FLAVOUR RELEASE FROM DRIED VEGETABLES

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Stellingen

1. Gaschromatografie van aroma-verbindingen m.b.v. vlamionisatiedetectie is minder geschikt voor correlatie met sensorische analyse dan die m.b.v. snuffelpoortdetectie.
   Dit proefschrift.

2. In aroma-isolatietechnieken voor groente is mechanische verkleining essentieel voor het nabootsen van het vrijkomen van vluchtige verbindingen in de mond.
   Dit proefschrift.

3. Vanwege anosmia bij proefpersonen is het gebruik van meerdere panelleden noodzakelijk voor toekenning van de geur(intensiteit) aan vluchtige verbindingen m.b.v. gaschromatografie/snuffelpoort-methoden.
   Dit proefschrift.

4. Bij de ontwikkeling van medisch toepasbare kunstspeeksels wordt helaas geen aandacht geschonken aan de invloed van de samenstelling op de gewaarwording van aroma door de patient.
   Levine, M.J. (1993). Development of artificial salivas. *Crit. Rev. Oral Biol. Med.*, **4**, 279-286.
   Dit proefschrift.

5. Voor de analyse van een aroma wordt door Preininger *et al.* (1994) en Schieberle (1994) ten onrechte voorbij gegaan aan de wijze van isoleren van de vluchtige verbindingen.
   Preininger, M., Rychlik, M. & Grosch, W. (1994). Potent odorants of the neutral volatile fraction of Swiss cheese (Emmentaler).
   Schieberle, P. (1994). Heat-induced changes in the most odour-active volatiles of strawberries. In *Trends in Flavour Research*, eds H. Maarse & D.G. van der Heij. Elsevier Science, Amsterdam, The Netherlands, resp. pp. 267-270 & pp. 345-351.

6. Bij de ontwikkeling van een model om het vrijkomen van vluchtige verbindingen in de mond na te bootsen moet men rekening houden met eet- en kauwgewoonten van volkeren.

7. Ook op de LUW-subsidieregeling t.b.v. het drukken van proefschriften is de wet van Sigstadt van toepassing: 'Als jij aan de beurt komt, veranderen ze de regels'.

8. Hoe planmatiger mensen optreden, des te ingrijpender treft ze het toeval.
F. Dürrenmatt.

9. Efficiëntie is een vorm van luiheid.

10. Wie samen kan reizen, kan ook samen leven.
S. Carmiggelt (1976). *Ze doen maar*. De Arbeiderspers, Amsterdam, The Netherlands.

Stellingen behorende bij het proefschrift
'Flavour Release from Dried Vegetables' door S.M. van Ruth.
Wageningen, 10 november 1995.

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

FLAVOUR

The flavour of food may be defined as the simultaneous perception of stimuli on the receptors of taste on the tongue and smell in the upper nasal cavity and of general pain, tactile and temperature receptors located throughout the mouth and throat (Flath *et al.*, 1981).

Many foods have highly complex chemical compositions, comprising both volatile and non-volatile substances, the sensory response to which may be significantly affected by their relative concentrations and the temperature of the product as consumed. The non-volatile components may either stimulate the taste-buds or activate the network of free sensory nerve endings and organised nerve terminations within the soft tissues of the mouth and throat, producing sensations such as astringency, pungency and cooling as well as tactile, kinaesthetic and pain responses in the relevant sensory areas (Heath, 1981). The volatile constituents are directly responsible for the (retronasal) perceived odour of the product (MonCrieff, 1970). Perceived odour and taste stimuli may differ in magnitude depending not only on the source of the stimulation and the sensory receptors involved but also on the overall receptivity of a subject (Heath, 1981).

Taste

Taste sensations result from stimulation of the microvilli of the taste receptor cells. Taste receptors are small oval structures, mainly located on the tongue but also on the palate and pharynx. Each taste-bud contains about 20 elongated receptor cells with hairlike tips that project beyond the surface through a pore (Fig. 1). The hairlike projections are the receptor surfaces. Chemical stimulation of these surfaces leads to generation of impulses in the sensory neurons that are wrapped around the receptor cell

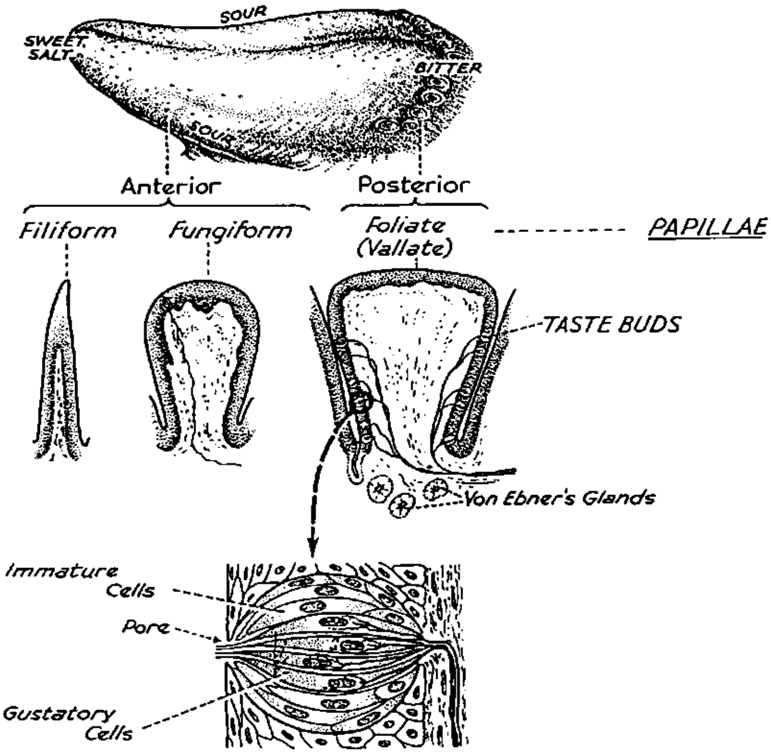

Fig. 1. Sweet, bitter, sour and salt taste receptor cells situated on the human tongue (McNaught & Callander, 1975).

(Griffiths, 1981). Transfer of potassium ions alters the electrical potential across the cell membrane, giving rise to an impulse, which is transmitted to the brain in the associated nerve fibre (MonCrieff, 1967; Kurihara, 1987). Parts of the tongue differ in sensitivity for taste stimuli, as taste-buds are located mainly on the edges and the rear of the tongue (Fig. 1). The middle top surface of the tongue is insensitive to all taste stimuli. The sweet and saline sensation is perceived primarily on the tip of the tongue, bitter at the base and sour (acid) at the sides (Beidler, 1968; McNaught & Callander, 1975).

Smell

The sense of smell plays a major part in recognition and in establishing the acceptance of foods (Beets, 1978). Stimulation of the olfactory receptors by foods via the retronasal route is a primary determinant of their flavour (Burdach & Doty, 1987). The importance of odour in flavour is well illustrated by the loss of flavour when subjects have a cold. The food seems tasteless yet the sense of taste is not impaired. However, the access to the olfactory sensors is blocked by nasal congestion. Persons who have lost their sense of smell frequently perceive the loss as one of taste rather than as one of smell (Schechter & Henkin, 1974; Doty & Kimmelman, 1986).

In mammals the sense of smell is located in the upper part of the nose; in man there are two nasal cavities separated by a septum. The lateral walls of each cavity are covered with vascular tissue in a series of two to six horizontal folds. The upper folds form three well-defined channels, known respectively as the inferior, median and superior meatus (Fig. 2). They form a common meatus opening directly into the olfactory cleft, which is also open to the pharynx (Griffiths, 1981; Wagner & Lawson, 1982). It is estimated that 5 to 10 % of inspired air passes directly over the

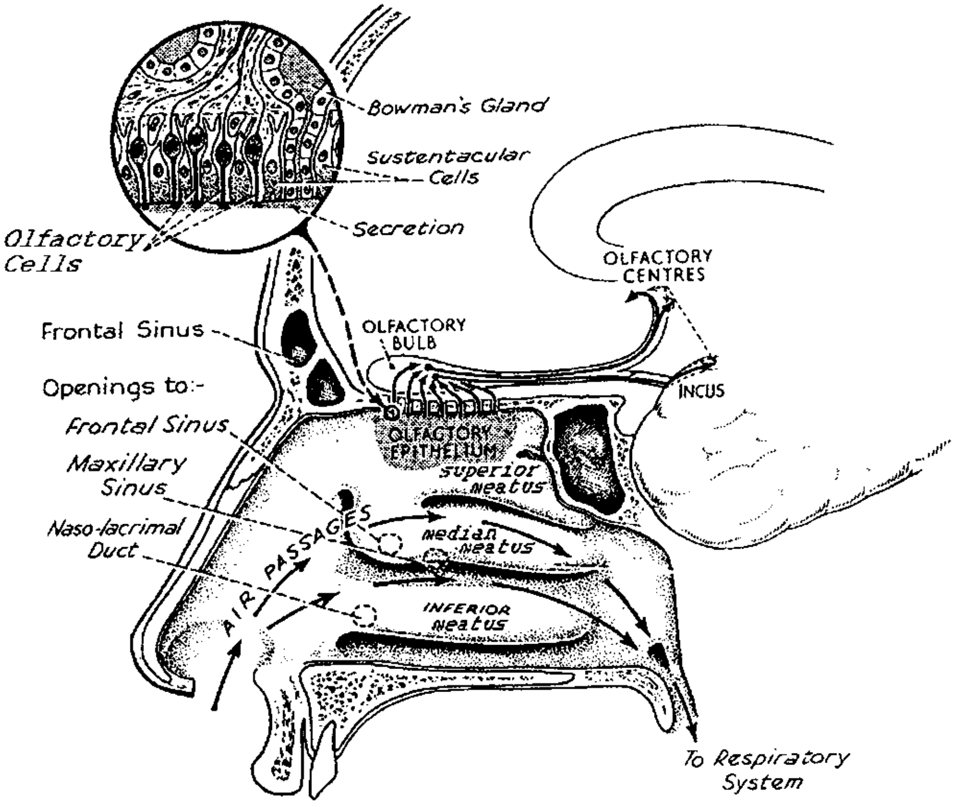

Fig. 2. Odour receptor cells in the nasal cavities (McNaught & Callander, 1975).

olfactory slit, although probably only as little as 2 % reaches the olfactory receptor region. This proportion may be substantially increased by sniffing, which causes a rapid movement of air through the upper nasal passages. Therefore, sniffing can greatly enhance the perceived odour effect (Heath, 1981; Engen, 1982).

The olfactory receptors (Fig. 2) are located in the olfactory epithelium, sited in the dorsal nasal cavities. The sensory cells are enlarged, rounded, olfactory vesicles, from the surface of which hairlike projections extend into the covering layer of mucus. The proximal pole of each sensory cell extends to form an axon, which continues as an olfactory nerve fibre. This is the only sense in which neural responses pass straight from the sensor to the brain, the neurone acting as both receiver and conductor (Harper, 1972; Griffiths, 1981).

The process of olfaction consists of the carrying of odour active compounds in a stream of moist air into the nose and over the sensitive receptor areas (Heath, 1981). However, before these aroma compounds get to the epithelium, molecules may be lost on the way because of adsorption on the other surfaces in the nasal cavities (Engen, 1982).

FLAVOUR RELEASE

The measurable flavour intensity, which is derived from a food during consumption, is determined by three aspects.

(1) The nature and amounts (ratios) of the volatile odour and non-volatile taste components present (above threshold value).

(2) The availability of these components to the sensory system as a function of time, which depends on a combination of two types of influences:

  (a) factors influencing flavour release, e.g. mastication, temperature, saliva composition and volume (hydration);

(b) factors influencing convective transport of the released volatiles via the respiratory cycle through the upper airways to the olfactory epithelium.

These processes are centered on human behaviour and anatomy. The food ingredients and particularly the structure of the food itself influence the time course of breakdown, the nature of the debris and specific interactions with flavour components.

(3) The mechanisms and strategies of determining flavour intensity over time (Haring, 1990; Overbosch *et al.*, 1991).

Physical aspects of flavour release

The driving force for flavour release of volatile compounds is the deflection from the thermodynamic equilibrium between the product phase and the vapour phase in the mouth. Equilibrium exists if the concentration in the respective phases obey the relation:

$$K_{gp} = C_g/C_p.$$

K_{gp} is the equilibrium partition coefficient between gas and product phase, which is determined by the volatility and solubility of the flavour compound. C_g and C_p represent the concentrations in the gas and product phase, respectively. As flavour compounds are usely present in highly diluted solutions, C_g and C_p are related by Henry's law when equilibrium exists. If the actual partition coefficient is smaller than K_{gp}, flavour will be released (Overbosch *et al.*, 1991).

Besides the partition coefficient, the resistance to mass transfer is a major factor determining the rate and extent of flavour release. Partitioning of flavour compounds is affected by the composition of the food. Non-equilibrium is the driving force for mass transport and the rate at which equilibrium is achieved, is determined by the resistance to mass transfer (De Roos & Wolswinkel, 1994). Most studies in this area dealt with partition phenomena, in particular with the effect of medium composition on the equilibrium headspace concentrations, but they were limited to simplified food models. The first studies on the partitioning of volatile compounds between air and water were conducted by Buttery *et al.* (1969 & 1971). They reported lipids to affect equilibrium headspace concentrations most of all food components. Hydrophilic food components revealed frequently a limited effect on the concentrations of flavour compounds in the headspace over aqueous solutions, in comparison with lipids. The presence of proteins (Overbosch *et al.*, 1991), purines, polyphenols (King & Solms, 1982) and sugars (Ebeler *et al.*, 1988; Godshall, 1988; Solms & Guggenbuehl, 1990) resulted in weak unspecific hydrophobic interactions only. Dalla Rosa *et al.* (1994) studied the influence of water activity on the headspace concentration of volatiles over model and food systems. In contrast with the numerous studies concerning the effect of partitioning on flavour release, the effect of mass transport received less attention. However, Pangborn & Szczesniak (1974), Godshall (1988) and Godshall & Solms (1992) studied the effect of thickeners on flavour intensity. In general, flavour release was decreased by thickeners.

McNulty & Karel (1973a, b & c) first described the release of volatile compounds during food consumption. Their model assumed that:

(1) flavour compounds are transferred from oil to water when the interphase equilibria are disturbed by dilution of saliva;

(2) only the aqueous concentrations stimulate perception.

Cussler *et al.* (1979) and Kokini (1985) developed a model for relating perceived sweetness intensity to diffusion coefficients in thickened aqueous systems. Generally, the calculations correlated well with the sensory scores for sweetness and sourness. As release and perception of taste compounds were performed in a single-phase system, the model did not account for partition phenomena. Overbosch *et al.* (1991) reviewed the current state of knowledge on flavour release in the mouth, including a number of mathematical relationships that related flavour release to both partition and diffusion coefficients.

De Roos & Wolswinkel (1994) developed a non-equilibrium partition model for predicting flavour release from foods as a function of chemical composition and texture. They described the fraction of a flavour compound retained in food after equilibrium as:

$$X_1/X_0 = P_{pg}/(P_{pg} + V_g/V_p)$$

where X_1 and X_0 are the quantity of a flavour compound in the product at equilibrium and the initial quantity in the system, respectively. P_{pg} is the concentration of the flavour compound in the product phase divided by the concentration in the gas phase. V_g and V_p are the volumes of gas and product phase. This static equilibrium model was applied to dynamic non-equilibrium systems by a multiple extraction model. The latter described flavour release, when phase equilibria are disturbed by continuous renewal of the headspace. During each successive extraction, product-to-gas equilibrium is achieved only at the gas-product interphase. Therefore, equilibrium exists between the volume fraction V_g^* of the gas phase and the volume fraction V_p^* of the product phase at the interphase. According to the authors mentioned, the fraction of a flavour compound remaining in the food after *n* extractions with a gas volume of V_g^* obeys the relation:

$$X_n/X_0 = [(V_p^*/V_p).\{P_{pg}/(P_{pg} + V_g^*/V_p^*)\} + (1-V_p^*/V_p)]^n.$$

In this equation V_p^*/V_p is the fraction of the food that is extracted during each extraction step. V_g^*/V_p^* denotes the ratio of the gas and product volumes that are in apparent equilibrium with each other. The ratio V_g^*/V_p^* reflects differences in mass transfer rates in the gas and product phase as a result of differences in mixing, rate of diffusion and gas flow rate. Increased resistance to mass transfer in the product will result in a smaller volume fraction V_p^*. Therefore, at high viscosity the value of the quotient V_g^*/V_p^* will be increased. If the value of V_g^*/V_p^* in this model is substantially larger than P_{pg}, the release is assumed mainly mass transfer controlled. The model was succesfully applied to chewing gums.

FLAVOUR ANALYSIS

Instrumental analysis

Flavour compounds can be divided into volatile and non-volatile compounds. Aroma is the key flavour component in many foods. Taste-buds are capable of differentiating four basic stimuli only, whereas the nose is capable of discerning hundreds of different odours. Therefore, it is not surprising that much flavour research was devoted to measuring volatile compounds (Hoff et *al.*, 1978).

An instrumental approach to aroma characterisation can be regarded as a two-phase arrangement. The first phase comprises representative isolation of volatile compounds (Dirinck & De Winne, 1994). Several analytical techniques were designed to measure two types of volatile profiles: the total volatile content of a food and the profile of volatile compounds present in the air above a food. The total volatile content is usually analysed by solvent extraction of the food material. To ensure that volatiles are extracted into the organic phase a combined steam and organic solvent distillation was frequently carried out using simultaneous distillation extraction in a specialised apparatus, like Likens-Nickerson. However, a general extraction technique has several disadvantages. Since samples are steam-distilled, cooking is performed, resulting in limited applications for fresh samples. In addition, artefacts can be formed due to the high temperature and long times needed for distillation, as was shown for marjoram by Fischer *et al.* (1988). Various modifications to the original process have been suggested using antioxidants or distilling under vacuum to improve the technique, but care is still needed when interpreting the results obtained from analysis of these steam-distilled extracts.

Whereas reliable analysis of the total volatile content may be difficult, the analysis of headspace presents additional challenges. The low levels of volatiles in air require a concentration procedure, which can distort the profile analysed, too (Taylor & Linforth, 1994). Purge-and-trap techniques and static or dynamic headspace collection methods are often used for concentration. Trapping may be on adsorbents like charcoal, Tenax or by cryogenic means (Wyllie *et al.*, 1978). The headspace methods provide volatile profiles which are expected to relate better to the profiles experienced by human subjects and are therefore nowadays widely used (Castelain *et al.*, 1994; Delahunty *et al.*, 1994; Legger & Roozen, 1994; Luning *et al.*, 1994). Headspace techniques do not present perfect profiles but they are capable of providing a profile under defined conditions (Taylor & Linforth, 1994).

Simulation of flavour release in the mouth by an instrumental technique was reported by Lee (1986). A mass spectrometer was coupled with a dynamic headspace model system to monitor the headspace model system continuously. In breath-by-breath analysis the release of volatiles from a food during consumption can be measured near to the nasal space. A membrane separator method using a direct mass spectrometry technique was developed by Soeting & Heidema (1988) for determining flavour profiles in the expired air of assessors as a function of time. However, in direct mass spectrometry only one volatile compound can be monitored at a time, although foods are usually more complex. Linforth *et al.* (1994) and Taylor & Linforth (1994) as well as Delahunty *et al.* (1994) reported expired breath sampling methods using Tenax traps, which were applied to whole foods. A procedure for determining flavour release from chocolate flakes by oral vapour gas chromatography was presented by Roozen & Legger-Huysman (1995).

The second phase of an instrumental approach to flavour characterisation involves selection of volatile compounds relevant to the flavour. This implicates correct determination of the relevant flavour compounds from the whole range of volatiles present in a particular food product (Dirinck & De Winne, 1994). An interesting approach is sniffing the gas chromatographic effluent in order to associate odour activity with the eluting compounds. Gas chromatography/sniffing port analysis (GC/SP) is the collection of techniques utilizing humans as detectors on gas chromatographs. GC/SP has been an essential bioassay used in the isolation and characterisation of odourants from complex natural products since the 1960s (Acree, 1990). Many detectors, i.e. flame ionisation, flame photometric, electron capture, mass spectrometric, fourier transform, infrared and thermal conductivity detectors are not as sensitive as the human nose for many odourants (Acree & Barnard, 1994). GC/SP techniques can be divided into four categories.

(1) Dilution analysis methods for producing titer or potency values based on stepwise dilution to threshold, e.g. CHARM (Acree *et al.*, 1984) and aroma extraction dilution analysis (Ullrich & Grosch, 1987).

(2) Response interval methods for recording time duration of perceived odours and the number of assessors with odour perception, also estimating a titer or potency (Linssen *et al.*, 1993).

(3) Time-intensity methods for producing estimates of perceived intensity recorded simultaneously with theelution of a chromatographic peak, e.g. OSME (Sanchez *et al.*, 1992).

(4) Posterior intensity methods for producing estimates of perceived intensity, which are recorded after a peak has eluted (Casimir & Whitfield, 1978).

Sensory analysis

A certain pattern of tastes, smells and texture may be recognized by subjects immediately as a certain product, but on request it can be analysed by them into several taste, smell and texture components (Kroeze, 1990). In sensory analysis, the human subject is used as an instrument, which registers stimuli, transforms them into sensations and measures these sensations (Köster, 1975). This sensory analysis implies attentional shifts between parts of a percept. People can be trained to adopt the analytical attitude and to use the judgment procedures required to analyse percepts. Analysis of a percept requires more effort if it is more complicated (e.g. a lot of parts), more tightly organized (i.e. the total impression of wholeness is so strong that the subjects can hardly direct their attention to a part of it), or more peripherally synthesized (as with yellow light, where subjects are not able to analyse it into relative contributions of a red and a green sensitive system) (Kroeze, 1990). For instance, humans have difficulty in describing complex aromas; they are able to identify only three or four individual aroma compounds in a mixture (Laing, 1994).

Sensory evaluation tests can be divided in analytical and hedonic tests. Analytical tests incorporate difference and descriptive tests. The principal of difference tests is simple, the assessors determine whether two products are similar or different. Many variants were developed: one product can be presented with the question whether it differs from a standard product, or two or more products can be provided with the question whether they are similar or different (paired comparison respectively, duo-trio, triangle or two-out-of-five tests) (Meilgaard *et al.*, 1991; Punter, 1991). Triangle tests are mostly used in difference tests (Brandt & Arnold, 1977; Frijters, 1984; O'Mahony, 1986). An application of difference tests are attribute difference tests. They are used to determine whether two or more samples differ with respect to one defined attribute (Meilgaard *et al.*, 1991).

Besides determination of differences between products, a second important task of sensory research is characterisation of the sensory attributes of a product. In descriptive analyses, assessors generate attributes for a type of product and provide intensity scores for these attributes for different products (Punter, 1991). The Flavour Profile Method is one of the techniques frequently performed in descriptive analysis (Powers, 1984; Meilgaard *et al*, 1991), besides Quantitative Descriptive Analysis (Stone & Sidel, 1985). In order to study aroma and taste release in-mouth a specialised form of sensory analysis is used, namely time intensity measurements. In this type of experiments assessors record the intensity of a particular attribute with time during eating (Cliff & Heymann, 1993).

The task in hedonic tests is to provide (subjective) preference responses. Hedonic tests involve preference tests in which the task is to arrange the products in order of preference. In acceptance tests, the task is to rate the product on a scale of acceptabilitiy and in 'attribute diagnosis' tests, the task is to rank or rate the principal attributes which determine a product's preference or acceptance (Meilgaard *et al.*, 1991; Punter, 1991).

FLAVOUR OF DRIED VEGETABLES

Vegetables are dried to extend shelf life from weeks to several years. In addition, as principally water is lost from the vegetables during dehydration, a weight reduction up to 90 % diminishes transportation costs. Dried vegetables are used in soups, sauces, convenience foods, infant foods, etc..

Some flavour compounds are present in the intact fresh vegetables, the composition of which depends on genus, cultivation, harvest and transport conditions. However, many volatiles are produced only when the tissue of the raw vegetable is broken, such as by cutting, chewing or blending (Buttery, 1981). In many plants, disruption of tissues gives rise to rapid hydrolytic and oxidative degradation of endogeneous lipids into various products, among which volatile compounds are found to be responsible for desirable and undesirable flavours (Tressl *et al.*, 1981; Hatanaka *et al.*, 1983). Blanching and the dehydration process can improve the formation of other volatile compounds, usually breakdown products of the vegetables major components, e.g. carbohydrates, proteins and lipids (Buttery, 1981). Due to the low water activity, dried vegetables are quite stable in a microbiological way, however, during storage chemical reactions still occur. Storage conditions may be of major importance in the formation of flavour compounds. As dried vegetables are rehydrated prior to consumption, rehydration conditions can influence flavour release from the vegetables as well.

Summarizing, the flavour of a rehydrated vegetable is determined by its genus, cultivation and transport conditions, cutting and blanching treatments prior to drying and dehydration, storage and rehydration conditions.

THE AIM AND OUTLINE OF THIS THESIS

The aim of this study was to develop an *in vitro* model system for isolation of volatile compounds from dried vegetables under mouth conditions, such as volume of the mouth, temperature, salivation and mastication. Instrumental analysis of these volatile compounds by gas chromatography combined with mass spectrometry, flame ionisation and sniffing port detection was to be correlated with descriptive sensory analysis. Furthermore some factors influencing flavour release from dried vegetables under mouth conditions were to be studied, as well as suitability of three types of the model system for simulation of flavour release in the mouth, using these instrumental and sensory techniques. In order to characterise the flavours of three rehydrated vegetables, as influenced by cultivar, origin, storage and rehydration conditions and to study the contribution of the volatile compounds to these flavours the same techniques were to be performed.
In the present study three types of the mouth model system are developed, which are described and compared for flavour release from diced bell peppers after rehydration in **Chapter 2**. Chapter 3 and 4 deal with factors affecting flavour release under mouth conditions, such as mastication, saliva composition and volume. The changes in flavour release from rehydrated bell peppers in the three types of the mouth model system by artificial saliva components are studied in **Chapter 3**. Flavour release from French beans influenced by artificial saliva components, in addition to the effect of saliva volume is described in **Chapter 4**. Suitability of the mouth model systems is studied in **Chapter 5**. In this chapter flavour release from rehydrated French beans, bell peppers and leeks in the mouth is compared with release in the three mouth model systems. The flavours of French beans, bell peppers and leeks are characterised by gas chromatography/sniffing port

analysis and sensory evaluation in **Chapter 6**. The flavours of commercially dried bell peppers of different origins are compared in **Chapter 7**, using similar techniques. The influence of storage conditions on the flavour of dried French beans is studied in **Chapter 8**. In conjunction, **Chapter 9** deals with the effect of rehydration conditions on the flavour of this vegetable. The implications of the results reported in Chapter 2-9 are discussed in **Chapter 10**.

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CHAPTER 2

COMPARISON OF DYNAMIC HEADSPACE MOUTH MODEL SYSTEMS FOR FLAVOUR RELEASE FROM REHYDRATED DICED BELL PEPPERS

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ABSTRACT

A mastication device was added to a dynamic headspace model system for studying flavour release from rehydrated diced red bell peppers at mouth conditions, such as volume of the mouth, temperature, salivation and mastication. The use of this new device in the dynamic headspace model system enlarged the peak areas of the volatile compounds, but the areas remained smaller than in the purge-and-trap model system.

INTRODUCTION

The release of flavour in the mouth is a very important but poorly understood area of investigation. Sensory techniques have been developed to measure time-related responses (Pangborn & Koyasako, 1981; Overbosch *et al.*, 1986). Soeting & Heidema (1988) developed a mass spectrometric method for determining profiles of flavour concentration as a function of time at the nostrils. The results demonstrated a very large variability among people. A sufficient number of panellists is needed to ensure accuracy for sensory techniques and breath-by-breath analyses (Overbosch *et al.*, 1986; Soeting & Heidema (1988). The quality of dried vegetables, such as bell peppers (*Capsicum annuum*), is largely determined by their flavour, the perception of which is mainly due to their volatile compounds. These compounds have been collected from vegetables by headspace methods, by steam distillation or by solvent extraction (Buttery *et al.*, 1969, Wu *et al.*, 1986; Shinohara *et al.*, 1991). Headspace methods overcome some of the problems going with distillation or solvent extraction methods, whereas the static headspace method does not generally provide sufficient amounts of the compounds needed for detection. Larger amounts are obtained with dynamic headspace methods using adsorbent traps. Lee (1986) coupled a mass spectrometer with a dynamic headspace model system to monitor the headspace concentration continuously. Never has a dynamic headspace model system with adsorbent traps been used to study the release of flavour at mouth conditions.

