

**Psychophysical Evaluation
of Interactive Effects between
Sweeteners and Aroma Compounds**



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**Psychophysical Evaluation
of Interactive Effects between
Sweeteners and Aroma Compounds**

**Psychofysische evaluatie
van interactieve effecten tussen
zoetstoffen en aromastoffen**

Proefschrift

ter verkrijging van de graad van doctor
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WAGENINGEN

STELLINGEN

1. Het gebruik van niet-lineaire psychofysische functies is af te raden als men zoetstofmengsels met een gelijke zoetheid wil samenstellen.
Dit proefschrift, hoofdstuk 4.
2. De synergie in meervoudige mengsels van zoetstoffen is een lineaire combinatie van de synergieën in de samenstellende tweevoudige mengsels.
Dit proefschrift, hoofdstuk 5.
3. Verschillen in de vluchtigheid van geurstoffen ten gevolge van de aanwezigheid van bepaalde zoetstoffen in een produkt zijn niet sensorisch waarneembaar voor panelleden/consumenten.
Dit proefschrift, hoofdstuk 6.
4. Het gebruik van de term 'uitzouten' is niet correct voor de toename in het vrijkomen van vluchtige verbindingen bij aanwezigheid van componenten anders dan zout.
5. Smaakonderzoek is in trek.
6. Ecotoerisme getuigt niet van veel respect voor de natuur.
7. De doeltreffendheid van vitamines in shampoo valt te betwijfelen, gezien de contacttijd van de shampoo met het haar.
8. Een snelkassa is alleen een snelle kassa wanneer je er slechts met contant geld kunt betalen.
9. De beste stellingen vind je bij de bouwmarkt.

Stellingen behorende bij het proefschrift

Psychophysical evaluation
of interactive effects between
sweeteners and aroma compounds

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Wageningen, vrijdag 3 september 1999

Abstract

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Keywords: sucrose, maltitol, aspartame, Na-cyclamate, Na-saccharinate, orange aroma, sweeteners, interactions, sensory analysis, flavour release

The presence of intense sweeteners in a light soft drink influences the preferences for, and the flavour profiles of these drinks to various extents, depending on the aroma and sweeteners present. In this study equisweet mixtures of sweeteners were composed at 10% Sucrose Equivalent Value. The sucrose/maltitol and sucrose/aspartame mixtures were additive, whereas equisweet binary sucrose/Na-cyclamate mixtures and ternary sucrose/aspartame/Na-cyclamate mixtures revealed synergistic effects. The synergy observed for the ternary mixture was a linear combination of the synergy of the binary mixtures. Sensory analysis (quantitative descriptive analysis) of different solutions containing sweetener mixtures and a water soluble orange aroma revealed that aspartame and Na-cyclamate both differed from sucrose on the attributes chemical and aftertaste. The addition of orange aroma levelled out the differences observed between the sweetener solutions. Instrumental analysis revealed that high sucrose concentrations changed the release of the volatile compounds, while Na-cyclamate did not show this effect. Modelling of volatile release showed that the gas/solution partition coefficient and the mass transfer coefficient are the main factors influencing the release.

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General introduction

Chapter 1

The taste of a classic soft drink is largely defined by its sweet taste from sucrose, while from a nutritional and marketing point of view there is a large and increasing demand for a reduced intake of sucrose. Therefore, the production of beverages containing less sucrose is of increasing importance to the beverage industry (O'Brien Nabors & Gelardi, 1991). Sucrose can be substituted by intense sweeteners to lower its content in soft drinks. Due to this substitution, however, the flavour of a soft drink changes. To obtain acceptable light beverages of optimum quality, their flavour should be similar to that of classic soft drinks. These drinks can be considered as flavour standards for beverages containing single sweeteners and combinations of them. The quality of the flavour of a beverage can be changed by the properties of the intense sweeteners themselves or because of interactive effects of the sweeteners with aroma compounds. Interactions between intense sweeteners and aroma-active compounds can lead to the selective release of these compounds during consumption. Information on this release is important for selecting the appropriate flavour compounds to be added to a beverage and the methods for dispersing or isolating them (Kinsella, 1988).

The research described in this thesis formed part of an EC-project *The mechanistic understanding of the sweetness response*. The aim of the research was to elucidate whether changes in the quality of the sweet taste are due to the properties of the sweeteners themselves and/or to interactive effects between these sweeteners and aroma compounds. Besides, the effect of the use of mixtures of sweeteners was studied. In **Chapter 2** two preliminary studies on commercial soft drinks are described. Chapters 3-5 deal with the sensory analysis of interactive effects between sweeteners and an orange aroma. Furthermore the techniques to obtain equisweet mixtures are discussed. Additive binary sucrose/maltitol and sucrose/aspartame mixtures were studied in **Chapter 3**. In **Chapter 4** the synergistic mixture of sucrose and Na-cyclamate is investigated. **Chapter 5** then describes a ternary mixture of sucrose, aspartame and Na-cyclamate. Chapters 6 and 7 deal with the flavour release from aqueous solutions containing sweeteners and an orange aroma. In **Chapter 6** the flavour release from mixtures of sucrose, Na-cyclamate and an orange aroma is described. In **Chapter 7** the parameters influencing the release of volatile compounds from aqueous sucrose solutions are revealed by modelling the release. Finally, the general discussion in **Chapter 8** integrates the studies described in this thesis. In the next paragraphs aspects relevant to this thesis are discussed.

1.1 Flavour stimuli

The sensory perception of foods comprises several impressions, among which the appearance, the texture, the taste and the smell. Flavour can be considered as being the sensation arising from the integration or interplay of signals produced as a consequence of sensations in the nose (aroma), on the tongue (taste) and in the mouth (mouthfeel and/or texture) (Laing and Jinks, 1996; Taylor and Linforth, 1998).

Odorants are volatile and their molecular weight is limited to a maximum of ~ 400 (Beets, 1978). When an odorant binds to a receptor protein, the chemical energy will be transduced into electrical energy by two pathways. Binding of an odorant to a receptor protein activates specific G-proteins to stimulate either an olfactory-specific adenylate cyclase, generating cyclic AMP (cAMP), or phospholipase C, which converts the membrane lipid phosphatidyl inositol biphosphate into the second messenger inositol triphosphate (IP₃) and diacylglycerol. The cAMP and IP₃ open different ion channels, changing the membrane potential of the cell and resulting in electrical signals to the brain (Laing and Jinks, 1996). Information about the identity of odorants is achieved by the production of spatial maps in the olfactory bulb and other brain structures. The perceived intensity of an odorant is probably a function of the responses of the different receptor cells (Chastrette *et al.*, 1998; Livermore and Laing, 1998).

Generally, sweet substances stimulating the sense of taste are non-volatile and vary in molecular weight from 120 (erythritol) to 22000 (thaumatin) (Van der Wel *et al.*, 1987). The reception and transduction of sugars and other sweeteners includes two pathways. Sugars bind to a receptor protein in the membrane of a taste receptor cell. Binding activates a G-protein to stimulate the enzyme adenylate cyclase to synthesize cyclic AMP, which activates protein kinase A to phosphorylate K⁺ channels; this induces their closure and a change in the membrane potential. Although not fully demonstrated experimentally, the prevailing view is that synthetic sweeteners such as saccharin may bind to receptor protein(s) but, unlike sugars, they stimulate phospholipase C to produce diacylglycerol, which may activate protein kinase C to phosphorylate and close the same K⁺ channels as are involved in transduction of the binding of sugars and alters the membrane potential. For the coding of taste information two major theories exist: the 'across fibre' or 'pattern' theory and the 'labelled line' theory (Laing and Jinks, 1996).

The model solutions studied in this thesis are made up of sweeteners and aroma compounds. The sweeteners used for the different studies were either bulk sweeteners or

intense sweeteners. Up to date sucrose is the most used sweetener, as it has a good sweet taste and no sidetastes. Next to the fact that sucrose is a tastant, it has a function as bulking agent, preservative and as fermentation substrate. However, the cariogenicity of sucrose and its role as energy-provider causes a demand for intense sweeteners (Frijters, 1989). The taste of sucrose is seen as the standard for sweet taste by the consumer. Up till now no intense sweetener was found having all the positive properties of sucrose. The ideal intense sweetener has the taste and functional properties of sucrose, has a low caloric density on a sweetness equivalency basis, is physiologically inert, non-toxic and non-cariogenic. Furthermore it should be colourless, odourless, water-soluble, chemically stable and competitively priced (Bakal, 1983; Van der Wel *et al.*, 1987; O'Brien Nabors and Gelardi, 1991; Verdi and Hood, 1993). Intense sweeteners have a sweetening power which is several times larger than that of sucrose. This sweetness depends on the used concentration. Besides, the price of most intense sweeteners based on equivalent sweetnesses is lower than the price of sucrose and other energy containing sweeteners (Table 1.1).

The use of mixtures of intense sweeteners helps in approaching the optimal sucrose taste. Combining sweeteners offers various advantages such as an improvement of the time-intensity profile, an improved stability, lower costs (even more in case of synergy) and a smaller daily consumption as compared to the use of a specific sweetener on its own (Verdi and Hood, 1993). Manufacturers can use combinations of sweeteners in their products. Each sweetener can be used in the applications for which it is best suited. Manufacturers can overcome limitations of individual sweeteners by using them in blends. Na-cyclamate and Na-saccharinate historically provided sweetness in a number of popular products. Their use

Table 1.1 Different sweeteners with their relative sweetnesses on weight basis at 10% SEV (sucrose = 1) and prices per kilo. Relative sweetnesses for maltitol, aspartame and Na-cyclamate were taken from Chapters 3, 4 and 5; - = no values possible at 10% SEV, because of off-tastes.

Sweetener	Relative sweetness	Price (Euro/kg)
Sucrose	1	1
Maltitol	0.7	3
Aspartame	100	44
Na-cyclamate	21	2
Na-saccharinate	-	3
Acesulfame-K	-	52

Chapter 1

the magnitude of response, R , to the concentration, C , of the applied chemical stimulus according to the following equation:

$$R = \frac{kR_s C}{1 + kC} \quad (1.1)$$

in which k = association constant and R_s = maximum response at very high concentrations. Monod *et al.* (1965) proposed a more complex model, in which the stimulants have to fit perfectly to the receptor. When the donor, acceptor and hydrogen atoms of a sweet molecule are colinear in the Shallenberg and Acree AH, B system, an optimal binding for sweetness is possible (Shallenberger & Acree, 1967).

Starting from Equation 1.1, Beidler (1971) presented the total response, R_T , to a mixture of stimuli A and B as:

$$R_T = \frac{k_A R_{SA} C_A + k_B R_{SB} C_B}{1 + k_A C_A + k_B C_B} \quad (1.2)$$

The value of k for Na-saccharinate may be 100 to 150 times greater than that for sucrose in man. Frijters and Oude Ophuis (1983) discussed three mixture models (addition, substitution and vector summation) predicting the intensity of a particular mixture and stated that these models were either internally inconsistent or lacked sufficient generality. They proposed an alternative model, a psychophysical equiratio mixture function:

$$R_{ABijpq} = C_{abijpq}^{pm+qn} \left(\frac{pk_A}{C_{AS}} + \frac{qk_B}{C_{BS}} \right) / \left(\frac{p}{C_{AS}} + \frac{q}{C_{BS}} \right) \quad (1.3)$$

in which i and j are the concentrations of stimuli A and B, respectively, m and n are the exponents in the respective psychophysical functions and p and q refer to the proportions of the stimuli within the mixture. The equiratio mixture model successfully predicted the sensory response to the sweetness intensity of complex mixtures of sugars and sugar alcohols (Frijters and De Graaf, 1987). Ennis (1991) distinguished the following molecular mixture models:

- Common receptors, simple binding, receptor-transducer model
- Common receptors, cooperative binding, receptor model
- Common receptors, cooperative binding, receptor-transducer model
- Independent receptors, simple binding, receptor model
- Independent receptors, simple binding, receptor-transducer model

Ennis (1991) stated that if a common receptor for two substances exists and if simple binding to the receptor occurs, there will be a linear relationship between the components of mixtures which have equal perceptual, or other, effects. Mixture models involving a common receptor are a generalization of the mixture model for taste proposed by Beidler (1971) (Equation 1.2). In the case of independent receptor models, each substance reacts with a unique receptor and/or unique transducer. Ayya and Lawless (1992) explained that the equiratio mixture model (Equation 1.3) assumes a single receptor mechanism. As different sweeteners may stimulate independent or at least partially independent receptor sites (Schiffman *et al.*, 1979; Faurion *et al.*, 1980; Lawless and Stevens, 1983; Van der Wel *et al.*, 1987; McBride, 1988; Frank *et al.*, 1989; Froloff *et al.*, 1998), the model is limited to the study of molecularly similar sweeteners. Schifferstein (1995) modified the equiratio mixture model and used equi-intense units to predict the mixture intensities for aspartame/sucrose mixtures. Schifferstein (1996) applied this equiratio mixture model to the non-additive aspartame/acesulfame-K mixtures by using equi-intense units as the measure of physical concentration, an interaction index and a nonlinear response output transformation.

1.3 Sweetness flavour interactions

When sucrose is replaced by intense sweeteners, the sweetness of a soft drink is kept the same as much as possible, but its flavour often changes. Interactive effects of aroma-active compounds as well as the mechanism of sensory perception could account for this result. Interactions can occur in the soft drink itself (physical and chemical interaction), at the periphery of the sense (receptor interaction) or in the brain (perceptual interaction).

1.3.1 Physical & chemical interaction

Several factors determine the rate of release of a volatile compound from an aqueous food during consumption. Homologous series of volatile compounds in aqueous solutions were used for preliminary research on their behaviour in drinks (Buttery *et al.*, 1969, 1971). A low solubility in these solutions increased the volatility of a compound (Buttery *et al.*, 1971). Drinks are complex mixtures of water, carbohydrates, lipids, proteins and other organic compounds, and all of them can interact with and/or bind flavours. The release of volatile compounds depends on the concentration of the volatile, its disposition in the drink (free,

entrapped, adsorbed, complexed), the composition (components) of the drink, the amount of saliva in the mouth and the influence of temperature on their partition coefficients. The properties of the volatile compound (functional groups, molecular size, shape, volatility etc.) and the physical and chemical properties of the components in the drink determine the relative importance of these factors (Kinsella, 1988; Taylor and Linforth, 1998).

Carbohydrates as polysaccharides contribute often to the viscosity of a beverage and therefore influence the diffusion of small volatile compounds. The effects of polysaccharides are usually of minor importance in soft drinks, because their concentrations are rather low (Overbosch *et al.*, 1991). Mono- and disaccharides affect the volatility by altering the activity coefficients of volatile compounds (Land, 1978). At relatively high concentrations, these components lower the amount of bulk water by structuring water, which increases the effective concentration of some volatile compounds and therefore can enhance their volatility (Nawar, 1971; Wientjes, 1968).

Lipids can adsorb or dissolve hydrophobic aroma compounds. In drinks these lipids are present in a dispersed form and mostly exist in distinct regions (for example droplets). The concentrations of hydrophobic volatiles in the aqueous and vapour phases are reduced, because of their physical partition between the lipid and aqueous phases of the drink (Buttery *et al.*, 1971).

Interactions between proteins and volatile compounds in drinks mainly depend on the type, amount and composition of the proteins, and temperature, pH and ionic strength of the medium (Kim & Min, 1988). The hydrophobicity of the protein influences the binding of volatile compounds. Small apolar compounds can diffuse into hydrophobic regions for further binding reactions (Solms *et al.*, 1973). Some compounds, such as aldehydes, can be bound irreversibly in covalent bindings with free sulphide and amino groups in the protein (Overbosch *et al.*, 1991). Van Ruth *et al.* (1995a) found a decreased release of volatile compounds from rehydrated bell peppers as well as from French beans, due to the presence of the protein mucin in saliva. The influence of pH and ionic strength of the medium is mainly evident from the isoelectric point, at which the protein can precipitate. Changes in conformation and solubility of the protein strongly alter the affinity to bind with volatile compounds (Dumont & Land, 1986).

Similar to carbohydrates, salts can increase the release of volatile compounds from drinks, due to a 'salting out' effect (Land, 1978). Salts in saliva did not affect the flavour release of

some rehydrated vegetables (Van Ruth *et al.*, 1995a), probably because of their low concentrations.

As binding of aroma molecules to receptors largely depends on its concentration in the vapour phase (nose) for olfaction and in the aqueous phase (saliva) for taste (Kinsella, 1988), the perception of these compounds will be influenced by their physical and chemical interactions with components of the soft drink.

1.3.2 Receptor interaction

The perception of the sweet taste of intense sweeteners largely depends on their binding affinity to the hydrophobic part of the receptor; it is also influenced by changes in the micro-environment. The relative binding strength of sweet molecules alters because of these changes (Van der Wel *et al.*, 1987). For example, Hoopman *et al.* (1993) noticed a decrease in perceived sweet taste due to an increased ethanol concentration in the solution. Kurihara (1992) has demonstrated taste modifications for the curculin protein due to changes in the micro-environment of its receptor. The protein induces a sweet taste when it binds to a sweet taste receptor. The sweet taste of this protein disappears in the presence of divalent cations of saliva and is regenerated by tasting water, probably by removing the cations. At acidic pH divalent cations of the saliva do not bind to the receptor and the sweetness of curculin will last longer in the presence of acids, as in soft drinks.

1.3.3 Perceptual interaction

The consumption of a food implies a simultaneous stimulation of several senses, e.g. vision, taste and smell. The character and acceptability of a drink is largely determined by the integration of percepts of these senses. Von Sydow *et al.* (1974) ascribed the enhancement of aroma perception by sucrose in beverages to psychological interactions rather than to receptor or chemical interactions. Frank and Byram (1988) examined the influence of strawberry aroma on sweetness. It was demonstrated that the modification of taste perception by olfactory stimuli was both tastant- and odorant-dependent. The influence of the strawberry aroma on sweetness seems to be more olfactory than gustatory. Murphy and Cain (1980) and Algom *et al.* (1993) proposed an additive model of taste-smell integration, in which the overall intensity of taste-smell mixtures is equal to the sum of the intensities of the unmixed components. Taste and smell behave in an independent manner, however, percepts of them

are often mixed up, whereby olfactory stimulation can evoke sensations of taste. The influence of the colour of a stimulus on its taste or smell is often reported (Maga, 1974; Dubose *et al.*, 1980; Johnson & Clydesdale, 1982). It is probable that colours are associated with particular tastes and smells. Booth (1994) reports that indications about calorie content and artificial nature on the label influence the sweetness and overall preferences for a soft drink.

Besides the integration of several senses, the interaction between different stimuli of one sense can be found on a psychological level, e.g. the interaction of sucrose and NaCl (McBurney & Bartoshuk, 1973; Smith, 1974). Kroeze (1978, 1979) and Lawless (1979) have shown that the interaction of sweet and salty or bitter tastes does not occur at the periphery, but at a higher level in the transduction process.

1.4 Techniques to study molecular interaction

The binding of flavours to components of soft drinks may be studied by sensory analysis and/or instrumental techniques (Kinsella, 1988). In order to obtain meaningful correlations of the data, sensory and instrumental measurements need to be considered together (Linthorpe *et al.*, 1999).

1.4.1 Sensory analysis

In sensory analysis, the human subject is used as an instrument (Köster, 1975). People can be trained to adopt an analytical attitude and to use judgment procedures which are needed for the analysis of percepts (Kroeze, 1990). As molecular interactions would change the perception of a soft drink, sensory analysis can be used for their study. Sensory analysis comprises difference and descriptive tests (Meilgaard *et al.*, 1991; Punter, 1991). In descriptive analyses, assessors assign attributes to a type of beverage and then give intensity scores for these attributes for different products (Punter, 1991). The amount of aroma compounds reaching the olfactory epithelium is different for each person. It varies with the adsorption along the respiration track, the flow of air and the amount of saliva in the mouth, the time, the temperature and the profile of the mouth (Rothe, 1988; Overbosch *et al.*, 1991). Measurements of the actual profile sensed by the olfactory bulb might improve the correlation between instrumental and sensory measurements (Taylor & Linthorpe, 1994). A

special type of sensory analysis is the time intensity measurement, which can be used to study aroma and taste release from soft drinks in the mouth. Assessors score the intensity of a particular attribute with time during consumption (Cliff & Heymann, 1993).

1.4.2 Instrumental analysis

The release of volatiles from a beverage can be estimated by static headspace analysis of the product (Buttery *et al.*, 1969, 1971; Franzen & Kinsella, 1974). The volatile compounds of most beverages are present in extremely low concentrations and therefore provide a near ideal state of infinite dilution. Under such conditions Henry's law can be applied, which means that the concentration of a volatile compound in the static headspace is proportional to its concentration in the aqueous phase of a soft drink, at equilibrium. However, the volatile profile of the static headspace can be below the detection limit of gas chromatography. Therefore, techniques were developed for isolation and concentration of aroma compounds from soft drinks, which are purge-and-trap, dynamic headspace analysis, steam distillation, vacuum distillation and extraction with solvents (Taylor & Linfoth, 1994). Oral vapour gas chromatography offers a possibility to analyse the release of volatiles in the mouth of human subjects, who had to follow instructions during eating (Legger & Roozen, 1994). *In vitro* mouth models can be used to obtain volatile profiles, which are closely related to the profiles of oral vapour gas chromatography, as Van Ruth *et al.* (1995b) found for rehydrated bell peppers. Oral conditions influencing the flavour release are saliva composition and volume, temperature, mouth volume and mastication.

The dynamic headspace techniques imply a transport of the volatile compounds by an inert gas, after which the volatiles are condensed and/or adsorbed on an appropriate medium (Taylor & Linfoth, 1994). Subsequently, the volatile compounds can be analysed by gas chromatography, using different detection methods (Van Ruth & Roozen, 1994). These methods are limited to qualitative analyses, because each adsorbance material has a different affinity for each volatile compound (Wyllie *et al.*, 1978). The identity of the volatile compounds can be determined by gas chromatography combined with mass spectrometry, flame ionisation detection (retention times and peak areas) and description of odours by assessors at the sniffing port (Acree *et al.*, 1984; Van Ruth & Roozen, 1994). This sensory technique is capable of associating flavour descriptors with chemical constituents (Acree *et al.*, 1984). Human subjects are used to detect aroma-active compounds at the outlet of a gas

chromatograph. They differ in sensitivity with chemical detectors and therefore they produce different chromatograms (Acree & Barnard, 1994). The number of assessors who smell an odour can be related to the concentration of odour-active volatile compounds at the sniffing port (Van Ruth *et al.*, 1995c), although an increase in concentration could also change the descriptions of an odour.

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2

Preliminary studies on commercial products

In the first study four types of commercial soft drinks were evaluated: a regular and a light version of both an orange and a cola soft drink. The aim of the tests was to determine the preference of the panellists being naive towards the nature of the soft drinks versus being aware of the presence of a light version of the soft drink. For orange soft drinks regular was not significantly preferred over the light version in the tests. Regular cola soft drinks, on the contrary, were significantly preferred over their light equivalents. The awareness of the presence of a light soft drink did not influence the preferences of the panellists in both cases.

In the second study a quaternary model of Beidler's mixture equation was used to describe the sweetness of a light blackcurrant soft drink, containing the intense sweeteners Na-saccharinate, Na-cyclamate, aspartame and acesulfame-K. The perceived sweetness of the light soft drink was lower than the sweetness of the original sucrose-sweetened soft drink. A proportional enhancement of the concentrations of the intense sweeteners was utilised to meet the sweetness of the classic soft drink. Consequently, the intensity of the aroma attribute strawberry increased, while the intensities of the currant and sour related attributes decreased.

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2.1 Preferences

Recent data from the Dutch association of the soft drinks industry (1998) show that 17.8% of the consumed soft drinks in the Netherlands were light soft drinks. Cola soft drinks were the most popular ones, showing a market share of 51.5% in 1998. Orange soft drinks represented 22.7% of the total consumption of soft drinks in 1998 (NFI, 1999). For soft drinks, cola is the number one preferred flavour all over the world and in Europe orange is, with a few exceptions, the second preferred flavour (Sinki, 1994). The assumption that taste differences between regular and light soft drinks exist inspired us to study interactive effects between sweeteners and aroma compounds (Nahon *et al.*, 1996). However, we do not know whether these differences are important enough to be noticed by regular consumers and to alter their choice for beverages. We wonder whether consumers have a marked preference for one of the soft drinks. Booth *et al.* (1987) explained that an absence of affective data in sensory analysis creates a risk of developing an expert gastronomy, or studying human perception, hereby wrongly predicting food selection in the market. In this study paired preference tests were used to compare four types of commercial soft drinks (of the same brand): a regular and a light orange soft drink and a regular and a light cola soft drink. The aim of the tests was to determine the preference of the panellists when naive towards the nature of the soft drinks and when aware of the presence of a light version of the soft drink. The habits of the panellists to drink regular or light soft drinks, as well as their gender and age were considered for their influence on the preferences.

The regular and the light version of each soft drink were compared in two sessions: the orange soft drink in 1996 and the cola soft drink in 1998. Each session comprised two paired preference tests. In test I two coded samples were presented, containing the regular and the light soft drink. The panellists did not know that they were tasting different sweeteners; they had no sample information. The panellists were asked to choose the sample they preferred most. In test II two differently coded samples were presented, containing the same soft drinks as before. The panellists were told that one of the samples was a light soft drink and that they had to choose their preferred sample again. Finally the panellists were asked to write down their age, gender and what kind of soft drinks (regular or light) they are used to drink. The regular versions of the soft drinks are sweetened by sucrose, whereas the light orange soft drink contains the intense sweeteners Na-saccharinate and Na-cyclamate and the light cola

Table 2.1 Panels for the orange and cola soft drink sessions: total number of people participating; numbers of men, women, young adults and adults (ages); percentage of panellists used to drink regular soft drinks, * = significantly different from 50% in two-tailed binomial test, $P < 0.05$.

	Orange soft drink session	Cola soft drink session
Total	119	63
Men	54	28
Women	65	35
Young adults	40 (age 18-27)	25 (age 11-27)
Adults	79 (age 41-61)	38 (age 41-80)
Habit = regular	80% *	73% *

soft drink contains aspartame and acesulfame-K. The soft drinks were presented at room temperature (20 °C) and had lost part of carbonation by the time of tasting. Students and their relatives joining the tests (Table 2.1), came from various parts of the country to visit the department's presentation on sensory evaluation during parent's days in 1996 (orange soft drink) and in 1998 (cola soft drink). The experiments were performed in an open classroom, imitating cafeteria conditions. In both sessions (orange and cola soft drink) significantly more panellists had the habit to drink regular soft drinks (Table 2.1). In the orange soft drink session more men than women had this habit (91 against 71%, two-tailed χ^2 test, $df = 1$, $P < 0.05$). Women might be used to drink light soft drinks, however, significant differences between men and women were not found in the cola soft drink session. Furthermore, age differences did not influence the distribution of these drinking-habits over the panellists, as differences between young adults and adults were negligible in both sessions (two-tailed χ^2 test, $df = 1$, $P < 0.05$).

2.1.1 Preferences without sample information

The preferences of the panellists in the tests I and II are given in Tables 2.2 and 2.3 for the orange and the cola soft drink session, respectively. Comparing the two soft drinks (orange and cola) shows that the "naive" preference for the regular soft drink is much more explicit for the cola than for the orange soft drink (compare Tables 2.2A and 2.3A). Research performed with the sweeteners sucrose and Na-cyclamate in combination with orange aroma showed that the orange aroma levels out original taste differences between sucrose and Na-cyclamate (Nahon *et al.*, 1998). The latter results might give rise to an absence of a

Table 2.2 Percentages of panellists preferring regular over light orange soft drinks within the segments men, women, regular- and light-drinkers. * = significantly different from 50% in two-tailed binomial test, $P < 0.05$.

A. Test I: without sample information.

Habit to drink	Men	Women	Total
Regular	59% (29 of 49)	65% (30 of 46)	62% (59 of 95) *
Light	60% (3 of 5)	37% (7 of 19)	42% (10 of 24)
Total	59% (32 of 54)	57% (37 of 65)	58% (69 of 119)

B. Test II: with sample information.

Habit to drink	Men	Women	Total
Regular	61% (30 of 49)	57% (26 of 46)	59% (56 of 95)
Light	20% (1 of 5)	32% (6 of 19)	29% (7 of 24)
Total	57% (31 of 54)	49% (32 of 65)	53% (63 of 119)

preference for one of the orange soft drinks. The regular cola soft drink is significantly preferred over the light version. A cola aroma is might show different interactive effects with the sweeteners present in the soft drink. Besides, the sweetener combinations in the two soft drinks were different, which leads to different tastes of the soft drinks.

In test I of both sessions, men and women did not differ in their preferences (two-tailed χ^2 test, $df = 1$, $P < 0.05$). Panellists who have the habit to drink regular soft drinks significantly prefer the regular soft drink over the light version, for orange as well as for cola (Tables 2.2A and 2.3A). Pliner (1982) reported that the likings for unfamiliar tropical fruit juices in her study increased monotonically with increasing exposure. It was assumed that mere exposure plays a role in the acquisition of human food preferences. However, in the study of Tuorila *et al.* (1990) the most discriminating aspect between regular and light soft drinks was the superiority of taste, either for users of regular soft drinks or for users of light soft drinks.

2.1.2 Preferences with sample information

In the second test of the sessions, the panellists were aware of the fact that a light soft drink was present. Again a marked preference for the regular version of the cola soft drink was found, whereas no significant preference was pronounced for one of the orange soft drinks (Tables 2.2B and 2.3B). For the orange soft drink as well as for the cola soft drink a shift in preference can be observed for the panellists who have the habit to drink light soft drinks.

Table 2.3 Percentages of panellists preferring regular over light cola soft drinks within the segments men, women, regular- and light-drinkers. * = significantly different from 50% in two-tailed binomial test, $P < 0.05$.

A. Test I: without sample information.

Habit to drink	Men	Women	Total
Regular	55% (11 of 20)	85% (22 of 26) *	72% (33 of 46) *
Light	63% (5 of 8)	44% (4 of 9)	53% (9 of 17)
Total	57% (16 of 28)	74% (26 of 35) *	67% (42 of 63) *

B. Test II: with sample information.

Habit to drink	Men	Women	Total
Regular	50% (10 of 20)	92% (24 of 26) *	74% (34 of 46) *
Light	13% (1 of 8)	67% (6 of 9)	41% (7 of 17)
Total	39% (11 of 28)	86% (30 of 35) *	65% (41 of 63) *

Their preference for regular soft drinks is smaller in test II than in test I of the sessions. When comparing Tables 2.2A and B with Tables 2.3A and B, we can observe a shift from 42 to 29% and from 53 to 41% for respectively the orange and the cola soft drinks. The information of a light drink being present might help these panellists in recognising their "own" drink and thus adjust their preferences. However, further study of the data of the group of light-drinkers showed that the observed shifts were not significant. Lähtenmäki and Tuorila (1997) reported that their panellists rated pleasantness almost identical before and after getting a brief description of the drinks. However, direct hedonic comparisons between the two sweeteners sucrose and aspartame were not possible in their analysis. These authors also noticed that the short time between the ratings might diminish differences in pleasantness ratings if subjects feel committed to their first evaluation. As test I and test II in our evaluation were also performed directly after each other, we might have a similar effect.

