumption and novelty- and intensity seeking behavior when they reach the middle childhood age.
4. Hypothetically, the high salivary flow, of those who prefer extreme sour taste, results in a faster disappearance of the sour stimuli from the oral cavity. In order to test this hypothesis, it needs to be investigated how long after ingestion the sour taste is still perceived in the oral cavity. Furthermore, the buffering capacity of saliva needs to be determined by means of titration in order to investigate whether there are differences between those who prefer sour taste and those who do not.
5. We hypothesize that decreasing the consumption of sweet foods in order to prevent the increase of preference for sweet taste, can only be effective if children do not perceive themselves as being restricted. We suggest an experimental design with three groups. One group needs to be restricted in their sugar consumption by applying strict rules. The second group needs to be encouraged to consume foods that are low in sugar contents and needs to be provided with low-sugar alternatives. The third group is a control group that can freely choose between sugar -and non-sugar containing products. Before and after the intervention children are tested for their preference for sweet taste and the food choices children make when parents or caretakers are not present. Furthermore, it needs to be determined whether sugar restriction rules are applied in a similar way across parents with different social economic and educational backgrounds. The results of these studies should result in advice that can be given to parents.
6. The influence of prenatal events on infants and children's preference for sour taste is unknown. Experimental studies such as carried out by Mennella and colleagues [99] are needed to investigate this matter.

7. Foods that are low in pH could cause teeth erosion. There is a need for additives that create a sour taste without lowering the pH of the product.

Answers to all these questions will able us to better understand children's taste preferences and understand better why children prefer the foods they do.

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Summary

In the industrialized countries children's food consumption depend largely on their taste preferences, which are formed by innate and learned responses. The present thesis focused on preferences for sweet and sour taste of young children (4- to 12-years of age) living in the US and the Netherlands. Preferences for sweet taste are of interest because of their association with energy density and therefore its possible role in childhood obesity. Preferences for sour taste are poorly investigated but may increase dietary variety and interact with the perception of sweet taste. Understanding how sweet and sour taste preferences are formed and modified can help health professionals and those working in industry, to develop strategies to decrease the consumption of sweet tasting foods and to increase the variety of children's diet with sour tasting foods.

Methodology

C*hapter 2* focused on the methodology concerning sensory testing with young children. We investigated whether rank-order and paired comparison tests gave consistent results during preference (0.14, 0.20, 0.29, 0.42 and 0.61M sucrose in orangeade) and discrimination tests (0.22, 0.25, 0.29, 0.34 and 0.39M sucrose in orangeade) with young adults (*n*=22), 4-year-olds (*n*=21) and 5-year-olds (*n*=47). The results showed that young adults and 5-year-old children were able to discriminate between all solutions and showed a high consistency between the rank-order and pair-wise tests for discriminatory ability (>76% consistency) and preference (>71% consistency). In contrast, 4-year-olds detected differences in sweetness during the preference tests, but failed to distinguish sweetness intensities during the discriminatory ability tests. The dissimilarity between 4- and 5-year-olds is thought to be due to differences in cognitive skills rather than sensory perceptual differences.

Taste preferences

C*hapter 3, 4 and 5* were dedicated to preferences for sour taste of young children. In *chapter 3* we investigated whether the level of sourness (0.0, 0.02, 0.08 and 0.25M added citric acid) most preferred in lemon flavored gelatin and the ability to discriminate differences in sour intensity differed between 5- to 9-year-old US-children (*n*=61) and their mothers. The results indicated that, although every mother and all but two of the children (92%) were able to rank the gelatins from most to least sour, more than one-third (35%) of the children, but none of the adults, preferred the high levels of sour taste in gelatin. Those children who preferred the extreme sour tastes were significantly less food neophobic ($P< 0.05$) and tended to experience a greater variety of fruits. A similar study with eighty-nine 7- to- 12-year-old Dutch children confirmed that 33% to 50% have a preference for extreme sour tastes (*chapter 4*). In addition this study showed that preference for extreme sour taste is not related to the perceived intensity, buffering

capacity of saliva or salivary pH. Preference for sour taste appeared to be related to preference for unfamiliar stimuli and intense colors.