The purpose of this work was to simulate the flavour release of rehydrated diced red bell peppers at mouth conditions. Therefore a mastication device was added to a dynamic headspace mouth model system (DHM). The effect of the device was compared with the dynamic headspace method as such (DH) and with the purge-and-trap technique (PT).

MATERIALS AND METHODS

Sample preparation

Commercially dried diced red bell peppers were supplied by Top Foods b.v. (Elburg, The Netherlands). They were packed in glass containers and stored at 5°C in the absence of light until sampling. The diced bell peppers (1.2 g) were rehydrated by adding 10 ml distilled water, heating in a waterbath at 100°C for 10 min and cooling in a waterbath at 25°C for 4 min.

Isolation and analysis of the volatiles

The bell pepper pieces were transferred into the sample flask of the PT/DH/DHM model system (Fig. 1) and 4 ml diluted artificial saliva added. The stock saliva consisted of 2.160 g mucin, 1.369 g $K_2HPO_4.3H_2O$, 0.877 g NaCl, 0.500 g NaN_3, 0.477 g KCl and 0.441 g $CaCl_2.2H_2O$ in 100 ml distilled water. The stock saliva was purged with purified nitrogen gas (50 ml/min) for 15 hours in order to diminish flavour compounds in the saliva. After purging, 5.208 g $NaHCO_3$ and 200,000 units α-amylase were added to 100 ml stock saliva and this saliva was diluted 1:9 with distilled water, adjusted to pH 7 with 2 M HCl and used throughout the study.

In the PT mode, a purified nitrogen gas flow (20 ml/min) passed through the bell peppers and saliva at 37°C for 1 h to trap the volatile compounds in Tenax TA (35/60 mesh, Alltech Nederland b.v., Zwijndrecht, The Netherlands). In the DH and in the DHM mode the headspace was flushed with nitrogen gas at the same rate, for the same time and at the same temperature as in the PT mode. In the DHM mode the plunger made about four up and down screwing movements to simulate mastication during isolation of the volatiles. A detailed set-up of DHM is shown in Fig. 2.

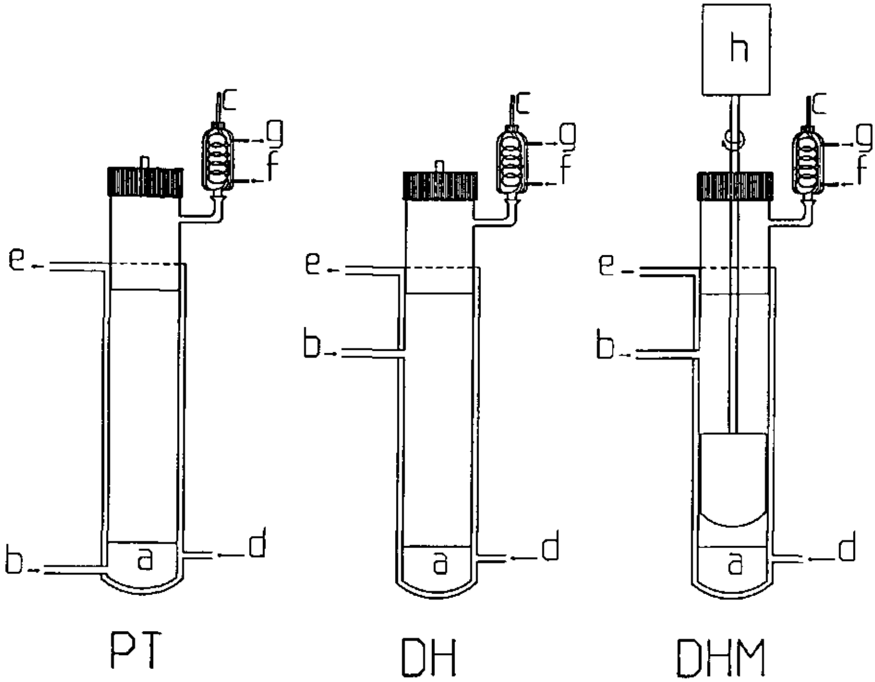
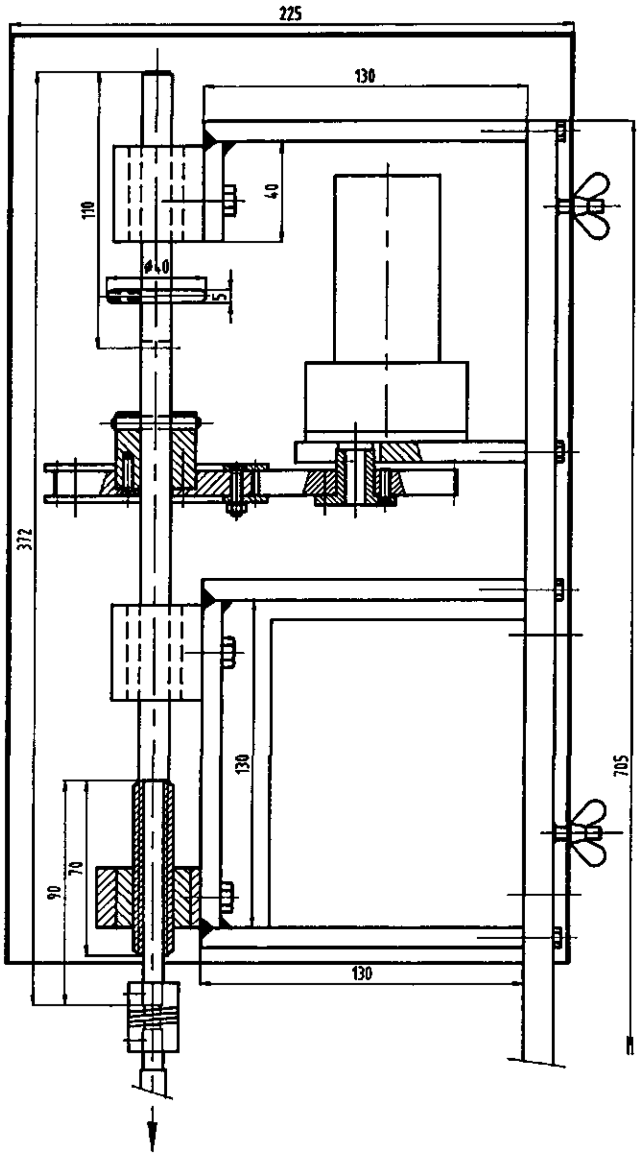
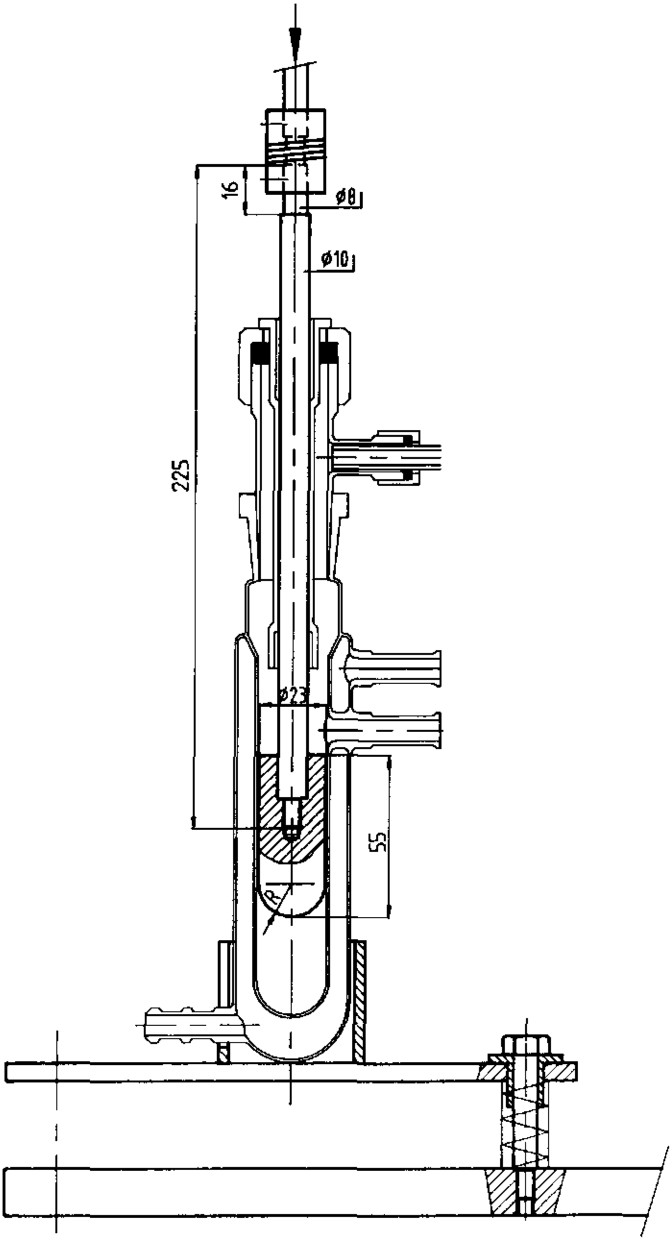


Fig. 1. Three types of flavour release mouth model systems. PT, purge-and-trap; DH, dynamic headspace; DHM, dynamic headspace and mastication. a, Sample flask (70 ml) containing bell peppers, water and saliva; b, nitrogen gas inlet; c, Tenax TA; d, 37°C water inlet; e, water outlet; f, -10°C ethanol inlet; g, ethanol outlet; h, engine.



(a)

Fig. 2. Detailed set-up of mouth model system dynamic headspace and mastication (DHM). (a) Engine part and (b) isolation part.



(b)

Fig. 2. (continued)

The volatile compounds were thermally desorbed from the Tenax tube by a thermal desorption/cold trap device (Carlo Erba TDAS 5000, Interscience b.v., Breda, The Netherlands). Gas chromatography (GC) was carried out on a Carlo Erba MEGA 5300 (Interscience b.v., Breda, The Netherlands) equipped with a Supelcowax 10 capillary column (60 m x 0.25 mm i.d.) and a flame ionisation detector (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 to 272°C at 6°C/min.
The volatile compounds trapped on Tenax TA were identified by Dr M.A. Posthumus, Department of Organic Chemistry, Wageningen Agricultural University, using combined GC (Pye 204, Unicam Ltd, Cambridge, UK) and mass spectrometry (MS; VG MM 7070 F, Fisons Instruments, Weesp, The Netherlands). A thermal desorption cold trap injector (Chrompack TCT Injector 16200, Chrompack, Middelburg, The Netherlands) was used as well as the capillary column and the temperature conditions described above. Mass spectra were recorded in the electron impact mode at an ionisation voltage of 70 eV and scanned from m/z=300 to 25 with a cycle time of 1.8 s.

RESULTS AND DISCUSSION

Chromatograms of the volatile compounds of the bell peppers isolated with the three flavour release model systems are presented in Fig. 3. The volatile compounds are listed together with their peak areas in Table 1. The sum of the peak areas of all volatile compounds, as well as the ones of the volatiles having retention times less than 15 minutes are listed in Table 1. Qualitatively, there is no difference in composition of the volatile compounds between the three model systems. Comparison of the peak areas showed that most volatile compounds were released in the PT model system, followed by DHM and DH, respectively. In model system DH the peak area of the most volatile compounds, peaks having retention times below 15 minutes,

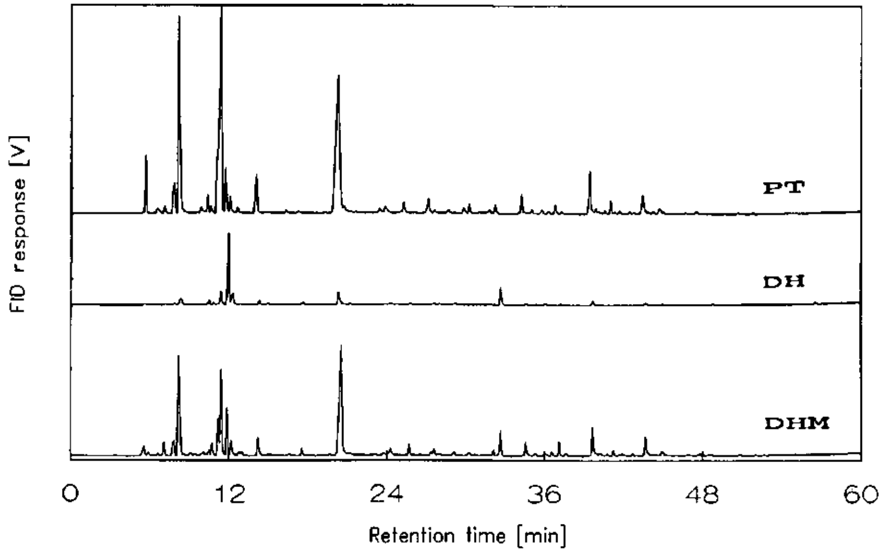


Fig. 3. Gas chromatograms of rehydrated diced red bell peppers isolated in three flavour release mouth model systems. For an explanation of codes see Fig. 1.

accounted for 79 % of the sum of the peak area of all compounds, in system DHM for 56 % and in system PT for 45 %. Mastication of the bell peppers (DHM) increased peak areas of all volatiles and caused a significant (Wilcoxon, $P<0.05$) relative increase in release of the less volatile compounds. Isolation of the volatile compounds in PT resulted in larger peak areas than in DH/DHM and the peak area percentage of the less volatile compounds increased

Table 1. Volatile compounds of rehydrated red bell peppers and their average peak areas (n=6)[a]

Compound	Peak area [0.1 V.s]		
	PT	DH	DHM
Alcohols			
2-Propanol	79.17	15.64	19.72
1-Methyl-1-propanol	0.23	tr[b]	tr
1-Butanol	27.51	0.90	5.02
1-Penten-3-ol	0.57	tr	tr
3-Methylbutanol	3.59	2.18	1.82
1-Pentanol	9.79	0.41	1.68
1-Hexanol	28.43	0.62	4.33
2-Hexen-1-ol	7.20	tr	0.69
1-Octen-3-ol	5.71	0.12	0.61
Aldehydes			
Propanal	108.59	1.32	28.68
2-Methylpropanal	500.19	16.80	83.65
Butanal	39.58	0.27	5.64
2-Methyl-2-propenal	10.33	0.36	3.21
2-Methylbutanal	259.38	2.30	32.06
3-Methylbutanal	646.32	4.65	75.91
Pentanal	130.32	1.73	17.65
Hexanal	1999.25	13.11	220.33
2-Ethyl-2-pentenal	7.95	tr	tr
Heptanal	tr	tr	tr
trans-2-Hexenal	24.05	0.55	4.52
Octanal	11.81	0.16	3.65
cis-2-Heptenal	11.27	0.90	1.93
Nonanal	18.46	0.75	8.04
2-Octenal	48.45	0.48	5.11
2,4-Heptadienal	22.57	0.41	3.84
Decanal	45.18	0.53	6.38
Benzaldehyde	15.53	0.89	4.20

Table 1. (continued)

Compound	Peak area [0.1 V.s]		
	PT	DH	DHM
Aldehydes (continued)			
2-Nonenal	6.45	tr	0.63
Ketones			
2-Propanone	0.63	tr	16.45
2-Butanone	15.91	0.69	2.94
2-Pentanone	20.00	0.27	2.71
2,3-Butanedione	50.12	0.66	6.79
1-Penten-3-one	1.36	tr	tr
3-Penten-2-one	1.22	tr	tr
2-Heptanone	55.75	0.63	7.89
4-Methyl-2-heptanone	7.45	tr	0.68
2-Octanone	2.55	0.14	0.13
6-Methyl-5-hepten-2-one	32.65	0.42	4.17
Phenylethanone	3.61	0.95	3.27
Sulphur compounds			
Dimethyl disulphide	8.68	tr	0.58
Dimethyl trisulphide	3.19	tr	tr
Others			
Ethyl acetate	28.56	0.48	3.61
Acetic acid	1.07	tr	0.69
2-Methoxy-3-isobutylpyrazine	tr	tr	tr
Limonene	12.27	1.25	0.20
1,2-Diethoxyethane	16.69	0.95	5.26
1-Methyl-1H-pyrrole	33.36	tr	6.77
Dimethyl sulphoxide	12.15	tr	0.32
Sum volatile compounds retention time < 15 min	1981.47	56.56	337.17
Sum volatile compounds	4374.10	71.52	601.76

[a]For an explanation of codes see Fig. 1.

[b]tr, peak area<0.01 V.s.

significantly (Wilcoxon, P<0.05) compared with DH/DHM. In PT the flavour compounds are obviously more strongly removed from the pieces than in the DH/DHM mode.

CONCLUSIONS

The mastication device enabled the determination of flavour release from rehydrated red bell peppers at mouth conditions with regard to temperature, salivation and mastication. The peak areas of the volatile compounds released in this model system were between those released in the purge-and-trap system and those released in the dynamic headspace system. During mastication of the rehydrated bell pepper pieces an overall increase in peak areas of the volatile compounds was observed as well as an increase in release of the less volatile compounds.

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CHAPTER 3

CHANGES IN FLAVOUR RELEASE FROM REHYDRATED DICED BELL PEPPERS (*CAPSICUM ANNUUM*) BY ARTIFICIAL SALIVA COMPONENTS IN THREE MOUTH MODEL SYSTEMS

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ABSTRACT

The effect of artificial saliva components on flavour release from rehydrated diced red bell peppers (*Capsicum annuum)* was studied in a mouth model system. This measured the dynamic headspace under oral conditions, such as temperature, volume of the mouth, salivation and mastication (DHM). The results were compared with a dynamic headspace (DH) and a purge-and-trap (PT) model system. Volatile compounds were analysed by gas chromatography, using flame ionisation detection (FID), mass spectrometry (MS) and sniffing port detection (SPD). SPD revealed that only 12 of the 47 compounds identified by MS and FID possessed odour activity. For all saliva compositions FID peak areas of volatile compounds were largest in the PT system, followed by DHM and DH, respectively. In the PT system the less volatile compounds were relatively better released than other volatile compounds in comparison with the DHM and DH system, for all salivas. The saliva components mucin and α-amylase caused a relative decrease in release of the less volatile compounds in all model systems. An overall decrease in FID peak areas of volatile compounds by the latter components was observed in model system DH only.

INTRODUCTION

Sensory techniques have been developed to measure time-related responses (McNulty, 1974; Larson-Powers & Pangborn, 1978; Birch *et al.*, 1980; Birch & Munton, 1981; Pangborn & Koyasako, 1981; Overbosch *et al.*, 1986). The release of volatiles from a food product during consumption can be measured near to the olfactory epithelium at the entrance of the nose (breath-by-breath analysis). This measurement of gases and volatile compounds is well known in medical science (Mackay & Hussein, 1978; Wilson & Ottley, 1981; Benoit *et al.*, 1983). However, the experimental techniques used were not suitable for the measurement of flavour release as they generally did not provide high sensitivity and short response times. Soeting & Heidema (1988) developed a membrane separator method for determining profiles of flavour concentration as a function of time in the nose. The results demonstrated a large variability in the release curves between different subjects, which was even larger than within the sensory intensity-time data. Flavour release seems to be enhanced by the breakdown of the food matrix through mastication (Haring, 1990). Linforth *et al.* (1994) demonstrated a technique of trapping expired air on Tenax (*p*-diphenylene oxide), followed by gas chromatography/mass spectrometry (GC/MS) which could be used for studying the volatile profile during eating of tomatoes, however, the variation in these analyses was higher than usual. A sufficient number of panellists is needed to secure reliability of sensory techniques and breath-by-breath analyses.

Lee (1986) developed an instrumental technique, in which a mass spectrometer was coupled with a dynamic headspace model system to monitor headspace concentration continuously. The model system replaced the column of a gas chromatograph within a combination of GC and MS. In the analyses, only one compound could be monitored at a time, while the volatile composition of foods is usually more complex.

Volatile compounds contribute considerably to the flavour of rehydrated diced red bell peppers, *Capsicum annuum* (Van Ruth & Roozen, 1994). Extraction and headspace analysis are the main methods for isolation of volatile compounds in vegetables (Buttery *et al.*, 1969; Wu & Liou, 1986; Wu *et al.*, 1986; Shinohara *et al.*, 1991). Headspace methods overcome some of the problems associated with extraction methods. Since the headspace concentrations of flavour compounds are low and the volume of a sample taken by a syringe is limited, the headspace sample usually must be concentrated prior to analysis. The most commonly used method for concentrating these samples is the application of adsorbents, of which Tenax has often been recommended (Kuo *et al.*, 1977; Boyko *et al.*, 1978; Ioffe & Vitenberg, 1984; Leahy & Reineccius, 1984; Ishihara & Honma, 1992).

In the present work a dynamic headspace model with Tenax TA trap and mastication device (DHM) was used to study the flavour release from rehydrated diced red bell peppers under mouth conditions, as reported previously (Van Ruth *et al.*, 1994). The effect of the artificial saliva components salts, mucin and α-amylase on the flavour release from rehydrated diced red bell peppers was studied in the DHM model system and was compared with the dynamic headspace method (DH) and with the purge-and-trap technique (PT).

MATERIALS AND METHODS

Plant material

Commercially dried diced red bell peppers, *Capsicum annuum*, from Hungary were supplied by Top Foods b.v. (Elburg, The Netherlands). The bell peppers were packed in glass jars and stored at 4°C in the absence of light until sampling.

Sample preparation

Diced bell peppers (1.2 g) were rehydrated by adding 10 ml distilled water, followed by heating in a waterbath at 100°C for 10 min and by cooling in a waterbath at 25°C for 4 min. The rehydrated bell pepper pieces were transferred into the sample flask (70 ml) of the PT/DH/DHM model system and 4 ml of one of the artificial salivas (W, WS, WSM and WSMA) were added (Van Ruth *et al.*, 1994). Saliva W consisted of distilled water only. Saliva WS consisted of 5.208 g $NaHCO_3$, 1.369 g $K_2HPO_4.3H_2O$, 0.877 g NaCl, 0.500 g NaN_3, 0.477 g KCl and 0.441 g $CaCl_2.2H_2O$ in 1 l distilled water (adjusted to pH 7). The composition of saliva WSM was identical to WS, but 2.160 g mucin was added and saliva WSMA was identical to saliva WSM, but 200,000 units α-amylase were added (Sigma Chemical Co., St Louis, MO, USA). The three types of flavour release model systems used have been described previously (Van Ruth *et al.*, 1994). In the PT model, a purified nitrogen gas flow (20 ml/min) passed through the bell peppers/saliva mixture at 37°C for 1 h to trap the volatiles in 0.10 g Tenax TA, (ϕ 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.. In the DH and DHM model the headspace was flushed with nitrogen gas at the same rate, at the same temperature and for the same time as in the PT model. In the DHM model the plunger made four up and down screwing movements per min in order to simulate mastication during isolation of the volatiles.

Instrumental analysis

Desorption of volatile compounds from Tenax was performed by a thermal desorption/cold trap device. The compounds were analysed by GC, using flame ionisation detection (FID), MS and sniffing port detection as reported

Table 1. Descriptors generated and used by the GC/sniffing panel

Bell pepper	Butter
Burned/rubber	Caramel
Cooked vegetables	Chocolate
Fresh vegetables	Coffee
Fruity	Fish
Grassy/green	Lemon-/orange-like
Mushrooms	Onion/leek
Sour	Plastic/chemical
Spicy	Rotten
Sweet	Sickly

previously (Van Ruth & Roozen, 1994). In preliminary sniffing experiments, 12 assessors generated flavour descriptors of rehydrated diced bell peppers, which were clustered after group sessions of the panel. Besides 'other/I do not know', one of these descriptors (Table 1) had to be used for each component detected by the assessors at the sniffing port. Tenax tubes without adsorbed volatile compounds were used as dummy samples for determining the signal-to-noise level of the group of assessors.

Statistical analysis

The FID data presented in the tables 2 and 5 represent the mean value of six replicates. When FID data were subjected to an analysis of variance, a Fisher's least significant difference (LSD) test was carried out to determine significant differences among treatments (O'Mahony, 1986). Student's *t*-tests were conducted on the FID peak areas as well (O'Mahony, 1986). A significance level of $P<0.05$ was used throughout the study.

RESULTS AND DISCUSSION

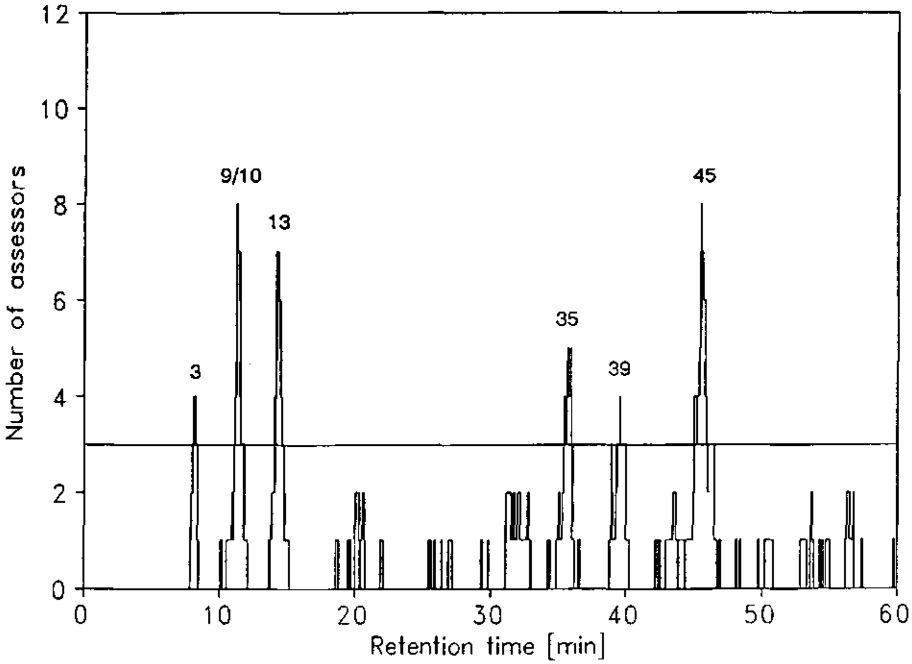
Volatile compounds of rehydrated red bell peppers were isolated in the three mouth model systems, to which four different compositions of artificial saliva were added. Fig. 1 represents the chromatograms obtained by sniffing port detection of volatile compounds isolated in mouth model system DH, DHM and PT. FID chromatograms were similar to chromatograms presented previously (Van Ruth *et al.*, 1994). The volatile compounds of rehydrated red bell peppers were identified by GC/MS (Table 2). The compounds were further characterised by their retention times, their peak areas and the odours described by the assessors at the sniffing port. The numbers above the peaks in Fig. 1 refer to the compounds listed in Table 2.

GC sniffing of dummy samples showed that detection of an odour at the sniffing port by three or less out of 12 assessors can be considered as 'noise'. Sniffing port analysis revealed that 12 out of the 47 compounds identified possessed detectable odours (Fig. 1). Model system DH shows fewer sniffing peaks than DHM and PT as the concentration of some compounds is probably below detection threshold. The sniffing chromatograms of DHM and PT are similar (Fig. 1(b)-(c)), although their FID peak areas differ considerably (Table 2). The concentrations of several volatile compounds isolated in both model systems probably did not exceed detection thresholds. Comparison of the odour descriptions provided by the GC/sniffing panel (Table 3) shows that some of the odours of volatile compounds changed at higher concentrations. Heptanal changed from fruity (DH) into fruity and rotten (DHM/PT) and ß-ocimene changed from spicy (DH) into spicy, fish and sickly (DHM) and subsequently into fish and sickly (PT). An increase in concentration of odour active volatile compounds at the sniffing port usually results in an increase in the number of assessors with an odour perception, however, it can also result in a change of the odour descriptions. This could be related to concentration alone but could also be due to simultaneous elution of more than one compound.

Wu *et al.* (1986) reported most of the odour active volatile compounds identified in our study, e.g. hexanal, heptanal, ß-ocimene, *trans*-3-hepten-2-one and 2-methoxy-3-isobutylpyrazine. Buttery (1981) reported 2-methoxy-3-isobutylpyrazine, ß-ocimene and limonene as major components of the vacuum steam volatile oil of green bell peppers, all three of them are identified in the present study. In comparison with other studies (Keller *et al.*, 1981; Chitwood *et al.*, 1983; Wu *et al.*, 1986) more small, highly volatile compounds were identified here, e.g. dimethyl sulphide, propanal, 2-methylpropanal, methyl acetate, 2-methylfuran, butanal, diethoxyethane and 3-buten-2-one. This is probably due to the dynamic headspace technique performed in the present study instead of the extraction procedures at elevated temperatures used in the studies mentioned before.