2.1.3 Preferences for light soft drinks

The commercial light orange soft drink used in the present study contained the intense sweeteners Na-saccharinate and Na-cyclamate. A study of the consumer acceptance of sweeteners in tea at room temperature (Sprowl and Ehrcke, 1984) showed a significant lower preference for Na-saccharinate as well as for aspartame compared to sucrose. The authors concluded that there was no completely acceptable low-calorie substitute for sucrose

available to consumers. As appears from the study of Moskowitz and Klarman (1975), the sweetness imparted by sucrose is considered to be more acceptable than the sweetness imparted by the intense sweeteners. At moderate concentration Na-cyclamate was almost pleasant, but showed a rapid drop-off into unpleasantness with increasing concentrations. Schiffman *et al.* (1985) investigated whether several sweeteners could be discriminated from one another in uncarbonated soft drinks. For colas they found that sucrose, aspartame and a Ca-cyclamate/Na-saccharinate blend, were statistically equivalent in "goodness". Acesulfame-K was rated least in "goodness" in their study. The commercial light cola soft drink used in the present study, contained the intense sweeteners aspartame and acesulfame-K.

2.2 Sweetness-flavour interactions in a light blackcurrant soft drink

2.2.1 Known interactions performed by intense sweeteners

Several studies report on the chemical reactions of intense sweeteners with volatile compounds. The data of Hussein *et al.* (1984) showed that aspartame reacts with aldehydes such as benzaldehyde, cinnamaldehyde, citral, *n*-decanal and vanillin. Also Le Quéré *et al.* (1994) found a decrease in the concentrations of several aldehydes in diet orange soft drinks containing aspartame, whereas Tateo *et al.* (1988) proved aspartame to be reactive with carbonyl compounds. Le Quéré *et al.* (1994) observed the formation of new volatile compounds in diet orange drinks containing cyclamate. These new volatiles were found to be structurally related to sodium cyclamate.

Concerning flavour enhancement, Higginbotham (1983) mentions special properties of thaumatin. In soft drinks, certain flavours such as blackcurrant, would allow a replacement level of sucrose up to 50 % as the enhancement of flavour masks the aftertaste of thaumatin. Baldwin and Korschgen (1979) found a significant higher sensory intensity of fruit-flavour in orange- and cherry-flavoured beverages sweetened with aspartame than in their standards sweetened with sucrose. The intense sweetener neohesperidine dihydrochalcone (NHDC) can improve the overall flavour profile and mouthfeel of certain soft drinks, even at very low concentrations (≤ 5 ppm) (Borrego & Canales, 1992). Lindley *et al.* (1993) recorded an intensification of fruity flavour attributes by addition of 1-4 ppm NHDC to sweet and non-

sweet beverages. In these cases NHDC acts as a flavour enhancer and modifier rather than as a sweetener.

2.2.2 Light blackcurrant soft drink

Substitution of sucrose by intense sweeteners demands a complete copy of taste and functional properties of sucrose. As none of the currently known sucrose substitutes possesses all of these qualities, manufacturers of soft drinks use combinations of intense sweeteners to solve flavour problems encountered with single sweeteners (Bakal, 1991; Houghton, 1988; Verdi & Hood, 1993). The sweetness of a light blackcurrant soft drink was evaluated as being lower than the sweetness of the classic soft drink. With the help of a quaternary model of Beidler's mixture equation the concentrations of the sweeteners in the light drink can be adjusted in order to meet the sweetness of the sucrose-sweetened soft drink. Descriptive sensory tests will allow a comparison of the classic and light soft drinks.

De Graaf and Frijters (1986) showed that Beidler's mixture equation can predict the intensity of a mixture of two substances. Beidler's mixture model describes the peripheral interaction between two taste substances; therefore, it is limited to mixtures of taste substances of similar taste qualities. Moskowitz *et al.* (1978) showed that psychological rules of sensory perception, which predict the responses to pure aqueous systems of sweeteners, hold for complex beverages. According to Beidler's mixture model, the magnitude of the response of a mixture containing particular concentrations W , X , Y and Z (mM) of respectively substances A, B, C and D is given by:

$$R_{ABCDWXYZ} = \frac{k_A R_{SA} W + k_B R_{SB} X + k_C R_{SC} Y + k_D R_{SD} Z}{1 + k_A W + k_B X + k_C Y + k_D Z} \quad (2.1)$$

in which R_{SA} , R_{SB} , R_{SC} and R_{SD} = maximum responses to substances A, B, C and D, respectively; k_A , k_B , k_C and k_D = association constants of substances A, B, C and D, respectively. Assuming that the responses to each mixture of a series of mixtures of substances A, B, C and D, containing the concentrations W (of A), X (of B), Y (of C) and Z (of D) (mM) are equal to the response magnitude R , the following equation can be derived (in analogy with the derivation by De Graaf and Frijters (1986) for a mixture of two substances):

$$W + \frac{C_{Ai}}{C_{Bi}} X + \frac{C_{Ai}}{C_{Ci}} Y + \frac{C_{Ai}}{C_{Di}} Z = C_{Ai} \quad (2.2)$$

C_{Ai} (mM) is the concentration of substance A that evokes the response of magnitude R when substances B, C and D are not present. This is the point of subjective equality (PSE) for substance A. Similarly C_{Bi} , C_{Ci} and C_{Di} (mM) can be determined for substances B, C and D, respectively.

The combination of intense sweeteners in the light blackcurrant soft drink should approximate the sweetness of a 10% sucrose soft drink as closely as possible. The light soft drink contains the sweeteners Na-saccharinate, Na-cyclamate, aspartame and acesulfame-K. Na-saccharinate is one of the cheapest sweeteners, however, the taste of this sweetener limits its use to a maximum dose (Mitchell & Pearson, 1991). Therefore the maximum concentration of Na-saccharinate in the light soft drink was kept at 0.18 mM. Several combinations of the other three sweeteners can be chosen to improve the taste and sweetness of the light soft drink.

The difference in taste between the various blackcurrant soft drinks was judged using quantitative descriptive analysis. This analysis was performed by a sensory panel of 12 selected and trained assessors (aged 20-23). A computer interactive interviewing system for composing questionnaires was used to gather survey information (Ci2 system, Sawtooth Software Inc., Ketchum, USA). In consultation with the panel, a vocabulary of 12 attributes was composed to describe the flavour of an assortment of blackcurrant soft drinks (Table 2.4). The sucrose-sweetened, light and adjusted light blackcurrant soft drinks were evaluated by tasting and the intensities of the attributes were marked on a 120 mm visual analogue scale on a portable computer screen.

The four axes of the quaternary model were respectively presented by Na-saccharinate (W), Na-cyclamate (X), aspartame (Y) and acesulfame-K (Z). PSEs were determined for the four sweeteners, compared to a 10% sucrose blackcurrant soft drink. The PSEs are derived from equisweet values reported by O'Brien Nabors and Gelardi (1991), they are presented in Table 2.5. Ketelsen *et al.* (1993) reported difficulties in determining PSEs for Na-saccharinate and acesulfame-K, due to their inherent bitterness and metal taste at that concentration. The PSE of aspartame was determined according to Bock and Jones (1968), using the method of constant stimuli (De Graaf & Frijters, 1986). As this PSE of 2.0 mM approximates (sufficiently) the value calculated from O'Brien Nabors and Gelardi (1991), it was assumed that the other PSEs could be used as well.

Table 2.4 Attributes describing the aroma of blackcurrant soft drinks (Bakker, 1995).

Attributes	
Strawberry	Sharp
Blackcurrant	Watery
Bitter	Tart
Refreshing	Sour
Metallic	Aftertaste
Musty	Sweet

Table 2.5 Points of subjective equality (PSEs) calculated from O'Brien Nabors and Gelardi (1991) for the intense sweeteners Na-saccharinate, Na-cyclamate, aspartame and acesulfame-K, related to a 10% sucrose-sweetened blackcurrant soft drink.

Intense sweeteners	PSE (mM)
Na-saccharinate (W)	1.6
Na-cyclamate (X)	16.6
Aspartame (Y)	1.9
Acesulfame-K (Z)	2.5

The PSEs are the intercepts in the four dimensional coordinate system representing the quaternary model by a tetrahedon. Combinations of the intensive sweeteners, on the peripheral triangles of this tetrahedon predict equal sweetness to the 10% sucrose blackcurrant soft drink. Beidler's mixture equation for this tetrahedon was

$$W + 0.096X + 0.842Y + 0.640Z = 1.6 \quad (2.3)$$

in which W , X , Y and Z are the concentrations (mM) of Na-saccharinate, Na-cyclamate, aspartame and acesulfame-K, respectively. This equation represents an additive model for concentrations of sweeteners needed to obtain a particular sweetness. However, synergistic effects occurred when combinations of intense sweeteners were tried out. The average score was 41 on a visual analogue scale instead of 28 found for the sucrose-sweetened drink. The tetrahedon in the four dimensional coordinate system was uniformly reduced to reckon with this synergy. After a reduction of 38% the tetrahedon (Figure 2.1) approximately fits with the sweetness of a 10% sucrose blackcurrant soft drink. The outcome of Beidler's mixture equation was then

$$W + 0.096X + 0.842Y + 0.640Z = 1.0 \quad (2.4)$$

Chapter 2

Beidler's mixture equation was found useful for sweetness prediction of binary mixtures of glucose and fructose by De Graaf and Frijters (1986). The response to intense sweeteners might be different; however, the quaternary model can provide a basis for the prediction of the sweetness of solutions. The sweetness of the light blackcurrant soft drink was predicted with the quaternary model of Beidler's mixture equation (Equation 2.4), which was 92% of the result for the sucrose-sweetened soft drink. A new combination of the intense sweeteners Na-saccharinate, Na-cyclamate, aspartame and acesulfame-K was used to meet the sweetness of the classic soft drink. The concentration of Na-saccharinate remained unchanged; the other sweeteners were increased about proportionally to their original ratios. In this way the limited shelf life of aspartame (Homler *et al.*, 1991) and the bitterness of acesulfame-K, were taken into account.

Figure 2.2 illustrates the evaluation of the three soft drinks. Sensory data were subjected to Student's *t* tests to determine significant differences between the three soft drinks. A significance level of $P < 0.05$ was used in this study. The light blackcurrant soft drink significantly differed from the classic soft drink for the attributes refreshing, sharp, tart, sour,

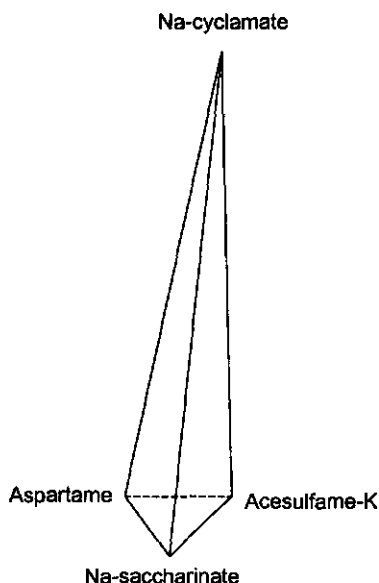


Figure 2.1 Tetrahedon, representing the quaternary model of Beidler's mixture equation;
 $W + 0.096X + 0.842Y + 0.640Z = 1.0$.

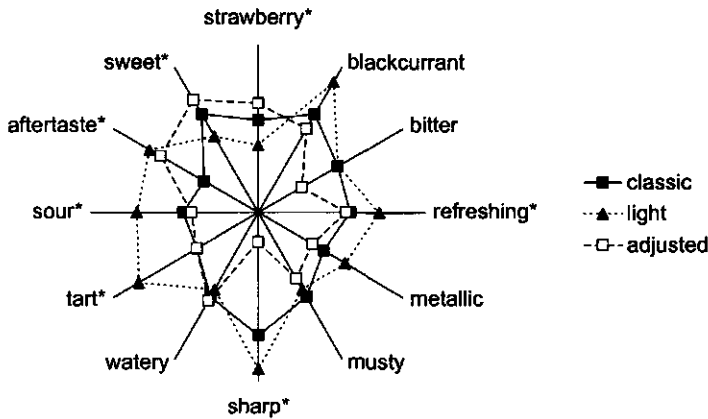


Figure 2.2 Spider web diagram of scores for sensory attributes of a classic (sucrose-sweetened), light and adjusted light blackcurrant soft drink; * = significant differences ($P < 0.05$).

aftertaste and sweet. The flavour of the light soft drink clearly differs from the one of the classic soft drink. When the new combination of intense sweeteners is applied, the character of the flavour shifts from currant to strawberry, together with a decrease of the sour related attributes.

2.3 Conclusions

The results of the first preliminary study showed that the groups of panellists were used to drink regular soft drinks more than light soft drinks. There was no significant preference for one of the orange soft drinks, but a regular cola soft drink is significantly preferred over its light equivalent. Telling these panellists that a light soft drink is present does not influence their preferences. In the second study a quaternary model of Beidler's mixture equation was used to modulate the concentrations of intense sweeteners in a light blackcurrant soft drink and to meet the sweetness of the classic soft drink. The model needs further elaboration of Beidler's mixture equation for its use in the future. Changing the concentrations of the intense sweeteners in the soft drink affected its flavour profile.

Apparently, the presence of intense sweeteners in a light soft drink influences the preferences for and the flavour profiles of these drinks to various extents, depending on the flavour and sweeteners present. More research on interactive effects between sweeteners and aroma compounds is therefore important.

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3

Sensory evaluation of mixtures of maltitol or aspartame, sucrose and an orange aroma

The suitability of Beidler's mixture equation for mixtures of sucrose and maltitol as well as for mixtures of sucrose and aspartame was examined in the presence of an orange aroma. The mean scores for the attribute sweet remained constant for each combination of sucrose and maltitol and for each combination of sucrose and aspartame. Therefore, Beidler's mixture equation can be used to choose combinations of sucrose and maltitol and combinations of sucrose and aspartame giving the same sweetness. Quantitative descriptive analysis of different solutions indicated that the flavour profiles of sucrose and maltitol did not differ significantly at a constant concentration of orange aroma. However, flavour profiles of solutions with increasing aspartame concentrations (but constant aroma levels) showed significantly higher scores for the attributes sour, chemical and aftertaste. Addition of orange aroma provided the different solutions with a more distinct flavour. The mean scores for the attributes orange, sour, fruity and aftertaste increased significantly for most of the sucrose/maltitol mixtures. This effect of orange aroma was even more pronounced in solutions containing combinations of sucrose and aspartame. Further comments on the attribute aftertaste showed similar terms for the different solutions, the most often mentioned being orange, sour, fruity and chemical for solutions containing the orange aroma. The aftertaste of solutions containing relatively more aspartame was mainly described as sweet and chemical.

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3.1 Introduction

The application of single sweeteners in beverages causes problems which can be solved by using combinations of intense sweeteners. The study of sweetness flavour interactions in soft drinks demands a fundamental understanding of the behaviour of these intense sweeteners. One important question concerns the amounts of sweeteners in complex mixtures necessary to produce a certain sweetness level, e.g. the sweetness equivalent to 10 w/v% sucrose. Ten years ago De Graaf and Frijters (1986) developed a simple method to predict equisweet combinations of sweeteners based on Beidler's mixture equation. A preliminary study (Nahon et al., 1996) proposed a quaternary extension of Beidler's mixture equation to modulate the concentrations of intense sweeteners used in a light black currant soft drink. It was concluded that the model needed further investigation of the conditions in which Beidler's mixture equation could be used (e.g. dependency of sweet taste receptors). The present study investigated the applicability of Beidler's mixture equation to mixtures of sucrose and maltitol, and mixtures of sucrose and aspartame. In both mixtures the composition of the mixture solutions as well as the concentration of orange aroma was varied.

Bulk sweeteners are assumed to compete for adsorption at the same receptor sites (De Graaf and Frijters, 1986; Ennis, 1996), which means that the sweetness of a solution of bulk sweeteners can be described with Beidler's mixture equation. Several authors (Rapaille and Van der Schueren, 1989; Sicard and Le Bot, 1990; Rapaille et al., 1995; Portmann and Kilcast, 1996) reported close similarities between sucrose and maltitol. Maltitol is mainly utilised for the production of sugarless confectionery. In experiment 1 of the present study, mixtures of sucrose and maltitol in water were studied in the presence of an orange aroma in order to examine the suitability of Beidler's model for these bulk sweeteners. Several combinations of sucrose and maltitol were chosen to study the sweetener contribution to the flavour perception. Also, the effect of different concentrations of orange aroma and interactions between the orange aroma and both bulk sweeteners were studied by descriptive analysis. The overall perception of the solutions was reflected in flavour profiles presenting attributes and their magnitudes.

Several investigations of mixtures of the bulk sweetener sucrose and the intense sweetener aspartame in water have been published. The sweetness of a mixture of sucrose and

aspartame was compared with the sweetness of sucrose or aspartame. The presence of synergy between sucrose and aspartame (-33 to 11%) was indicated by results of Frank et al. (1989) and Portmann and Kilcast (ECRO XII Symposium Zürich, August 25-31, 1996, unpublished data). However, Ayya and Lawless (1992) and Schifferstein (1995) showed that the sweetness of a mixture of sucrose and aspartame lies somewhere between the intensities of the composing compounds. Furthermore, Lawless and Stevens (1983) observed a partial cross adaptation with sucrose and aspartame, suggesting that these two sweeteners may share receptor site mechanisms (Ayya and Lawless, 1992). Descriptive analyses of solutions containing aspartame were carried out by S mundsen (1985), Redlinger and Setser (1987), Ott et al. (1991), Ketelsen et al. (1993) and Hanger et al. (1996). All these studies, except the one by Ott et al. (1991), reported a sweet aftertaste as well as bitter and off-flavour (after)tastes. Several sweeteners were arranged by Schiffman et al. (1979) in a three-dimensional space, which revealed that aspartame clusters with the sweet taste of sugars. However, at a high concentration (0.25 w/v%), a bitter component developed with time. DuBois and Lee (1983) demonstrated that aspartame is similar to sucrose in taste onset and persistence times. Wiet and Beyts (1992) noted a slight non-sweet aftertaste for aspartame in water compared to sucrose. Portmann and Kilcast (1996) found significantly higher scores for aspartame on the attributes liquorice and bitter (after)tastes, in comparison to sucrose or maltitol.

In experiment 2 of the present study, Beidler's mixture equation was studied with mixtures of sucrose and aspartame in water and in the presence of an orange aroma. Several combinations of sucrose and aspartame were chosen and interactions between the orange aroma and both sweeteners were studied and compared, as in experiment 1. Several authors described the interactions between sweeteners and flavours; however, they only presented the intensities of one to three attributes. Baldwin and Korschgen (1979) asked the panellists to judge fruit-flavour and found that an orange-flavoured beverage sweetened with aspartame had a more intense fruit-flavour than its counterpart sweetened with sucrose. Matysiak and Noble (1991) investigated the time-related perception of sweetness and fruitiness in model systems sweetened with aspartame or sucrose and flavoured with an orange extract. Their results show that aspartame has a longer sweetness duration and that its sweetness was enhanced by the orange flavour. Fruitiness persisted longer in aspartame-sweetened samples than in sucrose-sweetened ones. Bonnans and Noble (1993) varied the acid-content of these

beverages and found the same results as in the previous experiment. Larson-Powers and Pangborn (1978a) found time-intensity curves for the attributes sweet, bitter, sour and flavour of aspartame which were comparable to those of sucrose in all media. Van der Klaauw (1989) demonstrated that changes in perceived taste intensity can be instruction-dependent and that cognitive factors may affect judgements of chemosensory attributes. It is important to have appropriate descriptors available. Odour-induced enhancement of sweetness depends on the appropriateness of the stimulus attributes that subjects are instructed to rate. As fruitiness and sweetness appeared to be similar attributes, the fruitiness of a taste-smell mixture may be included in the working concept of sweetness under those conditions in which subjects were not asked to pay attention to the fruitiness. The quantitative descriptive analysis used in this study allows subjects to generate their own appropriate descriptors.

3.2 Experiment 1

This experiment was designed to examine the suitability of Beidler's mixture equation for mixtures of the bulk sweeteners sucrose and maltitol. Interactions between the orange aroma and both bulk sweeteners were also analysed.

3.2.1 Materials and methods

Subjects

Of the 61 applicants for the panel screened by a questionnaire, 48 candidates were further selected on such criteria as motivation, possession of good general perception, judgement of sweetness and the ability to generate and distinguish attributes and score these separately. From a pool of 35 available subjects, a panel of twenty-four paid subjects (4 men and 20 women) was chosen to be trained for experiment 1. Most subjects were students of Wageningen Agricultural University, ranging in age from 19 to 27 years and having no prior experience of psychophysical experiments. Informed consents were obtained from the subjects and the study was approved by the Medical Ethical Commission of Wageningen Agricultural University.

Stimuli

The stimuli were solutions of sucrose (CSM Suiker BV, Amsterdam, The Netherlands) and maltitol (Roquette Frères, Lestrem, France) and mixtures of these two substances in demineralised water. Solutions of limonene (Sigma, St. Louis, USA) and octanal (Merck, Hohenbrunnen, Germany) were used to generate attributes.

According to Portmann and Kilcast (1996), the 10% SEV (Sucrose Equivalent Value) for maltitol is 13.5 w/v%. These concentrations, 10 w/v% sucrose and 13.5 w/v% maltitol, were chosen as starting concentrations for the Beidler's mixture equation. This equation should then predict the concentration and composition of sucrose/maltitol mixtures having a constant perceived taste intensity of 10% SEV. The validity of the Beidler's mixture equation was assessed over a series of nine sucrose/maltitol ratios (100/0; 90/10; 75/25; 60/40; 50/50; 40/60; 25/75; 10/90 and 0/100). The overall sweetness of each mixture being equisweet at 10% SEV. The orange aroma added was a sample of the watery vapour phase of stripped orange juice (Cargill juice division, Amsterdam, The Netherlands). It was used in concentrations of 0, 15 and 30 g/L. The standard stimuli 'not sweet' and 'very sweet' consisted of 0 and 16 w/v% sucrose, respectively. The solutions were prepared at least 24 hours before evaluation and stored at 4 °C overnight. A stimulus consisted of 15 mL of solution, presented in a glass jar, covered by a plastic lid and aluminium foil to prevent interactions between plastic and orange aroma. The stimuli were presented to the panel at room temperature (22 °C).

Procedure

The relative sweetness factors (10% SEV) of sucrose (10 w/v%) and maltitol (13.5 w/v%) are introduced in Beidler's mixture equation. Beidler's mixture equation becomes then

$$W + 0.74X = 10 \text{ (w/v\%)} \quad (3.1)$$

for mixtures of sucrose ($=W$) and maltitol ($=X$) (De Graaf and Frijters, 1986). The panel performed a quantitative descriptive analysis to evaluate the solutions containing nine different combinations of sucrose and maltitol chosen with Equation 3.1 and three concentrations of orange aroma. With the help of several solutions, flavour attributes were generated during training of the subjects, which were ranked and clustered in consultation with the panel (Table 3.1). The panel was calibrated by tasting sucrose references of 0 and 16 % (w/v), which were the anchors of the visual analogue scale for sweetness. Similar scales

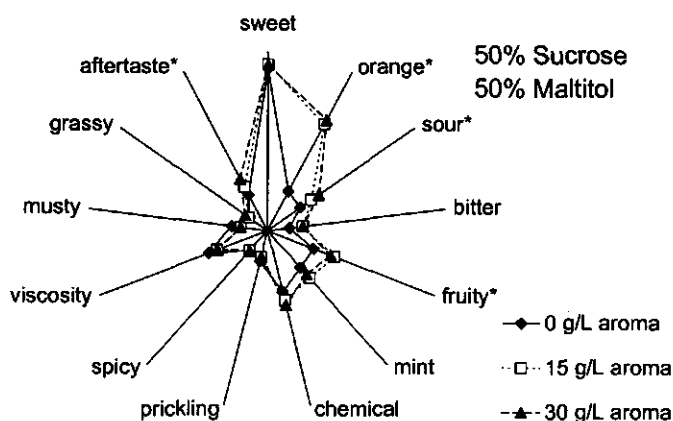


Figure 3.3 Spider web diagram representing the mean scores for sensory attributes of a mixture solution of a 50/50 sucrose/maltitol ratio, at three concentrations of orange aroma: 0, 15 and 30 g/L; * = significant differences ($P < 0.01$ for sweetness, $P < 0.05$ for other attributes).

The comments on aftertastes of the different solutions were similar to the attributes used. Solutions containing 0, 15 and 30 g/L orange aroma were compared across the nine different sucrose/maltitol mixtures. The number of subjects reporting the aftertastes sweet, orange, sour, fruity or chemical were counted and presented in Table 3.2. Solutions containing 15 or 30 g/L orange aroma gave stronger orange, sour, fruity and chemical aftertastes. An addition of 30 g/L orange aroma instead of 15 g/L provided a stronger orange aftertaste.

Table 3.2 Number of subjects reporting aftertastes sweet, orange, sour, fruity or chemical for solutions containing 0, 15 and 30 g/L orange aroma (experiment 1).

Orange aroma (g/L)	Aftertaste				
	Sweet	Orange	Sour	Fruity	Chemical
0	7-16	0-2	0-3	0-1	0-4
15	7-14	3-9	2-6	1-3	3-8
30	7-14	6-14	3-7	0-5	3-9

3.2.3 Conclusions

Beidler's mixture equation makes it possible to choose equisweet combinations of the bulk sweeteners sucrose and maltitol. The flavour profiles of the sweeteners sucrose and maltitol do not significantly differ at a constant concentration of orange aroma. Flavour profiles of different concentrations of orange aroma show significant differences for several attributes.

3.3 Experiment 2

The aim of this experiment was to examine the suitability of Beidler's mixture equation as in experiment 1, this time for mixtures of sucrose and aspartame.

3.3.1 Materials and methods

Subjects

Again the panel consisted of twenty-four paid subjects (4 men and 20 women), ranging in age from 19 to 27 years. Nineteen subjects also participated in experiment 1, the five additional subjects were taken from the pool made in experiment 1.

Stimuli

The stimuli were solutions of sucrose (CSM Suiker BV, Amsterdam, The Netherlands) and aspartame (Holland Sweetener Company, Maastricht, The Netherlands) and mixtures of these two substances in demineralised water.

Like in experiment 1, nine sucrose/aspartame ratios (100/0, 90/10, 75/25, 60/40, 50/50, 40/60, 25/75, 10/90 and 0/100) were chosen to assess the validity of the Beidler's mixture equation, with the overall sweetness of each mixture equisweet at 10% SEV. Again the orange aroma added was a sample of the watery vapour phase of stripped orange juice, used in concentrations of 0, 15 and 30 g/L. Further preparation, storage and presentation of the solutions were done as in experiment 1.

Table 3.3 Sucrose Equivalent Values (SEVs) for aspartame, as reported by several authors, the standard sucrose reference, details about the preparation of the solutions and the number of subjects used for the determination.

Authors	Year	10% SEV (w/v %)	Sucrose (w/v %)	Preparation solutions	# Subjects
Ayya and Lawless	1992	0.100	10	24h before	10-13
Baldwin and Korschgen	1979	0.065	9.5	flavoured beverage	8 (lab panel)
Bornstein <i>et al.</i>	1993	0.053	9	fresh	15?
Cloninger and Baldwin	1974	0.090	10	-	20
DuBois and Lee	1983	0.075	10	-	12
Faurion <i>et al.</i>	1980	0.085	9.6	frozen samples	9
Frank <i>et al.</i>	1989	0.080	8.6	24h before	18-20
Ketelsen <i>et al.</i>	1993	0.090	9	-	±30
Larson-Powers and Pangborn	1978a	0.190	10	16h before	13
Lawless and Stevens	1983	0.056	10.3	informal	
Matysiak and Noble	1991	0.085	10	48h before	25
Ott <i>et al.</i>	1991	0.113	10	16h before	8
Portmann and Kilcast	1996	0.120	10	24h before	12
Schifferstein and Frijters	1991	0.088	8.6	24h before	14
		0.094	9.2		
Theunissen and Kroeze	1995	0.130	10.5	24h before	15

Procedure

In several studies, the SEV of aspartame has been determined (Table 3.3). Whereas we found one SEV for maltitol in the literature, the values for aspartame were quite different, which may result from differences between panels or methods of preparation. The SEV for aspartame described by Nahon *et al.* (1996) was determined in a black currant soft drink, in which some additional sweetness from a fruit concentrate may be present. Therefore the 10% SEV of aspartame was again determined with the described panel and method of preparation. The 10% SEV was determined using the method of constant stimuli (Guilford, 1954) and weighted linear regression (Bock and Jones, 1968) as described previously (De Graaf and Frijters, 1986). Substituting the established 10 w/v% for sucrose and the resulting SEV of 0.096 w/v% for aspartame to Beidler's mixture equation gives

$$W + 104.06X = 10 \text{ (w/V\%)} \quad (3.2)$$

for mixtures of sucrose (=W) and aspartame (=X). Similar to the first experiment, nine sucrose/aspartame ratios were chosen and three different concentrations of orange aroma were added. Again the panel performed a quantitative descriptive analysis to evaluate the solutions containing several combinations of sucrose and aspartame chosen with Equation 3.2. The experimental design was the same as in the first experiment, except that the solutions were only tasted once. The attributes generated and used in experiment 1 also involved solutions containing aspartame (Table 3.1). Therefore, the same attributes were used in experiment 2. To stimulate a better use of the scales, 5% and 12.5% (w/v) sucrose solutions were evaluated in experiment 2. These solutions were randomly given with the other solutions to be evaluated.

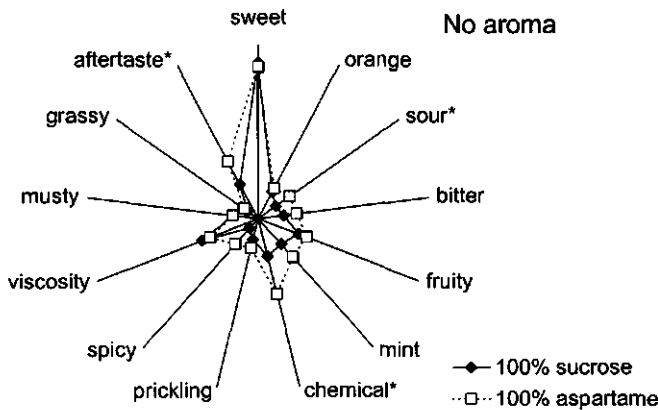


Figure 3.4 Spider web diagram representing the mean scores for sensory attributes of a sucrose and an aspartame solution at 10% SEV (in the absence of orange aroma); * = significant differences ($P < 0.01$ for sweetness, $P < 0.05$ for other attributes).