Besides the relationship between preferences for sour taste and the variety of fruits consumed (*chapter 3*), these taste preferences may also be related to the *amount* of fruits that are consumed. This hypothesis was tested in *chapter 5* (n=50, mean age 9.2 ± 0.9 yrs, rank-order test with 0.009, 0.013, 0.020, 0.029, 0.043 and 0.065M added citric acid to orangeade). Fruit consumption was assessed with a validated food-frequency questionnaire that was completed by the children's parents. Results showed that preference for sour taste of boys (standardized regression coefficient r=0.67), but not girls (standardized regression coefficient r=0.06) was positively correlated with their consumption of fruit (P<0.05).

Changing taste preferences

The studies reported in *chapter 6 to 8* focused on the effect of repeated exposure on sweet and sour taste preferences. In the first study (*chapter 6*) we investigated the role of early flavor experiences to sour tasting baby formula on sweet-and sour taste preferences during childhood. Eighty-three children were divided into four groups based on the type of formula fed during infancy and age. By using a paired comparison test we determined the level of sweetness (0.16-0.93M sucrose) and sourness (0-0.070M citric acid) preferred in juice. Children (4-5 yrs) who were fed protein hydrolysate formulas, which have a distinctive sour taste, preferred higher levels of citric acid in juice when compared to older children (6-7 yrs) who were fed similar formulas. No such difference was observed between the groups for sweet preference.

Taste preferences are not only influenced early in life, but repeated exposure during childhood also impact upon preferences for tastes (*chapter 8*). During an intervention study of eight days, 59 children (9.2 ± 0.9 yrs.) and 46 young adults (22 ± 2.0 yrs) received each day either orangeade with a sweet taste (0.42M sucrose), a sour taste (0.42M sucrose and 0.02 or 0.04 M added citric acid), or no orangeade (control). Before (baseline) and after the intervention, preferences for a series of orangeades and yoghurt that varied in balance of sweet and sour taste, were measured by means of a rank ordering procedure. After an 8-day exposure to the sweet-orangeade, children's preferences for this orangeade (0.42M sucrose) significantly increased (P<0.05). A similar trend was observed for the yogurt with 0.42M sucrose (P=0.09). An 8-day exposure to the sour-orangeade, did not have a significant effect on children's preference for this orangeade. The taste preferences of adults did not change after the intervention. The control group of children and adults did not show any change in preferences for sweet and sour tastes.

Although repeated exposure to sweet taste may increase children's preferences for sweet taste, restricting children's freedom to choose for these product may result in an increase in preference for sweet taste. This hypothesis was tested in *chapter 7*. To this end, 44 children (5.1 ± 0.5 yrs) performed a rank-order and paired-comparison test of preference for five orangeades (0.14, 0.20, 0.29, 0.42, 0.61M sucrose). Parents filled out a questionnaire concerning restriction rules and their children's consumption of mono and disaccharide (MDS)-containing foods. Stronger restriction rules were related to a lower consumption of beverages that contained MDS and to a lower consumption of MDS-containing foods during breakfast and lunch. Fifty-five percent of the children who were highly restricted showed a preference for the highest concentration of sucrose in orangeade. None of these children preferred the orangeade with the lowest concentration of sucrose. While 19% of the children who were little restricted preferred the beverage with the lowest concentration of sucrose, 33% preferred the beverage with the highest concentration.

In *chapter 9* we discus several methodological considerations as well as new insights gained by the present thesis concerning taste preferences and changing taste preferences of children. Furthermore, implications and recommendations for further research are given.

Conclusion

Sweet and sour taste preferences of young children can consistently be measured with paired comparison and rank-order methods if cognitive skills related to small age differences are taken into consideration. Young children prefer beverages with high concentrations of sucrose and a substantial part of children have a preference for extreme sour foods. The latter is related to intensity and novelty seeking behavior and consumption of fruit. Preferences for sweet and sour taste can be modified by learned experiences.