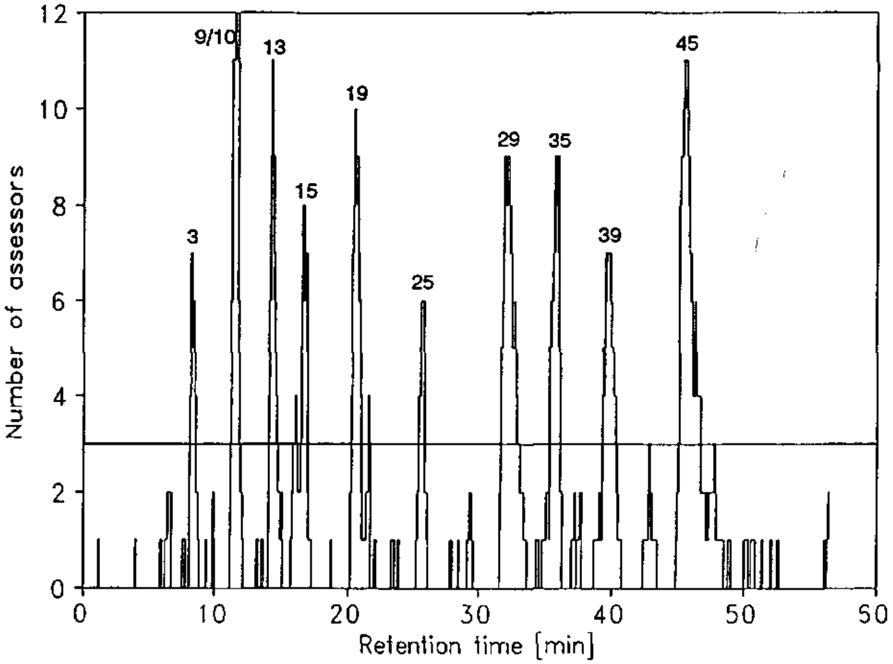
Overall, the same volatile compounds are present in the three mouth model systems and salivas (Table 2). However, analysis of variance shows that the average peak areas (n=6) of the volatile compounds differed significantly between the model systems ($F(2,419)=380$, $P<0.05$). For all salivas, model PT showed the largest peak areas, followed by DHM and DH, respectively, which confirms previous results (Van Ruth *et al.*, 1994). In the PT model the volatile compounds were obviously more strongly removed from the diced bell peppers than in the DH/DHM models. Mastication increased flavour release from the bell peppers. This is in agreement with sensory evaluations of Burdach & Doty (1987), who reported increased flavour scores of assessors by various mouth movements.

Analysis of variance of the four salivas shows that only the peak areas in model system DH differed significantly



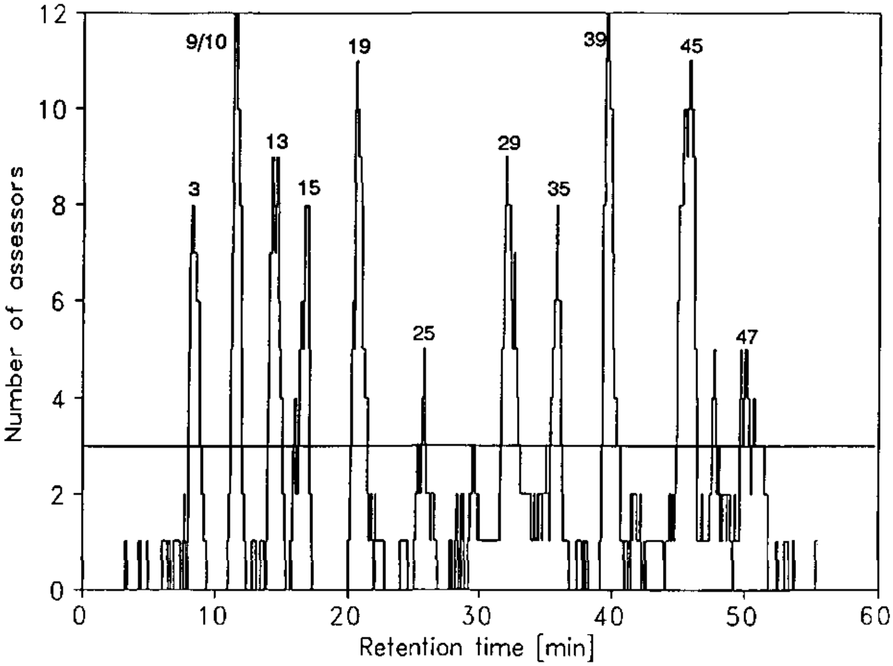
(a)

Fig. 1. Sniffing chromatograms of volatile compounds of rehydrated diced red bell peppers isolated in three mouth model systems containing saliva WSMA (water, salts, mucin and α-amylase). (a) Dynamic headspace model system (DH); (b) dynamic headspace and mastication model system (DHM); (c) purge-and-trap model system (PT). Numbers on the chromatogram refer to the compounds in Table 2.



(b)

Fig. 1. (continued)



(c)

Fig. 1. (continued)

Table 2. Volatile compounds of rehydrated *Capsicum* pieces isolated in three mouth model systems (DH/DHM/PT) with addition of four compositions of artificial saliva (W/WS/WSM/WSMA), their retention times, average peak areas and coefficients of variance ($n=6$)[a]

Peak no	Ret. time [min]	Compound	Peak area [V.s]											
			DH				DHM				PT			
			W	WS	WSM	WSMA	W	WS	WSM	WSMA	W	WS	WSM	WSMA
1	6.27	Dimethyl sulphide	0.26	0.09	0.01	0.04	0.22	0.15	0.02	tr	0.54	0.13	0.21	0.50
2	7.30	Propanal	0.45	0.67	0.09	0.30	2.97	4.26	4.97	3.96	3.87	6.23	8.20	10.50
3	7.83	2-Methylpropanal	2.09	1.40	0.52	1.15	13.29	13.87	13.24	12.58	21.63	30.09	15.00	33.06
4	8.11	Methyl acetate	0.01	0.01	tr	0.02	0.12	0.13	0.43	0.77	0.18	0.31	0.84	0.88
5	9.50	2-Methylfuran	0.07	0.06	0.02	0.03	0.45	0.45	1.52	1.34	2.37	2.88	3.46	3.53
6	9.62	Butanal	0.18	0.14	0.02	0.09	1.04	1.05	0.40	0.37	0.63	1.00	1.34	0.88
7	9.99	Diethoxyethane	0.02	tr	0.01	0.02	0.19	0.18	0.21	0.17	0.21	0.33	0.38	0.41
8	10.33	2-Butanone	0.21	0.28	0.12	0.11	0.62	0.73	1.20	0.76	0.88	1.12	1.49	2.02
9	10.79	2-Methylbutanal	0.97	0.66	0.14	0.47	7.29	6.68	7.44	8.24	16.76	17.33	23.34	19.99
10	11.24	3-Methylbutanal	2.38	1.61	0.37	1.08	17.33	15.86	17.00	19.32	43.04	42.93	50.83	48.63
11	12.69	3-Buten-2-one	0.07	0.09	0.05	tr	0.29	0.16	0.19	0.10	0.25	0.26	0.29	0.22
12	13.95	2-Pentanone	0.02	tr	tr	0.01	0.14	0.16	0.29	0.20	0.43	0.50	0.69	0.39
13	14.10	2,3-Butanedione	0.02	0.01	0.02	0.01	0.12	0.09	0.24	0.16	0.70	0.04	tr	tr
14	14.26	Pentanal	0.86	0.66	0.09	0.43	5.11	5.39	7.01	6.51	13.50	15.99	20.46	18.66
15	16.18	1-Penten-3-one	0.07	0.03	tr	0.02	0.58	0.55	0.37	0.39	1.25	1.18	0.67	0.72
16	16.48	Trichloromethane	0.07	0.03	tr	0.01	0.52	0.50	1.01	0.29	1.26	1.06	0.67	0.74
17	19.53	2,3-Pentanedione	tr	tr	tr	tr	0.23	0.22	0.02	0.05	0.56	0.54	0.14	0.14
18	20.10	Dimethyl disulphide	0.03	tr	tr	tr	0.20	0.10	0.18	0.13	0.27	0.48	0.49	0.30
19	20.55	Hexanal	7.04	5.64	1.14	2.98	48.09	49.24	58.66	54.04	140.04	161.80	191.78	166.87
20	20.95	3-Penten-2-one	tr	tr	tr	tr	tr	tr	0.01	0.01	0.13	0.04	0.13	0.07
21	23.42	1-Methyl-1H-pyrrole	0.14	0.10	tr	0.03	0.94	0.93	1.22	1.11	2.17	2.14	2.79	2.72
22	23.72	1-Butanol	0.09	0.05	tr	0.01	0.63	0.88	0.68	0.65	1.75	1.87	1.73	1.57
23	25.42	1-Penten-3-ol	0.30	0.20	0.03	0.19	1.33	1.18	1.55	1.48	2.53	2.16	2.96	3.30
24	27.06	2-Heptanone	0.08	0.03	tr	0.02	0.36	0.31	0.50	0.45	0.87	0.97	1.46	1.42
25	27.86	Heptanal	0.18	0.15	0.04	0.04	1.21	1.20	1.53	1.25	3.88	4.21	4.88	4.26

26	28.62	Limonene	1.22	0.98	0.27	0.01	7.91	10.70	0.73	0.65	29.22	29.47	1.46	1.48
27	29.76	3-Methyl-1-butanol	0.10	0.10	0.02	0.04	0.92	0.90	0.97	0.93	2.30	2.54	2.58	2.45
28	31.02	*trans*-2-Hexenal	0.01	tr	tr	tr	0.16	0.16	0.21	0.17	0.57	0.57	0.52	0.55
29	31.12	ß-Ocimene	0.01	tr	tr	tr	0.19	0.14	0.17	0.09	0.65	0.62	0.45	0.40
30	31.33	4-Methyl-2-hexanone	0.12	0.06	tr	0.05	0.57	0.51	0.48	0.49	0.96	1.13	0.84	1.01
31	31.74	1-Pentanol	1.03	1.14	tr	0.40	1.87	1.13	1.19	1.17	0.84	0.63	0.15	7.10
32	31.93	2,3-Hexanedione	tr	tr	tr	tr	tr	tr	tr	tr	0.01	0.04	0.06	0.06
33	34.01	5-Methyl-2-hexanone	tr	tr	tr	tr	0.07	0.06	0.08	0.07	0.21	0.24	0.28	0.27
34	34.26	Octanal	0.09	0.13	0.05	tr	0.41	0.43	1.04	0.35	0.84	0.91	0.84	0.81
35	34.97	*trans*-3-Hepten-2-one	0.02	tr	tr	tr	0.29	0.24	0.31	0.24	0.69	0.58	0.81	0.73
36	36.28	*cis*-2-Heptenal	0.12	0.12	0.01	0.03	0.84	0.90	0.91	0.87	2.40	2.76	2.70	2.50
37	36.83	6-Methyl-5-hepten-2-one	0.17	0.17	0.05	0.03	0.81	0.89	0.85	0.74	1.88	2.54	2.18	2.34
38	37.37	1-Hexanol	tr	tr	tr	tr	0.05	0.04	0.04	0.08	0.11	0.09	0.06	0.13
39	38.40	Dimethyl trisulphide	tr	tr	tr	tr	0.04	tr	0.01	0.02	0.24	0.32	0.41	0.31
40	39.43	Nonanal	0.33	0.38	0.16	tr	1.36	1.59	0.85	0.66	2.93	3.04	1.07	1.12
41	40.53	Dodecanethiol	0.04	tr	tr	0.01	0.53	0.27	0.31	0.32	0.80	0.93	0.96	0.93
42	41.04	1-Octen-3-ol	0.15	0.20	0.02	0.03	1.07	1.09	1.20	1.18	3.56	3.96	4.00	3.61
43	42.03	*trans,trans*-2,4-Hexadienal	0.14	0.13	0.01	0.02	0.68	0.62	1.00	0.83	1.72	1.41	1.77	1.52
44	43.46	Decanal	0.41	0.62	0.23	0.13	1.07	0.98	1.23	1.18	2.68	2.87	3.67	3.30
45	43.94	2-Methoxy-3-isobutylpyrazine	tr	tr	tr	tr	tr	tr	0.03	tr	tr	0.01	tr	tr
46	44.57	Benzaldehyde	0.02	tr	tr	tr	0.16	0.18	0.10	0.32	0.77	1.03	0.90	0.69
47	47.81	ß-Cyclocitral	0.05	0.07	0.01	tr	0.34	0.34	0.09	0.08	1.22	1.36	0.31	0.21
		CV [%]	43	32	45	37	23	16	21	27	29	23	26	26

[a] DH, dynamic headspace model; DHM, dynamic headspace and mastication model; PT, purge-and-trap model; W, distilled water; WS, W + salts; WSM, WS + mucin; WSMA, WSM + α-amylase; tr, peak area < 0.01 V.s;

CV, mean coefficient of variance of individual compounds.

Table 3. Odour active volatile compounds isolated in three mouth model systems and their descriptors provided by the GC/sniffing panel

Peak no	Compound	Model system[a]		
		DH	DHM	PT
3	2-Methylpropanal	Chocolate	Chocolate	Chocolate
9/10	2- & 3-Methylbutanal	Chocolate	Chocolate	Chocolate
13	2,3-Butanedione	Caramel, butter	Caramel, butter	Caramel, butter
15	1-Penten-3-one	BD[b]	Plastic/chemical	Plastic/chemical
19	Hexanal	Grassy/green	Grassy/green	Grassy/green
25	Heptanal	Fruity	Fruity, rotten	Fruity, rotten
29	ß-Ocimene	Spicy	Spicy, fish, sickly	Fish, sickly
35	3-Hepten-2-one	Mushroom	Mushroom	Mushroom
39	Dimethyl trisulphide	Rotten	Rotten	Rotten
45	2-Methoxy-3-isobutylpyrazine	Bell pepper	Bell pepper	Bell pepper
47	ß-Cyclocitral	BD	BD	Fruity

[a] For an explanation of codes see footnote to Table 2.

[b] BD, below detection.

Table 4. Volatile compounds of rehydrated red bell pepper pieces isolated in the dynamic headspace model system (DH) which decreased significantly in FID peak areas (Student's t-test, $P<0.05$) by artificial saliva components mucin and α-amylase

Peak no	Compound
2	Propanal
5	2-Methylfuran
6	Butanal
8	2-Butanone
9	2-Methylbutanal
10	3-Methylbutanal
14	Pentanal
19	Hexanal
21	1-Methyl-1H-pyrrole
22	1-Butanol
25	Heptanal
26	Limonene
27	3-Methyl-1-butanol
31	1-Pentanol
34	Octanal
36	*cis*-2-Heptenal
37	6-Methyl-5-hepten-2-one
40	Nonanal
42	1-Octen-3-ol
43	*trans,trans*-2,4-Hexadienal
44	Decanal
45	Benzaldehyde
47	ß-Cyclocitral

(*F*(3,138)=6.69, P<0.05). Peak areas of volatiles of bell peppers isolated in model DH containing saliva W and WS did not show mutual difference, but were larger than the peak areas of saliva WSM and WSMA. WSM and WSMA did not reveal mutual difference either. Table 4 presents the volatile compounds isolated in model system DH, which decreased significantly in peak areas (Student's *t*-test, P<0.05) in salivas containing mucin (WSM) as well as mucin in conjunction with α-amylase (WSMA). These results indicate a reduction in flavour release from the diced bell peppers when proteins are present in saliva. The effect of proteins agrees with the studies of Franzen & Kinsella (1974), Kim & Min (1988), Kinsella (1988) and O'Keefe *et al.* (1991). The peak areas of aldehydes decreased significantly in mucin-containing saliva (Student's *t*-test, P<0.05), which could be due to the binding of aldehydes to proteins, as was observed by Gremli (1974). The effect of mucin was not observed in model system PT or DHM, which could be due to disturbance of flavour-protein interactions by mechanical forces during sampling.

Comparison of the sum of the peak area percentages of compounds which eluted within 15 min (Table 5), shows that

Table 5. Sums of percentages of FID peak areas of highly volatile compounds (retention time < 15 min) (mean, *n*=6)

Model system[a]	Artificial saliva[a,b]			
	W	WS	WSM	WSMA
DH	$39^{x,II}$	$38^{x,II}$	$43^{y,III}$	$47^{z,III}$
DHM	$39^{x,II}$	$39^{x,II}$	$42^{y,II}$	$44^{z,II}$
PT	$34^{x,I}$	$34^{x,I}$	$35^{y,I}$	$40^{z,I}$

[a] For an explanation of codes see footnote to Table 2.

[b] x-z, values with different superscripts within a row are significantly different, Student's *t*-test P<0.05. I-III, values with different superscripts within a column are significantly different, Student's *t*-test P<0.05.

highly volatile compounds were relatively better released in the DH model system, followed by DHM and PT, respectively. This was probably due to increased release of less volatile compounds in the DHM/PT models. Comparison of the salivas showed that the relative release of highly volatile compounds was not influenced by salts (WS). Mucin (WSM) and mucin in conjunction with α-amylase (WSMA) caused a relative increase of highly volatile compounds. This could be due to binding of less volatile, more hydrophobic compounds to the proteins, as hydrophobic binding predominates in the flavour-protein interactions (Kinsella, 1988).

CONCLUSIONS

Flavour release from rehydrated diced bell peppers was largest in model system PT, followed by DHM and DH, respectively. Saliva components influenced the flavour release significantly. Release of volatiles, and in particular aldehydes, decreased in mucin-containing salivas, in system DH. Mucin-containing salivas caused a relative decrease in release of the less volatile compounds in all model systems.

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CHAPTER 4

FLAVOUR RELEASE FROM REHYDRATED FRENCH BEANS (*PHASEOLUS VULGARIS*) INFLUENCED BY COMPOSITION AND VOLUME OF ARTIFICIAL SALIVA

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ABSTRACT

Influence of saliva composition and volume on flavour release from rehydrated French beans (*Phaseolus vulgaris*) was studied in three types of mouth model systems; dynamic headspace (DH), dynamic headspace and mastication (DHM) and a purge-and-trap (PT) model system. Volatile compounds were analysed by gas chromatography, using flame ionisation detection (FID), mass spectrometry and sniffing port detection. FID peak areas were largest in the PT system, followed by DHM and DH, respectively. Saliva composition as well as volume influenced the release of volatile compounds from rehydrated French beans. Generally, FID data showed a decrease in release by the saliva component mucin, presumably because of volatile compound-protein interactions and an increase by its α-amylase, probably due to degradation of inclusion complexes of starch. The decrease in flavour release by enlarged saliva volume was evaluated by a model study. Sniffing patterns of odour active compounds were hardly influenced by both saliva composition and volume.

INTRODUCTION

The efficacy of a flavour molecule for olfaction is largely determined by its concentration in the nasopharynx. Stimulation of the olfactory receptors by foods via the retronasal route is a primary determinant of their flavour. Persons who have lost their sense of smell frequently perceive the loss as one of taste, rather than as one of smell (Mozell *et al.*, 1969; Schechter & Henkin, 1974; Doty & Kimmelman, 1986). To elicit an olfactory response, a flavour compound must achieve a sufficient concentration in the vapour phase (Kinsella, 1990). The perceived flavour is affected by differences in the rates and extent of flavour release in the mouth (McNulty, 1987). The rate of volatilization of a compound depends upon the partition coefficient of the compound, molecular interactions between flavour compounds, the ambient temperature, the composition and viscosity of the food material and of the binding to components of the food (Haring, 1990; Kinsella, 1990; Bakker *et al.*, 1994; De Roos & Wolswinkel, 1994). The binding of volatile compounds to food ingredients can have major effects on the perception of the total food product, as it may result in a distortion of the composition of volatile compounds (Overbosch *et al.*, 1991). This distortion can result in changes in appreciation (Van Ruth *et al.*, 1995a).

The physico-chemical environment changes markedly during the eating process, affecting the profile of volatile compounds and the perception of aroma (Linforth & Taylor, 1993). The breakdown of the food matrix through mastication enhances flavour release (Haring, 1990; Van Ruth *et al.*, 1994) as well as retronasal odour perception (Burdach & Doty, 1987). The physical form of foods affects the profile of volatile compounds and it changes during consumption, due to hydration and dilution of foods by saliva. Saliva plays a prominent role in perception of the physical and chemical properties of oral stimuli. In addition to protecting the oral mucosa against physical, chemical or thermal injury, saliva moistens food for bolus formation and provides the necessary lubrication for swallowing (Pangborn & Lundgren, 1978). Substances secreted in saliva including α-amylase, which functions in the initial digestion of starch, could alter the perception of the flavour of the food (McBurney & Pfaffmann, 1963). Previously, the influence of saliva components on the flavour release was shown in rehydrated bell peppers (Van Ruth *et al.*, 1995b). Beside factors influencing the amount and composition of saliva, such as the size of the food particles and variations within a day and day-to-day, there is a wide among-subject variation (Pangborn & Lundgren, 1978).

Extraction and headspace analysis are the main methods for isolation of volatile compounds from vegetables (Buttery *et al.*, 1969; Fischer & Grosch, 1987; Mtebe & Gordon, 1987; Shinohara *et al.*, 1991). The factors influencing flavour release under mouth conditions may create significant differences between the classical headspace profile of volatile compounds and the actual profile in the mouth (Taylor & Linforth, 1994).

Recently the authors introduced a dynamic headspace model system for isolation of volatile compounds from vegetables under mouth conditions, such as volume, temperature, salivation and mastication (Van Ruth *et al.*, 1994). The present study deals with the influence of artificial saliva components, salts, mucin and α-amylase, on the flavour release from rehydrated diced French beans in three types of the mouth model system. A mechanistic model was developed for changes in release of odour active compounds by saliva volume.

MATERIALS AND METHODS

Plant material

Commercially air-dried French beans (*Phaseolus vulgaris*) were supplied in pieces by Top Foods b.v. (Elburg, the Netherlands). The beans were blanched prior to drying. The vegetables were packed in glass jars and stored at 4°C in the absence of light until sampling.

Sample preparation

After storage, the dried beans (1.2 g) were rehydrated prior to analysis, by adding 10 ml distilled water, followed by heating in a waterbath at 100°C for 10 min and cooling down in a waterbath at 25°C for 4 min.

The diced French beans were transferred into the sample flask of the dynamic headspace (DH), dynamic headspace and mastication (DHM) and purge-and-trap mouth model system (PT) as described previously (Van Ruth *et al.*, 1994) and 4 ml of one of the artificial salivas were added, in all saliva composition experiments. Saliva W consisted of distilled water only. Saliva WS consisted of 5.208 g $NaHCO_3$, 1.369 g $K_2HPO_4.3H_2O$, 0.877 g NaCl, 0.500 g NaN_3, 0.477 g KCl and 0.441 g $CaCl_2.2H_2O$ in 1 l distilled water (adjusted to pH 7). The composition of saliva WSM was identical to WS, but 2.160 g mucin (Sigma Chemical Co., St Louis, MO, USA) were added. Saliva WSMA was identical to saliva WSM, but 200,000 units α-amylase (Merck, Darmstadt, Germany) were added. In the saliva volume experiments 0, 0.5, 1, 2, 3, 4, 5, 6, 7 or 8 ml of saliva WSMA were added to the rehydrated French beans. The headspace of the model systems DH and DHM was flushed with purified nitrogen gas in order to trap volatile compounds in Tenax TA, as described previously (Van Ruth *et al.*, 1995b). The plunger of the DHM model system made about four up and down screwing movements per min in order to simulate mastication during isolation of the volatiles. In the PT model nitrogen gas was purged through the vegetable/saliva mixture.

Instrumental flavour analysis

Desorption of volatile compounds from Tenax was performed by a thermal desorption/cold trap device. The compounds were analysed by gas chromatography with flame ionisation detection (GC/FID) as reported previously (Van Ruth & Roozen, 1994).

GC/sniffing port analysis (GC/SP) was carried out as described previously (Van Ruth *et al.*, 1995c). In preliminary GC/SP experiments 10 assessors (aged 20-50) generated odour descriptors for the volatile compounds of rehydrated French beans, which were clustered during group sessions of the panel. These descriptors included bell pepper, burned, caramel, chemical, chocolate, citrus, cooked vegetables, cucumber, detergent, fatty, French bean, fruity, garlic, grassy, herbal, leek, metal, mushroom, onion, rancid, rotten, sickly/musty, sour, spicy and sweet. Completed with 'other/I do not know', one of these descriptors had to be chosen for each compound detected by the assessors at the sniffing port. Tenax tubes without adsorbed volatile compounds were used as dummy samples for determining the signal-to-noise level of the group of assessors.

The volatile compounds trapped in Tenax TA were identified by combined gas chromatography/mass spectrometry (GC/MS) as reported previously (Van Ruth *et al.*, 1995d).

Starch determination

Starch contents were determined in 1.2 g dried French beans and 1.2 g dried red bell peppers after rehydration, using a Boehringer Starch test-kit (no 207748, Boehringer, Mannheim, Germany).

Starch degradation determination

High-performance anion-exchange chromatography (HPAEC) was performed using a Dionex Bio-LC system (Sunnyvale, CA) equipped with a Dionex CarboPac PA-100 (4 x 250 mm) and a Dionex pulsed electrochemical detector in the pulsed amperometric detection mode. In this system degradation products of starch (mono-, di-, tri-, tetra-, penta- and hexamers of glucose) were analysed in 1.2 g dried French beans after rehydration and isolation of volatile compounds, using a gradient of sodium acetate in 100 mM NaOH as follows: 0 to 30 min, 0 to 200 mM; 30 to 45 min, 200 to 600 mM; 45 to 50 min, 1000 mM; 50 to 65 min, 0 mM.

RESULTS AND DISCUSSION

Saliva composition

Volatile compounds of rehydrated French beans were isolated in three mouth model systems (DH/DHM/PT). Model systems DH and PT were studied as they are frequently used in instrumental flavour analysis. Previously it was shown, that release from rehydrated French beans in model system DHM did not differ from their release in the mouths of 12 assessors (Van Ruth *et al.*, 1995d). Four different compositions of artificial saliva were added (W/WS/WSM/WSMA). Fig. 1 and 2, respectively, represent the FID and SP chromatograms of diced rehydrated French beans, to which saliva W or WSMA was added in model system DHM. The volatile compounds were identified by GC/MS and their retention times. They were further characterised by their peak areas and the odour discriptors provided by the assessors at the sniffing port (Table 1 and 2). GC/SP of dummy samples revealed that detection of an odour at the sniffing port by three or less out of 10 assessors can be considered as 'noise'.

Similar volatile compounds were present in the three model

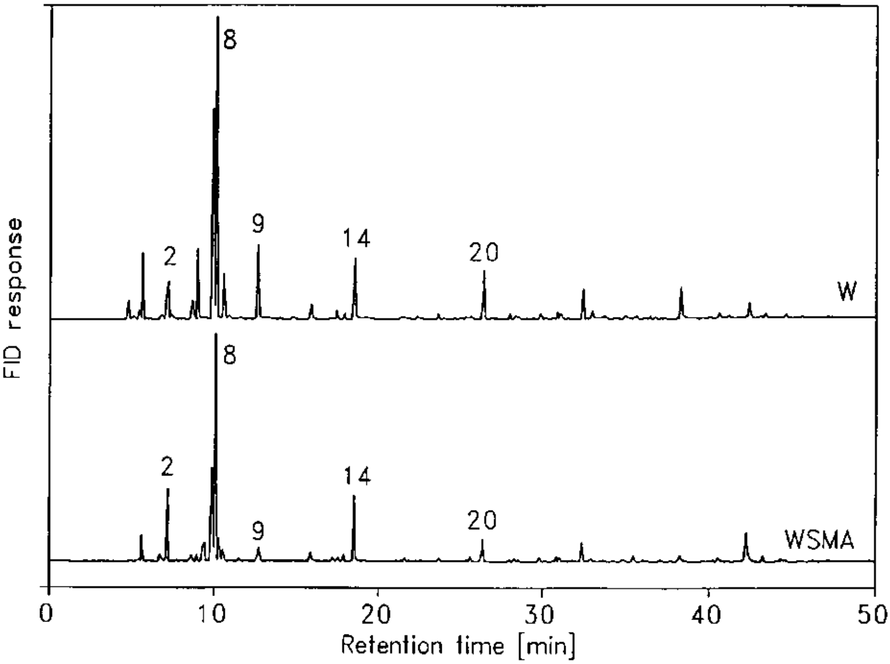

Fig. 1. Chromatograms of volatile compounds of rehydrated French beans in model system DHM with addition of saliva W and WSMA, obtained by flame ionisation detection. For an explanation of codes and numbers see Table 1.