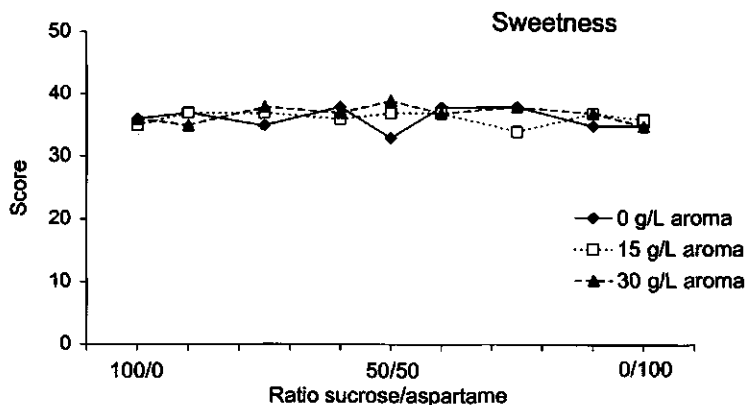


Figure 3.5 Mean scores for the sensory attribute sweetness for solutions containing mixtures of sucrose and aspartame. Three different concentrations of orange aroma (0, 15 and 30 g/L) were used.

3.3.2 Results

In Figure 4.4 a sucrose solution is compared to an aspartame solution at 10% SEV, both in the absence of orange aroma. In comparison to sucrose, the mean scores for aspartame are significantly higher for the attributes sour, chemical and aftertaste. The mean scores for the attribute sweet (Figure 4.5) remain constant for each combination of sucrose and aspartame. Apparently Beidler's model can be used to compose equisweet mixtures of these two sweeteners.

Almost all attributes show significant differences when different concentrations of orange aroma were compared for mixtures of a 50/50 sucrose/aspartame ratio (Figure 4.6). For every mixture solution containing sucrose and aspartame, the addition of orange aroma increases the mean scores for all attributes except sweetness, viscosity and musty. Addition of orange aroma again clearly gives the solution a more distinct flavour. In this experiment, subjects scaled 5 and 12.5 w/v% sucrose solutions as well. This probably introduced more variations in sweetness among solutions (compare Figures 4.2 and 4.5), although significant differences were not found. Standard deviations calculated for the attribute sweet were again low for each combination of sucrose and aspartame. As in experiment 1, it is difficult to find significant differences for the other attributes, but some tendencies may be recognised. The

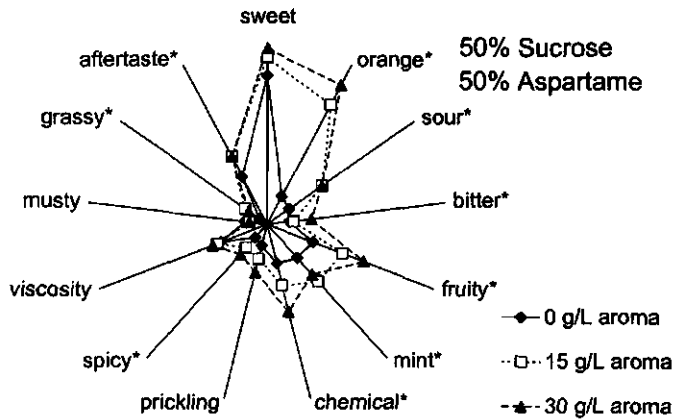


Figure 3.6 Spider web diagram representing the mean scores for sensory attributes of a mixture solution of a 50/50 sucrose/aspartame ratio, at three concentrations of orange aroma: 0, 15 and 30 g/L; * = significant differences ($P < 0.01$ for sweetness, $P < 0.05$ for other attributes).

mean scores for the attributes sour, bitter, chemical, prickling, spicy and aftertaste tend to be higher when more aspartame is present in the mixture (see for example Figures 4.7 and 4.8). The contribution of orange aroma to the mean scores of the attributes is of equal magnitude for every mixture solution. In case of interaction it is expected that the differences in mean scores of the attributes either decrease or increase when the ratio sucrose/aspartame changes. Therefore, interactions between aroma and sweetener are assumed to be absent. Besides, the mean scores for the attributes orange and fruity remain fairly constant for every possible combination of sucrose and aspartame. These attributes would change with the ratio sucrose/aspartame, if either sucrose or aspartame implied any of these tastes.

The terms used for the comments on the aftertastes of the different solutions did not differ from the attributes. However, solutions containing more aspartame tended to have more aftertaste, which is mainly described by the attributes sweet and chemical. Similar to solutions in experiment 1, solutions containing 15 or 30 g/L orange aroma provided stronger orange, sour, fruity and chemical aftertastes in comparison to solutions without orange aroma.

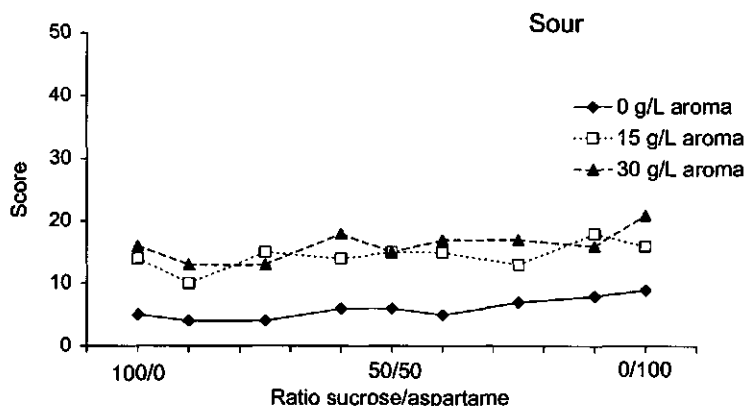


Figure 3.7 Mean scores for the sensory attribute sour for solutions containing mixtures of sucrose and aspartame. Three different concentrations of orange aroma (0, 15 and 30 g/L) were used.

3.3.3 Conclusions

Beidler's mixture equation can be used to choose combinations of the sweeteners sucrose and aspartame having the same sweetness. The flavour profiles of mixtures containing more aspartame as compared to those containing sucrose provide significantly higher scores on the attributes sour, chemical and aftertaste. Different concentrations of orange aroma provide significantly different flavour profiles.

3.4 Discussion

Beidler's mixture equation seems to be suitable for mixtures of sucrose and maltitol and for mixtures of sucrose and aspartame. Addition of orange aroma gives the solutions a more distinct taste. Solutions containing maltitol did not significantly differ from solutions containing sucrose in this study, whether in absence or in the presence of orange aroma. The same results were reported in the literature (Rapaille and Van der Schueren, 1989; Sicard and Le Bot, 1990; Rapaille *et al.*, 1995; Portmann and Kilcast, 1996). As expected, Beidler's mixture equation was appropriate for mixtures of these two sweeteners, the two bulk sweeteners seem to share the same receptor sites.

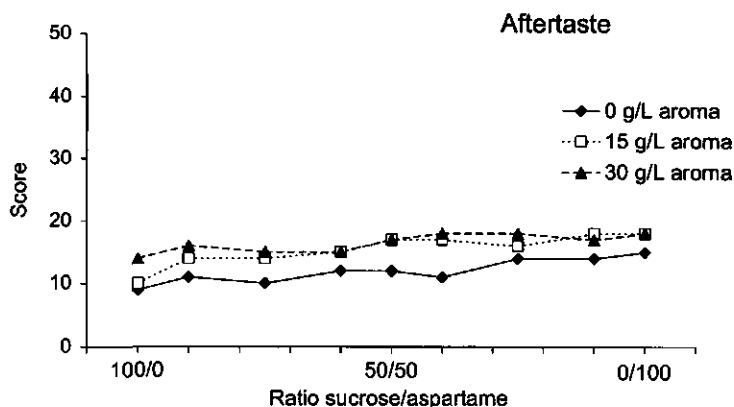


Figure 3.8 Mean scores for the sensory attribute aftertaste for solutions containing mixtures of sucrose and aspartame. Three different concentrations of orange aroma (0, 15 and 30 g/L) were used.

The 10% SEV determined for aspartame agrees with values reported in the literature which were determined with larger panels (panel >10). However, the sweetness values found for the mixtures of sucrose and aspartame were quite dissimilar. Our findings concerning the sweetness of mixtures of sucrose and aspartame agreed with those of both Ayya and Lawless (1992) and Schifferstein (1995). Beidler's mixture equation seems to be appropriate for these solutions. Once the use of Beidler's mixture equation for composing our equisweet mixture solutions had been established, the effects of added orange aroma could then be investigated. Larson-Powers and Pangborn (1978a), Baldwin and Korschgen (1979), Matysiak and Noble (1991) and Bonnans and Noble (1993) asked their subjects to judge one attribute at a time. Whereas we did not find any changes in the attribute orange aroma, all except Larson-Powers and Pangborn (1978a) found enhancement of fruitiness by aspartame. The applied quantitative descriptive analysis seems to be an appropriate method to avoid instruction-dependent changes in perceived taste intensity (Van der Klaauw, 1989).

Le Queré *et al.* (1994) evaluated several sweeteners in an orange soft drink by quantitative descriptive analysis. Comparison of aspartame with sucrose shows that synthetic and pineapple flavours are associated with high concentrations of aspartame. In contrast with their study, an unanchored descriptive analysis by Larson-Powers and Pangborn (1978b) showed a decrease in "fresh orange peel" and "orange-flavoured aspirin" aroma in orange

flavoured drinks, when comparing aspartame with sucrose. The flavour of drinks sweetened with sucrose or aspartame was judged as sweet-chemical and bitter. The aftertaste of samples containing sucrose or aspartame was described as "sweet-clean" (Larson-Powers and Pangborn, 1978b). In the present study, mixtures containing more aspartame were characterised by higher scores on attributes related to sour, bitter and chemical-related tastes. The aftertaste of these solutions was described as sweet-chemical. The quantitative descriptive analysis of the solutions did not reveal interactions between the sweeteners and the orange aroma. Mixtures of sweeteners showing synergistic effects might give more flavour effects. Orange aromas containing other flavour compounds, such as aromas based on peel oil, could also give different results.

In conclusion, it was shown that Beidler's mixture equation is valid for mixtures of maltitol or aspartame, sucrose and an orange aroma. Equisweet mixtures of them can be formulated to provide a basis for studying sweetness-flavour interactions. Furthermore, a quantitative descriptive analysis seems to be the most appropriate method for the study of these interactions.

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4.1 Introduction

Equisweet mixtures of sweeteners can be used as a basis for studying sweetness-flavour interactions. In previous experiments equisweet mixtures of maltitol or aspartame, sucrose and an orange aroma were formulated with the help of Beidler's mixture equation (Nahon *et al.*, 1998). A quantitative descriptive analysis (QDA) of the solutions did not reveal interactions between the sweeteners and the orange aroma. Mixtures of sweeteners showing synergy might affect the flavour profiles of these solutions (Nahon *et al.*, 1996). Literature data showed that mixtures of sucrose and Na-cyclamate give positive synergy and thus this mixture would be interesting to study.

Synergy between sucrose and cyclamate has been described before by Yamaguchi *et al.* (1970b), Hoppe (1981), Frank *et al.* (1989) and Portmann en Kilcast (1996b). The latter two determined a significant synergy; Frank *et al.* (1989) specified this synergy to be 15%. Hoppe (1981), on the contrary, reported a hindering between sucrose and Na-cyclamate, which would suggest a competition between sweetener molecules for receptor sites and thus an absence of synergy.

Showing synergy means that Beidler's mixture equation cannot be used to compose equisweet mixtures of the sweeteners sucrose and Na-cyclamate. A different approach has been chosen to equal their sweetness to the level of a 10 w/v% sucrose solution (denoted as 10% Sucrose Equivalent Value, SEV). Several authors reported quite different SEVs for Na-cyclamate (Table 4.1). The background of the panellists (e.g. age, food preferences) might account for different judgments in sensory evaluation.

The results obtained with mixtures of sucrose and maltitol or aspartame showed that sucrose fits in the linear equation of Beidler (Nahon *et al.*, 1998). Up to a concentration of 10 w/v% sucrose, the concentration of and response to this bulk sweetener are linearly related. The contribution of sucrose to the mixtures was thus fixed according to the chosen ratios. The next step was addition of Na-cyclamate until a sweetness equal to 10% SEV was reached.

Mixtures of sucrose and Na-cyclamate equisweet at 10% SEV were composed. QDA was used to find possible interactive effects between an orange aroma and mixtures of both sweeteners. The composition of the mixture solutions as well as the concentration of orange aroma was varied. The overall perception of the solutions was reflected in flavour profiles

presenting attributes and their magnitudes. Faurion *et al.* (1980) and DuBois and Lee (1983) both described similarities between sucrose and Na-cyclamate in multidimensional analysis and in temporal sensory properties, respectively. Additionally, Hanger *et al.* (1996) found no differences between Na-cyclamate and sucrose for the attributes sweet (aftertaste), bitter (aftertaste), off-flavour, mouth coating and drying. However, Portmann and Kilcast (1996a) reported a strong bitter flavour and bitter and metallic non-sweet aftertastes for Na-cyclamate. A caramel and burnt sugar flavour was also found, although these flavours are normally associated with nutritive sweeteners such as sucrose. Moskowitz and Klarman (1975) noticed that Na-cyclamate was rather pleasant at moderate concentrations but that this turned into unpleasantness at higher concentrations. In a lemonade product cyclamate was judged as sweet as and less tart than sucrose (Inglett *et al.*, 1969).

In this study, mixtures of sucrose and Na-cyclamate equisweet at 10% SEV were composed and interactive effects between both sweeteners and an orange aroma were studied by QDA.

Table 4.1 *Sucrose Equivalent Values (SEVs) for Na- and Ca-cyclamate, as reported by several authors, the standard sucrose reference, details about the preparation of the solutions and the number of subjects used for the determination; Na-cycl = sodium cyclamate, Ca-cycl = calcium cyclamate.*

Author(s)	Year	10% SEV (w/V %)	Sucrose ref. (w/V %)	Preparation / solutions	no. of subjects
DuBois and Lee	1983	0.5	10	Na-cycl	12
Faurion <i>et al.</i>	1980	0.35	9.6	frozen samples, Na-cycl	9
Frank <i>et al.</i>	1989	0.25	8.6	24h before, Na-cycl	18-20
Hanger <i>et al.</i>	1996	0.11	4	fresh daily, Na-cycl	25
Hoppe	1981	0.6	10.4	Na-cycl	
Ketelsen <i>et al.</i>	1993	0.42	9	Ca-cycl	± 30
Larson-Powers and Pangborn	1978a	0.86	10	16h before, Ca-cycl	13
Portmann and Kilcast	1996a	0.44	10	24h before, Na-cycl	12
Redlinger and Setser	1987	0.14	5	2.5h before, Ca-cycl	7
Yamaguchi <i>et al.</i>	1970a	0.2	5.84	Na-cycl	100

4.2 Materials and methods

Sensory evaluation was used to determine relative sweetnesses and to perform descriptive analyses. Whereas stimuli and subjects were the same in both cases, the methods (10% SEV determinations and QDA) are described separately.

4.2.1 Stimuli

Analogous with earlier experiments (Nahon *et al.*, 1998), the stimuli were solutions of sucrose (CSM Suiker BV, Amsterdam, The Netherlands) and of Na-cyclamate (Flevo chemie BV, Harderwijk, The Netherlands) and mixtures of these two substances in demineralized water. Nine sucrose/Na-cyclamate ratios (100/0, 90/10, 75/25, 60/40, 50/50, 40/60, 25/75, 10/90 and 0/100) were chosen to study possible interactive effects between sweeteners and aroma compounds. The pH of these solutions was measured with a Metrohm (Herisau, Switzerland) pH-meter. The overall sweetness of each mixture had to meet with a constant perceived taste intensity of 10% SEV. The standard stimulus for the SEV determinations was a 10 w/v% sucrose solution.

Solutions of sucrose, Na-cyclamate, acesulfame-K (Hoechst, Amsterdam, The Netherlands), orange aroma and octanal (Merck, Hohenbrunnen, Germany) were used to generate attributes for the QDA. The standard stimuli 'not sweet' and 'very sweet' used in this analysis consisted of 0 and 16 w/v% sucrose, respectively. The orange aroma added to evaluate interactive effects was a sample of the watery vapor phase of stripped orange juice (Cargill juice division, Amsterdam, The Netherlands). It was used in concentrations of 0, 15 and 30 g/L.

All solutions were prepared at least 24 h before evaluation and stored at 4 °C overnight. In all evaluations, a stimulus consisted of 15 mL of solution. These stimuli were presented in a glass jar, covered by a plastic lid and aluminium foil to prevent interactions between the plastic and the orange aroma. The stimuli were presented to the panel at room temperature (22 °C).

4.2.2 Subjects

The panel for both sensory evaluations (at least 18 subjects) was chosen from a pool of 25 paid subjects (7 men and 18 women), ranging in age from 19 to 26 years. These subjects were

selected and trained for the two experiments. Most subjects were students of Wageningen Agricultural University, some of them having prior experience of psychophysical experiments. Informed consents were obtained from the subjects and the study was approved by the Medical Ethical Commission of Wageningen Agricultural University. Subjects were instructed to taste according to the sip-and-spit method, the time intervals between stimuli being kept at 60 s. After tasting of a solution, the subjects neutralized their mouth with water and crackers. Information from the survey was gathered by a computer interactive interviewing system (Ci2 system, Sawtooth Software Inc., Ketchum, USA).

4.2.3 SEV determinations

Equisweet mixtures of the sweeteners sucrose and Na-cyclamate were composed by fixing the contribution of sucrose to the mixtures, according to the chosen ratios. Na-cyclamate was added until a sweetness of 10% SEV was reached (the partial SEV of Na-cyclamate). The extent of this addition was determined using the method of constant stimuli (Guilford, 1954) and weighted linear regression analysis (Bock and Jones, 1968). The panel was presented the standard stimulus, which is the 10 w/v% sucrose solution and seven comparison stimuli. These consist of a fixed amount of sucrose and amounts of Na-cyclamate that vary exponentially near the expected partial SEV for Na-cyclamate. The determination of this partial SEV further follows the method as described by De Graaf and Frijters (1986). At least 252 datapoints were used for the determination of the composition of one ratio mixture (correlation coefficients of weighted linear regressions > 0.91). This method was then repeated for all nine ratio mixtures, which provides the composition of mixtures matching a 10% SEV.

Table 4.2 *Mixtures of sucrose and Na-cyclamate matching a sweetness of 10% SEV, concentrations of sucrose and Na-cyclamate and pH of the solutions*

Mixture ratio sucrose/Na-cyclamate	Sucrose (w/v%)	Na-cyclamate (w/v%)	pH
100/0	10	0	6.5
90/10	9	0.015	6.2
75/25	7.5	0.043	6.5
60/40	6	0.086	6.0
50/50	5	0.111	6.0
40/60	4	0.140	6.2
25/75	2.5	0.236	5.9
10/90	1	0.335	6.0
0/100	0	0.443	5.9

4.2.4 Quantitative Descriptive Analysis

The solutions containing nine different combinations of sucrose and Na-cyclamate matching a sweetness of 10% SEV (Table 4.2) and three concentrations of orange aroma (0, 15 and 30 g/L) were evaluated by QDA. For this analysis, the subjects generated flavour attributes with the help of several solutions, which were ranked and clustered in consultation with the panel (Table 4.3). The panel was calibrated by tasting sucrose references of 0 and 16 w/v%, which were the anchors of the visual analogue scale for sweetness. Similar scales for other attributes were not anchored. The intensities of the attributes were marked on a 120 mm visual analogue scale (maximum score = 50) on a portable computer screen. Stimuli were presented randomly to the subjects and subjects were asked to comment on aftertastes. To monitor the

Table 4.3 *Attributes describing the flavour of several solutions containing sucrose (10 w/v%), Na-cyclamate (0.44 w/v%), acesulfame-K (0.087 w/v%), orange aroma (15 g/L) and/or octanal (0.05 g/L)*

Attributes	
Sweet	Fruity
Orange	Watery
Bitter	Mint
Sour	Musty
Sharp	Aftertaste
Chemical	

use of the scales, 5 and 12.5 w/v% sucrose solutions were evaluated as well. These solutions were randomly given with the other solutions to be evaluated.

Sensory data were subjected to Student's *t* tests to determine significant differences between solutions. A significance level of $P < 0.01$ was used for sweetness (anchored scale) and $P < 0.05$ was used for the other attributes.

4.3 Results

Figure 4.1 presents the results of the SEV determinations in comparison with linear expectations. The concentration of Na-cyclamate necessary to obtain equisweet mixtures at 10% SEV is smaller than follows from linearity.

The pH values measured for the mixture solutions used in the QDA are reported in Table 4.2. The pH values for the solutions containing orange aroma were in the same range. The results of the QDA show that the flavour profiles of a 100% sucrose solution compared with a 100% Na-cyclamate solution in the absence of orange aroma provide significant differences for the attributes chemical and aftertaste (Figure 4.2). A comparison between these two sweeteners in the presence of 30 g/L orange aroma shows similar profiles (Figure 4.3); no significant differences were found. For the attribute sweet, the mean scores remain

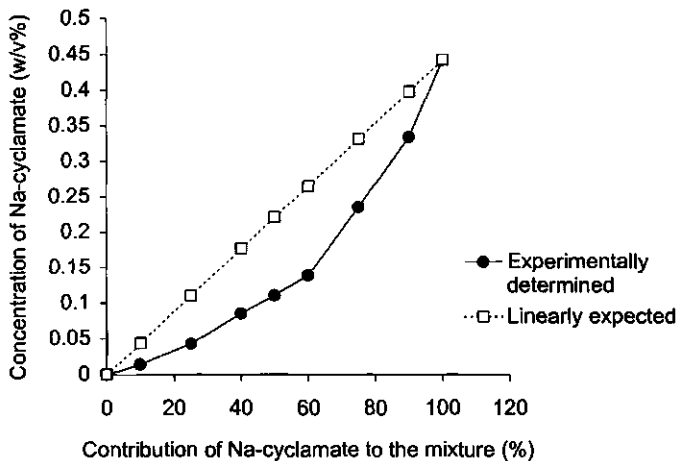


Figure 4.1 Concentration of Na-cyclamate (w/v%) in the mixture, as a function of the contribution of Na-cyclamate (%) to the mixture.

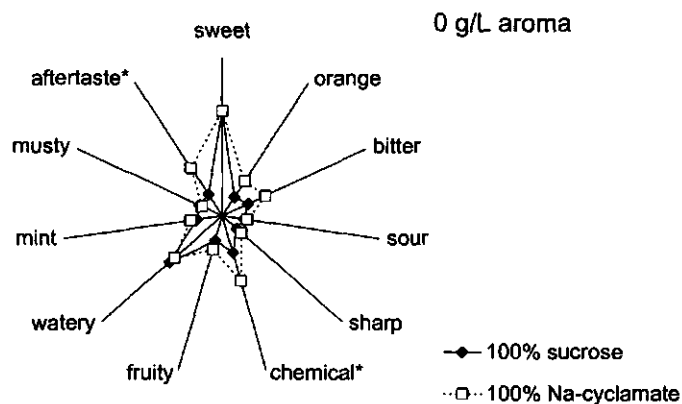


Figure 4.2 Spider web diagram representing the mean scores for sensory attributes of a sucrose and a Na-cyclamate solution at 10% SEV (in the absence of orange aroma); * = significant differences ($P < 0.01$ for sweetness, $P < 0.05$ for other attributes).

constant for each combination of sucrose and Na-cyclamate (Figure 4.4A). Figure 4.4B shows the mean scores for the attribute chemical. The contribution of orange aroma to the mean scores of this attribute is of a smaller magnitude for mixture solutions in which more Na-cyclamate is present. As in previous experiments (Nahon *et al.*, 1998), standard deviations calculated for the attribute sweet were low for each combination of sucrose and Na-cyclamate. Again, it is difficult to find significant differences for the other attributes.

The terms used for the comments on the aftertastes of the different solutions did not differ from the attributes. However, solutions containing more Na-cyclamate tended to have more aftertaste, which is mainly described by the attribute bitter. For all three concentrations of orange aroma, subjects mentioned a bitter aftertaste for mixtures containing more Na-cyclamate (Figure 4.5). Solutions containing 15 or 30 g/L orange aroma provided stronger orange, chemical and mint aftertastes than solutions without orange aroma.

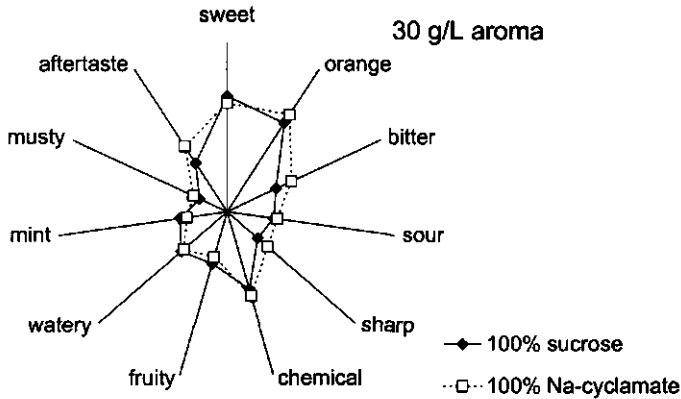


Figure 4.3 Spider web diagram representing the mean scores for sensory attributes of a sucrose and a Na-cyclamate solution at 10% SEV (in the presence of 30 g/L orange aroma); no significant differences.

4.4 Discussion

The 10% SEV determined for Na-cyclamate in this study (0.44 w/v%) perfectly agrees with the value given by Portmann and Kilcast (1996a). However, most values reported in the literature were lower (Table 4.1). It seems to be very important to determine the SEVs related to the panel worked with, as we noticed before (Nahon *et al.*, 1998). Figure 4.1 illustrates the difference between the linear expectations from Beidler's mixture equation and the results of the present experiments. Upon composing equisweet mixtures of sucrose and Na-cyclamate, one would expect to add fractions from the 10% SEV of Na-cyclamate according to Beidler's method. This linearity was found before for mixtures of sucrose and maltitol or aspartame (Nahon *et al.*, 1998). In case less Na-cyclamate is necessary than linearly expected, synergy has been found for these mixtures. Results from Table 4.2 and Figure 4.1 demonstrate a synergistic effect between sucrose and Na-cyclamate.

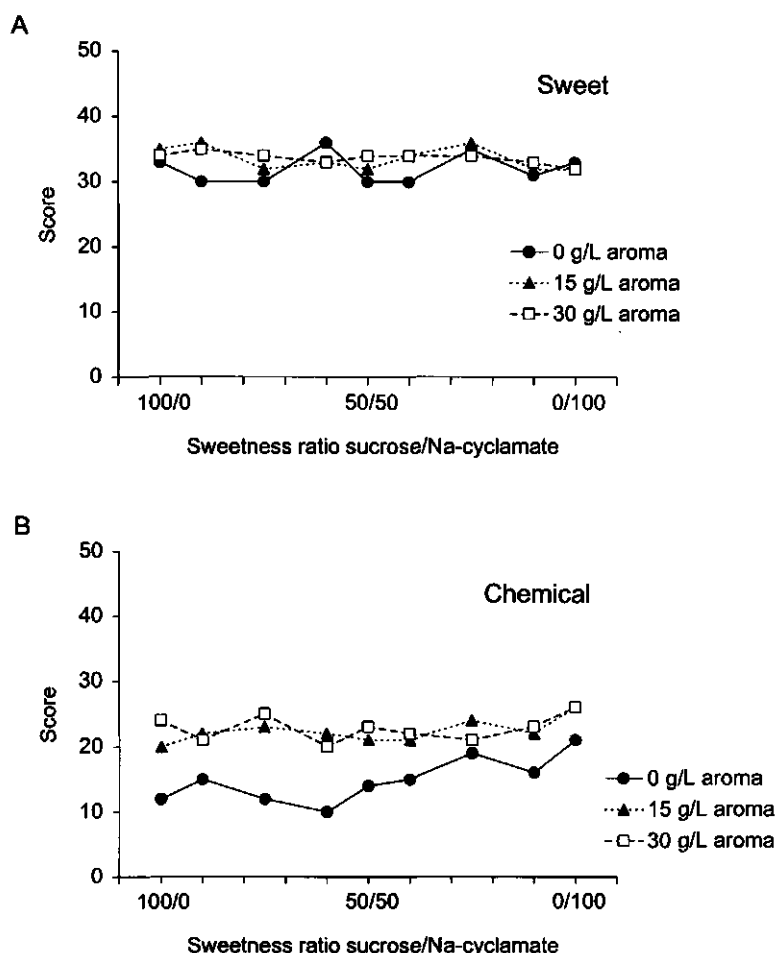


Figure 4.4 Mean scores for the sensory attributes sweetness (A) and chemical (B) for solutions containing mixtures of sucrose and Na-cyclamate. Three different concentrations of orange aroma (0, 15 and 30 g/L) were used.

The synergy between sucrose and Na-cyclamate has been reported before by several authors. Yamaguchi *et al.* (1970b) found that a mixture of 3 w/v% sucrose and 0.2 w/v% Na-cyclamate was equisweet to a 9.2 w/v% sucrose solution. As can be seen from Table 4.2, these values are just between the values found for the 25/75 and 40/60 sucrose/Na-cyclamate mixtures. Redlinger and Setser (1987) determined a 0.140 w/v% Ca-cyclamate solution to be as sweet as a 5 w/v% sucrose solution. As this concentration of Ca-cyclamate is higher than the concentration necessary to obtain the 50/50 sucrose/Na-cyclamate mixture at 10% SEV,

the presence of synergy between sucrose and (Na-)cyclamate was again confirmed. The synergy found by Portmann and Kilcast (1996b) was higher when less Na-cyclamate was present in the mixture. A look at their concentration-response relation for Na-cyclamate clarifies this effect, as this relation is negatively accelerated. Hoppe (1981) found a mixture of 75 g/L sucrose and 0.5 g/L Na-cyclamate to match the sweetness intensity of a 109.7 g/L sucrose solution. These values are similar to the ones reported in Table 4.2 for a 75/25 sucrose/Na-cyclamate mixture, which would suggest the presence of synergy. However, Hoppe (1981) ascribed these mixtures a hindering behavior. He used equations to quantify an expected mixture sweetness, which can then be compared with the experimentally determined mixture sweetness. The use of these equations introduces the reported hinderings, whereas a look at the actual concentrations in his Table 6 (Hoppe, 1981) indicates synergy. According to McBride (1988) a separate-sites model can account for the phenomenon of supplemental action. The sugars from a binary sugar system are transduced at independent receptor sites and then integrated in a common effector system to give a taste perception. Hutteau *et al.* (1998) showed that synergy can be correlated to an increase in water mobility and that an increased mobility of water molecules in the medium can be related to an increase in sweetness. They reported an increase in water mobility for sucrose/Na-cyclamate mixtures compared to sucrose. Synergistic effects observed, when two components are in mixture, are

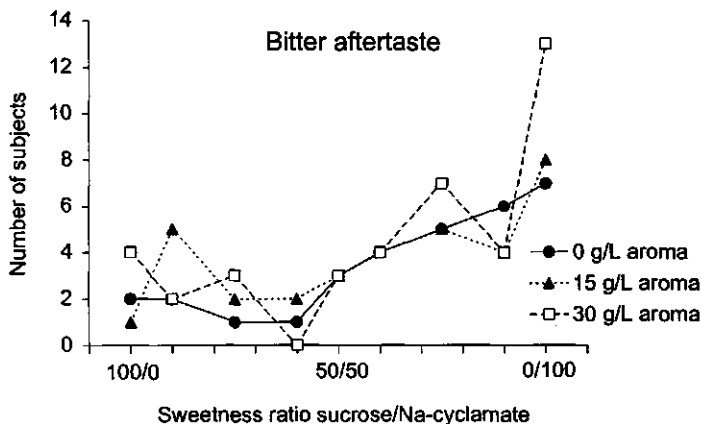


Figure 4.5 Number of subjects reporting a bitter aftertaste for solutions containing mixtures of sucrose and Na-cyclamate. Three different concentrations of orange aroma (0, 15 and 30 g/L) were used.

specific and depend on the compatibility of the hydration of each component and their influence on water structure.