Samenvatting

In de geïndustrialiseerde landen worden voedselvoorkeuren van kinderen voornamelijk bepaald door hun aangeboren en aangeleerde smaakvoorkeuren. Dit proefschrift richtte zich op voorkeuren voor zoete en zure smaak van jonge kinderen (4 tot 12jaar oud) die woonachtig waren in de Verenigde Staten of Nederland. Voorkeuren voor zoete smaak zijn interessant door de associatie van deze smaak met energiedichtheid en het mogelijke verband met zwaarlijvigheid van kinderen. Er is weinig onderzoek gedaan naar voorkeur voor zure smaak van kinderen. Mogelijk zorgt deze voorkeur voor een grotere verscheidenheid aan voedingsmiddelen die kinderen eten en is er een interactie tussen zure en zoete smaakwaarneming van kinderen. Door te begrijpen hoe de voorkeur voor zoete en zure smaak ontstaat en hoe deze zijn te beïnvloeden, kunnen wetenschappers en diegenen werkzaam in de industrie strategieën ontwikkelen die er op gericht zijn om de consumptie van het aantal zoete voedingsmiddelen te verlagen en het voedingspatroon van kinderen uit te breiden met zuur smakende voedingsmiddelen.

Methodologie

Hoofdstuk 2 richtte zich op de methodologie van sensorisch onderzoek bij jonge kinderen. We onderzochten of rangorde en paarsgewijze methoden consistente resultaten gaven tijdens testen gericht op voorkeur (0,14; 0,20; 0,29; 0,42 en 0,61M sucrose) en onderscheidend vermogen (0,22; 0,25; 0,29; 0,34 en 0,39M sucrose) van jong volwassenen (n=22), 4 jarigen (n=21), en 5 jarigen (n=47). Uit de resultaten bleek dat jong volwassenen en 5-jarigen alle oplossingen van elkaar konden onderscheiden. Verder was er een hoge consistentie tussen de rangorde en paarsgewijze methoden voor onderscheidend vermogen (>76% consistentie) en voorkeur (>71% consistentie). Dit in tegenstelling tot 4-jarigen, die in staat waren om verschillen tussen de oplossingen waar te nemen tijdens de voorkeurstest, maar dit niet konden weergeven tijdens de testen die gericht waren op onderscheidend vermogen. Het verschil tussen 4- en 5-jarigen is waarschijnlijk te wijten aan een verschil in cognitieve vaardigheden en niet aan verschillen in sensorische perceptie.

Smaakvoorkeur

De *Hoofdstukken 3, 4 en 5* waren gewijd aan voorkeur voor zure smaak van kinderen. In *hoofdstuk 3* onderzochten we of de meest geprefereerde concentratie citroenzuur (0,0; 0,02; 0,08; en 0,25M toegevoegd citroenzuur) in citroenpudding en het onderscheidend vermogen met betrekking tot zuur verschilden tussen 5-tot 9-jarige Amerikaanse kinderen (n= 61) en hun ouders. Uit de resultaten bleek dat alle moeders en kinderen, op twee na, (92%) in staat waren om de verschillende concentraties citroenzuur te onderscheiden. Echter, meer dan één derde (35%) van de kinderen, maar geen van de ouders, hadden een voorkeur voor extreem hoge concentraties citroenzuur in pudding. Kinderen die van extreem zuur hielden waren significant minder bang voor nieuwe

voedingsmiddelen ($P<0.05$) en het leek er op dat ze een grotere diversiteit aan fruit aten. Een soortgelijk onderzoek met 89 7 tot 12 jarigen bevestigde dat 33% tot 50% een voorkeur had voor extreem zure smaak (*hoofdstuk 4*). Verder toonde dit onderzoek aan dat voorkeur voor extreem zuur niet gerelateerd was aan waargenomen zuurintensiteit, bufferende werking van speeksel of de pH van het speeksel. Voorkeur voor zuur bleek wel gerelateerd te zijn aan voorkeur voor onbekende stimuli en intense kleuren.
Naast de relatie tussen zuurvoorkeur en de consumptie van een grote diversiteit aan fruit (*hoofdstuk 3*), zou voorkeur voor zuur ook gerelateerd kunnen zijn aan de hoeveelheid fruit die kinderen eten. Deze hypothese werd getest in *hoofdstuk 5* (n=50, gemiddelde leeftijd 9.2 ± 0.9 jaar, rangorde test met 0,009; 0,013; 0,020; 0,029; 0,043 en 0,065M toegevoegd citroenzuur aan sinaasappellimonade). Fruitconsumptie was gemeten met een gevalideerde voedselfrequentie vragenlijst die werd ingevuld door de moeders van de kinderen. De resultaten lieten zien dat de voorkeur voor zuur van jongens (gestandaardiseerde regressiecoëfficiënt r=0.67), maar niet meisjes (gestandaardiseerde regressiecoëfficiënt r=0.06), positief was gerelateerd aan fruitconsumptie ($P<0.05$).