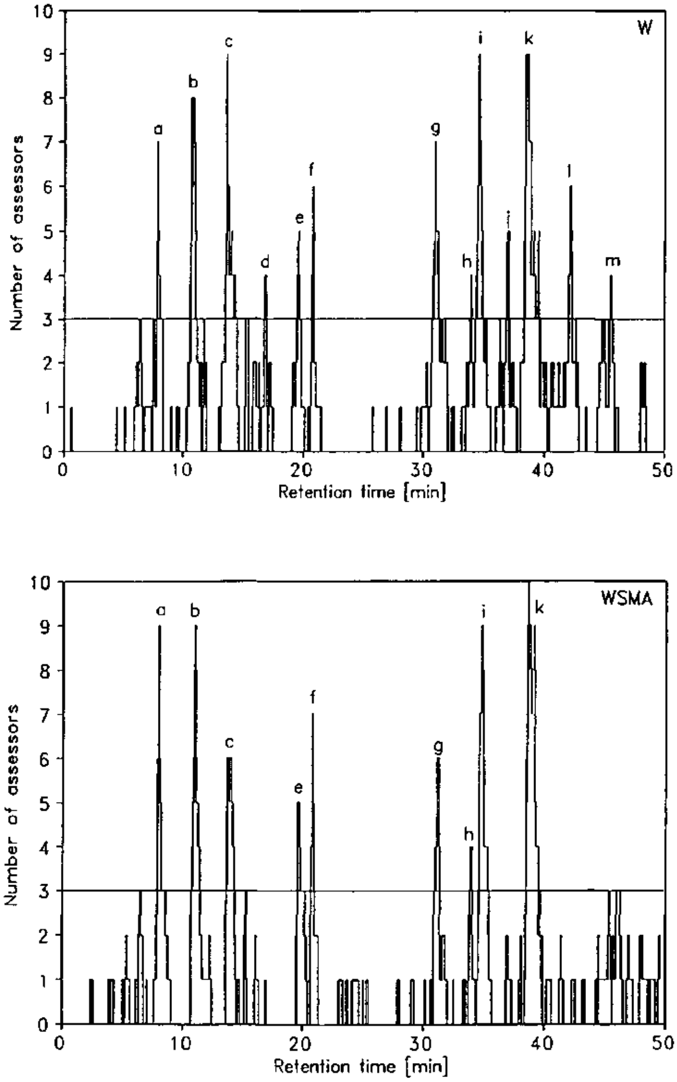

Fig. 2. Chromatograms of volatile compounds of rehydrated French beans in model system DHM with addition of saliva W and WSMA, obtained by sniffing port detection. For an explanation of codes and letters see Table 1 and 2, respectively.

Table 1. Volatile compounds of rehydrated diced French beans (*Phaseolus vulgaris*) isolated in three mouth model systems (DH/DHM/PT) with addition of four compositions of artificial saliva (W/WS/WSM/WSMA), retention times, average peak areas (flame ionisation detection) and coefficients of variance ($n = 6$)[a]

No	Ret. time [min]	Compound	Peak area [V.s]											
			DH				DHM				PT			
			W	WS	WSM	WSMA	W	WS	WSM	WSMA	W	WS	WSM	WSMA
1	6.68	Propanal	0.33	0.23	0.16	0.10	0.79	0.72	0.46	0.66	2.14	1.22	1.17	1.22
2	7.12	2-Methylpropanal	4.74	3.02	2.04	1.69	8.70	8.70	7.25	8.66	26.70	19.59	21.32	21.64
3	7.42	Methyl acetate	0.15	0.12	0.06	0.05	0.32	0.34	0.24	0.27	1.06	0.75	0.75	0.79
4	8.61	Butanal	0.27	0.14	0.12	0.07	0.40	0.39	0.38	0.41	1.04	0.86	1.19	1.03
5	9.22	2-Methyl-2-propenal	0.06	0.08	0.04	0.04	0.36	0.19	0.14	0.24	0.75	0.07	0.01	0.04
6	9.33	2-Butanone	0.59	0.20	0.18	0.13	0.48	0.45	0.46	0.52	0.18	1.11	1.05	1.22
7	9.85	2-Methyl butanal	9.83	2.87	2.06	1.37	9.00	9.60	8.60	9.83	29.79	25.48	26.95	26.94
8	10.04	3-Methyl butanal	11.21	3.62	2.73	1.74	10.52	10.90	9.90	10.49	36.59	30.36	32.57	31.67
9	12.61	Pentanal	2.59	1.20	1.05	0.65	3.20	2.83	2.70	2.66	8.83	6.88	8.56	6.05
10	14.69	1-Penten-3-one	0.03	0.04	0.01	tr	tr	0.01	0.05	0.01	0.13	0.03	0.10	0.04
11	17.09	2,3-Pentane-dione	0.16	0.07	0.06	0.02	0.16	0.15	0.15	0.16	0.48	0.37	0.48	0.44
12	17.38	2-Methyl-1-penten-3-one	0.35	0.20	0.22	0.08	0.37	0.66	0.68	0.54	1.00	1.26	2.80	1.54
13	17.78	Butyl acetate	0.22	0.16	0.09	0.06	0.25	0.17	0.18	0.30	0.51	0.66	0.84	1.00
14	18.44	Hexanal	4.08	1.40	1.17	0.67	3.65	3.34	3.25	3.10	13.82	11.04	13.56	10.97
15	19.11	2-Methyl-2-butenal	0.02	0.01	tr	tr	0.03	0.02	0.04	0.03	0.10	0.10	0.10	0.10
16	21.51	2-Pentenal	0.17	0.07	0.08	0.01	0.19	0.20	0.23	0.15	0.54	0.41	0.69	0.40
17	23.58	1-Penten-3-ol	0.22	0.12	0.13	0.07	0.26	0.23	0.22	0.26	0.64	0.51	0.82	0.66

18	25.29	2-Heptanone	0.04	0.07	tr	tr	0.01	0.05	0.02	0.04	0.23	0.21	0.18	0.17
19	25.52	Heptanal	0.07	0.03	0.02	tr	0.15	0.11	0.10	0.14	0.29	0.20	0.29	0.25
20	27.16	Limonene	0.10	0.05	0.01	tr	0.05	0.04	0.05	0.03	0.29	0.24	0.18	0.18
21	28.38	2-Hexenal	0.47	0.32	0.13	0.04	0.34	0.30	0.23	0.23	1.41	1.16	0.92	0.89
22	31.00	1-Pentanol	0.26	0.15	0.07	tr	0.19	0.16	0.14	0.13	0.83	0.69	0.55	0.51
23	33.55	2-Octanone	0.03	0.04	0.01	tr	0.03	0.03	0.04	0.04	0.12	0.09	0.10	0.08
24	33.65	Octanal	0.06	0.09	0.02	tr	0.04	0.06	0.04	0.03	0.19	0.15	0.13	0.12
25	34.78	*cis*-2-Heptenal	0.24	0.17	0.22	0.07	0.28	0.41	0.46	0.34	0.52	0.67	1.73	0.75
26	36.30	6-Methyl-5-hepten-2-one	0.06	0.07	0.01	tr	0.04	0.05	0.04	0.03	0.18	0.14	0.13	0.13
27	37.68	Dimethyl trisulphide	0.03	tr	tr	tr	tr	tr	tr	tr	0.14	0.10	0.13	0.16
28	38.15	Nonanal	0.16	0.14	0.08	0.10	0.85	0.37	0.35	0.64	0.39	0.27	0.25	0.34
29	39.67	1-Octen-3-ol	0.05	0.07	0.03	tr	0.03	0.06	0.07	0.05	0.17	0.13	0.29	0.13
30	42.10	Decanal	0.17	0.08	0.09	0.10	0.15	0.27	0.24	0.92	0.33	0.15	0.73	0.41
CV [%]			53	21	29	25	27	29	22	23	12	22	13	9

[a] DH, dynamic headspace model; DHM, dynamic headspace and mastication model; *PT*, *purge-and-trap model*; W, distilled water; *WS*, *W+salts*; *WSM*, *WS+mucin*; *WSMA*, *WSM+α-amylase*; *tr*, peak area<0.01V.s;

CV, mean coefficient of variance of individual compounds.

Table 2. Odour active volatile compounds of rehydrated French beans detected in gas chromatography/sniffing port analysis, retention times and odour descriptors

Code	Ret. time [min]	Odour active compound	Odour description
a	8.0	2-Methylpropanal	Chocolate, spicy
b	11.1	2/3-Methyl-butanal	Chocolate, spicy
c	13.6	2,3-Butanedione	Caramel, fatty
d	16.7	1-Penten-3-one	Chemical, sweet, sour, citrus, leek
e	19.5	Hexanal	Grassy, cucumber, bell pepper
f	20.6	2-Methyl-2-butenal	Chemical, fatty
g	31.1	Unknown	Chemical, rotten,rancid
h	33.9	Octanal	Chemical, citrus, fatty, sweet
i	34.9	1-Octen-3-one	Mushroom
j	36.6	Unknown	Rotten, sickly/musty
k	38.6	Dimethyl trisulphide	Metal, rotten, garlic
l	42.0	1-Octen-3-ol	Fatty, sickly/musty, mushroom, metal
m	45.1	Unknown	Cucumber, French bean, citrus, bell pepper

systems and saliva compositions (Table 1). Large differences in peak areas of volatile compounds between the model systems are obvious. For all salivas, model PT showed largest peak areas, followed by DHM and DH, respectively (sign tests, $P<0.05$). This is in agreement with previous results, which showed similar differences between the model systems in flavour release from rehydrated bell peppers (Van Ruth *et al.*, 1994). In particular the addition of mucin decreased the release of volatile compounds in model system DH (sign tests, $P<0.05$). This effect of protein agrees with our previous results concerning the flavour release from rehydrated bell peppers (Van Ruth *et al.*, 1995b) and with studies of Gremli (1974), Damodaran & Kinsella (1980), Kim & Min (1988) and O'Keefe *et al.* (1991). The effect of mucin was observed to a lesser extent in model system DHM and PT, which could be due to disturbance of volatile compound-protein interactions by mechanical forces during sampling.

The presence of α-amylase in artificial saliva (WSMA) in model system DHM increased the peak areas of many volatile compounds of French beans (Table 1). Comparison of rehydrated French beans and red bell peppers (Van Ruth *et al.*, 1995b) revealed that 24 volatile compounds were detected in both vegetables: propanal; 2-methylpropanal; methyl acetate; butanal; 2-butanone; 2- and 3-methylbutanal; pentanal;

Table 3. Number of volatile compounds present in both rehydrated red bell peppers and French beans which showed a change in peak area by addition of α-amylase to artificial saliva[a]

Alteration	French beans	Bell peppers
increase	15	6
decrease	7	18
no change	2	-

[a]Peak area data of rehydrated bell peppers were reported previously (Van Ruth *et al.*, 1995b).

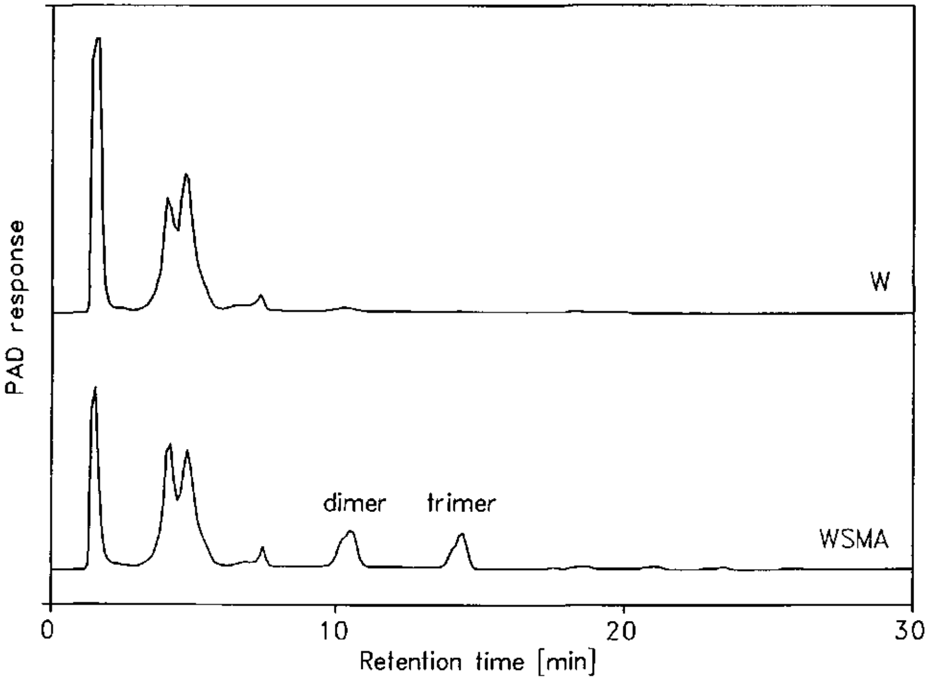

Fig. 3. High performance anion-exchange chromatograms of starch degradation products in rehydrated French beans after isolation of volatile compounds in model system DHM with addition of saliva W and WSMA. For an explanation of codes see footnote to Table 1.

Table 4. Peak areas (pulsed amperometric detection) of starch degradation products, mono-, di-, tri-, tetra-, penta- and hexamers of glucose, in rehydrated diced French beans after isolation of volatile compounds in three mouth model systems (DH/DHM/PT) with addition of four compositions of artificial saliva (W/WS/WSM/WSMA)[a]

	Peak area [V.s]											
	DH				DHM				PT			
	W	WS	WSM	WSMA	W	WS	WSM	WSMA	W	WS	WSM	WSMA
monomer	19.9	17.5	18.6	21.7	22.9	26.7	23.7	24.1	18.6	18.6	17.6	22.4
dimer	0.8	0.9	0.8	5.8	1.0	1.2	0.9	10.9	0.7	0.9	0.8	7.1
trimer	tr	tr	tr	4.1	tr	tr	tr	8.3	tr	tr	tr	5.1
tetramer	tr	tr	0.1	0.1	tr	0.2	tr	0.1	tr	tr	tr	0.2
pentamer	tr	tr	tr	0.1	tr	tr	tr	0.7	tr	tr	tr	0.3
hexamer	tr	tr	tr	0.1	tr	tr	tr	0.3	tr	tr	tr	0.2

[a] For an explanation of codes see footnote to Table 1; tr, peak area<0.1 V.s.

1-penten-3-one; 2,3-pentanedione; hexanal; 1-penten-3-ol; 2-heptanone; heptanal; limonene; 2-hexenal; 1-pentanol; octanal; 2-heptenal; 6-methyl-5-hepten-2-one; dimethyl trisulphide; nonanal; 1-octen-3-ol and decanal. The presence of α-amylase in artificial saliva (WSMA) resulted in French beans generally in increased peak areas of these volatile compounds (Table 3). In contrary, a general decrease in peak areas of these volatile compounds was shown in bell peppers. Their increased release from the French beans could be caused by degradation of inclusion complexes of starch, which were formed during the drying process. The amylose fraction of starch is able to include volatile compounds. Inside the helix structure is a relatively hydrophobic area, in which several ligands (alcohols, aldehydes, terpenes and fatty acids with apolar side chains) can be included. Drying of vegetables involves heating and cooling, which are ideal conditions for the formation of inclusion complexes (Osman-Ismail & Solms, 1972 & 1973). Degradation of inclusion complexes of starch by α-amylase could result in an increase in flavour release.

Fig. 3 represents the HPAEC chromatograms of the breakdown products of starch in rehydrated French beans after isolation of volatile compounds in model DHM, to which saliva W and WSMA was added. Based on the quantities and range of quantifiable maltodextrins (Table 4), the presence of α-amylase increased the degradation of starch in each of the model systems and most in model DHM. This is probably due to the mechanical effect of mastication, on the dried French beans, which contained a high level of starch (17.0 %). In contrary, the dried bell peppers consisted of 0.5 % starch. Therefore, no increase in flavour release due to starch degradation was to be expected in bell peppers. Flavour release from bell peppers was probably reduced by volatile compound-protein interactions of the α-amylase. Similar interactions of α-amylase could also predominate the enzymic effect in model DH, as addition of α-amylase resulted generally in a decrease in peak areas of volatile compounds in this model system (Table 1). In contrary with DHM and PT, no disturbance of these interactions by mechanical forces occurred in model system DH, during sampling. Previous comparison of the three model systems revealed that model system DH demonstrated largest decrease in flavour release from bell peppers by addition of α-amylase to the artificial saliva (Van Ruth *et al.*, 1995b).

Model system DHM simulated well the release of flavour in the mouth (Van Ruth *et al.*, 1995d). Saliva composition could affect flavour perception and therefore GC/SP analyses of rehydrated French beans in model system DHM with addition of the extremes in saliva composition (W and WSMA) were performed. The sniffing and FID chromatograms were compared: a slight decrease in the number of assessors perceiving an odour at the sniffing port was shown in GC/SP (Fig. 2). Odour descriptions were similar for both salivas. In Fig. 1, the FID data showed an overall decrease in peak areas of volatile compounds of the rehydrated French beans in the presence of salt, mucin and α-amylase in the artificial saliva (WSMA). Overall, saliva components seem to decrease flavour release from rehydrated French beans. In earlier work, sniffing port analysis correlated quite well with descriptive sensory analysis (Van Ruth *et al.*, 1995a & c), therefore only a slight effect of the composition of saliva on flavour perception is to be expected.

Saliva volume

The influence of saliva volume (WSMA) on flavour release from rehydrated French beans was studied in model system DHM, as mouth conditions were best simulated by this saliva and model system (Van Ruth *et al.*, 1995d). Table 5 presents the FID peak areas of four odour active compounds (2- and 3-methylbutanal, hexanal and octanal) released from the French beans with addition of different volumes of saliva WSMA.

Table 5. Peak areas (flame ionisation detection) and coefficients of variance (*n*=6) of four odour active compounds of rehydrated French beans influenced by saliva volume and parameter estimates for the description of changes in flavour release (W_r/kX_p and $1/kX_p$)[a]

Saliva volume [ml]	2-methyl-butanal	3-methyl-butanal	hexanal	octanal
0	10.18	14.77	11.90	0.42
0.5	9.05	12.92	10.19	0.25
1	9.21	13.80	9.77	0.28
2	6.20	8.95	7.38	0.13
3	5.98	8.09	7.21	0.15
4	6.11	8.50	8.32	0.15
5	5.81	8.07	7.19	0.12
6	4.91	6.58	6.22	0.11
7	3.66	5.20	4.85	0.08
8	3.63	5.46	4.92	0.08
CV [%]	20	20	18	24
W_r/kX_p	0.095	0.068	0.088	3.061
$1/kX_p$	0.021	0.015	0.014	1.168

[a] The model is described in Results and discussion.

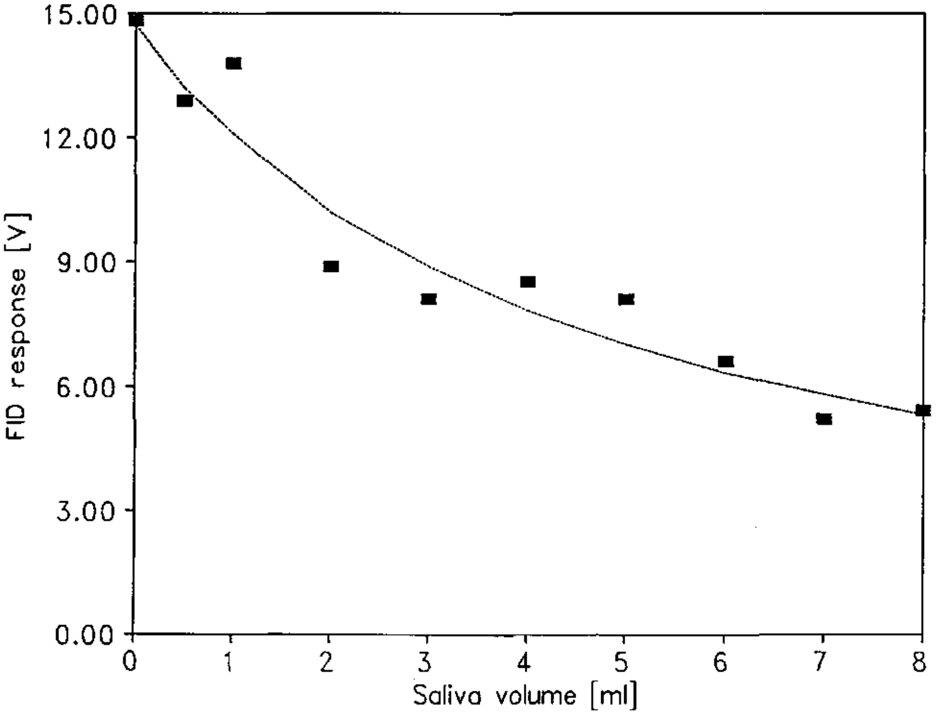

Fig. 4. Peak area (flame ionisation detection) of 3-methylbutanal of rehydrated French beans in model system DHM (■) influenced by saliva volume and (......) the model describing the changes in flavour release by saliva volume.

For an explanation of codes see footnote to Table 1.

McNulty (1987) and De Roos & Wolswinkel (1994) modelled flavour release in the mouth. According to these studies, dilution is not expected to affect product-to-air partition coefficients of volatile compounds as French beans possess low fat contents. The relationship between dilution factor and flavour release is supposed to be reversed proportional. The partition coefficient is defined as:

$$P_{pg}=X_p/X_g$$

where X_p and X_g are the quantities of flavour compounds in the product and gas phase, respectively. Table 5 justifies similar modelling as was proposed by the authors mentioned above and the data were fitted to the model:

$$1/X_g=W_r/kX_p + W_s/kX_p$$

W_r represents the quantity of rehydration water available for dilution, W_s is the quantity of saliva and k a constant. The estimates for W_r/kX_p and $1/kX_p$ are presented in Table 5 too. Both the *FID* responses determined for 3-methylbutanal and the curve of the corresponding model describing the changes in flavour release by saliva volume are shown in Fig. 4. High correlation coefficients were obtained for 2-methylbutanal (0.96), 3-methylbutanal (0.96), hexanal (0.94) and octanal (0.96), which indicated that the data fit well in the model. This implicates that the partition coefficients were hardly influenced by dilution. Saliva acted equivalent to water from a physico-chemical point of view, as was previously suggested by De Roos & Wolswinkel (1994). This confirms preliminary results which demonstrated no difference in flavour release from French beans between the addition of 8 ml saliva and the addition of 1 ml saliva diluted by 7 ml water.

A decrease in the release of flavour compounds from rehydrated French beans up to 70 % by 8 ml saliva could be of interest for flavour perception. Therefore GC/SP analyses of rehydrated French beans in model system DHM were carried out, with and without addition of 8 ml saliva WSMA. Despite of the 70 % FID response reduction, slight changes in GC/SP patterns were observed (data not shown). These results indicate that an effect of saliva volume on flavour perception can hardly be expected.

In conclusion, saliva composition as well as volume influenced flavour release from rehydrated French beans: overall mucin decreased, α-amylase increased and dilution decreased FID responses markedly. However, GC/SP patterns were hardly influenced by both saliva composition and volume.

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CHAPTER 5

VOLATILE COMPOUNDS OF REHYDRATED FRENCH BEANS, BELL PEPPERS AND LEEKS

PART I. FLAVOUR RELEASE IN THE MOUTH AND IN THREE MOUTH MODEL SYSTEMS

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ABSTRACT

Flavour release from three rehydrated vegetables, French beans, red bell peppers and leeks, was studied directly in the mouth of 12 assessors (oral vapour) and in three mouth model systems; purge-and-trap and dynamic headspace with and without mastication. Volatile compounds were analysed by gas chromatography and mass spectrometry, which resulted in 30, 52 and 42 identified compounds in French beans, bell peppers and leeks, respectively. Propanal, 2-methylpropanal, 2- and 3-methylbutanal, pentanal, hexanal, 2-pentenal, *trans*-2-hexenal, 2-heptenal, 2-butanone and 6-methyl-5-hepten-2-one were present in each of them. Flavour release from the three vegetables in model system 'dynamic headspace and mastication' did not differ significantly from release in the mouth. Peak areas of volatiles released in the mouth had larger coefficients of variance than the ones released in the model system. Although assessors released volatile compounds with different efficiencies, they showed a statistically consistent efficiency in flavour release across the vegetables.

INTRODUCTION

Many methods have been developed for analysing the flavour of foods over the last decades. Some methods have been used for quantitative extraction of volatile compounds, e.g. combined steam and organic solvent distillation techniques. Others have tried to link instrumental analysis to sensory properties of foods. Extraction and headspace analysis are the main methods for isolation of volatile compounds in vegetables (Buttery *et al.*, 1969; Wu & Liou, 1986; Fischer & Grosch, 1987; Mtebe & Gordon, 1987; Shinohara *et al.*, 1991).

Foods can be subjected to substantial changes during the eating process which affects the profile of volatile compounds markedly. The breakdown of the food matrix through mastication enhances flavour release (Haring, 1990; Van Ruth *et al.*, 1994) as well as retronasal odour perception (Burdach & Doty, 1987). The generation of volatile compounds, as some plant tissues are disrupted, changes the volatile composition (Fleming *et al.*, 1968; Hatanaka & Harada, 1973; Wu & Liou, 1986; Luning *et al.*, 1994). The physical form of food affects the volatile profile and it changes during consumption, e.g. due to hydration and dilution of (dry) foods by saliva. Saliva components, such as mucin and α-amylase, can influence the flavour release from foods (Van Ruth *et al.*, 1995). These factors may create significant differences between the classical headspace volatile profile and the actual volatile profile in the mouth. The ability to measure this actual profile in the mouth/nose is, therefore, of interest as it relates more directly to the human perception of flavour when food is consumed.

In breath-by-breath analysis the release of volatiles from a food during consumption can be measured near to the nasal space. A membrane separator method using a direct mass spectrometry technique was developed by Soeting & Heidema

(1988) for determining flavour profiles in the expired air of volunteers as a function of time. Lee (1986) developed an instrumental technique using dynamic headspace mass spectrometry to monitor continuously headspace concentrations of a model system. In direct mass spectrometry techniques only one volatile compound can be monitored at a time, although foods are usually more complex. Linforth *et al.* (1994) and Taylor & Linforth (1994), as well as Delahunty *et al.* (1994) reported expired breath sampling techniques using Tenax traps, which were applied to whole foods with lower levels of aromas. A procedure for determining flavour release from chocolate flakes by oral vapour gas chromatography was presented by Roozen & Legger-Huysman (1994).

Previously three mouth model systems were developed to study the effect of different mouth conditions such as volume, temperature, salivation and mastication on flavour release (Van Ruth *et al.*, 1994). The present study deals with flavour released from three rehydrated vegetables; French beans, red bell peppers and leeks in three mouth model systems in comparison with flavour released from them directly in the mouth of 12 assessors.

MATERIALS AND METHODS

Plant material

Three commercially air-dried vegetables; French beans and leeks from the Netherlands and red bell peppers from Hungary were supplied in pieces by Top Foods b.v. (Elburg, The Netherlands). The vegetables were packed in glass jars and stored at 4°C in the absence of light until sampling for 6 weeks max. Pieces of vegetables (1.2 g) were rehydrated by adding 10 ml distilled water, followed by heating in a waterbath at 100°C for 10 min and cooling down in a waterbath at 25°C for 4 min.

Isolation of volatile compounds

The rehydrated diced vegetables were transferred into the sample flask (70 ml) of one of the three mouth model systems; purge-and-trap (PT), dynamic headspace (DH) and dynamic headspace and mastication (DHM). Artificial saliva (4 ml) was added (Van Ruth *et al.*, 1994). This saliva consisted of 5.208 g $NaHCO_3$, 1.369 g $K_2HPO_4.3H_2O$, 0.877 g NaCl, 0.500 g NaN_3, 0.477 g KCl, 0.441 g $CaCl_2.2H_2O$, 2.160 g mucin and 200,000 units α-amylase (Merck, Darmstadt, Germany) in 1 l distilled water (adjusted to pH 7). In the PT model system the vegetable/saliva mixture was purged by purified nitrogen gas (250 ml/min) for 12 min at 37°C, in order to trap the volatile compounds in 0.1 g Tenax TA (35/60 mesh, Alltech Nederland b.v., Zwijndrecht, The Netherlands), packed in a glass tube, 3 mm i.d. and 100 mm long. In the DH and DHM model system, the headspace of the mixture was flushed with nitrogen gas at the same rate, for the same time and at the same temperature as in the PT model system. In DHM the plunger made about four up and down screwing movements/min in order to simulate mastication.

Twelve assessors (aged 20-50) participated in oral vapour analysis. Volatile compounds released from the rehydrated vegetable pieces in oral vapour, were directed from the mouth to a Tenax TA trap by a vacuum pump at 250 ml/min for 12 min as described by Roozen & Legger-Huysman (1994). The assessors were instructed to make four chewing movements at 1 min intervals during mouth sampling.