Synergistic mixtures of sweeteners might show interactive effects with aroma compounds. The pH values measured for mixture solutions containing orange aroma were in the range of the pH values reported in Table 4.2. Apparently, the addition of orange aroma has no influence on the pH of the solutions. The results of the QDA are presented in Figures 4.2-4.5. Figure 4.2 shows a comparison between a sucrose solution and a Na-cyclamate solution at 10% SEV, both in the absence of orange aroma. In comparison with sucrose, the mean scores for Na-cyclamate are significantly higher for the attributes chemical and aftertaste. The observed significant difference for the attribute aftertaste is quite common for intense sweeteners. The results agree with the findings of Portmann and Kilcast (1996a). Other authors (Faurion *et al.*, 1980; DuBois and Lee, 1983; Hanger *et al.*, 1996) reported more similarities between sucrose and Na-cyclamate. In the descriptive analysis by Larson-Powers and Pangborn (1978b) samples containing Ca-cyclamate were characterised as "sweet-chemical" and "bitter"; they also had a cloying, "sticky-sweet" and "medicinal" aftertaste. Overall differences between Ca-cyclamate and sucrose were not found by Ketelsen *et al.* (1993), although Ca-cyclamate tends to have a longer aftertaste than sucrose. When a 100% sucrose and a 100% Na-cyclamate solution in the presence of 30 g/L orange aroma were compared (Figure 4.3), no significant differences were found. Apparently, the addition of orange aroma levels out differences mentioned for sucrose and Na-cyclamate.

Sweetnesses for all mixtures were kept at 10% SEV, as can be deduced from Figure 4.4A. The mean scores for the attribute sweet remain constant for each combination of sucrose and Na-cyclamate. The addition of orange aroma has no effect on these mean scores, as subjects accurately separate the scores on the different attributes, following the applied QDA method. The lines representing the mean scores for the attribute chemical for the three concentrations of orange aroma (Figure 4.4B) converge when more Na-cyclamate is present in the mixture. The addition of orange aroma accounts for this effect; the significant differences between sucrose and Na-cyclamate were levelled out. Comparison of Figures 4.2 and 4.3 illustrates the effect of an addition of orange aroma. For solutions containing Na-cyclamate only three attributes (orange, sour and sharp) increase significantly, whereas for sucrose seven attributes change significantly (orange, sour, chemical, fruity, watery, mint and aftertaste). When more sucrose is present in the mixtures, initial scores are relatively low and an addition of orange

aroma substantially contributes to the mean scores. The addition of orange aroma then clearly gives the solutions a more distinct flavour. As Na-cyclamate gives higher mean scores on most of the attributes (Figure 4.2), an addition of orange aroma hardly increases these scores, according to the Weber ratio. For all mixture solutions, addition of orange aroma significantly increases the mean scores for the attribute orange. As expected, the addition of 30 g/L orange aroma did not double the mean scores obtained with 15 g/L orange aroma (Nahon *et al.*, 1998).

Comments on the aftertaste of the different solutions show that the significantly higher scores of Na-cyclamate on this attribute (Figure 4.2) can be ascribed to a lingering bitterness. The bitter aftertaste mentioned for mixtures containing more Na-cyclamate, appears especially for the sweetness ratios 25/75, 10/90 and 0/100 sucrose/Na-cyclamate (Figure 4.5). Time-intensity measurements by Larson-Powers and Pangborn (1978a) showed a greater sourness and a marked, persistent bitterness of Ca-cyclamate compared to sucrose. Hanger *et al.* (1996) and Portmann and Kilcast (1996a) reported bitter aftertastes as well. The mean scores for the attribute aftertaste decrease as soon as a slight amount of sucrose is present in the mixture. The presence of sucrose in the mixture solution might change the system in such a way that effects of Na-cyclamate are suppressed immediately. Addition of orange aroma increases the orange, chemical and mint aftertastes of solutions.

In conclusion, it was shown that mixtures of sucrose and Na-cyclamate are synergistic. With the help of SEV determinations equisweet mixtures were composed, which form an optimal basis for the study of sweetener-flavour interactions. A QDA then showed a levelling effect of orange aroma for significant differences observed between sucrose and Na-cyclamate.

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5

Sensory evaluation of ternary mixtures of sodium cyclamate, aspartame, sucrose and an orange aroma

Ternary mixtures of sucrose, aspartame and Na-cyclamate were studied in the presence of an orange aroma. Equisweet mixtures of the sweeteners were composed, which account for the observed synergistic effects between the sweeteners. The sweetness contributions of sucrose and Na-cyclamate were fixed on forehand according to their synergy, and the matching concentration of aspartame was determined. The composition of the equisweet ternary mixtures was described in a regression equation. The mixtures of aspartame and Na-cyclamate exhibited more synergy than the mixtures of sucrose and Na-cyclamate. Sucrose and aspartame were exchangeable in the sense that the synergy observed for the ternary mixture was a linear combination of the synergies of the binary mixtures.

The flavour profiles of combinations of the mixtures and an orange aroma were obtained with the help of quantitative descriptive analysis and indicated possible interactive effects between the sweeteners and the orange aroma. The sweetness contribution of Na-cyclamate to the ternary mixtures determined the flavour profile of this mixture to a large extent. The ternary mixtures containing mainly Na-cyclamate were described as being more chemical, bitter and musty and having more aftertaste. Further comments on this aftertaste revealed the descriptions bitter and chemical.

This chapter has been submitted as:

Nahon, D.F.; Roozen, J.P.; De Graaf, C. *Chemical Senses*

5.1 Introduction

Observed flavour differences between regular and light beverages gave rise to various studies involving sweeteners and aroma compounds. Muir *et al.* (1998) studied the influence of sweetener type on the sensory profiles of commercial fruit juice drinks. Differences noticed between the effects of sweetener combinations provide a basis for further studies of sweetener-aroma interactions. Instrumental methods were also used to determine the interaction of aroma compounds with sweeteners. Reineccius *et al.* (1998) indicated in such a study that significant differences observed in the headspace analysis of sweetened beverages needed to be checked for sensory significance. A rather direct comparison between sensory and instrumental analysis (Nahon *et al.*, 1998a) showed that this sensory significance was not found for beverages containing Na-cyclamate, sucrose and an orange aroma. In a preliminary experiment with a commercial light blackcurrant soft drink, a modulation of the concentrations of the four intense sweeteners in the drink caused shifts in the flavour descriptions of the drink (Nahon *et al.*, 1996).

Equisweet mixtures of sweeteners form an optimal basis for the study of sweetener-aroma interactions. It was demonstrated that Beidler's mixture equation can be used to describe the sweetness of the binary sucrose/maltitol and sucrose/aspartame mixtures (Nahon *et al.*, 1998b), which means that these mixtures behave linear: they do not show any synergistic effects. For mixtures of sucrose and Na-cyclamate synergy was observed and precisely described (Nahon *et al.*, 1998c). To approach the effect observed with the quaternary mixture of sweeteners, ternary mixtures are of interest for study. A combination of sucrose, aspartame and Na-cyclamate will be the next step towards the study of the complexity of mixtures containing more than two components. In this combination, the behaviour of the mixture of aspartame and Na-cyclamate is unknown. The ternary mixtures studied, will be chosen in such a way that possible synergistic effects between aspartame and Na-cyclamate or for the ternary mixture as a whole, will be revealed.

Ternary mixtures can be visualised by a plane in a three dimensional coordinate system, with the axes sucrose, aspartame and Na-cyclamate, respectively (Figure 5.1). The extremes of the triangle in this coordinate system, will be set by the 10% Sucrose Equivalent Values (SEVs) for the three sweeteners. The triangle between these points then indicates the linear expectations. As synergy between sucrose and Na-cyclamate was already shown and synergy

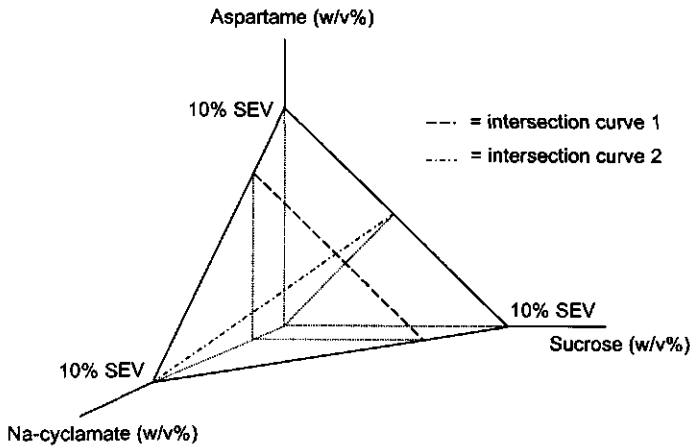


Figure 5.1 Linear expectations for equisweet ternary mixtures represented in a three dimensional coordinate system. Intersection curve 1: concentration of Na-cyclamate constant, intersection curve 2: ratio sucrose/aspartame constant.

between aspartame and Na-cyclamate can be expected, a concave triangle will probably represent the experimental plane at a total sweetness of 10% SEV. The experimental plane will be defined by equisweet mixtures of sucrose, aspartame and Na-cyclamate. To determine these equisweet mixtures the concentrations of sucrose and Na-cyclamate will be chosen on forehand, deducted from the results obtained in the sucrose-Na-cyclamate experiment (Nahon *et al.*, 1998c). With the help of partial SEV determinations (Nahon *et al.*, 1998c) we will determine how much aspartame should be added to the sucrose/Na-cyclamate mixture to obtain a sweetness equivalent to the sweetness of a 10 w/v% sucrose solution. In the first part of the experiment, the sweetness contribution of Na-cyclamate to the mixture will be fixed at 50%. In this way, intersection curve 1 (Figure 5.1) through the triangle plane in the three dimensional coordinate system will be studied. The second part of the experiment will deal with intersection curve 2 (Figure 5.1), which consists of increasing concentrations of Na-cyclamate, with a constant sucrose/aspartame ratio.

Synergy observed in ternary mixtures has been reported before (Schiffman *et al.*, 1996), however, a complete set of results has never been published. Within the ternary mixture sucrose/aspartame/Na-cyclamate, we have studied the binary mixtures sucrose/aspartame and sucrose/Na-cyclamate, but not aspartame/Na-cyclamate. Bakal (1983) indicated the presence of synergy between aspartame and cyclamates and reported that combinations of these two sweeteners in aqueous solutions exhibit superior taste qualities over the single sweetener

solutions (Bakal, 1991). Schiffman *et al.* (1979) studied qualitative differences among sweeteners with the help of multidimensional scaling techniques and found that Ca-cyclamate and aspartame fell closest to and thus tasted most like the sugars. These sweeteners also clustered in the multidimensional analysis by Faurion *et al.* (1980). Frank *et al.* (1989) established the presence of significant synergy (20%) between aspartame and cyclamate. Schiffman *et al.* (1996) suggested in their abstract that the synergy observed for binary mixtures was larger than that for ternary mixtures of sweeteners.

Mixtures of sweeteners were described in theoretical models (e.g. Beidler, 1971) and by empirical fitting equations (e.g. Moskowitz *et al.*, 1978). Laffort (1989) applied his empirical models for odor mixtures to the mixtures studied by De Graaf and Frijters (1986) and describes models for ternary mixtures as well (Laffort and Dravnieks, 1982). Most models start from the intensities of the components of the mixtures, for which the individual concentration-response relationships should be known. For the ternary mixture studied in the present experiment, an empirical fitting equation was established, which is useful for the calculation of the composition of equisweet mixtures in practice. Furthermore, this equation can be used to obtain information on the aspartame/Na-cyclamate mixtures.

The equisweet ternary mixtures obtained are essential to study the interactive effects with aroma compounds by quantitative descriptive analysis (QDA). Analogous to earlier experiments, the ternary mixtures were studied in the presence of a water soluble orange aroma. Sucrose/aspartame mixtures did not show interactive effects with the orange aroma added (Nahon *et al.*, 1998b). However, orange aroma levelled out taste differences detected between sucrose and Na-cyclamate solutions (Nahon *et al.*, 1998c).

In this study, ternary mixtures of sucrose, aspartame and Na-cyclamate, equisweet at 10% SEV were composed and described in a regression equation. Interactive effects between the sweeteners and an orange aroma were studied by QDA.

5.2 Materials and methods

Equisweet ternary mixtures were determined with the help of sensory evaluation and afterwards descriptive analyses were performed. Subjects and stimuli were the same in both cases, but the methods (10% SEV determinations and QDA) differed and are therefore described separately.

5.2.1 Subjects

The panel for both sensory evaluations (19-24 subjects) was chosen from a pool of 28 paid subjects (7 men and 21 women), ranging in age from 20 to 24 years. Some of these subjects (13) participated in earlier experiments, others were selected from a pool of 24 subjects participating in the selections for the present experiment. All subjects were trained for the two experiments. Most subjects were (former) students of Wageningen University and Research Centre, some of them having prior experience of psychophysical experiments. Informed consents were obtained from the subjects and the study was approved by the Medical Ethical Commission of Wageningen University and Research Centre. Subjects were instructed to taste according to the sip-and-spit method, the time intervals between stimuli were kept at 60 s. The subjects used water and crackers to neutralise their mouth after tasting of a solution. A computer interactive interviewing system (Ci2 system, Sawtooth Software Inc., Ketchum, USA) was used to gather survey information.

5.2.2 Stimuli

Analogous to earlier experiments (Nahon *et al.*, 1998b and c) the stimuli were solutions of sucrose, aspartame (Holland Sweetener Company, Maastricht, The Netherlands) and Na-cyclamate (Flevo Chemie BV, Harderwijk, The Netherlands) and mixtures of these three substances in demineralised water. Different sucrose/aspartame/Na-cyclamate ratios were chosen to study possible interactive effects between sweeteners and aroma compounds. The pH of the solutions was measured with a Metrohm (Herisau, Switzerland) pH-meter. The overall sweetness of each mixture had to meet with a constant perceived taste intensity of 10% SEV. The standard stimulus for the SEV determinations was a 10 w/v% sucrose solution.

Solutions of sucrose, aspartame, Na-cyclamate, orange aroma and octanal (Merck, Hohenbrunnen, Germany) were used to generate attributes for the QDA. The standard stimuli 'not sweet' and 'very sweet' used in this analysis consisted of 0 and 16 w/v% sucrose, respectively. The orange aroma added to evaluate interactive effects was a sample of the watery vapor phase of stripped orange juice (Cargill juice division, Amsterdam, The Netherlands). It was used in concentrations of 0, 15 and 30 g/L.

All solutions were prepared at least 24 h before evaluation and stored at 4 °C overnight. In all evaluations, a stimulus consisted of 15 mL of solution. These stimuli were presented in a

glass jar, covered by a plastic lid and aluminium foil to prevent interactions between the plastic and the orange aroma. The stimuli were presented to the panel at room temperature (22 °C).

5.2.3 SEV determinations

As a new panel and a new batch of Na-cyclamate was used in the present experiment, the 10% SEV values for aspartame and Na-cyclamate were determined once more (as compared to Nahon *et al.*, 1998b and c). The partial SEV for Na-cyclamate in the 50/50 sucrose/Na-cyclamate mixture was set again as well (Nahon *et al.*, 1998c). Next, equisweet mixtures of the sweeteners sucrose, aspartame and Na-cyclamate were composed by fixing the contributions of sucrose and Na-cyclamate to the mixtures, according to the chosen ratios. Aspartame was added until a sweetness of 10% SEV was reached (the partial SEV of aspartame). The extent of this addition was determined using the method of constant stimuli (Guilford, 1954) and weighted linear regression analysis (Bock and Jones, 1968). The panel was presented the standard stimulus, which is the 10 w/v% sucrose solution, and seven comparison stimuli. These consist of fixed amounts of sucrose and Na-cyclamate and amounts of aspartame which vary exponentially near the expected partial SEV for aspartame. The determination of this partial SEV further follows the method as described by De Graaf and Frijters (1986). According to this method, the standard and the comparison stimulus are tasted in both orders. McBride (1988) verified this procedure by finding a significant effect of order of tasting, with the first solution tasted being identified as sweeter. In this way, at least 266 datapoints were used for the determination of the composition of one ratio mixture (correlation coefficients of weighted linear regressions > 0.91). This method was then repeated for all ratio mixtures, providing the composition of ternary mixtures matching a 10% SEV.

5.2.4 Quantitative Descriptive Analysis

The solutions containing different combinations of sucrose, aspartame and Na-cyclamate matching a sweetness of 10% SEV (Table 5.1) and three concentrations of orange aroma (0, 15 and 30 g/L) were evaluated by QDA. For this analysis, 23 subjects generated flavour attributes with the help of several solutions, which were ranked and clustered in consultation with the panel (Table 5.2). The panel was calibrated by tasting sucrose references of 0 and 16

Table 5.1 Mixtures of sucrose, aspartame and Na-cyclamate matching a sweetness of 10% SEV in a given ratio, concentrations of sucrose, aspartame and Na-cyclamate and pH of the solutions.

Mixture ratio sucrose/aspartame/ Na-cyclamate	Sucrose (w/v%)	Aspartame (w/v%)	Na-cyclamate (w/v%)	pH
0 / 50 / 50	0	0.024	0.114	5.5
12.5 / 37.5 / 50	1.25	0.019	0.114	6.0
25 / 25 / 50	2.5	0.012	0.114	5.7
37.5 / 12.5 / 50	3.75	0.006	0.114	6.0
50 / 0 / 50	5	0	0.114	6.2
0 / 0 / 100	0	0	0.499	5.9
12.5 / 12.5 / 75	1.25	0.004	0.236	5.5
37.5 / 37.5 / 25	3.75	0.024	0.043	5.8
45 / 45 / 10	4.5	0.033	0.015	6.1
50 / 50 / 0	5	0.051	0	5.8

Table 5.2 Attributes describing the flavour of several solutions containing sucrose (10 w/v%), aspartame (0.101 w/v%), Na-cyclamate (0.499 w/v%), a mixture of these sweeteners (respectively 2.5 w/v%, 0.012 w/v% and 0.114 w/v%), orange aroma (15 g/L) and/or octanal (0.05 g/L).

Attributes	
Sweet	Sharp
Orange	Musty
Sour	Viscosity
Chemical	Fruity
Bitter	Aftertaste

w/v%, which were the anchors of the visual analogue scale for sweetness. Similar scales for other attributes were not anchored. The intensities of the attributes were marked on a 120 mm visual analogue scale (maximum score = 50) on a portable computer screen. Stimuli were presented randomly to the subjects and subjects were asked to comment on aftertastes. To monitor the use of scales, 5 and 12.5 w/v% sucrose solutions were evaluated as well. These solutions were randomly given with the other solutions to be evaluated.

5.2.5 Data analysis

Sensory data were subjected to Student's *t* tests to determine significant differences between solutions. A significance level of $P < 0.01$ was used for sweetness (anchored scale) and $P < 0.05$ for the other attributes.

The composition of the equisweet ternary mixtures was described in a regression equation with the help of a surface-fitting program (Tablecurve 3D, SPSS Inc., Chicago, USA). The equation was based on experimental data from the present experiment and on results obtained before (Nahon *et al.*, 1998b and c). A comparison was made with an equation based on information on the binary mixtures of the components. The fit of the empirical regression equation to the experimental results was determined.

5.3 Results

The starting points of all partial SEV determinations were set by the determinations of the 10% SEVs for aspartame and Na-cyclamate (compare Figure 5.1). For aspartame the same batch was used as before (Nahon *et al.*, 1998b) and with the panel used for the present experiment the 10% SEV for aspartame was 0.101 w/v% instead of 0.096 w/v%. In case of Na-cyclamate a new batch was used and the panel used for the present experiment determined its 10% SEV to be 0.499 w/v% compared to 0.443 w/v% found before (Nahon *et al.*, 1998c). As the partial SEV of Na-cyclamate in the 50/50 sucrose/Na-cyclamate mixture was the starting point for the study of ternary mixtures with a fixed sweetness contribution of Na-cyclamate of 50%, this partial SEV was determined once more. The value for this partial SEV was 0.114 w/v% instead of 0.111 w/v% (Nahon *et al.*, 1998c).

Given the results obtained for intersection curves 1 and 2 (Table 5.1) and the results on the mixtures of sucrose and aspartame (Nahon *et al.*, 1998b) and sucrose and Na-cyclamate (Nahon *et al.*, 1998c) the following ternary regression equation (Figure 5.2) was obtained:

$$Z = \frac{0.10 - 0.34X + 0.26X^2 - 0.016Y + 0.00058Y^2}{1 + 20.33X - 79.28X^2 + 197.18X^3 - 0.058Y} \quad (5.1)$$

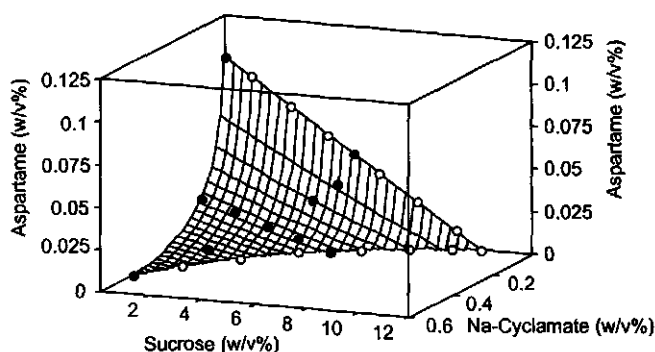


Figure 5.2 Representation of equisweet ternary mixtures in a three dimensional coordinate system. The grid plane represents Equation 1, the filled circles (●) are experimental results obtained from intersection curves 1 and 2 and the open circles (○) are obtained from Nahon *et al.* (1998b and c).

in which X = Na-cyclamate (w/v%), Y = sucrose (w/v%) and Z = aspartame (w/v%). After fixing the concentrations of Na-cyclamate and sucrose in the ternary mixtures, the concentrations of aspartame in these mixtures can be calculated with the help of this equation.

The outcome of each experiment can be illustrated by an intersection curve located on its two dimensional plane in the three dimensional coordinate system. As in earlier experiments (Nahon *et al.*, 1998c), the linear expectations were then compared to the experimental results. Intersection curves 1, based on linear expectations and experimental results and calculated from Equation 5.1, are all presented in Figure 5.3. The concentration of aspartame necessary to obtain equisweet mixtures at 10% SEV is linearly related to the concentration of sucrose in the ternary mixture, but is only half its linear expectation. Similarly, intersection curves 2 are presented in Figure 5.4. The concentration of aspartame necessary to get these mixtures equisweet at 10% SEV is smaller than linearly expected.

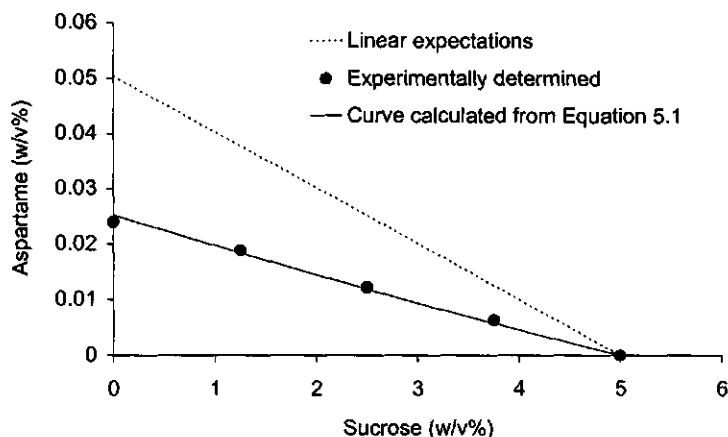


Figure 5.3 Intersection curve 1. The concentration of Na-cyclamate in the ternary mixture was kept constant at 0.114 w/v%, its contribution to the total sweetness being 50%. Concentration of aspartame (w/v%) in the mixture, as a function of the concentration of sucrose (w/v%) in the mixture; linear expectations, experimental results and curve obtained from Equation 5.1.

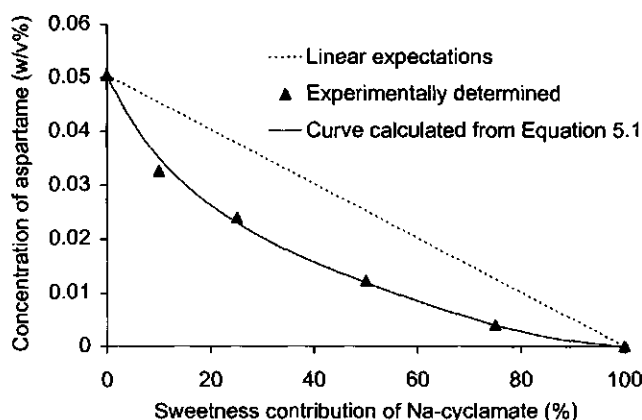


Figure 5.4 Intersection curve 2. The ratio between the sweetness contributions of aspartame and sucrose was kept constant at 50/50. Concentrations of aspartame (w/v%) in the ternary mixture, as a function of the contribution of Na-cyclamate (%) to the sweetness of the mixture; linear expectations, experimental results and curve obtained from Equation 5.1.

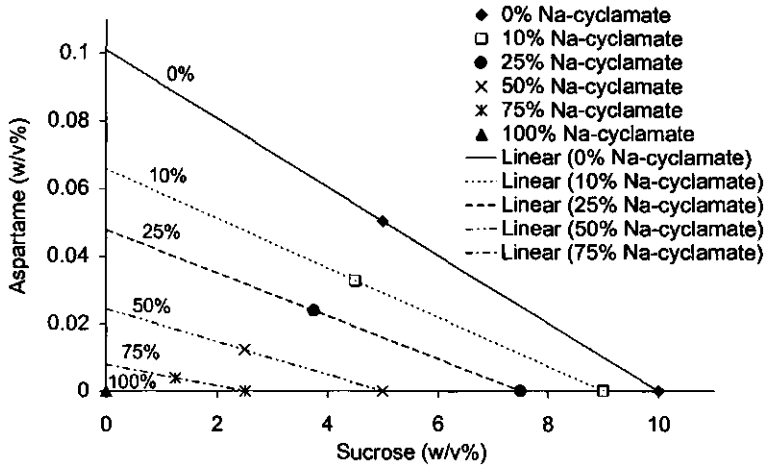


Figure 5.5 Linear extrapolations of intersections 1 and 2 (see Figure 5.1), which gives the relationships between sucrose (w/v%) and aspartame (w/v%) for various sweetness contributions of Na-cyclamate (0, 10, 25, 50, 75 and 100%).

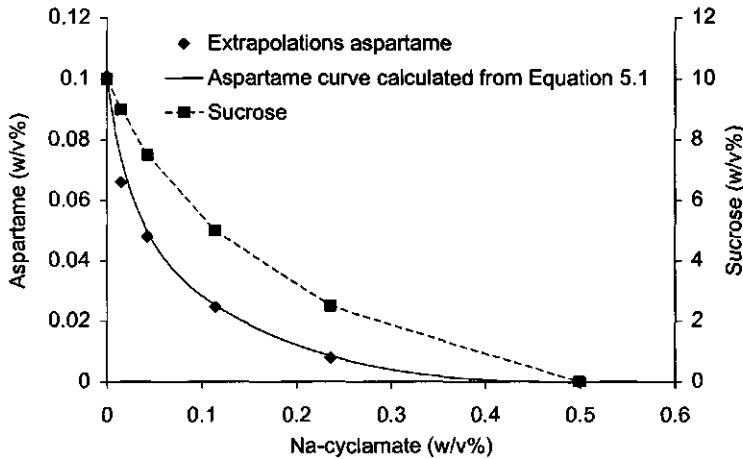


Figure 5.6 The concentrations of aspartame (w/v%) and sucrose (w/v%) in binary mixtures, as a function of the concentration of Na-cyclamate (w/v%) in these mixtures. The total sweetness of these mixtures being 10% SEV. For aspartame the curve obtained from Equation 5.1 is given, along with the results from linear extrapolations (Figure 5.5). The results for the sucrose/Na-cyclamate mixture are obtained from Nahon et al. (1998c).