Veranderen van smaakvoorkeuren

Het onderzoek dat beschreven staat in de *hoofdstukken 6 tot 8* richtte zich op het effect van herhaalde blootstelling op zoet en zuurvoorkeuren. In het eerste onderzoek (*hoofdstuk 6*) bestudeerden we de rol van vroege bloostelling aan zure babyvoeding op de voorkeuren voor zoet en zuur tijdens de kinderjaren. Drieëntachtig kinderen werden onderverdeeld in vier groepen gebaseerd op het type babyvoeding en leeftijd. Met een paarsgewijze methode bepaalden we het meest geprefereerde nivo van zoet (0.16-0.93M sucrose) en zuur (0-0.070M citroenzuur) in sap. Kinderen (4 en 5 jarigen) die met gehydrolyseerde babyvoeding (zure-bittere smaak) waren gevoed, hadden een voorkeur voor hogere concentraties citroenzuur in sap dan oudere kinderen (6 en 7 jarigen) die ervaring hadden met dezelfde soort babyvoeding. Een soortgelijk verschil tussen de leeftijdsgroepen werd niet gevonden voor zoet voorkeur of voor kinderen die een op melk gebaseerde baby voeding hadden gekregen
Smaakvoorkeuren zijn niet alleen beïnvloedbaar voor het eerste levensjaar, maar ook tijdens de kindertijd (*hoofdstuk 8*). Gedurende een interventiestudie van 8 dagen, kregen 59 kinderen (9,2 ± 0,9 jaar) en 46 jong volwassenen elke dag een sinaasappellimonade met een zoete smaak (0.42M sucrose), een zure smaak (0,42M sucrose en 0,02 of 0,04M toegevoegd citroenzuur) of geen sinaasappellimonade (controle). Voor (baseline) en na de interventie werd de voorkeur voor een reeks zoete en zure sinaasappellimonades en yoghurt gemeten met behulp van een rangorde methode. Na een blootstelling van 8 dagen aan de zoete limonades ging de voorkeur van kinderen voor deze limonade significant omhoog ($P<0.05$). Een zelfde trend werd gevonden voor de yoghurt met

0.42M sucrose (*P*= 0.09). Een 8-daagse blootstelling aan de zure limonade zorgde niet voor een significante verandering in de voorkeur voor deze limonade. De smaakvoorkeuren van volwassenen veranderden niet ten gevolge van de interventie. De controlegroep van kinderen en volwassenen lieten eveneens geen verandering zien in zoet-zuur preferenties.

Ondanks dat herhaalde blootstelling aan zoet mogelijk zorgt voor een verhoogde voorkeur voor zoete smaak, kan de restrictie van vrijheid van kinderen om voor deze producten te kiezen een zelfde effect hebben. Deze hypothese werd getest in *hoofdstuk 7*. In deze studie werden 44 kinderen (5.1 ± 0.5 jaar) getest op voorkeur voor een vijftal limonades (0.14, 0.20, 0.29, 0.42 en 0.61M sucrose) met behulp van een rangorder en paarsgewijze test. De ouders vulden een vragenlijst in over regels die tot doel hadden het suikergebruik (mono-en disacchariden) van hun kinderen in te perken. Strengere regels waren gerelateerd aan lagere consumptie van zoete dranken en een lagere consumptie van andere suikerhoudende voedingsmiddelen gedurende het ontbijt en de lunch. Vijfenvijftig procent van de kinderen die werden opgevoed met strenge regels omtrent suikergebruik hadden een voorkeur voor de hoogste concentratie sucrose in de limonade. Niemand in deze groep had een voorkeur voor de limonade met de laagste concentratie suiker. Dit in tegenstelling tot kinderen die minder streng waren opgevoed. Van hen had slechts 19% een voorkeur voor de hoogste suikerconcentratie en 33% een voorkeur voor de hoogste suikerconcentratie.

In *hoofdstuk 9* bediscussiëren we een aantal methodologische knelpunten en geven we aan welke nieuwe inzichten, met betrekking tot smaakvoorkeuren en verandering van smaakvoorkeuren van kinderen, verworven zijn op basis van het onderzoek dat in het proefschrift beschreven staat. Verder worden er implicaties en suggesties geven voor toekomstig onderzoek.