Analysis of volatile compounds

The volatile compounds were analysed on a Carlo Erba MEGA 5300 gas chromatograph (GC; Interscience b.v., Breda, The Netherlands) equipped with a flame ionisation detector

(FID) at 275°C. The volatiles were desorbed by a thermal desorption (210°C, 5 min)/cold trap (-120°C/240°C) device (Carlo Erba TDAS 5000, Interscience b.v., Breda, The Netherlands) and injected onto a Supelcowax 10 capillary column, 0.25 mm i.d. and 60 m long. Compounds were chromatographed using a linear temperature gradient from 40°C to 92°C at 2°C/min after a 4 min delay; followed by a 6°C/min linear gradient to 272°C.

The volatile compounds trapped in Tenax TA were identified by combined gas chromatography/mass spectrometry (GC/MS; Carlo Erba MEGA 3600, QMD 8000, Interscience b.v., Breda, The Netherlands) equipped with a thermal desorption unit (Carlo Erba Tekmar 5010, Interscience b.v., Breda, the Netherlands). Capillary column and oven temperature program were similar to those for GC/FID analyses. Mass spectra were recorded in the electron impact mode at an ionisation voltage of 70 eV.

Statistical analysis

The FID data presented in the tables, represent the mean value of six (mouth model systems) and 12 replicates (oral vapour). FID data were subjected to a Friedman analysis of variance by ranks, followed by sets of sign tests in order to determine significant differences between model systems and oral vapour. The Friedman analysis of variance was used to determine significant differences between assessors as well. A significance level of $P<0.05$ was used throughout the study.

RESULTS AND DISCUSSION

Volatile compounds of rehydrated French beans, red bell peppers and leeks were isolated in three mouth model systems and in the oral vapour of 12 assessors. Fig. 1 represents the FID chromatogram of volatile compounds of

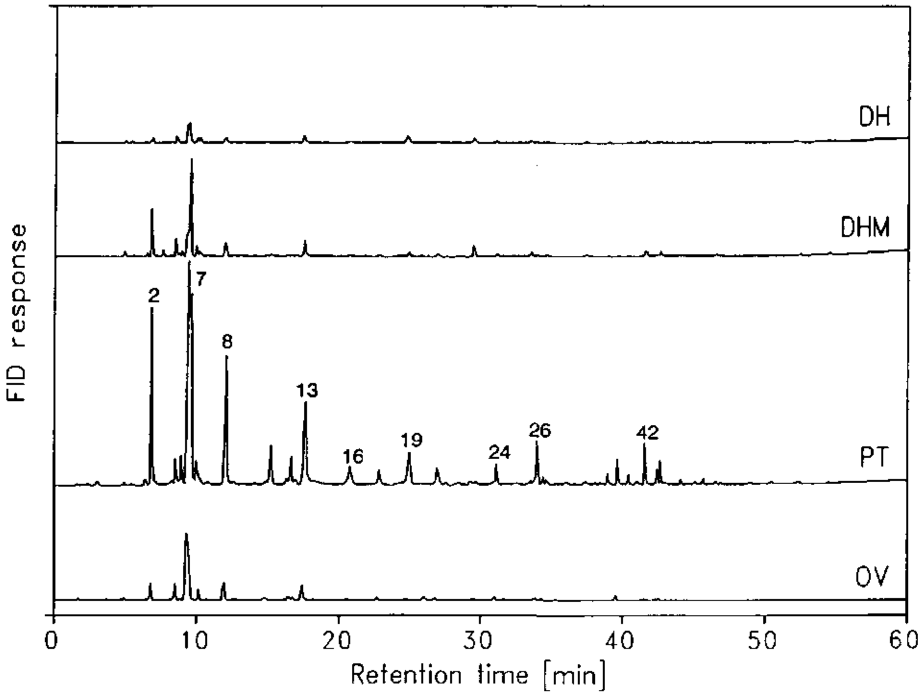

Fig. 1. Chromatograms of volatile compounds of French beans isolated in three mouth model systems (DH/DHM/PT) and in oral vapour (OV). For an explanation of codes see footnote to Table 1, numbers in the PT chromatogram refer to compounds in Table 1.

Table 1. Volatile compounds of rehydrated French beans isolated in three mouth model systems (DH/DHM/PT) and in oral vapour (OV), their retention times, average peak areas and coefficients of variance (DH/DHM/PT:n=6; OV:n=12)

Peak no	Retention time [min]	Compound	Peak area [V.s]			
			DH[a]	DHM	PT	OV
1	6.53	Propanal	0.04	0.31	0.61	0.18
2	6.92	2-Methylpropanal	0.62	3.49	12.62	5.79
3	8.23	Butanal	0.07	0.24	1.33	0.14
4	8.69	2-Methyl-2-propenal	0.07	0.39	0.14	0.13
5	8.94	2-Butanone	0.09	0.39	2.44	0.70
6	9.31	2-Methylbutanal	1.38	5.85	22.13	6.15
7	9.47	3-Methylbutanal	1.83	7.16	31.96	8.22
8	11.96	Pentanal	0.70	1.98	14.97	2.98
9	13.82	1-Penten-3-one	tr[b]	tr	0.22	0.08
10	16.23	2,3-Pentanedione	tr	0.17	1.06	0.16
11	16.49	2-Methyl-1-penten-3-one	0.11	0.32	3.83	0.70
12	16.72	Butyl acetate	0.03	0.22	0.55	0.36
13	17.38	Hexanal	0.84	1.93	16.76	2.36
14	18.19	2-Methyl-2-butenal	tr	0.10	0.27	0.19
15	19.97	Ethylbenzene	tr	0.03	0.18	0 14
16	20.97	2-Pentenal	0.03	0.10	1.54	0.15
17	22.74	1-Penten-3-ol	0.02	0.21	2.11	0.47
18	24.05	2-Heptanone	tr	0.03	0.18	0.04
19	24.18	Heptanal	0.02	0.03	0.34	0.01

20	25.61	Limonene	tr	tr	0.18	1.13
21	26.94	*trans*-2-Hexenal	0.15	0.21	2.73	0.56
22	28.32	6-Methyl-2-heptanone	tr	tr	0.40	0.15
23	31.80	2-Octanone	tr	tr	0.49	0.06
24	32.41	Octanal	tr	0.04	0.19	0.02
25	33.93	2-Heptenal	0.21	0.29	0.42	0.49
26	34.62	6-Methyl-5-hepten-2-one	0.09	0.13	0.87	0.35
27	35.92	Dimethyl trisulphide	tr	0.04	0.18	0.09
28	37.30	Nonanal	0.02	0.37	0.62	0.21
29	39.05	1-Octen-3-ol	tr	0.07	1.21	0.19
30	41.54	Decanal	0.27	0.69	0.24	0.34
		CV[c] [%]	64	27	22	67

[a] DH, dynamic headspace model; DHM, dynamic headspace and mastication model;

PT, purge-and-trap model.

[b] tr, peak area<0.01 V.s.

[c] CV, average coefficient of variance of individual compounds.

Table 2. Volatile compounds of rehydrated red bell peppers isolated in three mouth model systems (DH/DHM/PT) and in oral vapour (OV), their retention times, average peak areas and coefficients of variance (DH/DHM/PT:*n*=6; OV:*n*=12)

Peak no	Retention time [min]	Compound	Peak area [V.s]			
			DH[a]	DHM	PT	OV
1	6.07	Propanal	0.10	1.46	2.68	0.21
2	6.43	2-Methylpropanal	0.10	4.78	16.24	9.25
3	6.71	Methyl acetate	tr[a]	0.06	0.13	tr
4	7.73	Ethyl acetate	0.01	0.45	2.32	0.22
5	7.85	2-Methyl-2-propenal	tr	0.24	0.40	0.02
6	8.17	Diethoxyethane	tr	0.30	0.64	0.21
7	8.41	2-Butanone	0.05	0.28	1.87	0.45
8	8.83	2-Methylbutanal	0.22	2.57	14.18	2.63
9	9.06	3-Methylbutanal	0.45	7.46	39.26	7.03
10	9.40	Benzene	0.52	3.81	2.93	3.43
11	9.60	3-Buten-2-one	tr	0.15	2.18	0.35
12	10.13	2-Pentanone	tr	0.01	1.21	0.01
13	11.28	Pentanal	0.14	1.49	10.17	1.90
14	11.38	2,3-Butanedione	0.13	1.52	10.49	1.75
15	13.28	1-Penten-3-one	tr	0.40	2.31	0.46
16	14.39	Toluene	tr	0.64	1.60	0.86
17	15.48	2,3-Pentanedione	tr	tr	0.29	tr
18	16.08	Butyl acetate	tr	0.14	0.37	0.15
19	17.23	Hexanal	1.69	23.31	171.67	26.86

20	19.82	2-Pentenal	0.47	1.04	6.55	0.79
21	19.98	m-Xylene	tr	0.49	4.29	0.28
22	20.32	Pentyloxirane	tr	0.14	3.11	0.94
23	21.96	1-Penten-3-ol	0.22	1.26	8.68	2.44
24	23.37	2-Heptanone	tr	0.13	3.38	0.40
25	23.54	Heptanal	tr	0.45	2.89	0.47
26	24.01	Limonene	0.12	1.21	13.45	12.49
27	25.94	*trans*-2-Hexenal	tr	0.67	5.93	0.62
28	26.54	1-Methoxy-3-methylene-2-pentanone	tr	tr	1.47	0.28
29	27.25	6-Methyl-2-heptanone	tr	0.03	0.72	0.16
30	27.88	4-Heptenal	tr	0.01	0.18	tr
31	28.30	1-Pentanol	0.26	1.10	4.86	1.23
32	29.20	Methylisopropylbenzene	tr	tr	0.18	0.14
33	30.65	2-Octanone	tr	tr	0.40	0.12
34	30.89	Octanal	0.02	0.50	1.08	0.52
35	31.57	*trans*-3-Hepten-2-one	tr	0.17	1.37	0.18
36	32.48	2,2,6-Trimethylcyclohexanone	0.11	0.28	1.52	0.30
37	33.17	2-Heptenal	tr	0.59	5.36	0.69
38	33.90	6-Methyl-5-hepten-2-one	0.16	0.64	5.81	1.12
39	34.69	1-Hexanol	0.20	tr	4.01	1.91
40	36.71	Nonanal	0.21	2.04	1.71	0.30
41	37.03	3,5,5-Trimethyl-2-cyclohexen-1-one	tr	0.14	1.96	0.40
42	37.34	3-Octen-2-one	tr	0.09	1.21	0.16
43	37.68	5-Ethyl-1-cyclopentene-carbaldehyde	tr	0.17	1.88	0.15

Table 2. (continued)

Peak no	Retention time [min]	Compound	Peak area [V.s] DH[a]	DHM	PT	OV
44	38.34	2-Octenal	tr	0.35	7.66	0.75
45	39.01	1-Octen-3-ol	tr	0.25	3.47	0.60
46	39.78	2,4-Heptadienal isomer	0.14	0.85	7.17	0.62
47	40.88	2,4-Heptadienal isomer	0.56	3.17	11.95	0.97
48	41.42	3-Nonen-2-one	tr	0.12	0.22	0.06
49	41.89	Benzaldehyde	tr	1.94	4.23	0.80
50	41.95	2-Methoxy-3-isobutylpyrazine	tr	tr	tr	tr
51	42.40	Linalool	0.13	0.21	4.45	0.82
52	44.94	ß-Cyclocitral	tr	0.40	5.19	0.87
		CV[a] [%]	64	28	21	82

[a]For an explanation of codes see footnote to Table 1.

Table 3. Volatile compounds of rehydrated leeks isolated in three mouth model systems (DH/DHM/PT) and in oral vapour (OV), their retention times, average areas and coefficient of variance (DH/DHM/PT:*n*=6; OV:*n*=12)

Peak no	Retention time	Compound	Peak area [V.s]			
	[min]		DH[a]	DHM	PT	OV
1	4.92	Hexane	0.09	0.49	1.39	1.00
2	6.10	Propanal	6.70	30.07	105.15	12.30
3	6.49	2-Methylpropanal	0.36	1.47	13.45	8.45
4	6.76	Propanethiol	0.32	0.80	10.56	6.10
5	7.71	Butanal	0.03	0.20	3.92	0.16
6	8.38	2-Butanone	0.04	0.24	2.82	0.48
7	8.75	2-Methylbutanal	0.08	0.63	7.89	0.77
8	8.95	3-Methylbutanal	0.38	2.47	33.34	4.46
9	9.37	Ethanol	1.03	0.74	19.19	0.57
10	9.57	Benzene	0.97	0.73	10.57	3.11
11	10.09	3-Buten-2-one	0.03	0.10	2.76	0.80
12	10.65	2,4-Dimethylfuran	0.02	0.05	1.95	0.04
13	11.45	Pentanal	0.89	3.36	73.04	6.05
14	14.36	2-Butenal	0.16	0.56	11.14	2.92
15	15.86	Dimethyl disulphide	0.10	0.28	3.02	0.98
16	17.31	Hexanal	1.83	9.03	190.94	21.04
17	19.76	2-Methylthiophene	0.25	0.21	4.99	0.20
18	19.94	2-Pentenal	0.22	0.26	4.76	0.21

Table 3. (continued)

Peak no	Retention time [min]	Compound	Peak area [V.s]			
			DH[a]	DHM	PT	OV
19	21.43	2,5-Dimethylthiophene	0.19	0.24	4.45	1.36
20	21.89	2-Methyl-2-pentenal	0.51	1.86	38.98	4.88
21	23.69	2,4-Dimethylthiophene	0.40	0.57	20.16	4.86
22	25.95	*trans*-2-Hexenal	0.09	0.37	7.30	0.81
23	28.09	Dimethylthiophene	1.98	3.07	76.80	16.29
24	29.15	Methyl-1-propenyl disulphide isomer	0.46	0.91	27.30	4.49
25	31.01	Methyl-1-propenyl disulphide isomer	0.78	1.76	55.21	9.83
26	32.52	2,2,6-Trimethylcyclohexanone	0.16	0.12	0.94	0.10
27	33.27	2-Heptenal	0.20	0.38	22.67	1.91
28	33.90	2-Methyl-3-octanone	0.01	0.04	1.08	0.02
29	34.68	6-Methyl-5-hepten-2-one	0.05	0.03	1.85	1.59
30	35.76	Dipropyl sulphide	0.11	0.18	4.39	1.07
31	35.83	Dimethyl trisulphide	0.22	0.36	7.04	1.91
32	37.34	3-Octen-2-one	0.35	0.42	12.13	3.91
33	37.69	Propenyl propyl disulphide isomer	tr[a]	0.08	2.28	0.07
34	38.40	2-Octenal	0.54	0.69	22.21	5.51
35	39.04	Propenyl propyl disulphide isomer	tr	0.14	6.47	1.42
36	39.56	2-Ethylthiacyclohexane	0.06	0.06	2.93	0.70
37	40.34	Diallyl disulphide	0.11	0.13	4.60	1.11
38	40.89	Decanal	0.20	1.12	2.95	0.67

39	42.19	Benzaldehyde	0.37	0.49	5.21	0.14
40	42.37	2-Nonenal	tr	0.05	1.20	0.11
41	44.39	ß-Cyclocitral	0.07	0.29	0.68	0.20
42	49.02	1-Propenyl propyl trisulphide	0.01	0.03	2.41	0.86
		CV[a] [%]	53	30	22	66

[a] For an explanation of codes see footnote to Table 1.

French beans isolated in the three mouth model systems DH, DHM, PT and in oral vapour. FID chromatograms of leeks and bell peppers showed similar differences in patterns between isolation procedures as the French beans. The model system chromatograms of the bell peppers were similar to chromatograms presented previously (Van Ruth *et al.*, 1994). The volatile compounds of rehydrated beans, bell peppers and leeks identified by GC/MS are presented in Tables 1, 2 and 3, respectively. The compounds were further characterised by their retention times and their peak areas. The numbers above the peaks in Fig. 1 refer to the compounds listed in Table 1. In French beans, bell peppers and leeks, respectively 15 out of 30, 20 out of 42 and 37 out of 52 compounds have not been identified previously in the vegetables concerned (Maarse *et al.*, 1989).

The volatile compounds identified in French beans consisted of 16 aldehydes, 8 ketones and 2 alcohols. Self *et al.* (1963) and Kermasha *et al.* (1988) reported both the low-boiling volatiles propanal and methylpropanal in cooked French beans. Propanal, butanal and 2-pentenal were identified in cooked and rehydrated runner beans (MacLeod & Macleod, 1970), while Lovegren *et al.* (1979) identified methylpropanal, 2-butanone, methylbutanal and hexanal in dried beans. Toya *et al.* (1974) and Stevens *et al.* (1967) concluded that 1-octen-3-ol was among the volatile compounds of primary importance in the flavour of canned snap beans. In winged beans, the less volatile compounds such as hexanal, heptanal, octanal and nonanal were reported beside the low-boiling aldehydes (Mtebe & Gordon, 1987).

Decomposition of hydroperoxides, produced by lipoxygenase-catalysed oxidation of unsaturated fatty acids, is known to be a major pathway contributing to the flavour of various legumes. Hexanal and 2-heptanone are produced in these reactions in winged beans (Christopher & Axelrod, 1971). Hexanol, hexanal and 1-octen-3-ol have been reported as principal volatile compounds from linoleic acid in green beans and *trans*-2-hexenal and 1-penten-3-ol can be derived from linolenic acid (De Lumen *et al.*, 1978).

Wu *et al.* (1986) reported several of the 52 volatile compounds identified in the rehydrated diced bell peppers, e.g. ethyl acetate, hexanal, 2-heptanone, heptanal, *trans*-2-hexenal, *trans*-3-hepten-2-one, hexanol, 2-octenal, 1-octen-3-ol, 2,4-heptadienal and 2-methoxy-3-isobutyl-pyrazine. The latter compound possesses a distinct bell pepper aroma (Buttery *et al.*, 1969; Luning *et al.*, 1994; Van Ruth & Roozen, 1994). In general more small, low-boiling compounds were identified in the present study, in comparison with other studies (Keller *et al.*, 1981; Chitwood *et al.*, 1983; Wu *et al.*, 1986). This is probably due to the dynamic headspace technique performed here instead of extraction procedures at elevated temperatures used in the other studies.

Many saturated and unsaturated aldehydes and di- and trisulphides were present among the compounds identified in rehydrated leeks. 1-Propanethiol, 2-methyl-2-pentenal, *trans*-2-hexenal, dimethylthiophene, methyl propenyl disulphide, dimethyl trisulphide and propenyl propyl disulphide have been reported as odour active compounds of leeks (Schreyen *et al.*, 1976a). Several of the aldehydes and di- and trisulphides have been identified in leeks (Schreyen *et al.*, 1976b) as well as in onion (Boelens *et al.*, 1971). Propanal and dimethylthiophenes are the major volatiles of rehydrated leeks besides lipid oxidation products such as hexanal and pentanal. Schreyen *et al.* (1976b) noted propanal and 2-methyl-2-pentenal as the breakdown products of a lachrymatory factor in leeks. Dimethylthiophene isomers can be formed by heating alkenyl disulphides with a loss of hydrogen sulphide.

Comparison of the volatile compounds reveals 11 compounds which are present in each of the three vegetables: propanal; 2-methylpropanal; 2-butanone; 2- and 3-

methylbutanal; pentanal; hexanal; 2-pentenal; *trans*-2-hexenal; 2-heptenal and 6-methyl-5-hepten-2-one. This is in agreement with studies of Self *et al.* (1963), which showed similar patterns of low-boiling volatiles in cooked French beans and leeks, these volatiles varied in relative quantities only. 2-Methylpropanal, 2- and 3-methylbutanal can be formed in Strecker degradation reactions during the drying and cooking process, while most of the other volatiles can be derived from lipid oxidation reactions (Tressl *et al.*, 1981; Frankel, 1991; Whitfield, 1992).

In order to compare the isolation procedures, means of peak areas of volatile compounds released from the French beans, bell peppers and leeks in the three mouth model systems and oral vapour were subjected to a Friedman two-way analysis of variance. Sets of sign tests were carried out to

Table 4. Differences in peak areas of volatile compounds released from rehydrated French beans, bell peppers and leeks in three mouth model systems (DH/DHM/PT) and in oral vapour (OV) examined by Friedman two-way analysis of variance by ranks and sign tests

Vegetable	Friedman test	Sign test			
	X^{2a}	DH[b]	DHM	PT	OV
French bean	70	A[c]	B	C	B
Bell pepper	126	A	B	C	B
Leek	80	A	B	C	B

[a] $X^2>7.8$ indicates a significant difference in flavour release between sampling procedures (Friedman test, $P<0.05$).

[b] For an explanation of codes see footnote to Table 1.

[c] Different letters within a row indicate significant differences (Sign test, $P<0.05$).

Table 5. Differences between assessors in flavour release from French beans, bell peppers and leeks in oral vapour examined by the Friedman two-way analysis of variance by ranks

Vegetable	X^2[a]
French bean	93
Bell pepper	227
Leek	219

[a]X^2>19.7 indicates a significant difference in flavour release between assessors (Friedman test, P<0.05).

Table 6. FID peak areas (V.s) of hexanal released from three vegetables in oral vapour of 12 assessors, ranks in parentheses

Assessor	French bean	Bell pepper	Leek
A	1.0 (1)	7.8 (1)	4.1 (2.5)
B	2.3 (6)	11.7 (2)	0.6 (1)
C	2.5 (7)	14.5 (3)	4.1 (2.5)
D	1.6 (3.5)	16.1 (4)	25.4 (7)
E	1.2 (2)	35.6 (10)	22.7 (4)
F	2.0 (5)	28.0 (6)	24.9 (6)
G	1.6 (3.5)	19.5 (5)	27.9 (9)
H	3.1 (10)	32.2 (8)	25.6 (8)
I	3.0 (9)	30.3 (7)	30.7 (10)
J	2.8 (8)	34.0 (9)	31.6 (11)
K	3.2 (11)	56.4 (12)	22.9 (5)
L	4.0 (12)	36.2 (11)	32.0 (12)
X^2[a]		321	

[a]X^2>19.7 indicates a significant consistent efficiency in flavour release of assessors across the vegetables (Friedman test, P<0.05).

determine the significance of individual differences (Table 4). DHM and OV did not show significant differences in flavour release, although all other isolation procedures differed significantly, for the three vegetables studied. The flavour profile in the mouth is directly related to the human perception of a food. Headspace sampling techniques (without mastication) are often performed for determining volatile profiles of foods. However, significant differences in release between DH and OV, as well as between PT and OV indicate that these techniques are less suitable for simulation of flavour release in the mouth. Similarly, differences in volatile composition of headspace samples and expired breath for strong mints were reported by Linforth & Taylor (1993), for Cheddar cheese by Delahunty *et al.* (1994), for chocolate flakes by Legger & Roozen (1994) and for tomatoes by Linforth *et al.* (1994). Coefficients of variance (Table 1, 2 and 3) were larger for the assessors than for the model systems. Similar coefficients of variance for assessors were determined by Roozen & Legger-Huysman (1994) in oral vapour gas chromatography of chocolate flakes.

Comparison of flavour release of assessors by a Friedman two-way analysis of variance showed significant differences among them for each of the vegetables (Table 5), which is in agreement with studies of Linforth *et al.* (1994). They reported large differences between assessors in quantities of volatile compounds in expired breath during consumption of tomatoes. The FID peak areas of hexanal released from the three vegetables in the oral vapour of the individual assessors were subjected to a Friedman two-way analysis of variance (Table 6). Assessors released hexanal with statistically consistent efficiencies across the vegetables. Similar results were obtained for other mutual major volatile compounds, e.g. 2-methylpropanal, 2- and 3-methylbutanal.

In addition Table 7 presents the average ranks of assessors

Table 7. Average ranks of assessors for FID peak areas of volatile compounds released from three vegetables in oral vapour of 12 assessors as presented in Tables 1, 2 and 3

Assessor	French bean	Bell pepper	Leek
A	5.8	3.8	3.5
B	6.4	3.2	2.6
C	5.8	5.2	2.9
D	4.5	3.5	7.1
E	3.2	5.6	6.2
F	6.3	6.9	5.5
G	6.3	6.0	8.0
H	7.7	8.4	8.0
I	8.2	8.1	7.8
J	7.7	8.6	8.6
K	7.7	9.2	8.7
L	8.4	9.4	9.0

for FID peak areas of the individual volatile compounds released from each of the three vegetables, indicating an overall consistency in efficiency of flavour release across the three vegetables. As assessors were instructed to make four chewing movements/min, differences in flavour release could be due to different efficiency of mastication between people (Pierson & LeMagnen, 1970), as well as to differences in the rate of breathing (Overbosch *et al.*, 1991), the release rate of saliva (Christensen, 1986) and the saliva composition (Shannon *et al.*, 1974).

CONCLUSIONS

Flavour release from rehydrated French beans, bell peppers and leeks in mouth model system DHM, simulated flavour release in oral vapour quite well; no significant difference was determined between the two methods. Although assessors released volatiles with different efficiencies, a consistent efficiency in release rate of assessors was observed across the vegetables.

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CHAPTER 6

VOLATILE COMPOUNDS OF REHYDRATED FRENCH BEANS, BELL PEPPERS AND LEEKS

PART II. GAS CHROMATOGRAPHY/SNIFFING PORT ANALYSIS AND SENSORY EVALUATION

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ABSTRACT

The flavours of rehydrated diced French beans, red bell peppers and leeks were characterised by gas chromatography/sniffing port analysis (GC/SP) of volatile compounds released in a mouth model system and by descriptive sensory analysis. Volatile compounds were identified by combined gas chromatography/mass spectrometry. In French beans, bell peppers and leeks respectively 10, 16 and 22 compounds possessed detectable odours. A common odour profile was shown in the three vegetables. It comprised each of the odour active compounds present in French beans: i.e. 2-methylpropanal (chocolate), 2/3-methylbutanal (chocolate), 2,3-butanedione (caramel, fatty), hexanal (grassy, bell pepper), 2-methyl-2-butenal (chemical), octanal (sweet, sickly/musty, grassy, rancid), 1-octen-3-one (mushroom), dimethyl trisulphide (rotten, metal), 1-octen-3-ol (fatty, sickly/musty, mushroom) and one unknown compound (chemical, rotten, rancid). The three vegetables differed markedly in GC/SP patterns and in scores for sensory attributes. Use of nose-clips diminished the scores for attributes in sensory analysis. In principal component analysis, correlation of rehydrated vegetables with sensory attributes and volatile compounds showed considerable contribution of volatile compounds to the flavour of rehydrated vegetables.

INTRODUCTION

Foods have highly complex chemical compositions, containing both volatile and non-volatile substances. Their sensory responses may be significantly affected by their relative concentrations and the temperature of the product as consumed (Heath, 1981). Human taste-buds are capable of differentiating only four stimuli. As the nose is capable of discerning hundreds of different odours, it is not surprising that a major part of flavour research has dealt with analysis of volatile compounds (Hoff *et al.*, 1978).

An instrumental approach to characterise flavour can be regarded as a two-phase arrangement. The first phase involves representative isolation of volatile compounds, because gas chromatographic (GC) patterns are largely influenced by isolation procedures. Release rates of volatile compounds from a product depend upon partition coefficients of the compound, molecular interactions and ambient temperatures (Legger & Roozen, 1994). The second phase involves selection of volatile compounds relevant to the flavour. This implicates correct determination of the relevant flavour compounds from the whole range of volatiles present in a particular food product (Dirinck & De Winne, 1994). An interesting approach is sniffing the gas chromatographic effluent (GC/sniffing port analysis, GC/SP), in order to associate odour activity with the eluting compounds. Flame ionisation, flame photometric, electron capture, mass spectrometric, fourier transform, infrared and thermal conductivity detectors are not as sensitive as the human nose for many odourants (Acree & Barnard, 1994). The interest in determining the individual contribution of volatile compounds present in foods, has led to a new generation of GC/SP techniques, which can be classified into four categories.

(1) Dilution analysis methods for producing titer or potency values based on stepwise dilution to threshold e.g. CHARM (Acree *et al.*, 1984) and aroma extraction dilution analysis (Ullrich & Grosch, 1987).

(2) Response interval methods for recording time duration of perceived odours and the number of assessors with odour perception, it also estimates a titer or potency (Linssen *et al.*, 1993).

(3) Time-intensity methods for producing subjective estimates of perceived intensity recorded simultaneously with the elution of the chromatographic peak, e.g. OSME (Sanchez *et al.*, 1992).

(4) Posterior intensity methods for producing subjective estimates of perceived intensity, which are recorded after a peak has eluted (Casimir & Whitfield, 1978).

Correlation between sensory and instrumental flavour data has been attempted by several multivariate statistical techniques, like factor and principal component analyses (Noble, 1978). Such techniques have been used to reduce large numbers of sensory and instrumental variables to a smaller number of factors, or to study underlying patterns in the variables (Palmer, 1974; Guedes de Pinho *et al.*, 1994; Luning *et al.*, 1994a).