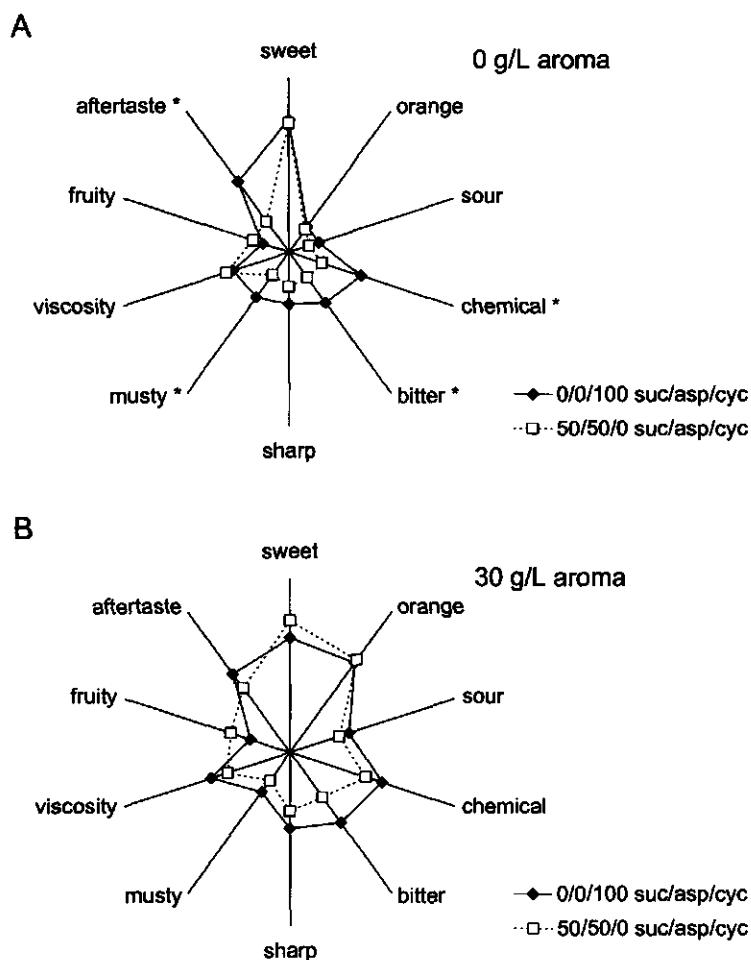


Figure 5.7 Spider web diagram representing the mean scores for sensory attributes of a Na-cyclamate (0/0/100) and a sucrose/aspartame (50/50/0) solution at 10% SEV. In the presence of **A.** 0 g/L and **B.** 30 g/L orange aroma. * = significant differences ($P < 0.01$ for sweetness, $P < 0.05$ for other attributes); suc = sucrose; asp = aspartame; cyc = Na-cyclamate.

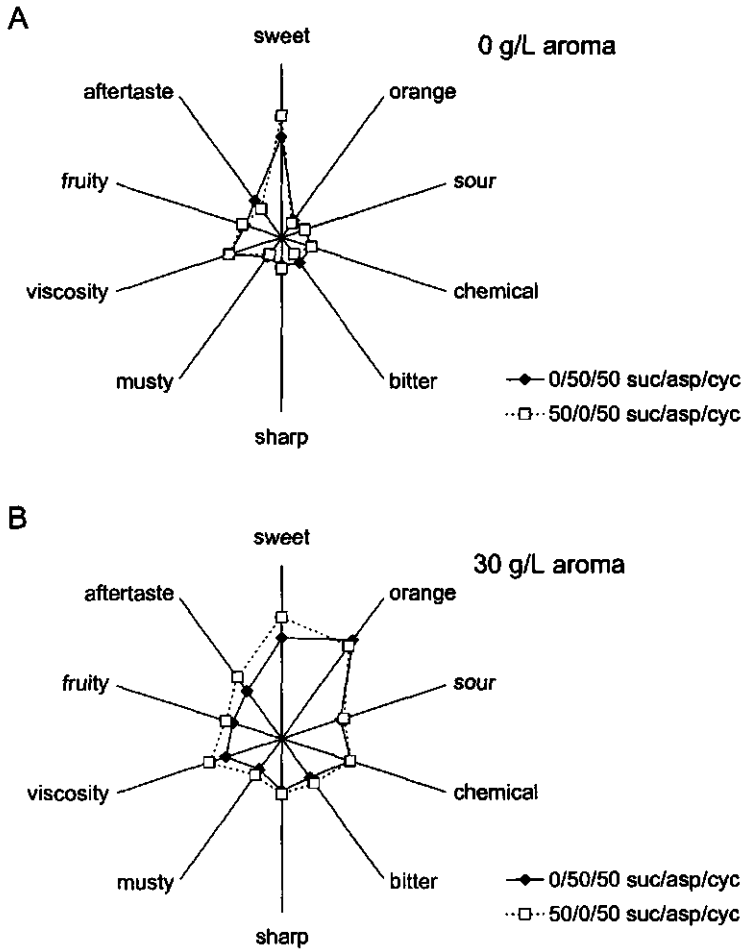


Figure 5.8 Spider web diagram representing the mean scores for sensory attributes of an aspartame/Na-cyclamate (0/50/50) and a sucrose/Na-cyclamate (50/0/50) solution at 10% SEV. In the presence of **A.** 0 g/L and **B.** 30 g/L orange aroma. No significant differences; suc = sucrose; asp = aspartame; cyc = Na-cyclamate.

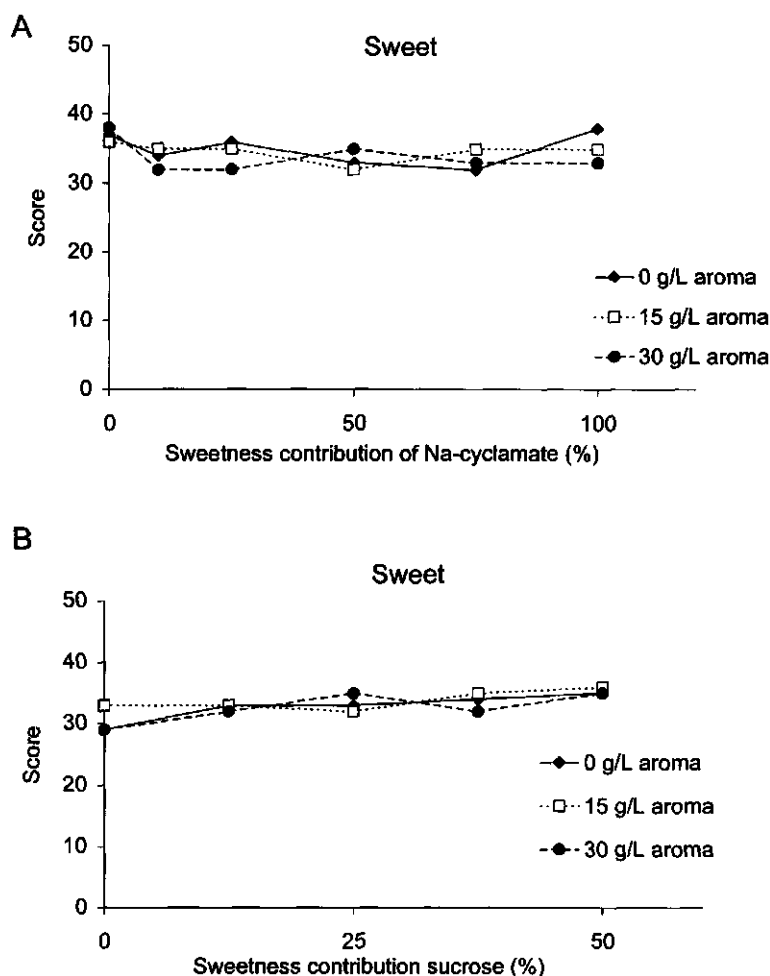


Figure 5.9 Mean scores for the sensory attribute sweetness for solutions containing ternary mixtures of sucrose, aspartame and Na-cyclamate. Three different concentrations of orange aroma (0, 15 and 30 g/L) were used. **A.** The sweetness contributions of sucrose and aspartame were kept constant at a ratio of 50/50, the mean scores were plotted against the contribution of Na-cyclamate (%) to the sweetness of the mixture (intersection curve 2). **B.** The sweetness contribution of Na-cyclamate was kept constant at 50%, the mean scores were plotted against the sweetness contribution of sucrose (%) (intersection curve 1).

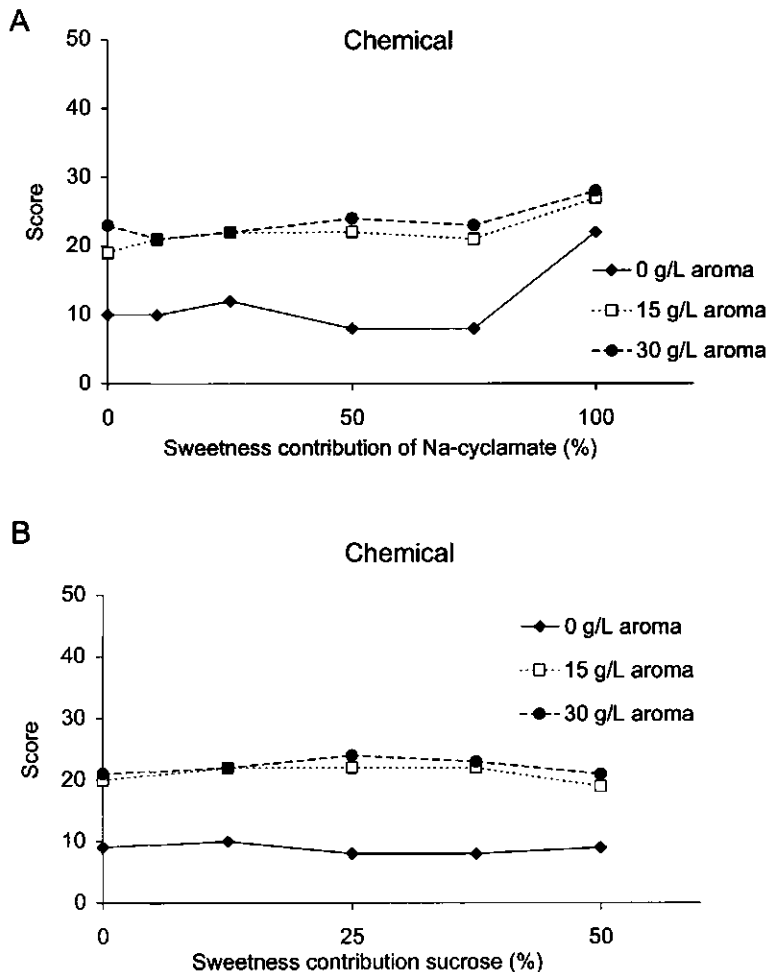


Figure 5.10 Mean scores for the sensory attribute chemical for solutions containing ternary mixtures of sucrose, aspartame and Na-cyclamate. Three different concentrations of orange aroma (0, 15 and 30 g/L) were used. **A.** The sweetness contributions of sucrose and aspartame were kept constant at a ratio of 50/50, the mean scores were plotted against the contribution of Na-cyclamate (%) to the sweetness of the mixture (intersection curve 2). **B.** The sweetness contribution of Na-cyclamate was kept constant at 50%, the mean scores were plotted against the sweetness contribution of sucrose (%) (intersection curve 1).

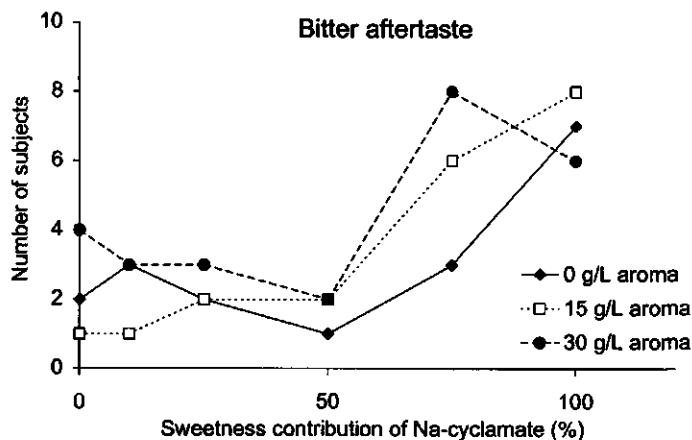


Figure 5.11 Number of subjects reporting a bitter aftertaste for solutions containing ternary mixtures of sucrose, aspartame and Na-cyclamate. Three different concentrations of orange aroma (0, 15 and 30 g/L) were used.

The deviation of the calculated curves (Equation 5.1) from the experimental results varied from 0.8–6.0%. This largest deviation of 6.0% was found for the 45/45/10 sucrose/aspartame/Na-cyclamate mixture. When calculating the composition of the ternary mixtures with the help of a regression equation based on the binary mixtures of the components, this deviation varied from 0.4–12.8%. Given the composition of equisweet mixtures of sucrose and Na-cyclamate (Nahon *et al.*, 1998c) and the results obtained with the intersections 1 and 2 (Figures 5.3 and 5.4), equisweet combinations of sucrose and aspartame at a given Na-cyclamate concentration, can be obtained by extrapolations (Figure 5.5). With the help of Equation 5.1, the (calculated) compositions of the equisweet mixtures of sucrose and Na-cyclamate and aspartame and Na-cyclamate can be compared (Figure 5.6).

The flavour profiles of the equisweet ternary mixtures were obtained by QDA. The pH values measured for the mixture solutions used in the QDA are reported in Table 5.1. The pH values for the solutions containing the orange aroma were in the same range (5.4–6.1). The comparison between the ternary mixtures 0/0/100 and 50/50/0 sucrose/aspartame/Na-cyclamate shown in Figure 5.7A, reveals significant differences for the attributes chemical, bitter, musty and aftertaste in the absence of orange aroma. When orange aroma is present (30 g/L) these two ternary mixtures do not differ significantly (Figure 5.7B). Comparison of mixtures containing relatively more sucrose or aspartame (50/0/50 and 0/50/50

sucrose/aspartame/Na-cyclamate) shows no significant differences between the two flavour profiles, either in the absence or in the presence of orange aroma (Figures 5.8A and B). For the attribute sweet, the mean scores are constant, for each possible mixture of sucrose, aspartame and Na-cyclamate (Figures 5.9A and B). Figures 5.10A and B show the mean scores for the attribute chemical for the ternary mixtures of sucrose, aspartame and Na-cyclamate. Figure 5.10A, showing the results obtained for intersection curve 2, illustrates the effect of relatively more Na-cyclamate in the ternary mixture. The contribution of orange aroma to the mean scores of the attribute chemical is of a smaller magnitude for solutions in which only Na-cyclamate is present. The standard deviations for the attribute sweet were low for all mixtures of sucrose, aspartame and Na-cyclamate. Standard deviations for the other attributes were larger and significant differences for these attributes were hardly found.

The comments on aftertastes of the different solutions resembled the attributes used. Solutions containing more Na-cyclamate tended to have more aftertaste, which is mainly described by the attributes bitter (Figure 5.11) and chemical. The number of subjects mentioning a bitter aftertaste increases when more Na-cyclamate is present in the ternary mixture, for all three concentrations of orange aroma. Solutions containing 15 or 30 g/L orange aroma provided stronger orange, chemical and bitter aftertastes than solutions without orange aroma.

5.4 Discussion

10% SEV determinations for aspartame and Na-cyclamate were repeated because of replacements in the sensory panel and the use of new product batches; they showed deviations of 5 respectively 13% compared to the determinations done before (Nahon *et al.*, 1998b and c). As the two determinations for aspartame were performed with the same sweetener batch but different panels, it is confirmed that SEV determinations performed with different panels can be compared to a certain extent only (Nahon *et al.*, 1998b). As the partial SEV of Na-cyclamate in the 50/50 sucrose/Na-cyclamate mixture was quite close to its value determined before (0.114 w/v% compared to 0.111 w/v%), the partial SEVs of Na-cyclamate for the other mixtures were taken from the results obtained in the earlier experiment (Nahon *et al.*, 1998c).

The linear relationship for sucrose and aspartame shown in Figure 5.3, implies an absence of extra synergy when comparing ternary mixtures with binary mixtures. As the contribution of Na-cyclamate to the ternary mixture was fixed for intersection curve 1, the synergy between sucrose and Na-cyclamate was already taken into account. A full exchangeability between sucrose and aspartame in the mixture, would result in the figure marked as 'linear expectations aspartame' (Figure 5.3). As a straight line was obtained, a certain amount of exchangeability will be present. Apparently, aspartame and Na-cyclamate show synergy as well and from Figure 5.6, we can conclude that aspartame and Na-cyclamate show more synergy than sucrose and Na-cyclamate. The difference between the linear expectations and the experimental results observed in Figure 5.3, can be ascribed to the extra synergy between aspartame and Na-cyclamate compared to the synergy between sucrose and Na-cyclamate. The exact figure for the synergy between aspartame and Na-cyclamate was not determined, but was approached by extrapolations of experimental results (Figure 5.5) and calculations using Equation 5.1 (Figure 5.6). Frank *et al.* (1989) reported more synergy for the aspartame/Na-cyclamate mixture (20%) as compared to the sucrose/Na-cyclamate mixture (15%). However, they also reported a synergy of 11% between sucrose and aspartame. Bakal (1983) reported synergies for the combination sucrose/Na-cyclamate and aspartame/Na-cyclamate, but also for the combination sucrose/aspartame. The results obtained in this experiment indicate that the resulting synergy in a ternary mixture, is the arithmetical sum of the synergies of the composing binary mixtures. Schiffman *et al.* (1996) suggested that binary mixtures might reach a greater degree of synergism than ternary mixtures. The synergistic effect reported refers to the effect that less aspartame or Na-cyclamate is needed than linearly expected. Lawless (1998) indicated in his short communication that various methods used for the determination of the presence or absence of synergy (ours included) may under- or overestimate the sweetness of a mixture. The reduction of concentrations found in our investigation might be a logical consequence of the method, but still this does not change the practical advantage of the fact that smaller amounts of sweeteners are needed to obtain the same sweetness.

In intersection 2, the sweetness contribution of Na-cyclamate to the mixture was varied and the ratio sucrose/aspartame was kept constant. The contributions of sucrose and Na-cyclamate were fixed on forehand, according to the synergistic effects observed by Nahon *et al.* (1998c). The linear expectations for the concentration of aspartame given in Figure 5.4,

imply a similar synergy for aspartame/Na-cyclamate mixtures and sucrose/Na-cyclamate mixtures. However, as mentioned before, the synergy between aspartame and Na-cyclamate is larger than the synergy between sucrose and Na-cyclamate and therefore, when 'substituting' sucrose by aspartame, less aspartame is needed than deducted from linear expectations. Including the results shown in Figure 5.4, we can imagine the equisweet concave triangle as a winding stairs with its axis the equisweet sucrose/Na-cyclamate mixtures (Figure 5.2). For each ternary mixture in which Na-cyclamate contributes to the sweetness to a certain extent, the sweetness contributions of sucrose and aspartame are linearly exchangeable. Given the data from intersections 1 and 2, linear regressions can be applied, to obtain the relationships between the concentration of sucrose and the concentration of aspartame, for different sweetness contributions of Na-cyclamate (Figure 5.5). Extrapolations to the points in which no sucrose is present in the mixtures, gives the data for binary mixtures of aspartame and Na-cyclamate (Table 5.3). As the sweetness contributions of sucrose and aspartame remain linearly exchangeable, whatever the sweetness contribution of Na-cyclamate, it is even more explicit that sucrose and aspartame share a common receptor mechanism (Ennis, 1991; Ayya and Lawless, 1992).

Using the surface-fitting program (Tablecurve 3D) it is relatively easy to calculate and visualise the composition of equisweet ternary mixtures (Figure 5.2). Therefore, this method would be a valuable tool to the industry, knowing the composition of some equisweet mixtures. A comparison of the range of equisweet mixtures used to establish such a fitting equation, reveals that the knowledge of the binary mixtures only, is not sufficient. Calculations based on the compositions of the binary mixtures revealed a deviation of 0.4-

Table 5.3 *Mixtures of aspartame and Na-cyclamate expected to match a sweetness of 10% SEV; calculated concentrations of aspartame and Na-cyclamate. The concentrations of aspartame were obtained with the help of the linear extrapolations shown in Figure 5.5.*

Mixture ratio aspartame/Na-cyclamate	Aspartame (w/v%)	Na-cyclamate (w/v%)
0/100	0	0.499
25/75	0.008	0.236
50/50	0.025	0.114
75/25	0.048	0.043
90/10	0.066	0.015
100/0	0.101	0

12.8%, whereas calculations based on the intersections and two binary mixtures gave a deviation of maximal 6.0%. With the help of Equation 5.1, the composition of other equisweet (binary) mixtures can be found. In this way, we could directly compare sucrose/Na-cyclamate and aspartame/Na-cyclamate mixtures (Figure 5.6). A more important synergy for the aspartame/Na-cyclamate mixtures was revealed.

Ternary mixtures of sweeteners might show interactive effects with aroma compounds, as we noticed similarly with quaternary mixtures (Nahon *et al.*, 1996). Again the pH values measured were in the same range for all ternary mixtures, in the absence and in the presence of orange aroma. This pH measurement additionally offers a control considering the quality of the orange aroma and the sweeteners present in the mixtures. The fluctuations in the pH can be ascribed to the low buffering capacity of the mixtures studied near neutral pH. The results of the QDA are shown in Figures 5.7-5.11. In Figure 5.7A a Na-cyclamate and a 50/50 aspartame/sucrose solution at 10% SEV are compared, both in the absence of orange aroma. The mean scores for the Na-cyclamate solution are significantly higher for the attributes chemical, bitter, musty and aftertaste. In a comparison between sucrose and Na-cyclamate we found the same results for the attributes chemical and aftertaste (Nahon *et al.*, 1998c). When the Na-cyclamate and 50/50 aspartame/sucrose solution in the presence of 30 g/L orange aroma (Figure 5.7B) were compared, no significant differences were found. As discussed in the same study, the addition of orange aroma levels out differences noticed for the solutions. An earlier comparison between sucrose and aspartame solutions (Nahon *et al.*, 1998b) showed significant differences for the attributes sour, chemical and aftertaste. The comparison between the 50/50 aspartame/Na-cyclamate and the 50/50 sucrose/Na-cyclamate solutions in the absence of orange aroma (Figure 5.8A) reveals no significant differences. Apparently the flavour profiles of these binary mixtures are dominated by the flavour profile of Na-cyclamate, which is after all present in the same amount in both binary mixtures. McBride and Finlay (1990) reported a similar effect for mixtures of sucrose and citric acid. The total intensity of the mixtures was determined by the intensity of the stronger, or dominant component alone. As expected, a comparison between the 50/50 aspartame/Na-cyclamate and the 50/50 sucrose/Na-cyclamate in the presence of 30 g/L orange aroma did not show significant differences either (Figure 5.8B).

Mixtures were prepared equisweet at 10% SEV, as can be deduced from Figures 5.9A and B. The mean scores for the attribute sweet remained constant for each ternary combination of

sucrose, aspartame and Na-cyclamate. Addition of orange aroma did not influence the mean scores for the attribute sweetness. Panellists were trained to accurately separate the scores on the different attributes, following the QDA method. The mean scores given for the attribute chemical (Figures 5.10A and B) illustrate a similar effect as noticed before (Nahon *et al.*, 1998c); the addition of orange aroma levels out the differences between the various mixture solutions. Again the presence of Na-cyclamate singly influences the mean scores on the attribute chemical to the largest extent (Figure 5.10A), whereas changes in the sweetness contributions of sucrose and aspartame do not influence these mean scores (Figure 5.10B). Muir *et al.* (1998) compared several fruit juice drinks, made up with different sweetener combinations. The type of sweetener influenced the perception on flavour, aftertaste, mouthfeel and in particular the perception of natural character. Their panellists separated the juice drinks mainly on the basis of sweetener type. However, the perception of aroma was not influenced by sweetener type, which might indicate that no sweetener-aroma interactions were present for these juice drinks. For solutions containing mixtures of sucrose, aspartame and Na-cyclamate, the addition of orange aroma increases the mean scores for all attributes except sweetness, musty and viscosity. The addition of orange aroma clearly gives the solution a more distinct flavour.

Comments on the aftertaste of the different mixture solutions reveal that the significantly higher scores of Na-cyclamate solutions on this attribute (Figure 5.7A) can be ascribed to a lingering chemical bitterness. Figure 5.11 shows that the number of subjects mentioning a bitter aftertaste is high for Na-cyclamate solutions (whether orange aroma is present or not) and decreases as soon as a certain amount of sucrose and/or aspartame is present in the solutions as well. The mean scores for the attribute aftertaste also decrease as soon as a slight amount of sucrose and/or aspartame is present in the ternary mixtures, just as described before by Nahon *et al.* (1998c). Addition of orange aroma increases the orange, chemical and bitter aftertastes of mixture solutions.

In conclusion, it was shown that equisweet ternary mixtures of sucrose, aspartame and Na-cyclamate can be composed when the compositions of the equisweet binary mixtures of these sweeteners are known. Describing the composition of the ternary mixtures in a regression equation, opens the possibility to compose ternary mixtures, knowing (maximal) contributions of one or two sweeteners. The equisweet ternary mixtures were used as a basis for the study of sweetness-flavour interactions, for which QDA revealed a levelling effect of

the orange aroma for significant differences between taste attributes of mixture solutions. The relative sweetness contribution of Na-cyclamate to the ternary mixtures seemed to influence the flavour profiles of these mixtures to the largest extent.

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6

Flavour release from mixtures of sodium cyclamate, sucrose and an orange aroma

Mixtures of sucrose and sodium cyclamate (Na-cyclamate) were studied to evaluate possible interactive effects between these sweeteners and orange aroma compounds. Equisweet mixtures (10% Sucrose Equivalent Value) of the sweeteners were prepared by taking into account the observed synergistic effects between sucrose and Na-cyclamate. The release of volatile compounds from the solutions containing these mixtures and a water soluble orange aroma was studied. The volatile compounds were quantified and identified by gas chromatography combined with flame ionisation detection (GC/FID) and mass spectrometry (GC/MS). The presence of sucrose significantly changes the release of 15 selected volatile compounds from a solution containing the aroma compared to the aqueous control. Increasing sucrose concentrations in the solutions (0-60 w/v%) caused an increased release of the volatile compounds with short GC/FID retention times and a decreased release of the compounds with longer retention times. Increasing Na-cyclamate concentrations (0-2.658 w/v%) did not change the release of volatile compounds.

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6.1 Introduction

Sweetness-flavour interactions in models for soft drinks can be studied by sensory and instrumental analysis. In a previous sensory experiment equisweet mixtures of sucrose and Na-cyclamate were evaluated (Nahon *et al.*, 1998). As the mixtures of sucrose and Na-cyclamate showed positive synergy, solutions containing these mixtures and a water soluble orange aroma were chosen to study sensory perceived properties by quantitative descriptive analysis (QDA). Comparison of a sucrose and a Na-cyclamate solution at 10% Sucrose Equivalent Value (SEV, a sweetness equivalent to a 10 w/v% sucrose solution) revealed significant differences for the attributes bitter and aftertaste (Figure 6.1). As 15 g/L orange aroma was present in the solutions, differences for some other attributes were found as well. Interactive effects between the sweeteners and volatile compounds of the orange aroma could be present in these solutions.

The possible interactive effects would change the release of volatile compounds from solutions made up from sweeteners and an aroma. An instrumental analysis by gas chromatography provides information on this release. Von Sydow *et al.* (1974) noted that adding sucrose to blueberry and cranberry juices gave only little change in the headspace composition and did not significantly alter the profile determined from "sniffing". According

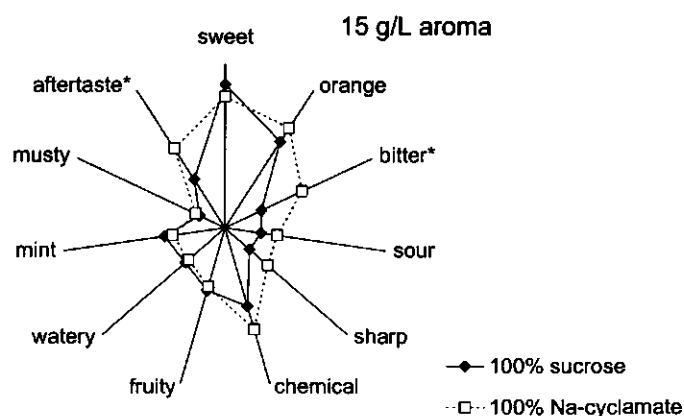


Figure 6.1 Spider web diagram representing the mean scores for sensory attributes of a sucrose and a Na-cyclamate solution at 10% SEV (in the presence of 15 g/L of a water soluble orange aroma); * = significant differences ($P < 0.05$).

to them, sucrose appears to enhance aroma on a psychological level, rather than acting upon chemical constituents and thus modifying the vapor composition. Their semiquantitative data showed that an increasing sucrose level in the juices increases the peak areas for the very low-boiling constituents in the gas phase. However, they reported that these compounds are not important for the desirable aroma of a fruit juice. Wiseman and McDaniel (1991) found that sucrose-sweetened solutions did not significantly affect the perception of orange or strawberry fruitiness. Le Quéré *et al.* (1994) observed chemical effects of intense sweeteners on the flavour of diet orange soft drinks. They mainly followed tendencies upon aging of the soft drinks. Johnson and Vora (1983) and Ohta *et al.* (1992) recognised the oxygen-containing components, including aldehydes such as octanal and alcohols, as the major contributors to the fresh and pleasant flavour of orange essences because of their low threshold and their olfactory characteristics. According to Moshonas and Shaw (1984), citrus juice volatiles concentrated in the aqueous fraction reflect both quantitatively and qualitatively the flavour and aroma of the parent juice. The aqueous fraction is thus a desirable flavouring material.

Mixtures of sucrose and Na-cyclamate equisweet at 10% SEV were prepared, and interactive effects between both sweeteners and a water soluble orange aroma were studied by instrumental analysis. The effect of sucrose on the flavour release was compared with the effect of Na-cyclamate by studying the release of the volatile compounds in solutions containing increasing concentrations of sucrose or Na-cyclamate.

6.2 Materials and methods

The release of volatile compounds from the solutions was studied by gas chromatography.

6.2.1 Stimuli

Stimuli were solutions of sucrose (CSM Suiker BV, Amsterdam, The Netherlands) and Na-cyclamate (Flevo Chemie BV, Harderwijk, The Netherlands) and mixtures of these two substances in demineralized water. Nine sucrose/Na-cyclamate ratios (100/0, 90/10, 75/25, 60/40, 50/50, 40/60, 25/75, 10/90 and 0/100) were chosen to study possible interactive effects between (mixtures of) sweeteners and aroma compounds. The overall sweetness of these mixtures met with a constant perceived taste intensity of 10% SEV (Nahon *et al.*, 1998). The

orange aroma added was a sample of the watery vapor phase of stripped orange juice (Cargill Juice Division, Amsterdam, The Netherlands). It was used in a constant concentration of 15 g/L. Increasing concentrations of sucrose (0, 5, 10, 20, 40 and 60 w/v%) and Na-cyclamate (0, 0.222, 0.443, 0.886, 1.772 and 2.658 w/v%) were chosen to study the effect of adding sweeteners on the release of volatile compounds from a solution containing the aroma.

All solutions were prepared at least 24 h before evaluation and stored at 4 °C overnight. In all evaluations, a sample consisted of 15 mL of solution. The samples were brought to room temperature (22 °C) before further analysis.