Conclusie

Zoet en zuur voorkeuren van kinderen kunnen consistent gemeten worden met behulp van een rangorde en paarsgewijze methode, mits de cognitieve vaardigheden die gerelateerd zijn aan kleine leeftijdsverschillen in acht worden genomen. Jonge kinderen houden van dranken met een hoge suikerconcentratie en een deel van de kinderen heeft ook een hoge voorkeur voor extreme zure voedingsmiddelen. Deze voorkeur is gerelateerd aan de voorkeur voor nieuwe en intense stimuli en de consumptie van fruit. Voorkeuren voor zoet en zuur kunnen worden aangeleerd.

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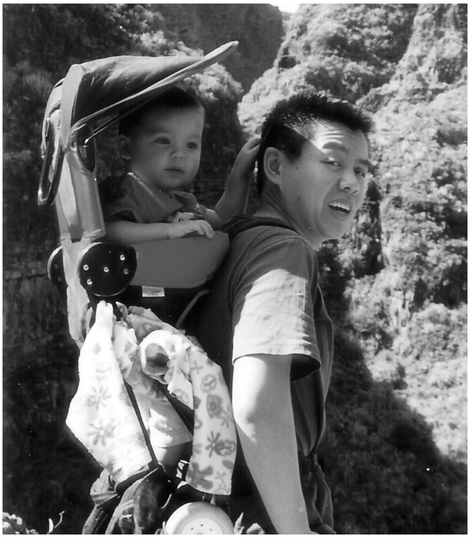
Curriculum Vitae

ABOUT THE AUTHOR



Djin Gie Liem was born on December 8th, 1974, in Amersfoort, The Netherlands. In 1994, he passed secondary school, Atheneum, at the Eemland College, Amersfoort and started the study 'Human Nutrition' at the Wageningen University. His BSc-thesis was on 'communication strategies in adolescence'. His MSc-thesis was on 'taste development in children'. During his MSc-program he conducted research studies at the Monell Chemical Senses Center in Philadelphia, PA, US, for 6 months. In collaboration with Dr. J.A. Mennella, he investigated the influence of early flavor experiences on taste preferences in children. Djin Gie graduated in 1999, after which he worked for the department of communication and public relations at the Wageningen University for half a year. In 2000, he designed his own PhD project in collaboration with the Monell Center and Wageningen University. This project was entitled: "Taste development in children". The first two years of this project were carried out at the Monell Center. Where Djin Gie conducted studies on food acceptance and taste development in infants and children. In 2002, he returned to Wageningen University were he continued his work on taste development in children. In 2004 he was selected to participate in the 10th European Nutritional Leadership Program, which took place in Luxembourg. He is currently working as a consumer-sensory scientist in the Consumer Perception & Behavior unit at Unilever R&D Vlaardingen.

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Liem DG, De Graaf C. Sweet and sour taste preferences in young children and adults: role of repeated exposure. Physiology & behavior, in press

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TRAINING AND SUPERVISION PLAN (SELECTION)

Course name	Year	Place	
Annual meeting of the Association for Chemoreception Sciences (AChemS)	1999	Sarasota, Florida, USA	Attendance
Annual meeting of the Association for Chemoreception Sciences (AChemS)	2000	Sarasota, Florida, USA	Poster presentation
International advanced course regulation of food intake and its implications for nutrition and obesity	2000	Wageningen, the Netherlands	VLAG course
Annual meeting of the Association for Chemoreception Sciences (AChemS)	2001	Sarasota, Florida, USA	Poster presentation
Annual meeting of the Association for Chemoreception Sciences (AChemS)	2002	Sarasota, Florida, USA	Poster presentation
Scientific writing	2003	Wageningen, the Netherlands	Course
Xth Food Choice conference	2003	Wageningen, the Netherlands	Oral presentation
Annual meeting of the Association for Chemoreception Sciences (AChemS)	2003	Sarasota, Florida, USA	Poster presentation
International advanced course on food perception & food preference	2003	Wageningen, the Netherlands	VLAG course
The 5th Pangborn sensory science symposium	2003	Boston, Main, USA	Poster presentation
Research seminars Monell Chemical Senses Center	2000-2002	Philadelphia, PA, USA	Seminars
European nutrition leadership program	2004	Luxembourg	Seminars and workshops

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