Recently, the authors reported a dynamic headspace model system for isolation of volatile compounds from vegetables under mouth conditions. Using this model system, release of volatiles from rehydrated French beans, red bell peppers and leeks did not differ significantly from their release in the mouth of 12 assessors (Van Ruth *et al.*, 1995). The present study deals with the relationship between GC/SP detected volatile compounds released in the reported mouth model system from rehydrated French beans, red bell peppers and leeks and the sensory properties of these vegetables. This relationship was examined by multivariate statistical techniques.

MATERIALS AND METHODS

Plant material

Commercially dried French beans and leeks from the Netherlands and red bell peppers from Hungary were supplied in pieces by Top Foods b.v. (Elburg, The Netherlands). The vegetables were packed in glass jars and stored at 4°C in the absence of light until sampling. Diced dried vegetables (1.2 g) were rehydrated by adding 10 ml distilled water, followed by heating in a waterbath at 100°C for 10 min and cooling down in a waterbath at 25°C for 4 min.

Instrumental analysis

The rehydrated vegetables were transferred into a dynamic headspace mouth model system with mastication device. Artificial saliva (4 ml) was added and the headspace was flushed with nitrogen gas (250 ml/min) for 12 min in order to trap volatiles in Tenax TA as described previously (Van Ruth *et al.*, 1995). A plunger made about four up and down screwing movements/min to simulate mastication. In GC/SP, desorption of volatile compounds from Tenax was performed by a thermal desorption (210°C, 5 min)/cold trap (-120°C/240°C) device (Carlo Erba TDAS 5000, Interscience b.v., Breda, The Netherlands). Gas chromatography was carried out on a Carlo Erba MEGA 5300 (Interscience b.v., Breda, The Netherlands) equipped with a Supelcowax 10 capillary column, 0.25 mm i.d. and 60 m long and a flame ionisation detector (FID) at 275°C. At the end of the capillary column the effluent was split 1:2:2 for FID, sniffing port 1 and sniffing port 2, respectively. Oven temperature programme and sniffing port detection were performed as reported previously (Van Ruth & Roozen, 1994). In preliminary GC/SP experiments, 10 assessors (aged 20-40) generated flavour descriptors for rehydrated French beans,

Table 1. Comparison of the descriptors of the gas chromatography/sniffing port panel (GC/SP) and the attributes of the analytical sensory panel for three rehydrated vegetables

Descriptors (GC/SP panel)	Attributes (sensory panel)
Bell pepper	Bell pepper
	Bitter
Burned	Burned
Caramel	
Chemical	Chemical
Chocolate	
Citrus	Citrus
Cooked vegetables	Cooked vegetables
Cucumber	
Detergent	
Fatty	
French bean	French bean
Fruity	
Garlic	
Grassy	Grassy
Herbal	
Leek	Leek
Metal	
Mushroom	Mushroom
Onion	Onion
Rancid	
Rotten	Rotten
	Sharp
Sickly/musty	
Sour	Sour
Spicy	Spicy
Sweet	Sweet

bell peppers and leeks, which were clustered after group sessions of the panel. Besides 'other/I do not know', one of these descriptors (Table 1) had to be used for each compound detected by the assessors at the sniffing port. Tenax tubes without adsorbed volatile compounds were used as dummy samples for determining signal-to-noise level of the group of assessors.
The volatile compounds trapped in Tenax TA were identified by combined GC (Varian 3400, Varian, Walnut Creek, CA, USA) and mass spectrometry (MS; Finnigan MAT 95, Finnigan MAT, Bremen, Germany) equipped with a thermal desorption/cold trap device (TCT injector 16200, Chrompack b.v., Middelburg, The Netherlands). Capillary column and oven temperature program were the same as those used in GC/SP analyses. Mass spectra were obtained with 70 eV electron impact ionisation, while the mass spectrometer was continuously scanning from m/z 24 to 400 at a scan speed of 0.7 s/decade (cycle time 1.05 s).

Sensory evaluation

A panel of 21 judges (aged 20-55) was selected and trained for quantitative descriptive analysis (QDA). A computer interactive interviewing system for composing questionnaires was used to gather survey information (Ci2 system, Sawtooth Software Inc., Ketchum, ID, USA). Samples of each of the diced dried vegetables (1.8 g) were rehydrated by adding 15 ml water and heating in a waterbath at 100°C for 10 min. They were cooled down and stored in a waterbath at 50°C until they were served to the panel and assessed for flavour evaluation. Flavour attributes were generated during training sessions, which resulted in a list of 16 attributes (Table 1). Sensory attributes of each of the rehydrated diced vegetables were evaluated with and without use of nose-clips (Jaeger Nederland b.v., Breda, The Netherlands) by scoring perceived intensities on a

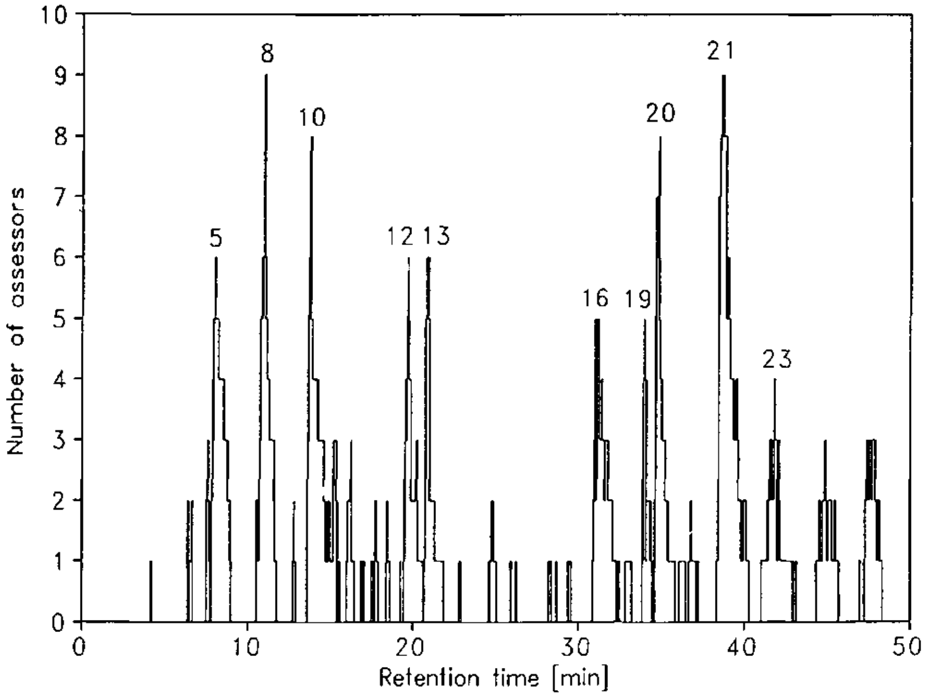
70 mm visual analogue scale on a portable computer screen.

Statistical evaluation

Sensory data were subjected to Student's t-tests to determine significant differences between the three vegetables. The SPSS/PC+ program (SPSS Inc., Chicago, IL, USA) was used for principal component analysis (PCA) of the sensory data as well as for correlation of instrumental and sensory data. A significance level of $P<0.05$ was used throughout the study.

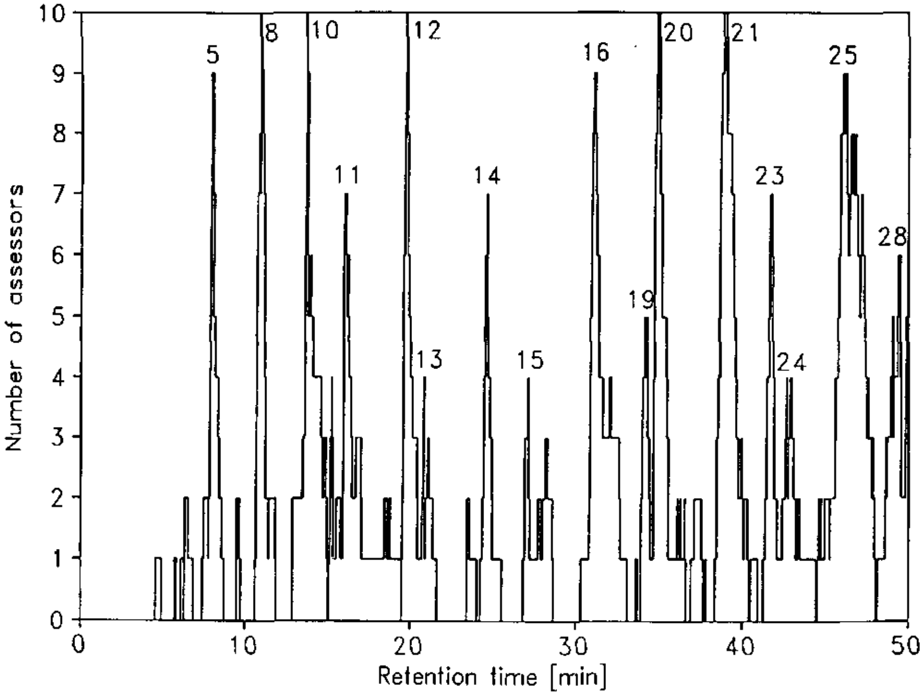
RESULTS AND DISCUSSION

Volatile compounds of rehydrated diced French beans, bell peppers and leeks were isolated in the mouth model system and analysed by GC/SP. Fig. 1 (a)-(c) represents the sniffing chromatograms of each of the three vegetables. The volatile compounds were identified by GC/MS and their retention times and characterised by their FID peak areas and the odours described by the assessors of the sniffing panel (Table 2). GC sniffing of dummy samples showed that detection of an odour at the sniffing port by three or less out of 10 assessors can be considered as 'noise'. GC/SP revealed that 10, 16 and 22 volatile compounds isolated from French beans, bell peppers and leeks, respectively, possessed detectable odours. Although differences in GC/SP patterns across the vegetables were obvious, a common GC/SP profile was shown in the three vegetables. It incorporated all odour active compounds isolated from French beans: 2-methylpropanal (chocolate), 2/3-methylbutanal (chocolate), 2,3-butanedione (caramel, fatty), hexanal (grassy, bell pepper), 2-methyl-2-butenal (chemical), unknown (chemical, rotten, rancid), octanal (sweet, sickly/musty, grassy, rancid), 1-octen-3-one (mushroom), dimethyl trisulphide (rotten, metal) and



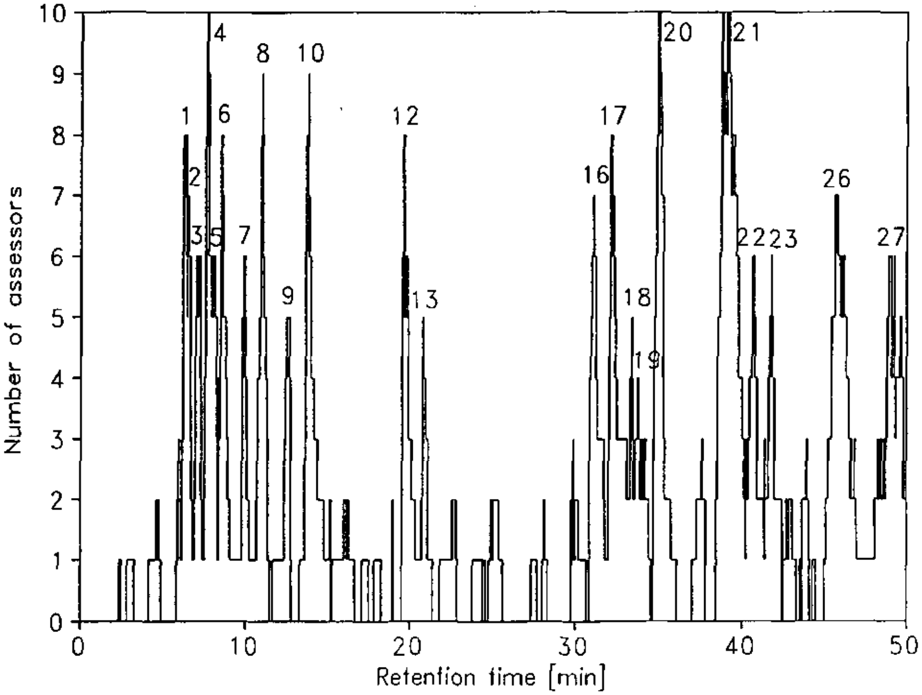
(a)

Fig. 1. Sniffing chromatograms of volatile compounds of rehydrated diced (a) French beans, (b) bell peppers and (c) leeks. Numbers in the chromatograms refer to compounds in Table 2.



(b)

Fig. 1. (continued)



(c)

Fig. 1. (continued)

Table 2. Odour active volatile compounds of rehydrated diced French beans, bell peppers and leeks, their retention times and odour descriptions

No	Ret. time [min]	Compound	Odour description		
			French beans	Bell peppers	Leeks
1	6.3	Methanethiol	-	-	Rotten
2	6.7	Carbon disulphide	-	-	Sweet, chemical
3	7.2	Dimethyl sulphide	-	-	Rotten, cooked vegetables, leek, spicy
4	7.7	Propanal	-	-	Cooked vegetables, grassy, chemical
5	8.0	2-Methylpropanal	Chocolate	Chocolate	Chocolate
6	8.7	1-Propanethiol	-	-	Onion
7	10.0	2-Propene-1-thiol	-	-	Garlic
8	11.1	2/3-Methylbutanal	Chocolate	Chocolate	Chocolate, spicy
9	12.5	3-(Methylthio)-1-propene	-	-	Onion, rotten, herbal, chemical
10	13.9	2,3-Butanedione	Caramel, fatty	Caramel	Caramel
11	16.1	1-Penten-3-one	-	Chemical, metal	-
12	19.8	Hexanal	Grassy, bell pepper	Grassy, bell pepper	Grassy
13	20.9	2-Methyl-2-butenal	Chemical	Chemical, metal, leek	Rancid, cooked vegetables, onion
14	24.9	Unknown	-	Citrus, fruity, rotten	-
15	27.2	2-Heptanone/ Heptanal	-	Bell pepper, cooked vegetables, cucumber	-
16	31.5	Unknown	Chemical, rotten, rancid	Rotten, rancid, sickly/musty, chemical, citrus, leek	Rancid, rotten, chemical, sickly/musty, onion
17	32.6	*cis*-1-Methyl-propenyl disulphide	-	-	Chemical, onion, rotten, herbal, burned

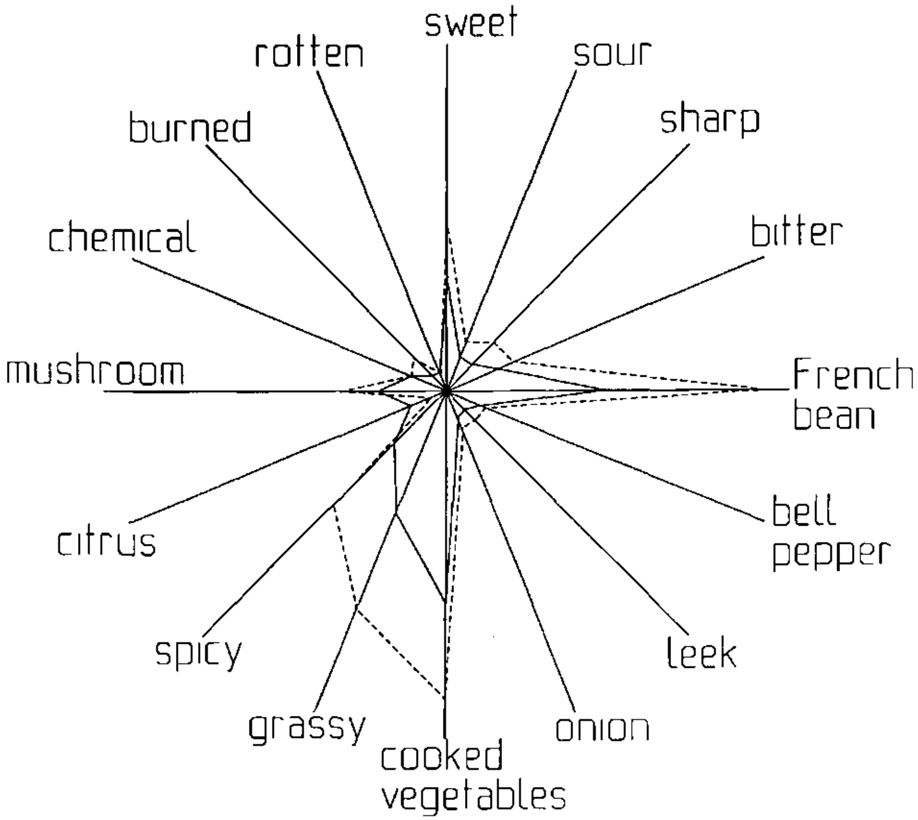
18	33.4	*trans*-1-Methyl-propenyl disulphide	-	-	Rotten, garlic, leek
19	34.0	Octanal	Sweet, sickly/musty, grassy, rancid	Citrus, cucumber, leek	Fatty, citrus, sweet
20	35.2	1-Octen-3-one[a]	Mushroom	Mushroom	Mushroom
21	39.3	Dimethyl trisulphide	Rotten, metal	Rotten, metal	Rotten, onion, leek, metal
22	40.6	Propenyl propyl disulphide	-	-	Herbal, garlic, leek, fatty
23	41.7	1-Octen-3-ol	Fatty, sickly/musty, mushroom	Sickly/musty, fatty, rancid, bell pepper	Fatty, herbal, leek
24	42.7	2,4-Heptadienal	-	Fatty, sickly/musty	-
25	46.1	2-Methoxy-3-isobutylpyrazine	-	Bell pepper	-
26	46.6	Unknown	-	-	Onion
27	49.1	Dipropyl trisulphide	-	-	Onion, garlic
28	49.6	2,4-Nonadienal	-	Cucumber, cooked vegetables, grassy	-

[a] 1-Octen-3-one eluted simultaneously with 3-hepten-2-one in bell peppers.

1-octen-3-ol (fatty, sickly/musty, mushroom). The presence of 2-methylpropanal and 2/3-methylbutanal is typical for dried vegetables. These volatile compounds can be formed by Strecker degradation of valine and (iso)leucine during the drying process. The other components of the common odour profile can be formed mostly in lipid oxidation reactions (Tressl *et al.*, 1981; Whitfield, 1992). Most compounds of the profile possess low thresholds in water (<5 mg/kg) (Leffingwell & Leffingwell, 1991).

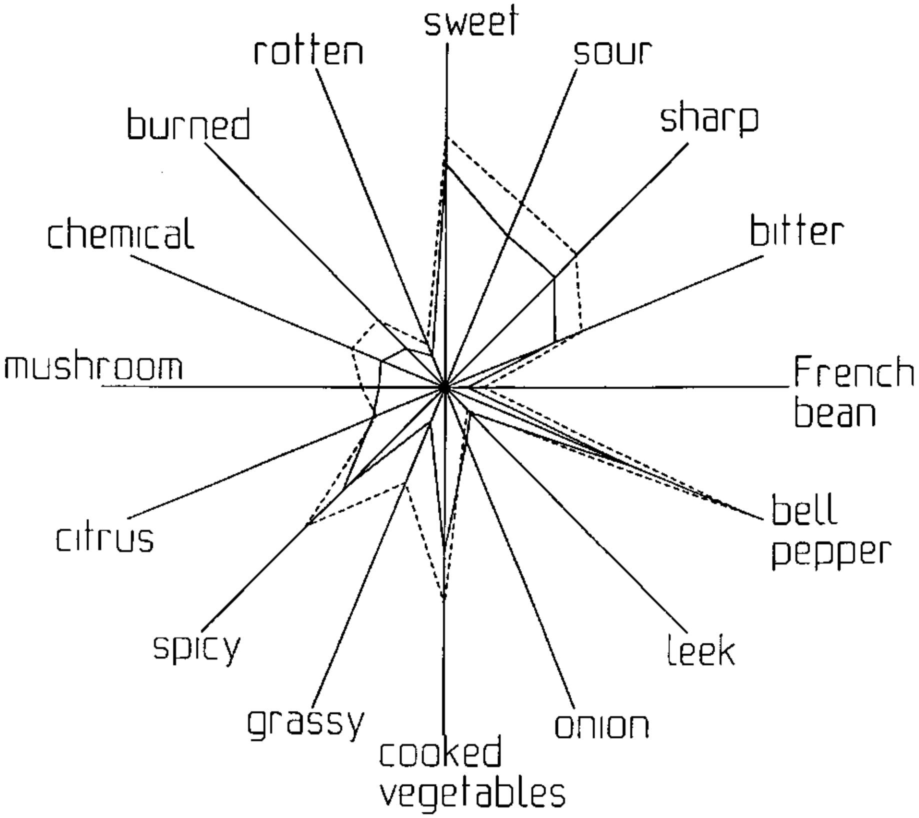
Many of the compounds with detectable odours were identified in Part 1 of the present study (Van Ruth *et al.*, 1995). Some of these compounds contribute to the flavour of the vegetables, despite their very low FID responses. 2-Methoxy-3-isobutylpyrazine is present in the rehydrated bell peppers and has a characteristic bell pepper odour. Several sulphur-containing volatile compounds in leeks possess leek odours. However, no volatile compounds identified in rehydrated French beans possessed a particular French bean flavour. The odour active compounds 2-methylpropanal and 2/3-methylbutanal, present in the French beans, have been reported in dried beans previously (Lovegren *et al.*, 1979). Stevens *et al.* (1967) and Toya *et al.* (1974) reported that 1-octen-3-ol belongs to the most important flavour compounds of canned snap beans. Several of the odour active compounds of bell peppers were reported previously (Wu *et al.*, 1986; Luning *et al.*, 1994a). The bell pepper odour of 2-methoxy-3-isobutylpyrazine is well known (Buttery *et al.*, 1969; Luning *et al.*, 1994b). Sulphur-containing compounds are the main odour active compounds of rehydrated leeks. Several of these compounds were reported by Schreyen *et al.* (1976a & b), i.e. 1-propanethiol, methyl propenyl disulphide, propenyl propyl disulphide and dipropyl trisulphide, each of them possessing leek/onion odours.

An analytical sensory panel of 21 judges evaluated the rehydrated diced French beans, red bell peppers and leeks.



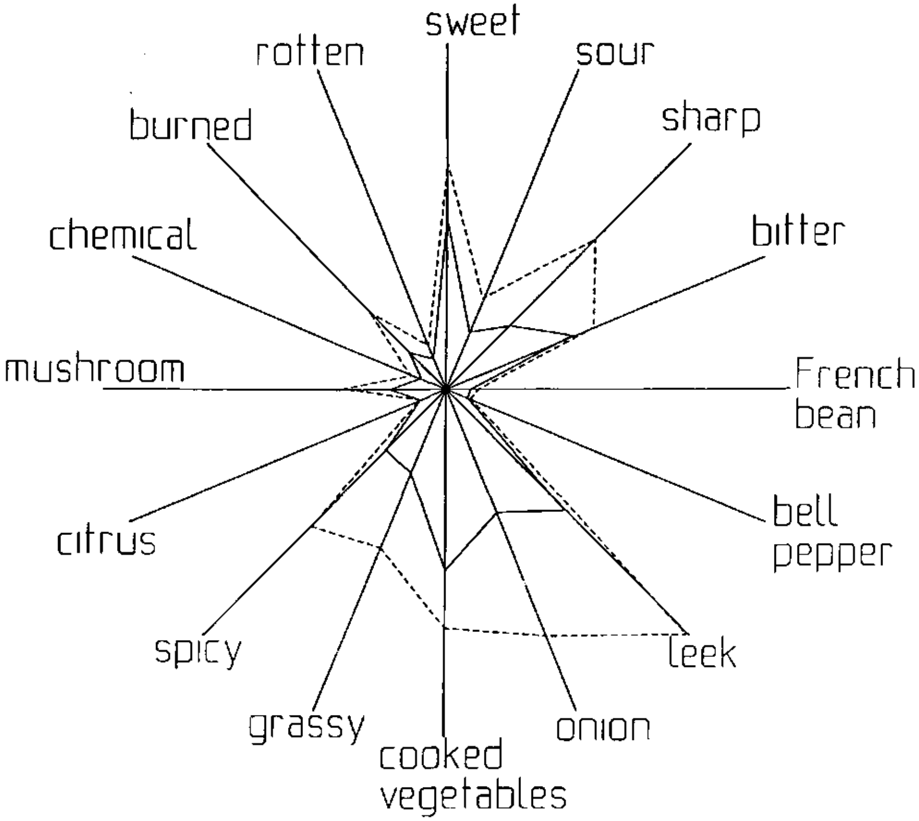
(a)

Fig. 2. Spider-web diagrams of scores for sensory attributes of rehydrated diced (a) French beans, (b) bell peppers and (c) leeks assessed (———) with and (-----) without nose-clips.



(b)

Fig. 2. (continued)



(c)

Fig. 2. (continued)

Although individual judges responded differently to the three vegetables, the panel was consistent across replications. Most of the attributes generated by the sensory panel were similar to the descriptors of the GC/SP panel (Table 1). Apparently, volatile compounds contribute to the flavour of French beans, bell peppers and leeks. The results of the sensory evaluation of each of the vegetables are presented in spider-web diagrams (Fig. 2(a)-(c)). Use of nose-clips diminished scores for sensory attributes in general, which is in agreement with packaging migration studies of Linssen *et al.* (1991). This confirms our suggestion that the contribution of volatile compounds to the flavour of the vegetables is significant. As expected, the scores for taste attributes, like 'sweet', 'sour', 'sharp' and 'bitter', as well as for the particular vegetable less relevant attributes (indicated by low intensity scores without use of nose-clips) decreased less than others.

Mean scores for the sensory attributes for each of the vegetables and the statistical significances of mutual differences (Student's *t*-test, $P<0.05$) are shown in Table 3. Intensity scores for the bell peppers were similar to those reported previously (Van Ruth & Roozen, 1994). French beans scored high in 'French bean', 'cooked vegetables', 'grassy', 'sweet' and 'spicy' attributes, the bell peppers in 'bell pepper', 'sweet', 'cooked vegetables', 'spicy', 'sour', 'sharp' and 'bitter' and leeks in 'leek', 'onion', 'cooked vegetables', 'sweet', 'sharp', 'spicy' and 'grassy'. The three vegetables did not differ significantly in scores for the attributes 'spicy' and 'mushroom', however, each of the other attributes revealed significant mutual differences between the vegetables. Sensory data were subjected to PCA, the first five principal components of which are shown in Table 4. PCA shows relationships between attributes: 'leek' and 'onion' loaded high on the first component, indicating that

Table 3. Scores for sensory attributes of rehydrated diced French beans, bell peppers and leeks assessed without nose-clips (mean, n=21)[a]

Code	Attribute	French beans	Bell peppers	Leeks
A	Sweet	30.2a	42.6b	39.3b
B	Sour	9.1a	35.8b	16.6a
C	Sharp	11.8a	33.4b	37.4b
D	Bitter	12.6a	26.4b	28.1b
E	French bean	56.6b	7.2a	6.3a
F	Bell pepper	7.1a	59.0b	4.9a
G	Leek	5.6a	5.9a	60.9b
H	Onion	5.5a	8.8a	47.5b
I	Cooked vegetables	54.0b	38.4a	42.3a
J	Grassy	41.1c	18.3a	30.0b
K	Spicy	28.7a	36.0a	34.3a
L	Citrus	4.0a	12.9b	4.5a
M	Mushroom	19.4a	16.2a	17.5a
N	Chemical	7.1a	17.5b	7.5a
O	Burned	7.9a	16.8b	19.3bc
P	Rotten	3.6a	8.1ab	8.2b

[a]Different following letters within a row indicate significant differences (Student's *t*-test, $P<0.05$).

these attributes were strongly associated with each other. 'Sweet', 'sour', 'citrus' and 'bell pepper' loaded high on the second component. Two associated attributes were determined for each of the other three components.