6.2.2 Isolation of volatile compounds

Samples consisting of 15 mL of the different mixtures mentioned (identical to the stimuli) were transferred in a so-called mouth model (Figure 6.2), consisting of a sample flask (50 mL) at a temperature of 37 °C (water bath), to which 4 mL of diluted artificial saliva containing salts, mucine and α -amylase in water was added (Van Ruth *et al.*, 1994). A purified nitrogen gas flow (20 mL/min) passed through the stirred solution for 10 min to trap the volatile compounds in 0.10 g of Tenax TA (poly(2,6-diphenyl-*p*-phenylene oxide, Ø

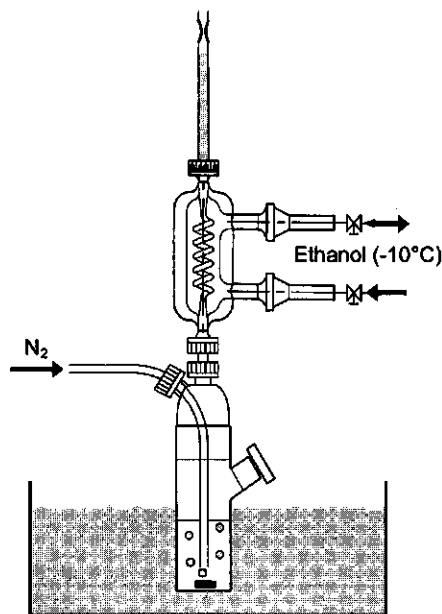


Figure 6.2 Mouth model used for the purge and trap method. Nitrogen gas passes through the stirred solution to trap the volatile compounds in Tenax TA.

0.25-0.42 mm, Alltech Nederland b.v., Zwijndrecht, The Netherlands), positioned in a glass tube, 100 mm long and 3 mm i.d.. One measurement consists of four repetitions of this method.

6.2.3 Gas Chromatography (GC)

The volatile compounds were desorbed from Tenax by a thermal desorption/cold trap device (Carlo Erba TDAS 5000, Interscience b.v., Breda, The Netherlands). The compounds were analyzed by GC on a Carlo Erba HRGC 5300 (Interscience b.v.), equipped with a Supelcowax 10 capillary column (60 m \times 0.25 mm \times 0.25 μ m) and a flame ionisation detector (FID) at 275 °C. The oven temperature was 40 °C for 4 min and then programmed to 92 °C at a rate of 2 °C/min and then to 272 °C at 6 °C/min.

The volatile compounds were identified with the help of a gas chromatograph/mass spectrometer (GC/MS, Varian 3400/Finnigan MAT 95, Bremen, Germany), equipped with a Chrompack 16200 thermal desorption/cold trap unit (Chrompack, Middelburg, The Netherlands). The capillary column and the temperature program were the same as described above. The mass spectrometer was operated in the 70 eV EI ionisation mode and scanned from mass 24 to 300 with a cycle time of 1.0 s.

6.3 Results

Flavour release from nine equisweet sucrose/Na-cyclamate mixtures was studied. Figure 6.3 shows an example of the gas chromatograms obtained by purging 10% SEV sucrose solutions, containing 15 g/L orange aroma. With the help of GC/FID for quantity evaluation and GC/MS for identity evaluation, 15 volatile compounds were selected (Table 6.1). To observe possible interactive effects between sweeteners and aroma compounds, graphs of the average peak area versus the sweetness ratio were plotted for each volatile compound. In Figure 6.4 the results obtained for (*E*)-2-pentenal are given, which are representative for results obtained for the other volatile compounds. In Table 6.1, the average peak areas for the extremes, that is, sucrose (100/0 mixture) and Na-cyclamate (0/100 mixture) are compared. For six volatile compounds (peaks 1-6), the release was significantly higher from the sucrose solution. Afterwards, flavour release was studied, using increasing concentrations of sucrose or Na-cyclamate. The fifteen selected volatile compounds were divided into three groups of

retention times (RT), based on the results obtained in the GC/FID analysis and on the polarity of the volatile compound as calculated by the method of Rekker (1977) (Table 6.1; peak 1-5, 6-13 and 14-15). Log P represents the hydrophobicity of the volatile compound; a negative value implies hydrophilicity. In Figure 6.5A the relative sum of the peak areas presented was obtained by summing the average peak areas within one retention time group and then relating to the 0 w/v% solution, which is the control. Increasing the concentration of sucrose enlarged the release of the volatile compounds with smaller RT; it did not change the release of the volatile compounds with medium RT and decreased the release of the volatile compounds with larger RT. Increasing the concentration of Na-cyclamate did not reveal any differences among the three retention time groups (Figure 6.5B).

Table 6.1 Volatile compounds of an orange aroma, released from solutions containing sucrose or Na-cyclamate at 10% SEV, their peak numbers, retention times (RT), hydrophobicity constants log P as calculated (Rekker, 1977), average areas and standard deviations ($n=4$). Short (< 15 min), medium (15-31 min) and long (> 31 min) retention time groups are distinguished. * = significant differences ($P < 0.05$), CV [%] = overall coefficient of variance.

Peak	RT (min)	Volatile compound	log P	Average areas and standard deviations (mVs)	
				Sucrose	Na-cyclamate
1	9.1	1-Ethoxy-1-methoxyethane	-0.291	103 ± 11	85 ± 7 *
2	10.4	Ethyl acetate	0.642	460 ± 31	396 ± 22 *
3	10.6	1,1-Diethoxyethane	0.239	867 ± 86	724 ± 70 *
4	14.7	2-Pentanone	1.033	56 ± 6	39 ± 4 *
5	15.0	Methyl butanoate	1.172	20 ± 2	17 ± 1 *
6	17.0	1-Penten-3-one	0.736	51 ± 4	42 ± 2 *
7	18.1	Ethyl butanoate	1.702	519 ± 32	473 ± 25
8	18.1	2-Methyl-3-buten-2-ol	1.123	106 ± 7	97 ± 5
9	20.9	Hexanal	1.476	220 ± 18	204 ± 11
10	21.4	2-Methyl-1-propanol	0.678	14 ± 1	13 ± 1
11	24.3	(<i>E</i>)-2-Pentenal	0.356	14 ± 2	12 ± 1
12	29.8	3-Methyl-1-butanol	1.208	29 ± 10	27 ± 8
13	30.8	(<i>E</i>)-2-Hexenal	0.886	67 ± 11	64 ± 6
14	31.8	Ethyl hexanoate	2.762	9 ± 2	10 ± 0
15	35.3	Octanal	2.536	206 ± 37	238 ± 9
CV [%]				11.9	8.2

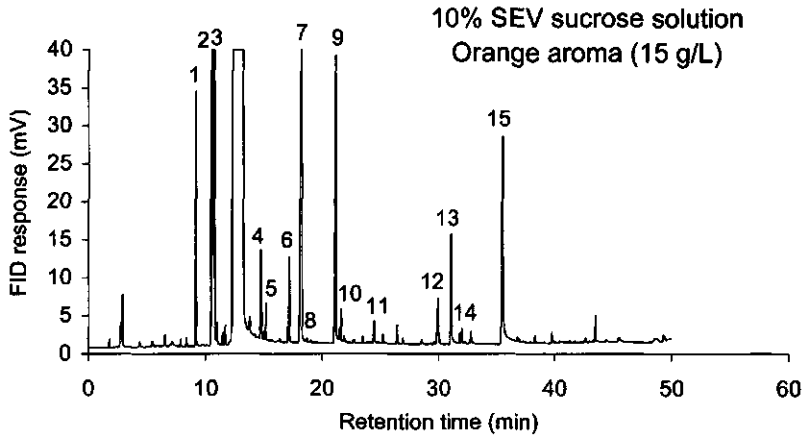


Figure 6.3 Gas chromatogram obtained by purging a 10% SEV sucrose solution, containing 15 g/L of a water soluble orange aroma. Peak numbers refer to the volatile compounds listed in Table 6.1.

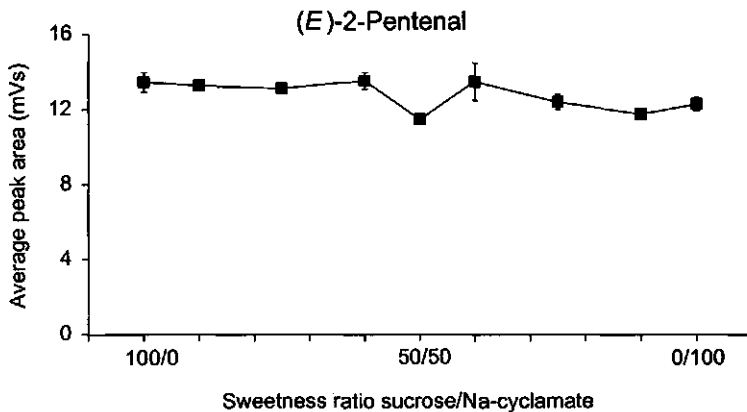


Figure 6.4 Average peak areas and standard deviations ($n=4$) for (E)-2-pentenal, released from solutions containing mixtures of sucrose and Na-cyclamate, for different sweetness ratios, all equalling to a total sweetness of 10% SEV.

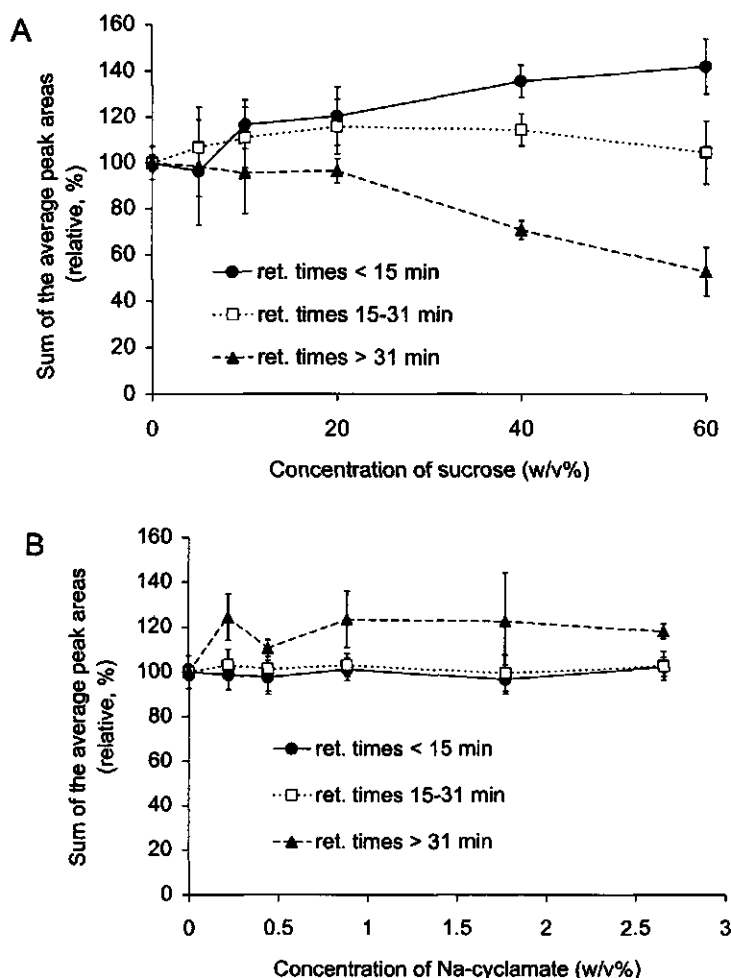


Figure 6.5 Volatile compounds of an orange aroma, released from solutions with increasing concentrations of sucrose (A) or Na-cyclamate (B). Sum of average peak areas and mean coefficients of variance, both related to a control solution of orange aroma in water, for volatile compounds with short (< 15 min), medium (15 - 31 min) and long (> 31 min) retention times (Table 6.1).

6.4 Discussion

The release of volatile compounds from solutions containing a water soluble orange aroma and sucrose and/or Na-cyclamate was studied; 15 volatile compounds were selected for the instrumental analysis (Table 6.1). Although ethanol is the volatile compound present in the largest quantity in fresh orange juice (Shaw, 1977, 1991), this compound was not selected for

the analysis. Ethanol is mainly used as a solvent and might enhance the fruity character of the aroma without contributing to a distinct aroma by itself (Williams and Rosser, 1981; Shaw, 1991). Moshonas and Shaw (1984, 1987, 1989, 1994) and Lin *et al.* (1993) showed typical gas chromatograms of various orange aromas with peak identifications. For some volatile compounds the retention times were different in this study, which could be ascribed to the use of a stationary phase made up of 5% phenyl - 95% methyl silicone in their GC analysis. The chromatograms and compounds shown by Moshonas *et al.* (1972), Ohta *et al.* (1992) and Tønder *et al.* (1998) gave the same sequence and similar retention times as shown in Figure 6.3; they all used poly(ethylene glycol) as a stationary phase in the GC analysis. All volatile compounds found in this study were reported by other authors as well (Table 6.2). In fresh juice octanal, ethyl butanoate and hexanal were among the most important volatile compounds. Ethyl butanoate, octanal and 2-pentanone were the most odour-active compounds (Tønder *et al.*, 1998). Of the 15 volatile compounds selected, methyl butanoate, ethyl butanoate and octanal give strong fruity or orange flavours (Ahmed *et al.*, 1978; Shaw, 1991). Other volatile compounds present in the orange aroma might contribute indirectly to the orange flavour through additive or synergistic effects with other components. Ethyl acetate is one of the major esters in fresh orange juice, but it is mostly present at less than its flavour threshold value in water and probably does not make a direct contribution to orange flavour. In some perfumes ethyl acetate shows an azeotropic effect to citrus notes (Shaw, 1991).

Six volatile compounds with short retention times give significantly higher peak areas when sucrose is present in the solution; they were significantly more released in solutions containing sucrose compared to Na-cyclamate (Table 6.1). Purging simple solutions containing sweeteners but no orange aroma did not release any volatile compounds. Apparently the presence of sweeteners, and especially sucrose, influences the release of volatile compounds from an orange aroma. To fully understand the effect of the addition of sweeteners, their concentrations were increased.

Parts A and B of Figure 6.5 show the changes in peak area with increasing concentrations of sucrose and Na-cyclamate, respectively. Volatile compounds with short, medium and long RT are distinguished. The influence of sugars on solute volatility varies with the specific sugar and specific volatile as well as with their concentrations (Ebeler *et al.*, 1988). When the sucrose concentration increases, the peak areas of the volatile compounds with short RT

Table 6.2 Volatile compounds present in orange aroma/juices, as reported by several authors.^a

Volatile compounds	1	2	3	4	5	6	7	8	9	10	11	12
1-Ethoxy-1-methoxyethane							x					x
Ethyl acetate			x	x	x	x	x	x	x	x	x	x
1,1-Diethoxyethane	x	x	x				x		x			x
2-Pentanone											x	x
Methyl butanoate	x		x	x	x	x	x	x	x	x		x
1-Penten-3-one			x	x	x							x
Ethyl butanoate	x	x	x	x	x	x	x	x	x	x	x	x
2-Methyl-3-buten-2-ol					x		x		x		x	x
Hexanal	x	x		x			x		x	x	x	x
2-Methyl-1-propanol			x	x	x				x			x
(E)-2-Pentenal		x				x						x
3-Methyl-1-butanol				x	x		x		x			x
(E)-2-Hexenal	x	x		x	x				x	x		x
Ethyl hexanoate					x			x				x
Octanal	x	x	x	x	x	x		x	x	x	x	x
(Ethanol)		x	x	x	x	x	x	x	x		x	x

^a1 = Moshonas *et al.* (1972) - volatile compounds in orange, 2 = Moshonas and Shaw (1984) - aqueous orange essence, 3 = Moshonas and Shaw (1987) - fresh orange juice, 4 = Moshonas and Shaw (1989) - aseptically packaged orange juice, 5 = Moshonas and Shaw (1994) - fresh-squeezed unpasteurized orange juice, 6 = Shaw (1991) - volatiles important to orange flavour, overview, 7 = Johnson and Vora (1983) - major components of orange aroma, 8 = Ohta *et al.* (1992) - orange essences, 9 = Lin *et al.* (1993) - typical natural orange aroma, 10 = Le Quéré *et al.* (1994) - diet orange soft drinks, 11 = Tønder *et al.* (1998) - orange juice, 12 = Shaw (1977) - aqueous essences, overview article

increase significantly. Increases in the concentration of sucrose enhance the release of volatile compounds with short retention times. The hydrophobicity constants, $\log P$, for these compounds (Table 6.1) indicate that these are rather hydrophilic and thus better soluble in water (Espinosa-Díaz *et al.*, 1996). De Roos and Wolswinkel (1994) explained that the addition of sucrose increases the hydrophobic character of the solution. The quality of the solvent then changes. Wientjes (1968) reported an increase in peak height for ethyl butanoate upon addition of rather high concentrations of invert sugar (73.1 w/w%) and fructose (79.1 w/w%). The headspace concentration of acetone increased by addition of sucrose to solutions (Nawar, 1971; Voilley *et al.*, 1977). For one of the volatile compounds with short retention

times, ethyl acetate, Voilley and Bosset (1986) determined the partition and activity coefficients in pure water compared with a 50 w/w% glucose solution. Both partition and activity coefficients were larger in the 50 w/w% glucose solution. Chandrasekaran and King (1972) measured the activity coefficients of ethyl acetate and hexanal as a function of sucrose concentration. The experimentally determined activity coefficients of the volatile compounds increased with increasing sucrose concentration. Kieckbusch and King (1979) found the partition coefficients for C₁-C₅ acetates between air and solutions of sucrose to increase sharply with increasing sucrose contents. This effect could be attributed qualitatively to a loss of free water due to hydration of sugar molecules. Only the free water is available as a solvent for the acetates. Several other authors (Nawar, 1971; Darling *et al.*, 1986) described this effect as "salting-out". According to Nawar (1971), the effects on the headspace concentration of volatiles are related to the interactions of the solids with water. Changes in headspace concentrations of volatiles upon addition of sucrose to aqueous volatile solutions do not involve a direct interaction between sugar and volatile but mostly occur via interaction of the sugar with the water molecules.

The peak areas of the volatile compounds with medium RT remain constant and those of the volatile compounds with long RT decrease significantly. This decrease in the release of the volatile compounds with longer retention times might be due to an effect of an increasing viscosity of the solution. According to Fick's law, the release of volatile compounds is linearly related to their diffusion coefficients, which are inversely proportional to the viscosities. The diffusion of volatile compounds then decreases with increasing viscosity of the solution (Roberts *et al.*, 1996). As we used a purge and trap method to analyse the release of volatile compounds, the viscosity of the solutions is probably less important. Nawar (1971) reported viscosities for 0, 20, 40 and 60 w/w% sucrose solutions to be 0.8, 1.5, 4.3 and 33.8 cP, respectively. As we composed sucrose solutions of 0 to 60 w/v%, which is 0 to 48.75 w/w%, the viscosities obtained will increase less. In the experiments of Nawar (1971) the headspace concentrations of heptanone and heptanal decreased with increasing sucrose concentrations in the solutions. Contradictory findings by Wientjes (1968) show an increase in peak height for ethyl hexanoate upon addition of invert sugar or fructose, both in rather high concentrations (73.1 and 79.1 w/w%, respectively).

De Roos and Wolswinkel (1994) reported a retention of hydrophobic volatile compounds, such as α -ionone and naphthalene, upon addition of sucrose. The volatility did not change for

esters and carbonyl-compounds, such as methyl butanoate, 2-hexanone, 1-hexanol, methylbenzoate and methylcinnamate. Bakker *et al.* (1996) found a detectable decrease in release of rather large volatiles with high volatility when increasing viscosity and thus sucrose concentrations. For the less volatile flavours no detectable effect was found by them. According to Roberts *et al.* (1996), the volatility of a flavour molecule may be affected by the reduction of diffusion of flavour molecules, by the formation of barriers occurring in high-viscosity matrices, or by specific binding interactions with the solute/thickening agent. Highly volatile compounds are most affected by a change in viscosity. Less volatile flavours do not show a significant decrease in volatility. Roberts *et al.* (1996) suggest also that inclusion complexes may be present in the sucrose solutions for hydrophobic molecules. They determined whether any decreases in aroma release were due to mass transfer alone or to odorant binding by the thickening agents. From a comparison between the thickening agents sucrose, carboxymethylcellulose and guar gum they concluded that binding interactions are probably present, giving a different flavour release for the various thickening agents. As sucrose solutions are concentrated, they become more glassy or crystalline in state. Trapping of volatiles in these solutions could occur due to partial crystalline formation. Hydrophobic complexes will be formed, which results in a greater depression of volatility by sucrose. Pangborn and Szczesniak (1974) investigated the effect of hydrocolloids and viscosity on flavour and odour intensities of flavour compounds, and also found effects that were specific for the gum/odorant combination. The addition of hydrocolloids generally decreased both odour and flavour intensities.

Increasing concentrations of Na-cyclamate do not significantly change the peak areas for either of the three groups of volatile compounds (Figure 6.5B). Beck (1956) determined the increase of viscosity of a solution with an increasing concentration of Na-cyclamate. The properties found are radically different from those of a sucrose solution of comparable sweetness. The relative viscosity η/η_0 at 25 °C for a 10% SEV Na-cyclamate solution (0.44 g/L) is indistinguishable from 1. The viscosity of this solution is thus equal to the viscosity of water. Beck (1956) also reported Na-cyclamate to have the properties of a strong electrolyte salt and to act as NaCl would do. Then, at higher concentrations, Na-cyclamate could have the same effect as salts and salt-out volatile compounds. Bopp and Price (1991) reported cyclamate to enhance fruit aromas. In the results obtained in the GC analysis (Figure 6.5B) no specific changes could be observed by increasing the concentration of Na-cyclamate.

Apparently the significant differences observed within the instrumental analysis cannot be confirmed by the panellists in the quantitative descriptive analysis (Nahon *et al.*, 1998), as the sensory analysis did not show significant differences for any of the aroma-related attributes when a water soluble orange aroma was present in the solutions (Figure 6.1). Ebeler *et al.* (1988) found that an addition of up to 40 w/v% sucrose did increase the headspace concentrations but did not significantly affect the perceived aroma intensity of either menthone or isoamyl acetate. Their GC analysis also showed that the GC was much more sensitive to small changes in the headspace than the sensory panel was. As expected from Fechner's law, the perceived aroma intensity is logarithmically related to the concentration of the volatile compound. Godshall (1995) concluded that the aroma of a beverage could, theoretically, be intensified by increasing the volatility of its trace aroma constituents by increasing the sucrose content. The results obtained in the described experiment, however, show that increasing the sucrose content will influence the release of the volatiles differently, depending on the properties of the volatile compound itself. In contrast to the desired effect, a retention of volatile compounds might result. Besides, threshold values for odour and flavour may differ in such a way that a volatile compound might be detected as a flavour (e.g., mouthfeel) but not as an odour. In the study of Ahmed *et al.* (1978), octanal was found to have a significantly higher odour threshold when compared to the corresponding flavour threshold. Their panellists found octanal to possess an orange-like flavour and aroma and a slightly bitter taste. Shaw and Wilson III (1980) observed such significant differences between aroma and taste thresholds for octanal, citral, nonanal and (*E*)-2-hexenal. Apparently, the flavour of an aroma can be more important to the overall perception than the odour.

In conclusion, it was shown that increasing concentrations of sucrose significantly alter the release of volatile compounds, but that these changes are probably not noticeable at the sweetness level normally found in soft drinks (i.e., 10% SEV). Increasing concentrations of Na-cyclamate have no influence on the release of volatile compounds from a water soluble orange aroma. Flavour differences observed among various sweeteners and aroma combinations can probably be ascribed to differences in flavour among the sweeteners.

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7

Modelling flavour release from aqueous sucrose solutions, using mass transfer and partition coefficients

The penetration theory of interfacial mass transfer was used to model flavor release from aqueous solutions containing different concentrations of sucrose. The mass transfer coefficient as well as the gas/solution partition coefficient are the main factors of the model influencing the release in time. Parameters governing the isolation by a purge and trap method at mouth conditions (volume, temperature, artificial saliva) were used in the model description of the flavor release. Viscosities of the different sucrose solutions (0-60 w/V%) at 37 °C were estimated and their influence on the mass transfer coefficients was determined. The gas/solution partition coefficients for ethyl acetate, methyl butanoate, ethyl butanoate, hexanal and octanal were measured for the different sucrose concentrations at 37 °C. At lower sucrose concentrations the partition coefficient primarily controls flavor release during a purge time of ten minutes, whereas at higher sucrose concentrations the influence of the mass transfer coefficient is more important.

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7.1 Introduction

The instrumental analysis of the release of volatile compounds from solutions containing sucrose and an orange aroma formed part of the study of sweetness-flavour interactions in models for soft drinks (Nahon *et al.*, 1998). In the presence of sucrose, volatile compounds from a watery vapor phase of stripped orange juice (orange aroma) were isolated by a purge and trap method at mouth conditions (volume, temperature, artificial saliva), during ten minutes. The volatile compounds were trapped onto an adsorbance material (Tenax) and then quantified by gas chromatography coupled with flame ionisation detection (GC/FID). To understand the effect of the addition of sucrose (as compared with an addition of e.g. Na-cyclamate), the release of volatile compounds was studied in solutions containing increasing concentrations of sucrose. The latter caused an increased release of the volatile compounds with short GC/FID retention times and a decreased release of the volatile compounds with longer retention times (Nahon *et al.*, 1998). Modelling of these results will improve the understanding of the system worked with and elucidate the parameters influencing the flavour release.

Harrison and Hills (1997) modelled the dynamic flavour release from liquid emulsions in the mouth. Their model revealed the most important parameters influencing the release. One important parameter is the viscosity, which determines the diffusion coefficient, D and thus the interfacial mass transfer coefficient, h_d (Harrison *et al.*, 1997). The mass transfer coefficient is a measure for the rate of release (De Roos and Wolswinkel, 1994). The other important parameter is the gas/solution partition coefficient, K_{gs} , which is influenced by the sucrose concentration as well and reflects the concentration ratios at equilibrium (De Roos and Wolswinkel, 1994).

In the literature, several tables can be found, reporting the viscosities for different sucrose solutions, at different temperatures (e.g. Bates, 1942). However, these tables do not give the exact viscosity values for the sucrose concentrations relevant to the present study and moreover not for the specific temperature of 37 °C. Bretsznajder (1971) and Génotelle (1978) presented equations and figures reflecting relationships between temperatures and viscosities for various sucrose solutions.

Table 7.1 Authors reporting partition coefficients ($\times 10^3$) for ethyl acetate, methyl butanoate, ethyl butanoate, hexanal and/or octanal. A. Specifications of the determinations; author number, author, volatile concentration, temperature, equilibration time, method. SHGC = Static headspace gas chromatography. B. Partition coefficients determined in water and sucrose/glucose solutions; author number between brackets.

A.

no.	author	volatile concentration (ppm)	temp. (°C)	equilibration time (min)	method
1	Amoore and Buttery (1978)	solubility level	25		calculation
2	Buttery <i>et al.</i> (1965)	5	25	15	SHGC
3	Buttery <i>et al.</i> (1969)	5-200	25	> 30	SHGC
4	De Roos and Wolswinkel (1994)		37	60	stripping
5	Guitart <i>et al.</i> (1989)	20-5000	37	30	SHGC
6	Hall and Andersson (1983)	0.25-1.2	40	60	SHGC
7	Kieckbusch and King (1979b)	400	30	< 30	SHGC
8	Kolb <i>et al.</i> (1992)	± 1000	37	60	extrapolated /calculated
9	Landy <i>et al.</i> (1995)	20-1000	25		exp. dilution mol fractions
10	Voilley and Bosset (1986)	1000/10000	25	5	SHGC

B.

	ethyl acetate		methyl butanoate		ethyl butanoate	hexanal		octanal	
water	9.2	(7)	8.4	(3)	22.4 (9)	6.9	(2)	5.4	(2)
	9.0	(5)	11.0	(1)		8.7	(3)	14.0	(1)
	9.6	(9)	15.8	(4)		11.4	(1)	21.0	(3)
	11.6	(10)				36	(6)	79	(6)
	14.8	(8)							
+ sucrose	12.0-30.3	(7)	<15.8	(4)					
+ glucose	38.1	(10)							

Table 7.1 shows an overview of the authors reporting partition coefficients for five volatile compounds studied in the present experiment. These volatile compounds were taken from the three retention time groups as distinguished by Nahon *et al.* (1998); ethyl acetate and methyl butanoate (short GC/FID retention times), ethyl butanoate and hexanal (medium retention times) and octanal (long retention times). Several gas/solution partition coefficients were reported in literature, but not for all sucrose solutions and at temperatures different from 37 °C. The results of Kieckbusch and King (1979a) indicated that the partition coefficients are linearly related to the reciprocal temperature (in K). Overbosch *et al.* (1991) also reported that the partition coefficients increase with increasing temperatures. Hall and Andersson (1983) determined the influence of temperature on the volatility of various aldehydes. They reported different linear temperature dependencies of partition coefficients for different compounds in the media: vegetable oil, dioctyl phthalate, paraffin oil and water. These authors found that varying the temperature does not only change the overall concentration level of volatile compounds in the gas phase, but also the relative composition of the gas phase. In the course of time, different methods for the determination of gas/solution partition coefficients were given in literature. Chaintreau *et al.* (1995) and Chai and Zhu (1998) proposed indirect headspace gas chromatographic methods, for the determination of partition coefficients independently of the concentration of the reference. This method uses two sample vials filled with different volumes of an identical solution.

In the present study, the dependency of the mass transfer coefficient on the viscosity was described in an equation. The gas/solution partition coefficients for ethyl acetate, methyl butanoate, ethyl butanoate, hexanal and octanal over the different sucrose solutions were determined. Then the release of these volatile compounds from sucrose solutions was described by a model. The mass transfer coefficient in water was used as a fitting parameter to approach experimental results published before (Nahon *et al.*, 1998). Modelling these experimental results will reveal the parameters influencing the flavour release.

7.2 Materials and methods

Viscosities of different sucrose solutions were calculated, partition coefficients of volatile compounds over these solutions were determined and the release of the volatile compounds was described by a model.

7.2.1 Sample preparation

Samples were solutions of sucrose (CSM Suiker BV, Amsterdam, The Netherlands) and volatile compounds in demineralized water. The sucrose concentrations were 0, 5, 10, 20, 40 and 60 w/v%. The volatile compounds were ethyl acetate (E. Merck, Darmstadt, Germany), ethyl butanoate (Janssen Chimica, Geel, Belgium), methyl butanoate, hexanal and octanal (all Merck-Schuchardt, Germany). The purities of the volatile compounds were all over 98% (synthesis-grade). Preparation of solutions was at room temperature (22 °C), 6 to 15 hours before analysis at 37 °C.

7.2.2 Viscosity

The required viscosities at 37 °C can be determined by using equations given in literature. Génotelle (1978) established the following relationship between the dynamic viscosity (η), the sucrose concentration and the temperature of the solution;

$$\log \eta = 22.46 N - 0.114 + \Phi (1.1 + 43.1 N^{1.25}) \quad (7.1)$$

in which $N = (B/(1900 - 18 B))$ is the molar fraction of sucrose, B being °Brix and $\Phi = (30 - T)/(91 + T)$, T being the temperature. The data generated with the help of Equation 7.1 deviate maximal 1% from the data for standard solutions as published by the National Bureau of Standards (Bates, 1942). Given the experimental temperature of 37 °C, the weight fractions or °Brix for the sucrose solutions were determined to be able to calculate the viscosities for the different sucrose solutions with the help of Equation 7.1. An Abbe refractometer (Atago Type No. 302 with Power Source) was used for this determination.