PCA was performed on combined GC/SP (number of assessors perceiving an odour active compound) and QDA sensory data sets (intensities of sensory attributes perceived without nose-clips) (Fig. 3). The first two principal components explained 75 % of the variance in the data sets. Vegetable sample scores in the PCA map show that leeks (LEEK) were

Table 4. Attributes of the first five principal components concerning rehydrated diced French beans, bell peppers and leeks (loadings in parentheses)

First 25%[a]	Second 16%	Third 13%	Fourth 9%	Fifth 7%
Leek (0.9)	Sweet (0.7)	Grassy (0.9)	Burned (0.8)	Mushroom (0.7)
Onion (0.9)	Sour (0.7)	Cooked vegetables (0.8)	Chemical (0.7)	Rotten (0.7)
	Citrus (0.7)			
	Bell pepper (0.7)			

[a]Percentage of explained variance.

separated from bell peppers (BELL) and French beans (BEAN) along the first component. BELL and LEEK were separated along the second component. The diagram reveals three sites in which vegetable sample, volatile compounds and sensory attributes are closely related to each other. The following numbers and capitals in parentheses refer to volatile compounds in Table 2 and sensory attributes in Table 3, respectively. 'French bean' (E) and 'cooked vegetables' (I) showed high negative loadings on both the first and second component and correlated well with BEAN. BELL correlated well with 2-methoxy-3-isobutyl-pyrazine (25) and 'bell pepper' (F). The compound 2-methoxy-3-isobutylpyrazine was described by the GC/SP panel as bell pepper. Sensory attributes 'onion' (H) and 'leek' (G), as well as several volatile compounds having leek or onion odours according to GC/SP, have high loadings on the first component and low

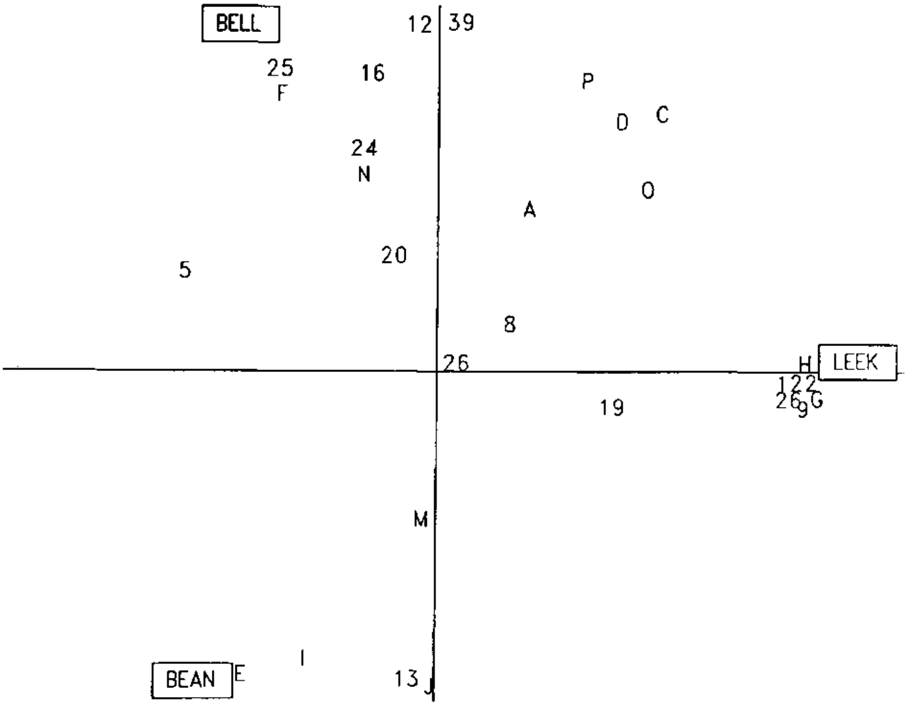


Fig. 3. Scores of vegetable samples (BEAN, French beans; BELL, bell peppers; LEEK, leeks) and loadings of volatile compounds and sensory attributes on the first (horizontal) and second (vertical) principal component axes. Numbers refer to volatile compounds in Table 2 and capitals to sensory attributes in Table 3.

loadings on the second. These sensory attributes and volatile compounds, among which are methanethiol (1), 3-(methylthio)-1-propene (9) and propenyl propyl disulphide (22), correlated with LEEK. Correlation between the rehydrated vegetables, sensory attributes and several volatile compounds having corresponding odours according to GC/SP, showed that volatile compounds contribute considerably to the flavour of rehydrated vegetables. This is in agreement with studies of Noble (1978), in which a correlation between sensory and instrumental data of wines was determined and Rothe *et al.* (1994), who reported relationships between volatile compounds and Blue cheese flavour. Investigations of Teule & Crouzet (1994) showed a relationship between volatile compounds and formation of cooked aroma in dehydrated apple products. Burdach & Doty (1987) reported that persons who have lost their sense of smell, frequently perceive this loss as one of taste, rather than as one of smell. This indicates that stimulation of the olfactory receptors by foods via the retronasal route is a primary determinant of their flavour. In GC/SP the volatile compounds are sampled separately by the assessors in GC elution sequence. Nevertheless the instrumental data of GC/SP of volatiles released in a mouth model system with mastication device correlated quite well with sensory data.

CONCLUSIONS

The presence of a common odour profile was shown in the three rehydrated vegetables by GC/SP. This profile incorporates each of the odour active compounds of rehydrated French beans. Correlation between rehydrated vegetables, sensory attributes and several volatile compounds isolated in a mouth model system, showed major contribution of volatile compounds to the flavour of rehydrated vegetables.

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CHAPTER 7

GAS CHROMATOGRAPHY/SNIFFING PORT ANALYSIS AND SENSORY EVALUATION OF COMMERCIALLY DRIED BELL PEPPERS (*CAPSICUM ANNUUM*) AFTER REHYDRATION

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ABSTRACT

Cultivated, cut and commercially dried red bell peppers (origins Chile, Hungary and Turkey) were rehydrated and then evaluated by descriptive and hedonic panels. The types of pepper did not differ significantly in sensory values for the attributes 'cucumber', 'cooked vegetables', 'burned', 'mushrooms', 'bell pepper', 'fruity', 'grassy/green vegetables', 'fresh', 'spicy' and 'sweet'. The bell peppers of Chilean origin were rated higher in 'sour', 'bitter', 'sharp' and 'pungent' attributes than the Turkish and higher in 'bitter' and 'pungent' attributes than the Hungarian. The Hungarian rated higher in 'sour' than the Turkish. Volatile compounds were analysed by gas chromatography, using flame ionisation detection, mass spectrometry and sniffing port detection. Forty-six compounds were identified, 12 of which possessed odours: 2-methylpropanal (chocolate); 2- and 3-methylbutanal (chocolate); 2,3-butanedione (caramel/ butter); 1-penten-3-one (plastic/chemical); hexanal (grassy/green); heptanal (lemon/orange); ß-ocimene (fish/rotten/sickly); *trans*-3-hepten-2-one (mushrooms); dimethyl trisulphide (rotten/ onion/leek); 2-methoxy-3-isobutyl-pyrazine (bell pepper) and ß-cyclocitral (fruity). The compositions of the volatiles were similar for the three origins, although the Hungarian one generally exhibited largest peak areas. The bell peppers from Chile were appreciated more by the panel than the ones from Turkey. This was probably due to the higher intensity of taste attributes ('sour', 'bitter', 'sharp' and 'pungent') because composition of the volatile compounds and intensity of odour attributes were similar for both origins.

INTRODUCTION

Fruits of the genus *Capsicum*, both fresh and dried, are widely accepted for use as spices and as staples in the diet. Bell peppers differ from most other members of the genus in that they generally do not have the 'hot' taste associated with the Chili and Tabasco types. Bell peppers possess a characteristic pleasant aroma, although various varieties have distinctive aromas (Heath, 1981).

Some aroma compounds are present in the intact bell peppers, many others are produced only when the tissue of the raw vegetable is broken, such as by cutting, chewing or blending (Buttery, 1981). In many plants, disruption of tissues gives rise to rapid hydrolytic and oxidative degradation of endogeneous lipids into various products, among which are volatile compounds found to be responsible for desirable and undesirable flavours (Tressl *et al.*, 1981; Hatanaka *et al.*, 1983). Wu & Liou (1986) showed that tissue disruption stimulated enzymic formation of hexanal, *trans*-2-hexenal, hexanol, *cis*-3-hexen-1-ol and *trans*-2-hexen-1-ol, while the contents of 2-methoxy-3-isobutylpyrazine, linalool, *trans*-ß-ocimene and benzaldehyde were similar before and after tissue disruption. Cooking or processing of the vegetable gives rise to other volatile flavour compounds, usually breakdown products of its major components, e.g. carbohydrates, proteins and lipids (Buttery, 1981). The aroma of fresh bell peppers, mainly due to 2-methoxy-3-isobutylpyrazine, is modified to a cooked bell pepper aroma by the increased amounts of C_9-ketones in particular (Govindarajan, 1986). As a result the flavour of rehydrated bell pepper cuttings is due to compounds present in the intact vegetable and to compounds formed during processing and rehydration.

Some investigations have examined hedonic (like-dislike) responses to peppers (Weisenfelder *et al.*, 1978; Saldana & Meyer, 1981). Studies of Rozin & Schiller (1980) and Rozin *et al.* (1981) noted that people typically like the widely varying flavours of the different varieties of the fruit, in addition to acquiring a liking for the intensity of the pungency of Capsicum. According to Chitwood *et al.* (1983) the volatile fraction of fresh *C. annuum* yielded 11 components, nine of which were described by a gas chromatograph effluent sniffing panel. Descriptors used were: green; grassy; bitter almond; floral; green bell pepper; wintergreen-like; apple-like; fruity; violet-like and woody.

The present study deals with the relationship between the volatile constituents and flavour characteristics of bell peppers cultivated and dried in Chile, Hungary and Turkey.

MATERIALS AND METHODS

Commercially dried red bell pepper cuttings from Chile, Hungary and Turkey were supplied by Top Foods b.v. (Elburg, The Netherlands). The bell peppers were stored for 2 months max in glass jars at 4°C and in absence of light.

Instrumental analysis

Bell pepper cuttings (3 g) were rehydrated by adding 35 ml water and 0.1 ml 0.3 % anti foam agent Polysiloxan (A. Smit & Zn b.v., Weesp, The Netherlands), then heating in a waterbath at 100°C for 10 min and cooling in a waterbath at 25°C for 4 min. The cuttings were transferred into the sample flask of a purge-and-trap mouth model system and 25 ml artificial saliva were added (Van Ruth *et al.*, 1994). This saliva consisted of 5.208 g $NaHCO_3$, 2.160 g mucin, 1.369 g $K_2HPO_4.3H_2O$, 0.877 g NaCl, 0.500 g NaN_3, 0.477 g KCl, 0.441 g $CaCl_2.2H_2O$ and 200,000 units α-amylase (Sigma Chemical Co., St Louis, MO, USA) in 1 l distilled water (adjusted to pH 7). A flow of purified nitrogen (20 ml/min) passed through the bell pepper cuttings/saliva mixture at

37°C for 2 h to trap the volatiles in 0.10 g Tenax TA, (35/60 mesh, Alltech Nederland b.v., Zwijndrecht, The Netherlands), positioned in a glass tube, 3 mm i.d. and 10 cm long.

Thermal desorption of the volatiles from Tenax was performed by a thermal desorption (200°C, 10 min)/cold trap (-100°C) device (Carlo Erba TDAS 5000, Interscience b.v., Breda, The Netherlands). Gas chromatography (GC) was carried out on a Carlo Erba MEGA 5300 (Interscience b.v., Breda, The Netherlands) equipped with a Supelcowax 10 capillary column, 0.25 mm i.d. and 60 m long and a flame ionisation detector (FID) at 275°C. An initial oven temperature of 40°C for 4 min was used followed by a rate of 2°C/min to 92°C and then of 6°C/min to 272°C.

GC/sniffing analyses were performed with a Carlo Erba gas chromatograph, type 6000 VEGA series (Interscience b.v., Breda, The Netherlands). A thermal desorption/cold trap device (Chrompack TCT injector 16200, Chrompack, Middelburg, The Netherlands) was used as well as the column and temperature conditions described before. At the end of the capillary column the effluent was split 30:35:35 for FID, sniffing port 1 and sniffing port 2, respectively (Linssen *et al.*, 1993). Twelve assessors were selected on their availability, sensitivity, memory and ability to recognise odours. Prior to sniffing bell pepper samples, the assessors were trained on the technique of sniffing. Assessors used portable computers with a program in Pascal for data collection. The data were converted from the field disks into Lotus 123 software in order to process the raw data. Flavour descriptors were generated during preliminary GC/sniffing experiments and clustered after group sessions of the panel, resulting in a list of 20 descriptors (Table 1). These descriptors and 'other/I do not know' had to be used for each component detected by the assessors at the sniffing port. Tenax tubes without adsorbed volatile compounds were used as dummy samples for determining the

Table 1. Comparison of the descriptors of the GC/sniffing panel and the attributes of the analytical sensory panel

Descriptors (GC/sniffing panel)	Attributes (analytical sensory panel)
Bell pepper	Bell pepper
Burned/rubber	Burned
Cooked vegetables	Cooked vegetables
Fresh vegetables	Fresh
Fruity	Fruity
Grassy/green	Grassy/green vegetables
Mushrooms	Mushrooms
Sour	Sour
Spicy	Spicy
Sweet	Sweet
Butter	Bitter
Caramel	Cucumber
Chocolate	Pungent
Coffee	Sharp
Fish	
Lemon-/orange-like	
Onion/leek	
Plastic/chemical	
Rotten	
Sickly	

signal-to-noise level of the group of assessors.
The volatile components trapped on Tenax TA were identified by Dr M.A. Posthumus, Department of Organic Chemistry, Wageningen Agricultural University, using combined GC (Pye 204, Unicam Ltd, Cambridge, United Kingdom) and mass spectrometry (MS; VG MM 7070 F, Fisons Instruments, Weesp, The Netherlands). The thermal desorption device, capillary column and oven temperature program were the same as

described for the GC/sniffing analyses. Mass spectra were recorded in the electron impact mode at an ionisation voltage of 70 eV and scanned from *m/z*=300 to 25 with a cycle time of 1.8 s.

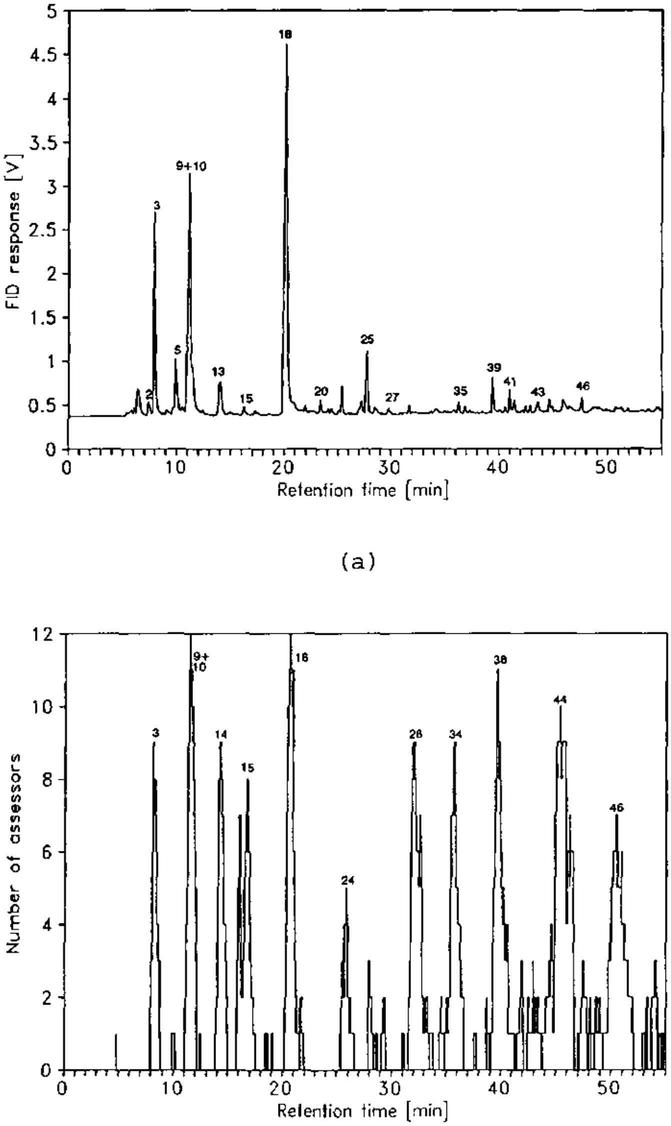
Sensory evaluation

A panel of 24 judges (aged 20-65) was selected and trained for analytical sensory analysis. Part of the training was utilisation of a 70 mm visual analogue scale on a portable computer screen for scoring perceived flavour intensities. A computer interactive interviewing system for composing questionnaires was used to gather survey information (Ci2 system, Sawtooth Software Inc., Ketchum, ID, USA). The SPSS/PC+ program was used for statistical evaluations (Linssen *et al.*, 1991). Samples of 2.4 g dried red bell pepper cuttings were rehydrated by adding 25 ml water and heating in a waterbath at 100°C for 10 min. They were subsequently served to the panel at room temperature and assessed for flavour evaluation. Flavour attributes were generated during training sessions and clustered after group sessions, which resulted in a list of 14 attributes (Table 1).

In a paired comparison test a hedonic panel of 203 assessors (students and their parents) provided flavour preference responses to bell peppers from Chile and Turkey. The red bell pepper cuttings were rehydrated and served as described before.

RESULTS AND DISCUSSION

Dynamic headspace gas chromatography of rehydrated bell pepper samples was used to analyse the release of volatiles at mouth conditions (artificial saliva, 37°C). Fig. 1 represents the chromatograms of rehydrated Hungarian bell pepper cuttings, obtained by FID and sniffing port detection. The numbers above the peaks in Fig. 1 refer to the compounds listed in Table 2. GC/sniffing of dummy samples revealed that detection of an odour at the sniffing port by three or less out of 12 assessors can be considered as 'noise'. FID and sniffing chromatograms of Chilean and Turkish bell peppers were similar to the Hungarian bell pepper chromatograms as presented in Fig. 1. The volatile compounds of dynamic headspace samples of rehydrated red bell pepper cuttings from Chile, Hungary and Turkey were identified by GC/MS (Table 2). The compounds were further characterised by their retention times, their peak areas and the odours described by the assessors at the sniffing port. Overall there is no difference in composition of volatiles between the bell peppers. However, the average peak areas (*n*=6) of the volatile compounds differ substantially between the different origins. In general, Hungarian bell peppers exhibit largest peak areas. More smaller, highly volatile compounds were identified in comparison with other studies (Buttery *et al.*, 1969; Keller *et al.*, 1981; Chitwood *et al.*, 1983; Wu *et al.*, 1986), i.e. dimethyl sulphide, propanal, 2-methylpropanal, methyl acetate, 2-methylfuran, butanal, diethoxyethane, 3-buten-2-one, 2-pentanone and 2,3-butanedione. Most of the odour active volatile compounds, as hexanal, heptanal, ß-ocimene, *trans*-3-hepten-2-one and 2-methoxy-3-isobutylpyrazine, were also reported by Wu *et al.* (1986). In the study of Buttery (1981) 2-methoxy-3-isobutylpyrazine, *trans*-ß-ocimene and limonene contributed to the vacuum steam volatile oil of green bell peppers for 37 %. The only similar odour active compound of bell peppers described by a GC effluent sniffing panel is 2-methoxy-3-isobutylpyrazine, which was reported by Chitwood *et al.* (1983). This characteristic 2-methoxy-3-isobutylpyrazine was identified in sweet bell peppers, as well as in Chili peppers at a lower level (Murray & Whitfield, 1975).



(a)

(b)

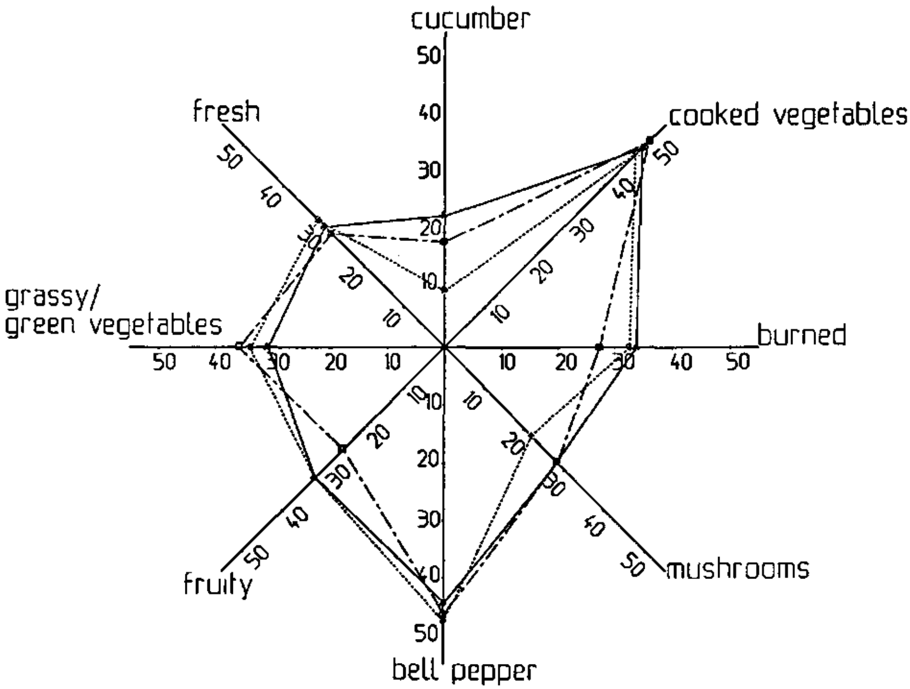
Fig. 1. Gas chromatograms of volatile components of rehydrated Hungarian bell pepper cuttings, (a) FID chromatogram and (b) chromatogram of the components detected at the sniffing port.

Table 2. Volatile components of *Capsicum* cuttings of three different origins, their retention times, odour descriptions and average peak areas (*n*=6)

Peak no	Ret. time [min]	Component	Odour description	Peak area [V.s]		
				Chile	Hungary	Turkey
1	6.27	Dimethyl sulphide		1.84	0.94	5.61
2	7.30	Propanal	Chocolate	1.03	3.35	1.17
3	7.83	2-Methylpropanal		12.94	26.97	12.02
4	8.11	Methyl acetate		0.09	0.08	0.27
5	9.50	2-Methylfuran		0.76	3.10	1.25
6	9.62	Butanal		0.78	1.28	1.04
7	9.99	Diethoxyethane		0.16	1.17	0.95
8	10.33	2-Butanone		1.77	1.69	4.39
9	10.79	2-Methylbutanal	Chocolate	21.06	54.53	29.96
10	11.24	3-Methylbutanal	Chocolate	45.00	75.73	61.07
11	12.69	3-Buten-2-one		1.61	0.53	0.85
12	13.95	2-Pentanone		0.11	tr[a]	0.67
13	14.05	Pentanal		5.01	25.43	4.99
14	14.26	2,3-Butanedione	Caramel, butter	0.60	2.96	0.33
15	16.18	1-Penten-3-one	Plastic/chemical	0.69	3.94	0.29
16	19.53	2,3-Pentanedione		0.12	1.78	0.29
17	20.10	Dimethyl disulphide		1.24	0.10	3.32
18	20.55	Hexanal	Grassy/green	16.99	212.37	15.69
19	20.95	3-Penten-2-one		0.06	0.04	0.05
20	23.42	1-Methyl-1H-pyrrole		3.82	7.88	5.01
21	23.72	1-Butanol		0.03	1.39	tr
22	25.42	1-Penten-3-ol		0.64	3.10	0.26
23	27.06	2-Heptanone		0.42	7.85	0.78

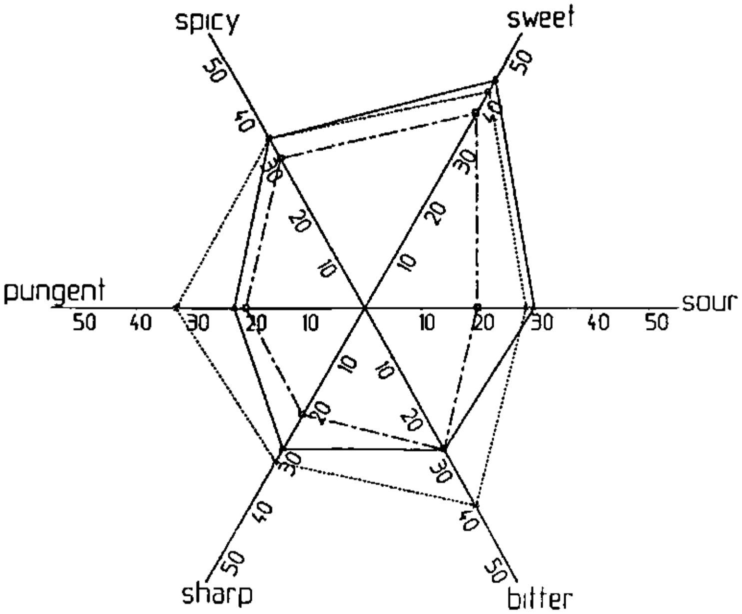
24	27.86	Heptanal	Lemon/orange	2.31	81.32	1.47
25	28.62	Limonene		0.10	14.21	0.28
26	29.76	3-Methyl-1-butanol		1.28	2.68	0.99
27	31.02	*trans*-2-Hexenal		0.08	0.88	0.05
28	31.12	ß-Ocimene	Fish, rotten, sickly	0.96	0.41	0.31
29	31.33	4-Methyl-2-hexanone		0.19	1.49	0.15
30	31.74	1-Pentanol		0.27	2.33	0.20
31	31.93	2,3-Hexanedione		0.18	0.08	0.08
32	34.01	5-Methyl-2-hexanone		0.14	0.66	0.11
33	34.26	Octanal		0.58	1.69	0.47
34	34.97	*trans*-3-Hepten-2-one	Mushrooms	0.69	1.10	1.19
35	36.28	*cis*-2-Heptenal		1.26	3.45	0.52
36	36.83	6-Methyl-5-hepten-2-one		1.40	3.30	1.44
37	37.37	1-Hexanol		0.54	0.67	0.36
38	38.40	Dimethyl trisulphide	Rotten, onion/leek	0.48	0.13	0.53
39	39.43	Nonanal		0.20	2.37	0.20
40	40.53	tert-Dodecanethiol		0.11	1.00	0.07
41	41.04	1-Octen-3-ol		1.07	5.37	0.52
42	41.77	Acetic acid		0.84	0.15	0.20
43	43.46	Decanal		1.45	4.61	0.16
44	43.94	2-Methoxy-3-isobutylpyrazine	Bell pepper	0.19	0.38	0.09
45	44.57	Benzaldehyde		0.62	6.99	0.61
46	47.81	ß-Cyclocitral	Fruity	0.02	0.44	0.05

[a] tr, peak area<0.01 V.s.



(a)

Fig. 2. Spider-web diagrams of rates for sensory attributes of rehydrated red bell pepper cuttings from (.....) Chilean, (———) Hungarian and (-.-.-) Turkish origin. (a) Odour attributes and (b) taste attributes.



(b)

Fig. 2. (continued)

Table 3. Number of assessors preferring rehydrated red bell pepper cuttings from Chilean or Turkish origin in a hedonic paired comparison test (n=203)

Country of origin	Number of assessors
Chile	127[a]
Turkey	76

[a]Binomially significant (P<0.05).

An analytical sensory panel of 24 judges evaluated the bell peppers from Chile, Hungary and Turkey. Averages for the intensities of sensory attributes of three origins of *Capsicum* are presented in spider-web diagrams in Fig. 2. Although individual judges responded differently to the three origins, the panel was consistent across replications. The origins of bell peppers did not differ significantly for the attributes 'cucumber', 'cooked vegetables', 'burned', 'mushrooms', 'bell pepper', 'fruity', 'grassy/green vegetables', 'fresh', 'spicy' and 'sweet' (Student's *t*-test, P<0.05). The Chilean origin was rated significantly higher than the Turkish in 'sour', 'bitter', 'sharp' and 'pungent' and also higher than the Hungarian in 'bitter' and 'pungent'. The attribute 'sour' rated higher in the Hungarian than in the Turkish origin. Therefore the bell peppers were more different in 'taste' attributes like 'sour', 'bitter', 'sharp' and 'pungent' than in 'odour' attributes. This could be due to differences in concentration of capsaicinoids, which contribute to the pungency of red pepper (Bennett & Kirby, 1968; Huffman *et al.*, 1978; Rowland *et al.*, 1983).

Most of the attributes generated by the sensory panel were similar to the descriptors of the sniffing panel (Table 1). Apparently, volatile compounds contribute considerably to the flavour of rehydrated bell peppers. This is in agreement with studies of Chitwood *et al.* (1983) in which a similarity between sniffing descriptors and sensory attributes of bell peppers was observed. Although FID responses of the odour active compounds 1-penten-3-one, hexanal and heptanal showed a considerable difference between the Hungarian and the other bell pepper origins (Table 2), this difference was apparently not sufficient to cause significant changes in the values of sensory attributes. This is probably due to the fact that sensory intensity is related to the log physical concentration as in the Fechner equation (Meilgaard *et al.*, 1991). In preliminary sensory experiments the intensity rate of the attribute 'grassy/green vegetables' increased 1.3 times per doubled concentration of hexanal. Hexanal concentration detected at the FID was about 13 times ($2^{3.7}$) higher for the Hungarian peppers compared with the other two origins. Therefore, a difference in intensity of 4.8 could be expected. For a significant difference (Student's *t*-test, $P<0.05$) the increase in intensity rate of the attribute 'grassy/green vegetables' should have been about 10 in the bell pepper experiments. Thus, the difference is not likely to cause a significant difference in the intensity rate for the attribute mentioned.