7.2.3 Gas/solution partition coefficients

The samples for the measurements of the gas/solution partition coefficients were prepared by adding 14-17 ppm of volatile compound to the various sucrose solutions. Sealed volumetric flasks with solutions containing ethyl butanoate, hexanal or octanal were placed in a sonification bath (Sonicator Ultrawave CE) at room temperature for 30 min, to fully solubilize these more apolar volatile compounds. The homogeneity of the solutions was checked visually, according to the method described by Buttery *et al.* (1969).

The equilibrium concentrations of the volatile compounds in the gas phase were measured at 37 °C using static headspace gas chromatography (SHGC). The sample solution (3 mL)

was transferred into a 12 mL vial, which was capped and incubated at 37 °C for 15 or 30 min in the headspace unit (Fisons HS800) of the gas chromatograph. The gas/solution equilibrium is considered to be reached when the volatile concentration in the headspace remains constant. For ethyl acetate and octanal an incubation time of 15 min was testified to be sufficient; for methyl butanoate, ethyl butanoate and hexanal an equilibrium was reached after an incubation time of 30 min. After incubation, 300 μ L of the sample headspace passed a MFA 815 cold trap (Fisons Instruments) for cryofocussing and was then injected into a HRGC 5300 Mega Series gas chromatograph (Carlo Erba Instruments, Interscience BV, Breda, The Netherlands). The GC was equipped with a DB-wax column (30 m \times 0.542 mm \times 1.0 μ m) and a flame ionisation detector at 220 °C. The oven temperature was 40 °C for 5 min and then programmed to 110 °C at a rate of 3 °C/min and further to 170 °C at 20 °C/min. One measurement consisted of at least three repetitions of this method.

For calibration curves, 2 or 10 μ L of a pure volatile compound were dissolved in 10 mL solvent and different volumes of these two solutions were manually injected on the column. The solvent used for ethyl acetate and methyl butanoate was demineralized water, and the solvent for ethyl butanoate, hexanal and octanal was hexane. The method was repeated two times, to obtain significant and reliable calibration curves ($R^2 > 0.84$).

The gas/solution partition coefficient K_{gs} is given by the following equation:

$$K_{gs} = \frac{C_g^{eq}}{C_s^{eq}} \quad (7.2)$$

in which C_g^{eq} is the concentration of the volatile compound in the gas phase at equilibrium and C_s^{eq} is the concentration of the volatile compound in the solution at equilibrium. As the volatile compound in the system distributes between the gas phase and the solution until equilibrium has been reached, the concentration in the solution can be calculated from the initial volatile concentration, the concentration in the gas phase and the volumes of the gas phase and solution;

$$C_s = C_s(0) - C_g \times \left(\frac{v_g}{v_s} \right) \quad (7.3)$$

in which $C_s(0)$ is the initial volatile concentration in the solution and v_g and v_s are the volumes of the gas phase and the solution, respectively.

7.2.4 Flavour release model

Parameters in the model of Harrison and Hills (1997) were adjusted to approximate the experimental design used in the study of Nahon *et al.* (1998). The ratios of release in time are then given by the following model:

$$\frac{C_g(t)}{C_s(0)} = \frac{A_{gs} h_d}{v_g} \left[\frac{\exp r_1 t - \exp r_2 t}{r_1 - r_2} \right] \quad (7.4)$$

in which $r_1, r_2 = f(h_d, K_{gs}, Q, A_{gs}, v_g, v_s)$. Apparently, the concentration of the volatile compound in the headspace in time, $C_g(t)$, is a function of the initial concentrations in the solution, $C_s(0)$, the viscosity-dependent mass transfer coefficient, h_d , the gas/solution partition coefficient, K_{gs} , the nitrogen gas flow rate, Q , the interface surface area, A_{gs} , the volume of the headspace, v_g and the volume of the solution, v_s (sample + artificial saliva).

Parameters were substituted in the model presented by Equation 7.4. Similar to the expressions reported by Harrison and Hills (1997), r_1 and r_2 in Equation 7.4 are given by

$$r_1 = -\frac{\alpha}{2} + \frac{\sqrt{\alpha^2 - 4\beta}}{2} \quad (7.5)$$

and

$$r_2 = -\frac{\alpha}{2} - \frac{\sqrt{\alpha^2 - 4\beta}}{2} \quad (7.6)$$

with

$$\alpha = \frac{Q}{v_g} + \frac{h_d A_{gs}}{v_g K_{gs}} + \frac{h_d A_{gs}}{v_s} \quad (7.7)$$

and

$$\beta = \frac{Q h_d A_{gs}}{v_g v_s} \quad (7.8)$$

The following parameters, necessary for Equations 7.4, 7.5, 7.6, 7.7 and 7.8, were known from the set-up of the release experiments:

$$\begin{aligned} Q &= 20 \text{ mL/min} & v_g &= 31 \text{ mL} \\ v_s &= 19 \text{ mL} \end{aligned}$$

The partition coefficients, K_{gs} , were determined for the different volatile compounds, dissolved in the different sucrose solutions. As the volatile concentrations in and above the solutions (C_s and C_g) are unknown, the relative concentrations given by $C_g(t)/C_s(0)$ will be studied in time.

The mass transfer coefficient, h_d , is an unknown parameter, which can be described as a function of the viscosity, η . Harrison *et al.* (1997) and Bakker *et al.* (1998) reported the following relationship:

$$h_d \propto \frac{1}{\sqrt{\eta}} \quad (7.9)$$

Starting from this relationship, the mass transfer coefficient can be described as a function of the mass transfer coefficient in water, $h_d(0)$, the viscosity in water, $\eta(0)$ and the viscosity for a specific sucrose solution, with concentration, C_{suc} .

$$\frac{h_d(C_{suc})}{h_d(0)} = \sqrt{\frac{\eta(0)}{\eta(C_{suc})}} \quad (7.10)$$

As $\eta(0)$ and $\eta(C_{suc})$ will be calculated using Equation 7.1, the mass transfer coefficient for a specific volatile compound at a given sucrose concentration depends on $h_d(0)$ for that volatile compound. The interface area, A_{gs} , concerns the complete interface between the gas phase and the solution, including the gas bubbles in the solution caused by the nitrogen flow. As A_{gs} can only be approximated, it was included in the mass transfer coefficient to give hda ($= h_d \times A_{gs}$). Then hda depends on the sucrose concentration and the magnitude of $h_d(0)$. The latter

Table 7.2 Average and standard deviations ($n=10$ for 0 and 60 w/v% sucrose; $n=3$ for 5, 10, 20 and 40 w/v% sucrose) for gas/solution partition coefficients ($\times 10^3$) of ethyl acetate, methyl butanoate, ethyl butanoate, hexanal and octanal for different sucrose solutions (w/v%) at 37 °C (Verset, 1998).

sucrose (w/v%)	ethyl acetate	methyl butanoate	ethyl butanoate	hexanal	octanal
0	10.3 ± 0.33	11.2 ± 0.35	14.8 ± 0.67	12.9 ± 0.99	21.1 ± 1.80
5	11.2 ± 0.26	13.8 ± 0.37	18.9 ± 0.59	13.0 ± 0.08	20.0 ± 0.32
10	11.3 ± 0.61	14.9 ± 0.12	19.7 ± 0.50	13.5 ± 0.04	19.2 ± 0.38
20	13.2 ± 0.78	16.6 ± 0.65	22.1 ± 1.98	13.3 ± 0.10	17.5 ± 0.99
40	16.4 ± 0.74	21.0 ± 0.08	28.2 ± 1.18	13.9 ± 0.15	16.1 ± 1.49
60	22.8 ± 1.66	28.4 ± 7.76	29.8 ± 1.72	13.1 ± 1.09	14.3 ± 3.17

was used as the fitting parameter, to approach the experimental results with the model description (Equation 7.4).

Using a partition coefficient measured for a certain volatile compound in a sucrose solution (Table 7.2) and given the relationship between the mass transfer coefficient and the sucrose concentration, the release of this volatile compound in time can be determined with the use of Equation 7.4. To be able to compare this predicted release with that determined previously (Nahon *et al.*, 1998), the time-release curves were integrated with the help of the technical computing program Mathematica (Version 3.0, Wolfram Research Inc., Champaign, IL, USA). For each volatile compound, the amount of volatile released from the sucrose solution in ten minutes (i.e. the purge time) was related to the amount of volatile released from water. These ratios of release predicted by the model (integration areas) were compared with the ratios of release obtained in the purge and trap experiment (GC/FID peak areas). The $h_d(0)$ was used to adjust the ratios of release predicted by the model. The optimal $hda(0)$ was given by the optimal least squares solution for the fit of the model integration ratios to the experimental peak area ratios.

7.3 Results & discussion

The release of volatile compounds from aqueous solutions containing sucrose was described in a model (Equation 7.4). One of the parameters used in the model was the viscosity of the different sucrose solutions. Another parameter used in the model is the gas/solution partition coefficient.

7.3.1 Viscosity

The viscosities of the sucrose solutions were calculated from the sucrose concentrations in °Brix (Equation 7.1). The weight fractions or °Brix for the different sucrose solutions and the calculated viscosities are given in Table 7.3. These viscosities are in the same range as values reported in literature (Bates, 1942; Nawar, 1971; Voilley and Bosset, 1986), when considering the different sucrose concentrations and the different temperatures. The °Brix was determined at room temperature and then used for the calculation of the viscosity at 37 °C. This temperature difference introduces a deviation in °Brix of about 0.5%, resulting in a deviation of maximal 2.5% for the calculated viscosity according to Génotelle (1978).

Table 7.3 Weight fractions / °Brix (w/w%) and dynamic viscosities η (cP) for different sucrose solutions (w/v%) at 37 °C. The dynamic viscosities were calculated from Equation 7.1 (Génotelle, 1978).

sucrose (w/v%)	sucrose (w/w%)	viscosity (cP)
0	0	0.67
5	4.50	0.76
10	9.25	0.88
20	18.25	1.20
40	34.50	2.55
60	48.75	6.99

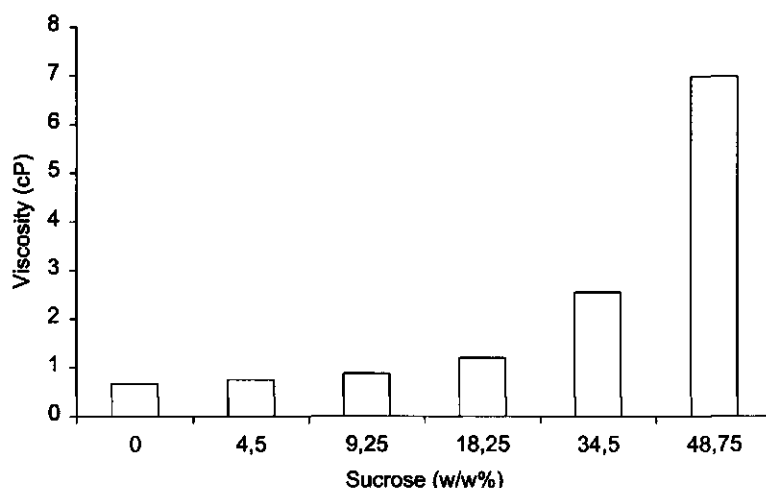


Figure 7.1 Viscosity η (cP) for different sucrose concentrations (w/w%), calculated from Equation 7.1 (Génotelle, 1978).

Richardson *et al.* (1987) studied the mobility of water in sucrose solutions. They distinguished several regions. In the first region, sucrose concentrations varying from 0 to 40 w/w%, water mobility decreases linearly. The second concentration range of 40 to 60 w/w% sucrose shows a non-linear decreasing water mobility, due to the formation of a network by intermolecular hydrogen bonds between water and sucrose, hydrogen bond bridging of water between sucrose molecules and sucrose-sucrose hydrogen bonding. The calculated viscosities (Equation 7.1) show a near-linear increase up to a concentration of 20 w/w% and next a non-linear increase (compare Figure 7.1).

7.3.2 Gas/solution partition coefficients

Figure 7.2 shows the gas/solution partition coefficients for the five volatile compounds as a function of the sucrose concentration in the solutions. Apart from ethyl butanoate, the evolution of these partition coefficients shows a grouping of volatile compounds which is similar to the division in retention time groups made in the results of the instrumental analysis reported before (Nahon *et al.*, 1998).

In the present experiment, we choose specific volatile concentrations (14–17 ppm) for measuring the partition coefficients over the different sucrose solutions. Buttery *et al.* (1969) stated for hexanal that the partition coefficient was constant up to its point of saturation, at 5000 ppm. Land (1978) also clarified in his paper that the gas/solution partition coefficient is constant up to the saturated vapor pressure at the solubility limit. The equilibration time used for the determination of the gas/solution partition coefficients varied often from 15 to 30 min. (e.g. Buttery *et al.*, 1965, 1969; Chaintreau *et al.*, 1995; Chai and Zhu, 1998). An exception was the equilibration time chosen by Voilley and Bosset (1986), which was set at 5 min. Whereas the model describes the release of a single volatile compound, the release in the purge and trap system concerned a mixture of orange aroma compounds. Guitart *et al.* (1989) indicated that the composition of the mixture has very little influence on the partition of its constituents. Chaintreau *et al.* (1995) proved that the influence of other volatiles in a model mixture of flavours on the individual component concentrations in the gas phase was not

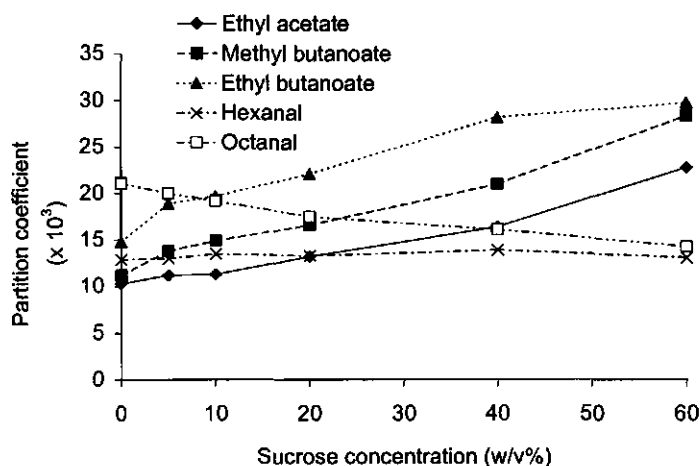


Figure 7.2 Partition coefficients ($\times 10^3$) of ethyl acetate, methyl butanoate, ethyl butanoate, hexanal and octanal as a function of the sucrose concentration (w/v%).

noticeable. However, Bohnenstengel *et al.* (1993) investigated the influence of interactions between substances on the SHGC. They found that even small changes in the sample composition can cause changes in the resulting headspace composition. The determination of the partition coefficients of volatiles in the presence of other volatile compounds can therefore still be of interest for study.

The authors mentioned in Table 7.1 determined partition coefficients which were in the same range as those determined by in the present experiment, especially when considering the temperature used for the determination (compare Tables 7.1 and 7.2). Buttery *et al.* (1969) found a gradual increase in volatility for the higher molecular weight homologs, with alcohols having the lowest volatilities, ketones intermediate, and esters and aldehydes somewhat higher. Amoores and Buttery (1978) calculated the partition coefficients for a series of aldehydes with the help of their vapor pressures and solubilities. They observed a clear increase of the partition coefficients with the carbon chain length of these aldehydes. Overbosch *et al.* (1991) also indicated that, for a polar solvent, the partition coefficient increases with increasing chain length of the flavour compound. Landy *et al.* (1995) determined vapor-liquid partition coefficients for ethyl acetate, ethyl butanoate and ethyl hexanoate as a ratio of molar fractions. They found that the natural logarithm of these partition coefficients, $\ln K$, increased linearly with the number of carbon atoms in the series. Their partition coefficient for ethyl acetate was smaller than the one determined in this study, whereas the partition coefficient reported for ethyl butanoate was larger.

7.3.3 Flavour release model

The release of ethyl acetate from solutions containing sucrose and an orange aroma, increases with increasing sucrose concentrations as predicted by Equation 7.4 (Figure 7.3A). For octanal, the release decreases with increasing sucrose concentrations (Figure 7.3B). Increasing the sucrose concentration, makes the solvent character of a solution more hydrophobic, which explains the increased and decreased partition coefficients for ethyl acetate and octanal, respectively. The maximal ratio of headspace and initial concentration reflects the evolution of the partition coefficients. As the partition coefficient in water is smaller for ethyl acetate than for octanal, its maximal release ratio is smaller. At 40 w/v% sucrose, the partition coefficients of ethyl acetate and octanal are equal (Figure 7.2 and Table

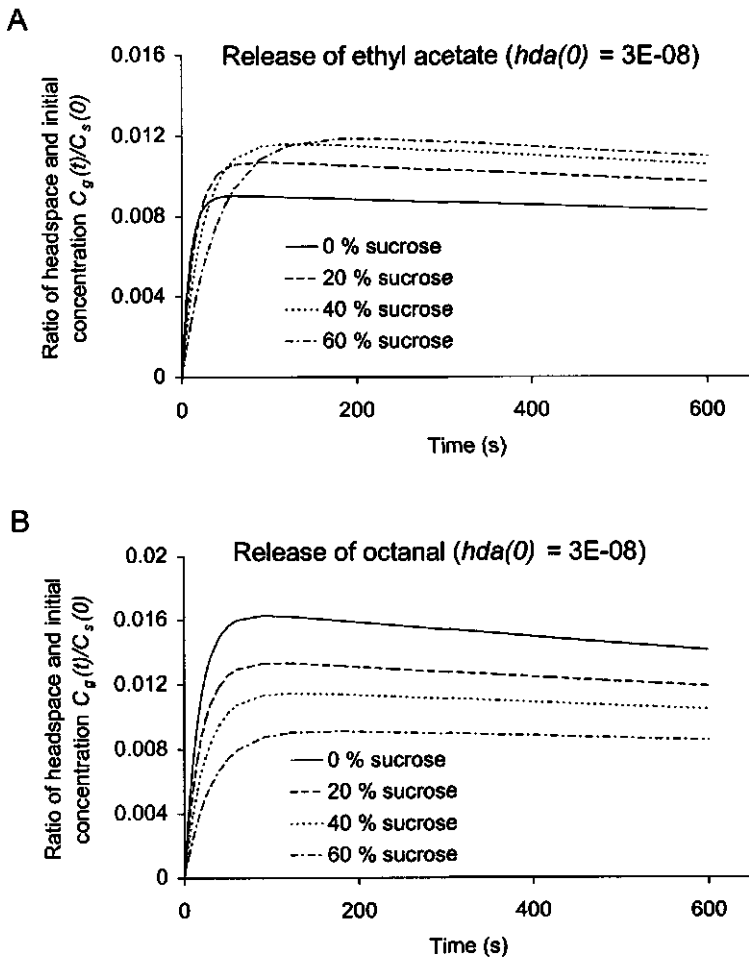
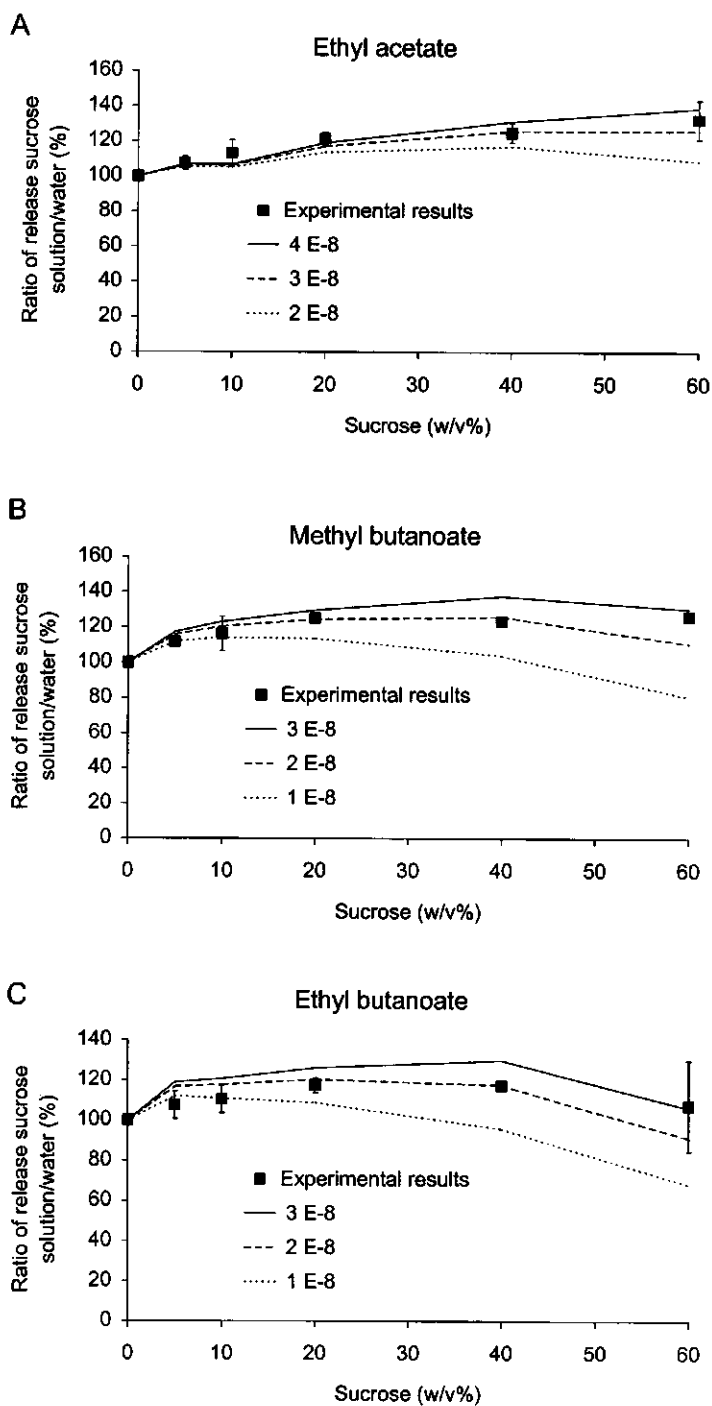


Figure 7.3 Predicted release (Equation 7.4) of **A.** ethyl acetate and **B.** octanal into headspace from solutions containing sucrose (0, 20, 40 and 60 w/v%) and an orange aroma, represented by the ratio of headspace and initial concentration of the volatile compound as a function of time. $hda(0)$ was set at 3×10^{-8} (m^3/s) for both compounds.

7.2), resulting in an equal maximal release ratio for a 40 w/v% sucrose solution (compare Figures 7.3A and B).



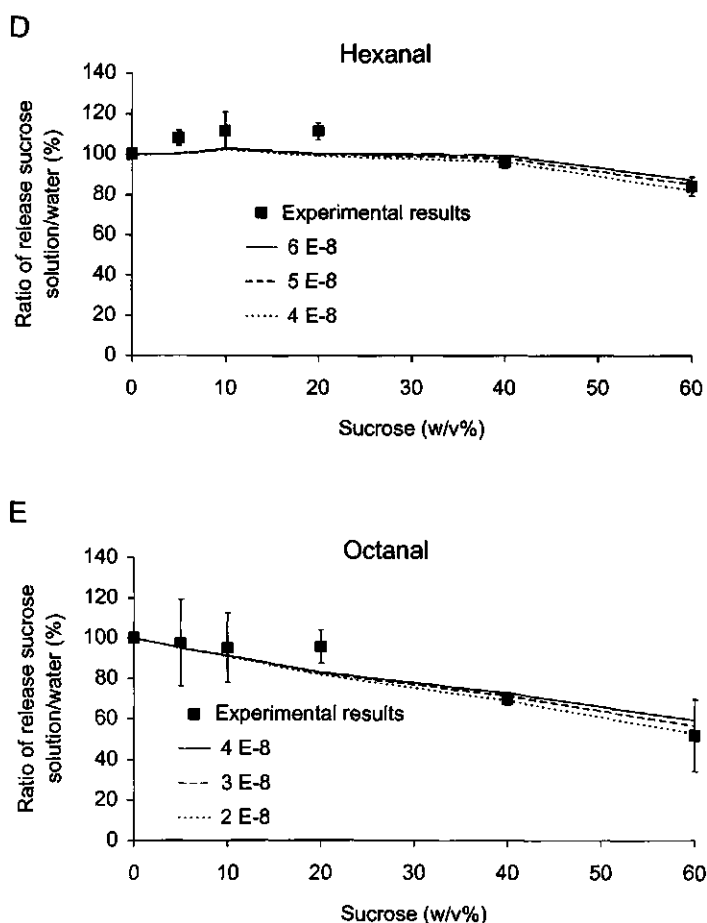


Figure 7.4 Peak area and integration ratios representing the release of *A.* ethyl acetate, *B.* methyl butanoate, *C.* ethyl butanoate, *D.* hexanal and *E.* octanal, for different sucrose concentrations. The peak area ratios (■) and their standard deviations were deducted from experimental observations (Nahon *et al.*, 1998); the integration ratios were obtained by modelling (Equation 7.4). The model predictions are given for three $hda(0)$'s, the middle providing the optimal fit.

The mass transfer coefficient decreases with increasing sucrose concentrations. In Figures 7.3A and B this is demonstrated by a smaller initial slope of the time-release curve (Bakker *et al.*, 1998). These authors also reported that the initial release rates for diacetyl decreased as the concentration of gelatin increased, i.e. as the viscosity increases. Chandrasekaran and King (1972) found that the activity coefficients for ethyl acetate and hexanal increased with

increasing sugar concentrations. The diffusion coefficients of these volatile compounds decreased with increasing sucrose concentrations. Darling *et al.* (1986) observed for isopentyl acetate that for higher sucrose concentrations (> 30%) the viscosity of the solution increased steeply and consequently the diffusion coefficient fell.

In order to compare the release described by the model (Equation 7.4) with the release in the purge and trap system, the areas under the release curves were integrated. In Figures 7.4A-E the integration ratios as obtained with the model (lines) and the peak area ratios as obtained in the experiments (marks) (Nahon *et al.*, 1998) are compared. For each volatile compound, the model predictions for three $hda(0)$'s are given, the middle $hda(0)$ being the optimal least squares solution for the fit of the model to the experimental results. The optimal values of $hda(0)$ for ethyl acetate, methyl butanoate, ethyl butanoate, hexanal and octanal were 3×10^{-8} , 2×10^{-8} , 2×10^{-8} , 5×10^{-8} and 3×10^{-8} m³/s, respectively. Figures 7.4A-E reflect the fit of the model to the experimental results. Figure 7.4A shows that the predicted and observed release of ethyl acetate increase with increasing sucrose concentration. Choosing a higher mass transfer coefficient (and thus hda) increases the release of for all volatile compounds (Figure 7.4). Comparing Figures 7.4A-C with 7.4D-E shows that the influence of the mass transfer coefficient/ hda , is much smaller for the volatile compounds hexanal and octanal. Increasing the sucrose concentration and thus the mass transfer coefficient, hardly influences the ratios of release for these compounds. Therefore, changes in the fitting parameter hda will not influence these ratios either. The optimal values of $hda(0)$ for the five volatile compounds are similar. Overall they vary from 2×10^{-8} to 5×10^{-8} m³/s. There is no special trend when looking at more polar or more apolar volatile compounds. The purge and trap method used for the instrumental analysis of the flavour release included stirring of the solution. Bakker *et al.* (1998) reported an effect of stirring rate on the dynamic release of diacetyl. Therefore, stirring is one of the factors introducing variation in the experimental data. As the fit of the model to the experiments is adjusted per volatile compound, the fitting parameter hda will vary as well. According to Overbosch *et al.* (1991), the diffusion coefficient does not vary significantly between flavour compounds. As the diffusion coefficient determines the mass transfer coefficient, this latter coefficient will not vary much with the flavour compound either. De Roos and Wolswinkel (1994) also neglected differences in diffusion coefficients between volatile compounds in their release study.

However, Harrison and Hills (1997) stated that the $h_d(0)$ will vary between volatile compounds.

Knowing the hda giving the best fit of the model to the experimental results, the expected mass transfer coefficient h_d can be deducted from an approximation of the interface surface area A_{gs} . This area is composed of the surface area of the solution and the surface areas of the nitrogen gas bubbles purged through the solution. The diameter of the tube through which the nitrogen gas is let into the solution is 3×10^{-3} m, which produces bubbles with a calculated volume of 1.4×10^{-8} m³. Given the experimental nitrogen flow of 20 mL/min, 14147 bubbles will pass through the solution during the ten minutes of the analysis. This gives an interface area of 0.4 m² as provided by the bubbles. The surface area of the solution can be assumed neglectable ($\cong 4.8 \times 10^{-4}$ m²). As the average best fitting hda was 3×10^{-8} m³/s in the present experiment, the best estimate of the mass transfer coefficient in this system is 7.5×10^{-8} m/s.

When comparing the model predictions (Figure 7.4) with the evolution of the partition coefficients (Figure 7.2), it appears that the ratio-curves at low sucrose concentrations, are mainly controlled by the partition coefficient of a specific volatile compound. When the sucrose concentration and thus the viscosity of the solution increases, the mass transfer coefficient becomes smaller (as given by Equation 7.10). Then the partition coefficient loses impact on the release during a purge time of ten minutes. In accordance with the explanations of Richardson *et al.* (1987) the viscosity of the solutions increases especially for the two highest sucrose concentrations (40 and 60 w/v%) in Figure 7.1. A comparison of Figures 7.2 and 7.4 demonstrates that the partition coefficient has the largest influence on the ratios of release up to a sucrose concentration of 40 w/v%. De Roos and Wolswinkel (1994) also found that the effect of the resistance to mass transfer on the relative release rate was relatively small and that flavour release was mainly partition-controlled for water and an aqueous solution of 25% sucrose.

The model describing the flavour release from the sucrose solutions, includes the mass transfer coefficient as being dependent of the viscosity. It is questionable whether it is actually the viscosity or the sucrose concentration influencing the diffusion coefficient and thus the mass transfer coefficient. Roberts *et al.* (1996) studied flavour release for different thickeners at equal viscosities and found that the three thickened systems did not have the same flavour release profiles. Thickener-specific influence on the flavour release was revealed. Apparently, viscosity-dependent mass transfer does occur, but other mechanisms

such as changes in the water activity are also important. Darling *et al.* (1986) explained that interactions between long chain polymers will induce the 'macro' viscosity of solutions, while still very large pores of 'free' water are available to small molecules for diffusion. Diffusion in this water phase is not related to the viscosity of the bulk solution. For solutions thickened by large quantities of small molecules (e.g. sucrose) the 'macro' viscosity extends down into the 'micro' environment, which will be experienced by the diffusing aroma molecules. Richardson *et al.* (1987) reported a decreasing water mobility when the sucrose concentration passes 40 w/w%. In this study, the mass transfer coefficient could be better described as a function of the sucrose concentration instead of the viscosity.

In conclusion, the model described the dynamic flavour release for five volatile compounds from aqueous sucrose solutions. By determination of the viscosities and the partition coefficients, the model provided an acceptable fit to the experimental data obtained with instrumental analysis. The model description revealed that at low sucrose concentrations the partition coefficient primarily controls the flavour release, whereas at higher sucrose concentrations the mass transfer coefficient has more influence.

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Chapter 7

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8

General discussion

In this thesis the presence or absence of interactive effects between sweeteners and aroma compounds has been studied with the help of sensory and instrumental analysis. The results of the sensory analysis reveal whether panellists are able to perceive differences in the flavour profiles of several stimuli. The results of the instrumental analysis clarify whether the presence of (intense) sweeteners influences the release of volatile compounds from a solution. Mathematical modelling of this release reveals the parameters influencing the release.

The two preliminary studies on commercial products (Chapter 2) demonstrate that the presence of intense sweeteners in a light soft drink influences the preferences for and the flavour profiles of these drinks to various extents, depending on the flavour and sweeteners present. The results of the preference study show that regular orange soft drinks are not significantly preferred over their light equivalents. Regular cola soft drinks, on the contrary, were significantly preferred over the light version in the tests. Informing the panellists about the presence of a light soft drink does not influence their preferences. In the sensory study (quantitative descriptive analysis, QDA) a commercial light blackcurrant soft drink, sweetened with a combination of four intense sweeteners, has been investigated. A change in the sweetener composition of the soft drink causes a shift in the character of the flavour from blackcurrant to strawberry, together with an intensity decrease of the sour-related attributes (Chapter 2).

The aim of the research described in the present thesis is to determine whether changes in the quality of sweet taste are due to the properties of the sweeteners themselves and/or to interactive effects between these sweeteners and aroma compounds. To be able to study possible interactions, the starting point for all investigations should be similar, i.e. the solutions containing the sweeteners should be equisweet. Sensory and instrumental analysis will elucidate interactive effects between sweeteners and an orange aroma.

8.1 Equisweet mixtures

The sweetness level used in the research described in this thesis, is the one commonly present in sucrose-sweetened soft drinks, i.e. equivalent to the sweetness of a 10 w/v% sucrose solution (10% SEV). In each study (Chapters 3-5) the 10% SEV for a certain sweetener is determined with the panel involved in that study. Differences in individual subjects'

sensitivities to various taste compounds can be quite pronounced and this results in very different responses to mixtures of compounds (Pangborn and Chrisp, 1964; Pangborn and Trabue, 1964). Pangborn (1960) has suggested that many of the discrepancies among laboratories may be due to the employment of expert panels in some studies and naïve panels in others. Gregson and McCowen (1963) also attribute inter-laboratory differences to variations in the sensitivities of the different populations of panellists. In Chapter 5 SEV determinations are repeated because of replacements in the sensory panel and the use of new product batches. Comparison with the determinations reported in Chapter 3 and 4 confirm that SEV determinations performed with different panels can be compared to a certain extent only.

When studying combinations of sweeteners in solutions, the equisweetness of 10% SEV can be attained in different, sweetener-specific ways. For mixtures of bulk sweeteners, Beidler's mixture equation can be used, as demonstrated with mixtures of sucrose and maltitol in Chapter 3. Given the 10% SEV of maltitol, a linear equation could be derived from Beidler's mixture equation. All mixtures of sucrose and maltitol meeting with this equation are equisweet. The mean scores on the attribute sweetness, taken from the quantitative descriptive analysis, are constant for all different sucrose/maltitol mixtures (Chapter 3, Figure 3.2).

For additive mixtures of bulk and intense sweeteners, such as the mixtures of sucrose and aspartame, the validity of Beidler's mixture equation has been studied (Chapter 3). After determining the 10% SEV for aspartame, again a linear equation has been obtained from Beidler's mixture equation. Mixtures composed with the help of this equation are equisweet, as confirmed by their constant mean scores on the attribute sweetness from the QDA (Chapter 3, Figure 3.5).

Equisweet mixtures of sweeteners showing synergy were obtained by using a new method, developed for mixtures of sucrose and Na-cyclamate (Chapter 4). The contribution of sucrose to the mixtures was fixed, according to the chosen ratios of sweetness. Next Na-cyclamate was added until a sweetness of 10% SEV was reached. The magnitude of this addition was determined with the help of the method of constant stimuli in combination with weighted linear regression. A comparison between the linear expectations derived from Beidler's mixture equation and the additions determined in this way, then illustrated the synergy which occurred in sucrose/Na-cyclamate mixtures during tasting.

For the ternary mixtures of sucrose, aspartame and Na-cyclamate, a similar method as mentioned for binary mixtures was applied. In Chapter 5 the concentrations of sucrose and Na-cyclamate were fixed and deducted from the results given in Chapter 4. Then the necessary concentration of aspartame for reaching 10% SEV was determined. The composition of the equisweet ternary mixtures demonstrated that the synergy observed in these mixtures is a combination of the relationships found for the composing binary mixtures.

The method used for composing the equisweet ternary mixtures is suitable for industrial applications. The determination of some relevant equisweet binary and ternary sweeteners mixtures is sufficient for the constitution of an equation representing equisweet mixtures of sweeteners. An optimal sweetener-combination can be chosen with the help of such an equation, taking into account possible taste limitations (as mentioned for Na-saccharinate in Chapter 2), differences in costs and maximal doses (Allowed Daily Intake). Moskowitz *et al.* (1978) also used this approach in their study of the sweetness and acceptance optimization of cola flavored beverages, although they did not start from equisweet mixtures.

8.2 Methodology in sensory analysis

The use of QDA for the evaluation of different stimuli allows subjects to generate their own appropriate descriptors. Only then subjects pay attention to all aspects of a solution, which prevents cognitive factors affecting the judgments of chemosensory attributes (Van der Klaauw, 1989). The instructional set given to subjects can have a substantial impact on odor-induced enhancement of sweetness (Frank *et al.*, 1990). The instructions and prototypes offered can influence the subjects in their judgments to be in- or excluded in a particular sensory attribute. In the present work no changes in the scores of the attribute sweetness were observed, whether in the absence or in the presence of orange aroma (Chapters 3-5). Baldwin and Korschgen (1979), Matysiak and Noble (1991) and Bonnans and Noble (1993) found enhancement of fruitiness by aspartame. However, the results reported in Chapter 3 do not reveal any changes in the attribute orange upon the addition of aspartame. Mixture-induced taste quality enhancement is reduced or eliminated when appropriate attribute rating scales are provided. Odor- or taste-induced enhancement of specific taste qualities in mixtures of chemosensory stimuli is strongly influenced by the instructions given to subjects and the specific taste and odor qualities of a mixture. As subjects are provided with additional,

appropriate rating categories, either absence of interaction or mixture suppression replaces enhancement (Frank *et al.*, 1993). According to Noble (1996) the enhancement of fruitiness by sweetness and the enhancement of sweetness by fruitiness will occur, even after rigorous training and rating of separate taste and aroma attributes. However, it should not be concluded that mixture experiments should always use multiple, simultaneous, independent ratings of 'appropriate' sensory qualities in order to avoid the 'biases' observed with the other rating procedures. The choice of the method clearly depends on the aim of the experiment (Frank *et al.*, 1993).

8.3 Sensory analysis of interactive effects

Starting from the equisweet mixtures of sweeteners, interactive effects of these mixtures with aroma compounds can be studied. Considering sensory analysis, changes in the flavour profiles of the different solutions containing mixtures of sweeteners and an orange aroma (watery vapour phase of stripped orange juice) were investigated using QDA. The contribution of the different sweeteners to the sweetness of the mixture was varied to elucidate possible effects of sweeteners and/or aroma compounds. The flavour profiles of sucrose and maltitol do not differ significantly at a constant concentration of orange aroma (Chapter 3). The intense sweeteners aspartame and Na-cyclamate both differ from sucrose on the attributes chemical and aftertaste (Chapters 3 and 4). In the ternary sucrose/aspartame/Na-cyclamate mixture, the relative sweetness contribution of Na-cyclamate to this mixture determines the flavour profile to the largest extent (Chapter 5).

The graphs of the mean scores for each attribute, over the different mixtures of sweeteners and for different concentrations of orange aroma, demonstrate whether interactions with the orange aroma are present. Possible interactions with orange aroma would cause these mean scores to either diverge or converge upon changes in the composition of the sweetener mixture. The contribution of orange aroma to the mean scores of the attributes is similar for the sucrose/maltitol and the sucrose/aspartame mixtures (Chapter 3). For these sweeteners the difference in taste between the sweetener/aroma solutions is due to the sweeteners themselves and not to a possible interaction with aroma compounds. For the binary sucrose/Na-cyclamate mixture and the ternary sucrose/aspartame/Na-cyclamate mixture the added orange

aroma levels out taste differences observed between the sweetener solutions without orange aroma (Chapters 4 and 5).

For all solutions, the addition of orange aroma provides a more distinct flavour profile. In general the addition of orange aroma increases the mean scores for the attributes orange, sour, chemical, fruity and aftertaste. Chastrette *et al.* (1998) compared the models of Fechner, Stevens, Beidler and Hill for their prediction of the human olfactory stimulus-response function. These authors concluded that the model of Hill fitted the experimental data for 20 odorous compounds as well as, or better than, the other models. According to the latter model, a twofold increase of the original concentration of orange aroma increased the mean scores of the attributes involved only slightly, as we observed in Chapters 3-5.

8.4 Instrumental analysis of the flavour release

The presence or absence of interactive effects between sweeteners and an orange aroma has also been studied by an instrumental analysis of the flavour release. Volatile compounds were isolated by a purge and trap method at mouth conditions (volume, temperature, artificial saliva) and then quantified and identified by gas chromatography combined with different detection methods. A comparison of a sucrose and a Na-cyclamate solution at 10% SEV reveals significant differences in the release of some volatile compounds from the solution (Chapter 6). However, sensory analysis of these solutions (Chapter 5) shows that the scores for the different attributes remain constant when comparing the sweeteners in the presence of an orange aroma. Differences observed in the instrumental analysis are not perceived by the panellists. To be able to fully understand possible effects of adding sweeteners to the orange aroma solution, the concentrations of the sweeteners were enhanced. At high sucrose concentrations (60 w/v%) the release of the volatile compounds with short retention times in the GC/FID analysis increased and the release of the volatile compounds with large retention times in the GC/FID analysis decreased. The hydration of the sucrose molecules in the solution diminishes the availability of free water for the rather polar volatile compounds, therefore increasing their concentration/release. The addition of sucrose to the solutions changes the solvent character of these solutions, thus changing the release of the more apolar volatile compounds. At higher Na-cyclamate concentrations (2.658 w/v%) the release of volatile compounds from the orange aroma solutions does not significantly change (Chapter

6). Apparently, at this Na-cyclamate concentration no physical and/or chemical interactions with the volatile compounds of the orange aroma occur. However, in the sensory analysis (Chapter 5) a levelling effect of the orange aroma has been observed.

The release of volatile compounds from aqueous solutions containing increasing sucrose concentrations was modelled to improve the understanding of the system worked with. The mass transfer coefficient as well as the gas/solution partition coefficient are the main parameters influencing the release. The viscosities of the different sucrose solutions were estimated and their influence on the mass transfer coefficients was determined. By measuring the gas/solution partition coefficients for five volatile compounds, the model provides an acceptable fit to the experimental flavour release data. The model description reveals that at low sucrose concentrations primarily the partition coefficient determines the release of the volatile compounds, whereas at higher sucrose concentrations the interfacial mass transfer coefficient is more important (Chapter 7).

8.5 Concluding remarks

The studies presented in the thesis on *Psychophysical evaluation of interactive effects between sweeteners and aroma compounds*, show that

- sensory perceived equisweetness is an evident condition for understanding sweetness-flavour interactions
- composition of equisweet mixtures of synergistic sweeteners can only be obtained by determining the partial SEVs and not from the psychophysical concentration-response relationships
- the synergy observed in sweet taste perception of ternary mixtures is a combination of the synergies observed in the composing binary mixtures of sweeteners
- taste differences among sweeteners cause flavour differences found among the various sweetener/aroma solutions
- modelling of the flavour release emphasises the role of partition and mass transfer coefficients in the release.

Substitution of sucrose by intense sweeteners at a sweetness level commonly present in soft drinks (i.e. 10% SEV), will cause differences in the release of volatile compounds, which are

probably not noticeable by panellists/consumers. Flavour differences observed among the sweeteners and aroma combinations studied in this thesis can probably be ascribed to differences in taste among the sweeteners. However, aromas containing flavour compounds in micro emulsions, such as oil based aromas, could give quite different results. Sensory analysis revealed a levelling effect of the orange aroma. Possibly this can be ascribed to perceptual interaction, the specific mechanism of this is currently being unknown; it is extremely unlikely that taste-odour interactions occur at the receptor level.

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Summary

Summary

Sucrose can be substituted by intense sweeteners to lower its content in soft drinks, however, their flavour often changes. The aim of the research described in this thesis is to elucidate whether these changes are due to the properties of the intense sweeteners themselves and/or to interactive effects between sweeteners and aroma compounds. The effect of mixtures of sweeteners and the presence or absence of interactive effects have been studied with the help of sensory and instrumental analysis. The results of the sensory analysis reveal the ability of panelists to perceive differences in the flavour profiles of several stimuli. The results of the instrumental analysis clarify whether the presence of (intense) sweeteners influences the release of volatile compounds from a solution. Mathematical modelling of this release revealed the parameters influencing the release of volatiles.

In Chapter 1 the outline of the thesis and aspects relevant to the thesis are presented. A general introduction to flavour stimuli and psychophysical models for mixtures of sweeteners is given. Furthermore three levels of sweetness flavour interactions are discussed: physicochemical, receptor and perceptual interactions. Finally techniques to study molecular interaction are briefly presented.

Chapter 2 deals with two preliminary studies on commercial products. In a study on sensory preferences the panelists did not significantly prefer regular orange soft drinks (sweetened with sucrose) over their light equivalents (sweetened with intense sweeteners). For cola soft drinks, on the contrary, the regular soft drink was significantly preferred over the light version. In a sensory study (quantitative descriptive analysis, QDA) the concentrations of intense sweeteners in a light blackcurrant soft drink were changed, which affected the flavour profile of this drink. It was concluded from these studies that the presence of intense sweeteners in a light soft drink influences the preferences for and the flavour profiles of these drinks to various extents, depending on the flavour and sweeteners present.

In Chapter 3 Beidler's mixture equation was used to compose equisweet mixtures of sucrose and maltitol as well as equisweet mixtures of sucrose and aspartame at 10% Sucrose Equivalent Value (SEV). QDA of different solutions containing combinations of these sweeteners and a water soluble orange aroma revealed that the mean scores for the attribute sweet remained constant for each solution. The flavour profiles of sucrose and maltitol did not significantly differ at a constant concentration of orange aroma. Flavour profiles of solutions with increasing aspartame concentrations (at constant aroma levels) showed

significantly higher scores for the attributes sour, chemical and aftertaste. Addition of orange aroma provided the different solutions with a more distinct flavour. Requested comments on the attribute aftertaste showed similar expressions for the different solutions. The aftertaste of solutions containing relatively more aspartame was mainly described as sweet and chemical.

Chapter 4 deals with mixtures of sucrose and Na-cyclamate in the presence of orange aroma. Equisweet mixtures at 10% SEV were composed, which accounted for the observed synergistic effects between sucrose and Na-cyclamate. Sensory analysis (QDA) revealed that the flavour profiles of a sucrose solution and a Na-cyclamate solution, both in the absence of orange aroma, significantly differed for the attributes chemical and aftertaste. Addition of orange aroma provided the solutions with a more distinct flavour and levelled out differences observed between sucrose and Na-cyclamate solutions. Comments on the attribute aftertaste showed that the aftertaste of solutions containing mainly Na-cyclamate was characterized as bitter.

In Chapter 5 the results of Chapters 3 and 4 are used in the study of the ternary sucrose/aspartame/Na-cyclamate mixtures in the presence of an orange aroma. Equisweet mixtures were composed, which account for the observed synergistic effects between the sweeteners. The sweetness contributions of sucrose and Na-cyclamate were fixed on forehand according to their synergy and the matching concentration of aspartame was determined. The composition of the equisweet ternary mixtures was described in a regression equation. The binary mixtures of aspartame and Na-cyclamate exhibited more synergy than the mixtures of sucrose and Na-cyclamate. Sucrose and aspartame were exchangeable in the sense that the synergy observed for the ternary mixture was a linear combination of the synergy of the binary mixtures. The flavour profiles of combinations of the mixtures and an orange aroma were obtained with the help of QDA. In the ternary mixtures Na-cyclamate contributed mostly to their flavour profiles, i.e. they were described as being more chemical, bitter and musty and having more aftertaste. Comments on the attribute aftertaste revealed the descriptions bitter and chemical.

Instrumental analysis, as described in Chapter 6, was used to evaluate possible interactive effects between sucrose/Na-cyclamate mixtures and orange aroma compounds. The release of volatile compounds from the solutions containing equisweet mixtures of these sweeteners (Chapter 4) and a water soluble orange aroma was studied. The volatile compounds were quantified and identified by gas chromatography combined with flame ionisation detection

Summary

(GC/FID) and mass spectrometry. The presence of sucrose significantly changed the release of 15 selected volatile compounds compared to the aqueous control. Increasing sucrose concentrations in the solutions (0-60 w/v%) caused an increased release of the volatile compounds with short GC/FID retention times and a decreased release of the compounds with longer retention times. Increasing Na-cyclamate concentrations (0-2.658 w/v%) did not change the release of volatile compounds.

In Chapter 7 the flavour release from aqueous solutions containing sucrose was described in a mathematical model. Parameters involved in the isolation by a purge and trap method at mouth conditions (volume, temperature, artificial saliva) were used in the model description of the flavour release. The viscosities and the gas/solution partition coefficients determined for the volatile compounds were included in the model, which provided an acceptable fit to the experimental data obtained with the instrumental analysis in Chapter 6. The model description revealed that at low sucrose concentrations primarily the partition coefficient controls the flavor release, whereas at higher sucrose concentrations the mass transfer coefficient has more influence.

In Chapter 8 the main results of this thesis are discussed. It can be concluded that substitution of sucrose by intense sweeteners at a sweetness level commonly present in soft drinks (i.e. 10% SEV), will influence the flavour release by changes in the partition or mass transfer coefficients. However, these changes in the release of volatile compounds at the 10% SEV level are probably not noticeable by panelists/consumers. Flavour differences observed among the sweeteners and aroma combinations studied in this thesis can probably be ascribed to differences in flavour among the sweeteners. Sensory analysis revealed a levelling effect of the orange aroma (Chapters 4 and 5). Possibly, this can be ascribed to perceptual interaction, of which the specific mechanism is currently being unknown.

Samenvatting

Samenvatting

Sucrose ofwel suiker kan in frisdranken vervangen worden door intensieve zoetstoffen om zijn aandeel te verlagen. Echter, ten gevolge van deze vervanging verandert het aroma van een frisdrank. Het doel van het onderzoek, dat beschreven is in dit proefschrift, is het beantwoorden van de vraag of de veranderingen in de kwaliteit van de zoete smaak toe te schrijven zijn aan de eigenschappen van de intensieve zoetstoffen zelf en/of aan interactieve effecten tussen deze zoetstoffen en aromacomponenten. Daarnaast is het effect van mengsels van zoetstoffen bestudeerd. De aan- of afwezigheid van interactieve effecten is bestudeerd met behulp van sensorische en instrumentele analyse. De resultaten van de sensorische analyse laten zien of panelleden in staat zijn om verschillen waar te nemen tussen de aromaprofielen van verscheidene combinaties van zoetstoffen. De resultaten van de instrumentele analyse maken duidelijk of de aanwezigheid van (intensieve) zoetstoffen invloed heeft op het vrijkomen van vluchtige verbindingen uit een oplossing. Dit vrijkomen van vluchtige verbindingen wordt in een model beschreven om te zien welke parameters het vrijkomen beïnvloeden.

In Hoofdstuk 1 worden de opbouw van het proefschrift en het doel van het onderzoek gepresenteerd. Er wordt een algemene inleiding gegeven over geur- en smaakwaarneming en psychofysische modellen voor mengsels van zoetstoffen. Bovendien worden drie niveau's van zoetstof-aroma interacties behandeld: fysisch-chemische, receptor en perceptuele interacties. Tenslotte worden technieken ter bestudering van moleculaire interacties in het kort besproken.

Hoofdstuk 2 betreft twee inleidende sensorische onderzoeken met commerciële producten. In een preferentie onderzoek bleken de panelleden geen significante voorkeur te hebben voor gewone frisdranken (gezoet met sucrose) ten opzichte van hun 'light' equivalenten (gezoet met intensieve zoetstoffen). Voor cola frisdranken daarentegen, werd de gewone frisdrank significant geprefereerd ten opzichte van de 'light' versie. In een profiel onderzoek werden de concentraties van de intensieve zoetstoffen in een 'light' cassis frisdrank verandert, hetgeen het aromaprofiel van deze drank beïnvloedde. Er werd geconcludeerd dat de aanwezigheid van intensieve zoetstoffen in een 'light' frisdrank de preferenties en ook het aromaprofiel van deze frisdranken in verschillende mate beïnvloedt. Dit is afhankelijk van het aanwezige aroma en de aanwezige zoetstoffen.

In Hoofdstuk 3 is Beidler's mengvergelijking gebruikt voor het samenstellen van mengsels van sucrose en maltitol, evenals van sucrose en aspartaam, met een zoetheid gelijk

aan die van een 10 w/V% sucrose oplossing (10% SEV). Kwantitatieve beschrijvende sensorische analyse (QDA) van verschillende oplossingen van combinaties van deze zoetstoffen en een water oplosbaar sinaasappelaroma liet zien dat de gemiddelde scores voor het attribuut zoet gelijk bleven voor elke oplossing. De aromaprofielen van sucrose en maltitol verschilden niet significant van elkaar bij gelijke concentraties van het sinaasappelaroma. De aromaprofielen van oplossingen met toenemende concentraties aspartaam (maar gelijke concentraties sinaasappelaroma) lieten significant hogere scores zien voor de attributen zuur, chemisch en nasmaak. Een toevoeging van sinaasappelaroma gaf de verschillende oplossingen een meer uitgesproken aromaprofiel. Nadere omschrijvingen van het attribuut nasmaak waren gelijk voor de verschillende oplossingen. De nasmaak van de oplossingen, die relatief meer aspartaam bevatten, werd voornamelijk beschreven als zoet en chemisch.

Hoofdstuk 4 behandelt mengsels van sucrose en Na-cyclamaat in de aanwezigheid van sinaasappelaroma. Mengsels met een gelijke zoetheid van 10% SEV werden samengesteld, waarbij rekening gehouden werd met de waargenomen synergistische effecten tussen sucrose en Na-cyclamaat. Sensorische analyse (QDA) liet zien dat de aromaprofielen van een sucrose oplossing en een Na-cyclamaat oplossing, beide in afwezigheid van sinaasappelaroma, significant van elkaar verschilden voor de attributen chemisch en nasmaak. De toevoeging van sinaasappelaroma gaf de oplossingen een meer uitgesproken aromaprofiel en nivelleerde de waargenomen verschillen tussen sucrose en Na-cyclamaat oplossingen. Nadere omschrijvingen van het attribuut nasmaak toonden aan dat de nasmaak van oplossingen met relatief meer Na-cyclamaat voornamelijk bitter was.

In Hoofdstuk 5 werden de resultaten van de Hoofdstukken 3 en 4 gebruikt in het onderzoek van het tertiaire sucrose/aspartaam/Na-cyclamaat mengsel in de aanwezigheid van sinaasappelaroma. Mengsels met een gelijke zoetheid van 10% SEV werden samengesteld, waarbij rekening gehouden werd met de waargenomen synergistische effecten tussen de zoetstoffen. De zoetheidsbijdrages van sucrose en Na-cyclamaat werden vastgevoren vastgelegd volgens hun synergie, en daarna is de aanvullende aspartaam concentratie bepaald. De samenstelling van de tertiaire mengsels met gelijke zoetheid (10% SEV) is beschreven in een regressievergelijking. De mengsels van aspartaam en Na-cyclamaat vertoonden meer synergie dan de mengsels van sucrose en Na-cyclamaat. Sucrose en aspartaam waren uitwisselbaar, in zoverre dat de waargenomen synergie in de tertiaire

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mengsels een lineaire combinatie was van de synergie in de binaire mengsels. De aromaprofielen van combinaties van deze mengsels en een sinaasappelaroma werden verkregen met behulp van QDA. Het aandeel van Na-cyclamaat in de tertiaire mengsels bepaalde in grote mate het aromaprofiel van deze mengsels, die hoger scoorden op de attributen chemisch, bitter, muff en nasmaak. Verdere omschrijvingen van deze nasmaak waren weer bitter en chemisch.

De instrumentele analyse, die in Hoofdstuk 6 beschreven wordt, is gebruikt om mogelijke interactieve effecten tussen sucrose/Na-cyclamaat mengsels en componenten van sinaasappelaroma te bestuderen. Het vrijkomen van vluchtige verbindingen uit de even zoete waterige mengsels van deze zoetstoffen (Hoofdstuk 4) en een water oplosbaar sinaasappelaroma is bestudeerd. De vluchtige verbindingen werden gekwantificeerd en geïdentificeerd met behulp van gas chromatografie gecombineerd met vlamionisatie detectie (GC/FID) en massaspectrometrie. Het vrijkomen van 15 geselecteerde vluchtige verbindingen uit een sucrose oplossing met sinaasappelaroma veranderde significant ten opzichte van de standaard (water + sinaasappelaroma). Hoge sucrose concentraties in de oplossingen (60 w/V%) veroorzaakten een toename in het vrijkomen van de vluchtige verbindingen met korte GC/FID retentietijden en een afname in het vrijkomen van de verbindingen met langere retentietijden. Hoge Na-cyclamaat concentraties (2.658 w/V%) veranderden het vrijkomen van vluchtige verbindingen niet.

In Hoofdstuk 7 werd het vrijkomen van vluchtige verbindingen uit sucrose oplossingen beschreven in een model. De parameters, die van belang zijn voor de isolatie onder mondomstandigheden (volume, temperatuur, kunstspeeksel), werden gebruikt in een modelbeschrijving voor het vrijkomen van vluchtige verbindingen. Met viscositeiten en gas/oplossing verdelingscoëfficiënten voor de bestudeerde vluchtige verbindingen, gaf het model een goede benadering van de experimentele data van de instrumentele analyse in Hoofdstuk 6. De modelbeschrijving maakte duidelijk dat het vrijkomen van vluchtige verbindingen bij lagere sucrose concentraties voornamelijk bepaald wordt door de verdelingscoëfficiënt, terwijl de massatransport coëfficiënt meer invloed heeft bij hogere sucrose concentraties.

In Hoofdstuk 8 worden de belangrijkste resultaten van dit proefschrift besproken. Er kan geconcludeerd worden dat bij het vervangen van sucrose door intensieve zoetstoffen, op een zoetheidsniveau zoals dat gebruikelijk is voor frisdranken (10% SEV), het vrijkomen van

vluchtige verbindingen beïnvloed wordt door veranderingen in de verdelingscoëfficiënt of de massatransport coëfficiënt. Echter, deze veranderingen in het vrijkomen van vluchtige verbindingen op het 10% SEV niveau zijn niet zo zeer waarneembaar voor panelleden/consumenten. Verschillen in smaak, die waargenomen worden in de bestudeerde combinaties van zoetstoffen en aroma, kunnen waarschijnlijk geheel worden toegeschreven aan verschillen in de smaak van de zoetstoffen. De sensorische analyse liet een nivellerend effect van het sinaasappelaroma zien (Hoofdstukken 4 en 5). Waarschijnlijk kan dit toegeschreven worden aan een perceptuele interactie, waarvan het specifieke mechanisme (nog) onbekend is.

Dankwoord

JA, we zijn er: het meest gelezen deel van het proefschrift..... vrees ik. Oh help, nu moet ik echt mijn best doen. Alhoewel, zo moeilijk is het niet! Fons Voragen, een fijnere promotor kan ik me niet indenken. Ik vond de tussentijdse evaluaties altijd erg plezierig, bedankt! Jacques Roozen, hartstikke bedankt voor je begeleiding de afgelopen jaren. Ik vind het toch echt ongelooflijk hoe je elke keer weer, in de zoveelste versie van een schrijfsel precies de zwakke punten weet aan te stippen! Ook je enorme kennis van de levensmiddelenchemische processen heeft ons door vele discussies heen geleid. Ik wil Cees de Graaf graag bedanken voor zijn rol als mede-begeleider.

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Dankwoord

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Aan het eind van mijn promotieonderzoek weet ik weer helemaal waarom ik voor de sectie Levensmiddelenchemie koos: collega's bedankt voor de leuke tijd! Familie en vrienden die soms urenlang gezeur van mij moesten doorstaan en dat flink de kop in wisten te drukken door een enorme portie gezelligheid: hartstikke bedankt!

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Curriculum Vitae

Denise Francisca Nahon werd op 30 januari 1971 geboren te Rijswijk Z.H. Halverwege haar lagere school tijd verruilde zij de 'meesters' van het Westen voor de 'meneren' in het Zuiden des lands, en daarom volgde zij haar VWO-opleiding aan het Mgr. Frencken College te Oosterhout N.B. Na het behalen van het diploma in 1989 begon zij met de studie Levensmiddelentechnologie aan de Landbouwniversiteit Wageningen (LU). Binnen deze studie deed zij een afstudeervak Wiskundige Statistiek bij Coberco Arnhem, een afstudeervak Levensmiddelenchemie bij de betreffende leerstoelgroep van de LU en een stage bij ENSBANA in Dijon, Frankrijk. Op 30 januari 1995 studeerde Denise af, en drie dagen later begon zij als Assistent In Opleiding bij de leerstoelgroep Levensmiddelenchemie van het Departement Levensmiddelentechnologie en Voedingswetenschappen aan de LU. In het kader van dit promotieonderzoek heeft zij een door het COST action 96 program gefinancierde wetenschappelijke stage uitgevoerd bij het Institute of Food Research te Norwich, Engeland. Het in januari 1999 afgeronde promotieonderzoek staat beschreven in dit proefschrift.

List of publications

- Nahon, D.F., Harrison, M. and Roozen, J.P. (1999) Modelling flavor release from aqueous sucrose solutions, using mass transfer and partition coefficients. *J. Agric. Food Chem.*, submitted.
- Nahon, D.F., Roozen, J.P. and De Graaf, C. (1999) Sensory evaluation of ternary mixtures of sodium cyclamate, aspartame, sucrose and an orange aroma. *Chem. Senses*, submitted.
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