In the paired comparison test the hedonic panel provided preference responses to bell peppers from Chile and Turkey (Table 3). The bell peppers from Chile were significantly more appreciated (binomial probability < 0.05) than the Turkish bell peppers. The two origins differed in 'taste' attributes like 'sour', 'bitter', 'sharp' and 'pungent' only, according to the sensory analysis results. This is in agreement with the GC/FID and GC/sniffing results, which indicated only small differences in composition of the volatile compounds between the two origins.

CONCLUSIONS

Compositions of the volatile compounds were similar for Chilean and Turkish bell peppers, as well as the rates of 'odour' attributes in sensory analysis. Therefore 'taste' attributes are expected to be responsible for the difference in appreciation of Chilean and Turkish bell peppers.

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CHAPTER 8

INSTRUMENTAL AND SENSORY EVALUATION OF THE FLAVOUR OF DRIED FRENCH BEANS (*PHASEOLUS VULGARIS*) INFLUENCED BY STORAGE CONDITIONS

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ABSTRACT

The influence of storage conditions on the flavour of stored French beans after rehydration was evaluated by gas chromatography/sniffing port analysis (GC/SP) and GC/mass spectrometry of volatile compounds and by descriptive (QDA) and hedonic sensory evaluation. The dried beans were stored at three water activities (aw), two different temperatures and in presence or absence of light. In French beans 18 odour active volatile compounds were detected and a common odour profile of 10 compounds was shown. At elevated temperature and aw 0.3 or 0.5, GC/SP showed an increase in the number of assessors perceiving chemical and rotten odours at the sniffing port. The number of assessors perceiving chemical odours at the sniffing port was increased by light exposure at aw 0.1. QDA revealed a significant decrease in intensities for the attributes 'French bean' and 'sweet' and an increase for 'chemical', 'burned', 'musty' and 'bitter' at the elevated temperature and aw 0.3 or 0.5. Exposure to light at aw 0.1 decreased 'French bean' scores and increased scores for 'chemical' and 'burned'. French beans stored at 20°C in absence of light and at aw 0.3 were more appreciated by the hedonic panel than those stored at 40°C. GC/SP and QDA revealed relationships between differently stored French beans, sensory attributes and volatile compounds. Therefore, volatile compounds are expected to be responsible for the change in flavour and appreciation of dried French beans by storage conditions.

INTRODUCTION

The French bean (*Phaseolus vulgaris*) is an important food source and extensively cultivated in France. French beans are not only consumed as a shoot, but also as an immature green pod, or as an immature or mature green seed, both fresh and processed (Kermasha & Metche, 1986).

The oil present in French beans (Hollard *et al.*, 1991) makes them prone to the development of off-flavours during storage (Kermasha *et al.*, 1988). These off-flavours are considered to be due to (enzymic) degradation of unsaturated fatty acids. Linoleic and linolenic acids are the precursors of volatile short-chain aldehydes (Jadhav *et al.*, 1972; Arens *et al.*, 1973) and of both volatile and non-volatile longer-chain aldehydes (Grosch & Schwarz, 1971; Gaillard *et al.*, 1976). The isolation and partial characterization of lipoxygenase in fresh green beans (Hale *et al.*, 1969; De Lumen *et al.*, 1978), in snap beans (Klein, 1976) and in bean seeds (Grosch *et al.*, 1976) have been reported previously. Ketols and aldehydes resulting from hydroperoxide isomerase activity, are responsible for the desirable, fresh-vegetable flavours associated with normal metabolism of the growing plant. However, they are also responsible for undesirable off-flavours that occur during processing and storage (Kermasha & Metche, 1987). Lipoxygenase-mediated conversion of polyunsaturated fatty acids is a major contributor to the off-flavours in legume protein products (Sessa, 1979). It has also been well established that enzyme systems can produce off-flavours in fruits and vegetables even at subzero temperatures and at low moistures (Scott, 1975; Kermasha *et al.*, 1988). Consequently, most vegetables which are preserved by canning, freezing or even dehydration, are subjected to blanching treatments to inactivate these enzymes (Kermasha *et al.*, 1988).

Occasionally, off-flavours developed during storage of dry beans caused the rejection of large quantities of beans for human purposes (Buttery *et al.*, 1975). Off-flavour generation despite of enzyme inactivation, suggests that oxidation of the unsaturated fatty acids in these dried stored beans results from autoxidation rather than enzymic activity (Kermasha & Metche, 1987).

In conjunction, off-flavours generated in dried beans stored at elevated temperatures could be due to formation of volatile compounds in Maillard reactions (Strecker degradation). Some of the Strecker aldehydes could be responsible for off-flavours in beans as they possess chocolate odours, e.g. 2-methylpropanal and 2- and 3-methylbutanal (Van Ruth *et al.*, 1995a). In conclusion, both lipid oxidation and browning reactions could affect the flavour of dried French beans during storage.

Recently the authors reported a dynamic headspace model system for isolation of volatile compounds from vegetables under mouth conditions (Van Ruth *et al.*, 1994). Release of volatiles from rehydrated French beans did not differ significantly from their release in the mouths of 12 assessors (Van Ruth *et al.*, 1995b). The present study deals with the flavour of stored dried French beans, influenced by water activity (aw), temperature and light intensity. The flavour was characterised by gas chromatography/ sniffing port analysis (GC/SP) of volatiles released in the mouth model system previously described and by quantitative descriptive sensory analysis (QDA). The correlation of the instrumental and analytical sensory analyses was examined by multivariate statistical techniques. Differences in preferences for the French beans stored under different conditions were determined after rehydration in a hedonic sensory evaluation.

MATERIALS AND METHODS

Plant material and storage

Commercially air-dried French beans (*Phaseolus vulgaris*), were supplied in pieces by Top Foods b.v. (Elburg, The Netherlands); the beans were blanched before drying. Prior to the main storage in this study, the beans were stored in dessicators containing 1 kg silicagel at 20°C for 60 hours, in order to lower their aw to about 0.1. Afterwards the diced dried beans were stored in dessicators at aw 0.1, 0.3 or 0.5 and 20°C or 40°C in the absence of light and at the same water activities and 20°C in the presence of light (Osram L13W/41, Lumilux Interna, Italy) for 2 months. Water activities were maintained by saturated salt solutions: aw 0.1 by 112.5 g LiCl in 64 ml distilled water, aw 0.3 by 213.3 g $MgCl_2.6H_2O$ in 12.5 ml water and aw 0.5 by 115.2 g $Mg(NO_3)_2.6H_2O$ in 10 ml water.

After storage the dried beans (1.2 g) were rehydrated prior to analysis, by adding 10 ml distilled water, followed by heating in a waterbath at 100°C for 10 min and cooling down in a waterbath at 25°C for 4 min.

Instrumental analysis

The rehydrated vegetables were transferred into the sample flask of the mouth model system with mastication device (Van Ruth *et al.*, 1994). Artificial saliva (4 ml) was added and the headspace was flushed with nitrogen gas in order to trap volatile compounds in Tenax TA, as described previously (Van Ruth *et al.*, 1995a). In preliminary GC/SP experiments 10 assessors (aged 20-40) generated flavour descriptors for the volatile compounds of rehydrated French beans, which were clustered during group sessions of the panel. Completed with 'other/I do not know', one of these descriptors (Table 1) had to be chosen for each compound

Table 1. Comparison of the descriptors of the gas chromatography/sniffing port panel (GC/SP) and the attributes used by the analytical sensory panel for rehydrated French beans

Descriptors (GC/SP panel)	Attributes (sensory panel)
Burned	Burned
Chemical	Chemical
French bean	French bean
Grassy	Grassy
Mushroom	Mushroom
Sour	Sour
Spicy	Spicy
Sweet	Sweet
Anise	Bitter
Caramel	Cooked vegetables
Chocolate	Crunchy
Citrus	Fibrous
Fruity	Herbal
Metal	Mealy
Rancid	Musty
Rotten	
Soapy	

detected by the assessors at the sniffing port. Tenax tubes without adsorbed volatile compounds were used as dummy samples for determining the signal-to-noise level of the group of assessors. The volatile compounds trapped in Tenax TA were identified by combined gas chromatography/mass spectrometry (GC/MS) as described previously (Van Ruth et al., 1995a).

Sensory evaluation

For quantitative descriptive analysis a panel of 21

assessors (aged 20-55) was selected and trained. A computer interactive interviewing system for composing questionnaires was used to gather survey information (Ci2 system, Sawtooth Software Inc., Ketchum, ID, USA). Samples of the stored dried French beans (1.8 g) were rehydrated by adding 15 ml water and heating in a waterbath at 100°C for 10 min. They were cooled down and stored in a waterbath at 50°C for 10 min max, subsequently served to the panel and assessed for flavour evaluation. Flavour attributes were generated during training sessions, resulting in a list of 15 attributes (Table 1). Sensory attributes of the rehydrated beans were evaluated with and without the use of nose-clips (Jaeger Nederland b.v., Breda, The Netherlands). Perceived intensities were scored on a 70 mm visual analogue scale on a portable computer screen and data were processed as reported previously (Van Ruth *et al.*, 1995a).
In paired comparison tests, a hedonic panel of 157 assessors (students and their parents) provided preference responses to rehydrated stored French beans. A group of 53 assessors compared beans stored at aw 0.3 in absence of light at 20°C and 40°C, another group of 53 assessors compared beans stored at aw 0.3 and 20°C in the presence and absence of light and a group of 51 assessors compared beans stored at 20°C in presence of light at aw 0.3 and aw 0.5.

Statistical analysis

Sensory data were subjected to Student's *t*-tests in order to determine significant differences between storage conditions. Principal component analysis (PCA) was conducted on the sensory data sets as well as on the combined GC/SP and sensory data sets, using the SPSS/PC+ program (SPSS Inc., Chicago, IL, USA). A significance level of $P<0.05$ was used throughout the study.

RESULTS AND DISCUSSION

Dried French beans were stored at aw 0.1, 0.3 or 0.5 and 20°C or 40°C in absence of light or at 20°C in presence of light. After rehydration their volatile compounds were isolated in a mouth model system and analysed by GC/SP. Furthermore, these volatile compounds were identified by GC/MS, by their retention times, FID peak areas and by the descriptors used by the assessors of the sniffing panel. The results of the identification, the retention times, the odour descriptions and the number of assessors perceiving an odour at the sniffing port, are presented in Table 2. GC sniffing of dummy samples indicated that detection of an odour at the sniffing port by one or two out of ten assessors can be considered as 'noise'. GC/SP revealed that 18 compounds isolated from rehydrated French beans possessed detectable odours. Several of these compounds have been identified more often in beans. Propanal and butanal were detected in cooked and rehydrated runner beans by MacLeod & MacLeod (1970). Lovegren *et al.* (1979) identified methylpropanal, methylbutanal and hexanal in dried beans and Mtebe & Gordon (1987) reported hexanal, octanal and nonanal among the volatile compounds in winged beans. Although differences in GC/SP patterns across the storage conditions were obvious, a common GC/SP profile was shown. It comprised the compounds 2-methylpropanal (chocolate), 2- and 3-methylbutanal (chocolate), 2,3-butanedione (caramel), hexanal (grassy), an unknown compound (peak no 12; rancid, rotten, French bean, fruity), octanal (fruity, citrus, burned), 1-octen-3-one (mushroom), dimethyl trisulphide (rotten, rancid, burned) and nonanal (chemical, rotten). This profile is similar to the common odour profile of rehydrated French beans, bell peppers and leeks reported previously (Van Ruth *et al.*, 1995a). The presence of 2-methylpropanal and 2- and 3-methylbutanal is typical for dried vegetables. These volatile compounds can be formed by Strecker degradation of valine and (iso)leucine during the drying process. Most of the other compounds of the common profile can be derived from lipid oxidation reactions (Tressl *et al.*, 1981; Whitfield, 1992). In the present study, the number of assessors perceiving an odour at the sniffing port was considered to correspond with odour intensity, as previously was shown that the number of assessors perceiving an odour in GC/SP correlated significantly (Spearman's ranked correlation test, $P<0.05$) with odour intensity scores for eluting compounds (Van Ruth *et al.*, 1995c).

At aw 0.5 the influence of temperature is shown by the GC/SP chromatograms of French beans stored in absence of light at 20°C or 40°C (Fig. 1). At this aw and the higher temperature, the number of assessors perceiving an odour at the sniffing port changed for several compounds. The number of assessors increased for hexanal (grassy), 2-methyl-2-butenal (chemical, rancid, rotten), octanal (fruity, citrus, burned), nonanal (chemical, rotten) and an unknown compound (peak no 8; chemical, rotten, caramel). At the conditions mentioned, the increased rates of lipid oxidation and Strecker degradation are expected to be responsible for the formation of volatile compounds. The lipids of French beans are highly unsaturated (Hollard *et al.*, 1991) and therefore susceptible to oxidation. As the beans were blanched prior to drying, lipid oxidation may result from autoxidation rather than enzymic activity (Kermasha & Metche, 1987).

Fig. 2 represents the GC/SP chromatograms of French beans stored at aw 0.1 and 20°C in absence and presence of light. The latter resulted in increased numbers of assessors with an odour perception at the sniffing port for several compounds eluting within 35 minutes. These compounds are propanal (chemical, sour, sweet), butanal (chocolate), 2-methyl-2-butenal (chemical, rancid, rotten), octanal (fruity, citrus, burned) and an unknown compound

Table 2. Volatile compounds of differently stored French beans after rehydration, retention times, odour descriptions and number of assessors with an odour perception at the sniffing port

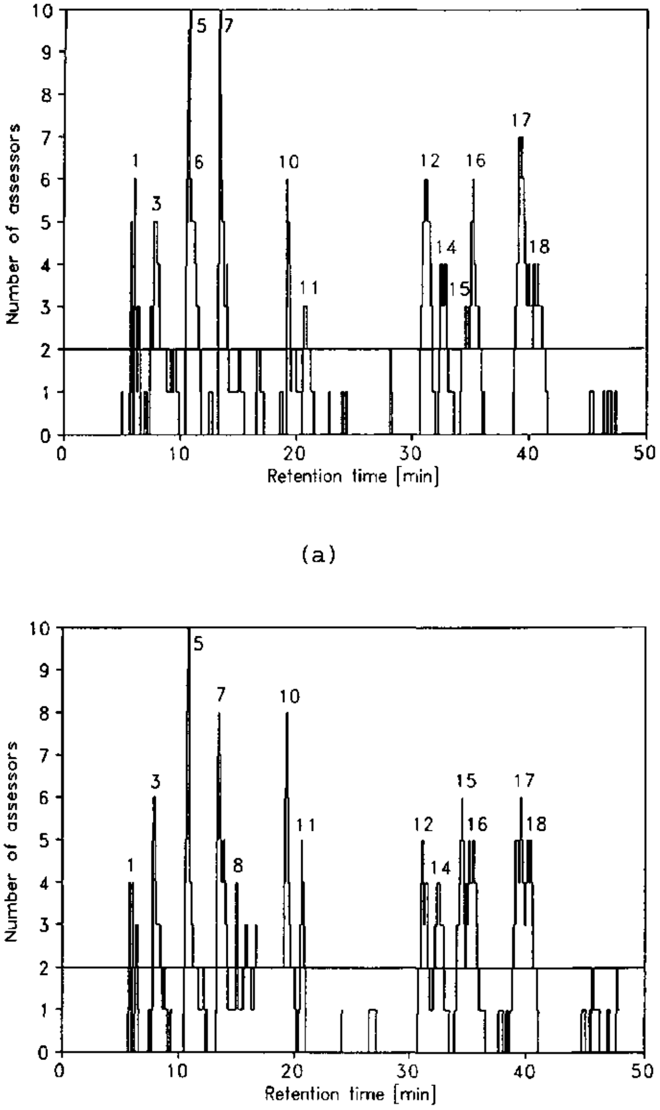
No	Ret. time [min]	Compound	Odour description	Water activity[a]								
				0.1			0.3			0.5		
				D40	D20	L20	D40	D20	L20	D40	D20	L20
1	6.2	Unk[b]	Chemical	5	-[c]	5	4	5	3	4	6	4
2	7.7	Propanal	Chemical, sour, sweet	-	-	4	3	-	7	-	3	4
3	8.0	2-Methylpropanal	Chocolate	6	6	6	6	6	6	6	5	6
4	9.4	Butanal	Chocolate	-	-	5	-	-	-	-	-	-
5	10.8	2-Methylbutanal	Chocolate, rotten, sweet	10	8	10	7	10	8	10	10	9
6	11.4	3-Methylbutanal	Chocolate	6	4	5	7	6	7	5	5	5
7	14.4	2,3-Butanedione	Caramel	9	10	9	6	8	10	8	10	6
8	15.1	Unk	Chemical, rotten, caramel	3	-	4	5	5	5	4	-	3
9	16.8	2-Butenal	Chemical	4	-	-	-	-	3	3	-	3
10	20.0	Hexanal	Grassy	6	8	9	7	6	5	8	6	6
11	20.6	2-Methyl-2-butenal	Chemical, rancid, rotten	-	-	3	5	-	3	5	3	4
12	31.0	Unk	Rancid, rotten, French bean, fruity	6	6	7	3	8	6	5	6	6
13	32.0	Unk	Burned, rancid	3	-	4	5	3	4	4	4	5

14	32.6	2-Octanone	Chemical, rotten, spicy	-	-	3	5	-	-	4	4	-
15	34.0	Octanal	Fruity, citrus, burned	3	4	5	4	4	5	6	3	4
16	35.5	1-Octen-3-one	Mushroom	6	7	7	6	6	7	5	6	6
17	38.9	Dimethyl trisulphide	Rotten, rancid, burned	5	7	5	5	7	7	6	7	5
18	40.0	Nonanal	Chemical, rotten	5	5	6	5	8	5	5	4	6

[a] Storage of dried French beans at water activities (0.1, 0.3 or 0.5) in absence of light, at 20°C (D20) or 40°C (D40) or in presence of light at 20°C (L20).

[b] Unknown compound.

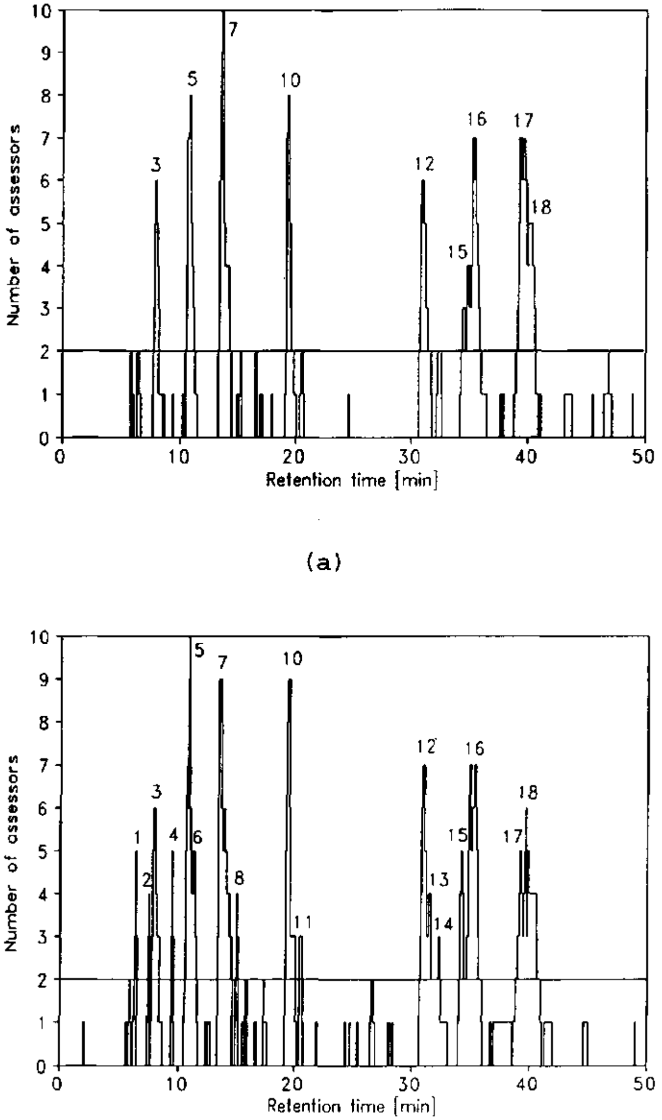
[c] Below detection level.



(a)

(b)

Fig. 1. Sniffing chromatograms of volatile compounds of dried French beans which were stored at water activity 0.5, in absence of light and (a) 20°C or (b) 40°C. Numbers on the chromatograms refer to the compounds in Table 2.



(a)

(b)

Fig. 2. Sniffing chromatograms of volatile compounds of dried French beans which were stored at water activity 0.1 and 20°C, (a) in absence or (b) presence of light. Numbers on the chromatograms refer to the compounds in Table 2.

(peak no 8; chemical, rotten, caramel). At aw 0.1, exposure to light increased particularly the number of assessors perceiving volatile compounds with chemical odours. These compounds are known to be generated by lipid oxidation reactions (Tressl *et al.*, 1981; Ullrich & Grosch, 1987; Frankel, 1991). The rate of light induced lipid oxidation was probably increased at low water activity, because the effect of light exposure was less noticed for aw 0.3 and 0.5.

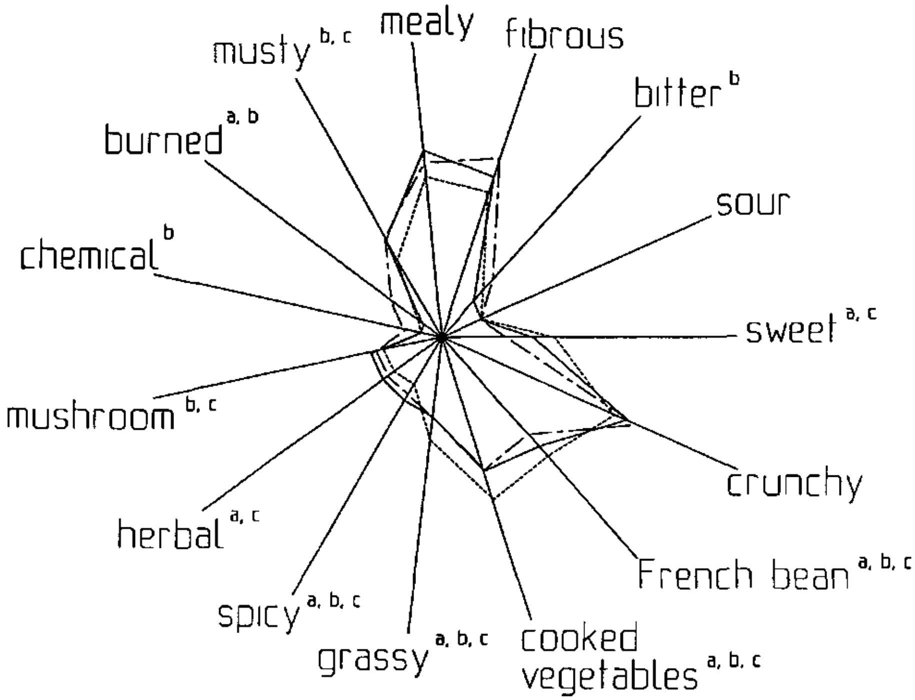
A QDA panel evaluated the dried and stored French bean samples after rehydration. Although individual assessors responded differently to the samples of French beans, the panel was consistent across replications. Many of the attributes generated by the QDA sensory panel were similar to the descriptors of the GC/SP panel (Table 1). Fig. 3 presents the scores of sensory attributes assessed with and without nose-clips for French beans stored under different conditions. Comparison of Fig. 3 (a)-(b) reveals that the use of nose-clips diminished the scores for sensory attributes in general, which is in agreement with previous studies (Linssen *et al.*, 1991; Van Ruth *et al.*, 1995a). Apparently, volatile compounds contribute to the flavour of rehydrated French beans. Therefore, scores for taste and mouthfeel attributes like 'mealy', 'sweet', 'sour', 'bitter', 'crunchy' and 'fibrous' decreased less than scores for odour attributes.

Averages of the scores for the sensory attributes of French beans for each of the storage conditions and the significances of mutual differences (Student's *t*-test, $P<0.05$) are shown in Table 3. Intensity scores for French beans which were stored at aw 0.3, in absence of light and at 20°C, were similar to those reported previously for rehydrated French beans (Van Ruth *et al.*, 1995a). Under these storage conditions the flavour of the dried beans is expected to be quite stable. The flavour of these beans was characterised by the attributes 'French bean', 'cooked vegetable', 'crunchy', 'grassy' and 'mealy'. At aw 0.5 and 40°C a significant decrease in scores was obtained for several odour attributes which were probably positively related to the flavour of the vegetable, such as 'French bean', 'cooked vegetables', 'grassy' and 'sweet'. The higher temperature resulted in a significant increase of the intensities for less vegetable-like attributes as well, such as 'chemical', 'burned', 'musty', 'mealy', 'fibrous' and 'bitter'. In analogy, GC/SP showed an increase in the number of assessors perceiving volatile compounds described with chemical and rotten, which implicates a contribution of volatile compounds to the change of flavour of dried French beans during storage at an elevated temperature.

As already shown in GC/SP (Fig. 2), influence of light was mainly observed at aw 0.1. Exposure to light caused a decrease in 'French bean' scores in QDA and an increase in scores for the attributes 'chemical' and 'burned'. This is in agreement with GC/SP results, which showed an increase in the number of assessors perceiving chemical odours at the sniffing port. Apparently, volatile compounds contribute to the change in flavour in dried French beans during storage in light.

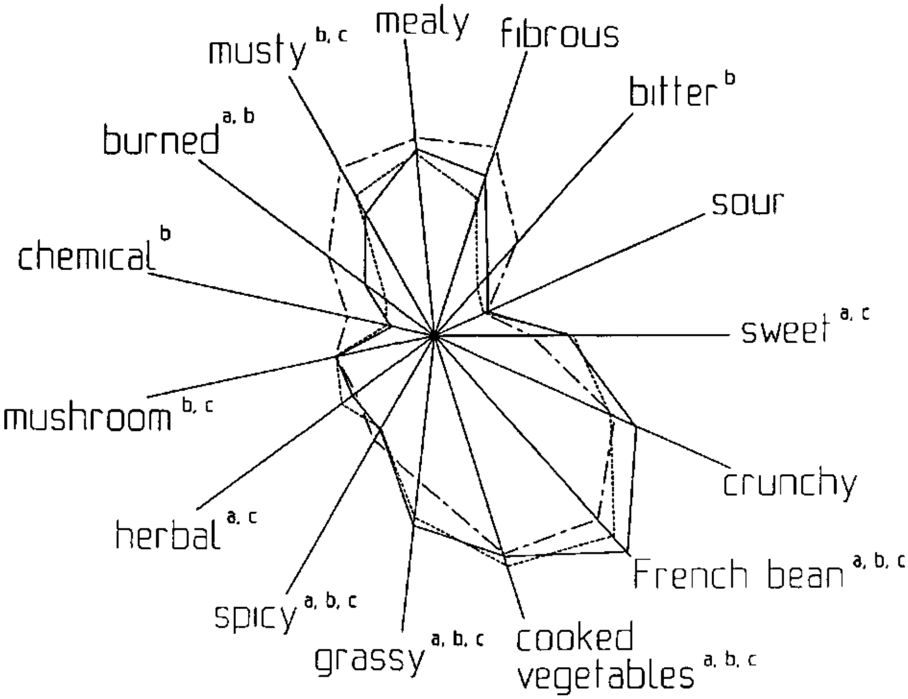
French beans stored at aw 0.3 and 20°C in absence and presence of light did not differ significantly in scores for sensory attributes. GC/SP showed similar patterns for both storage conditions, however, FID chromatograms differed considerably (Fig. 4). These results implicate that GC/SP is more suitable than GC/FID for correlation with sensory analysis. This confirms our previous studies concerning the flavour of two origins of rehydrated red bell peppers. These origins differed considerably in FID patterns, but differed neither in GC/SP patterns, nor in scores for sensory attributes (Van Ruth & Roozen, 1994).

Sensory data were subjected to PCA, the first six principal components of which are shown in Table 4. PCA shows relationships between attributes, e.g. 'mealy', 'mushroom'



(a)

Fig. 3. Spider-web diagrams of scores for sensory attributes of dried French beans which were stored at water activity 0.3 assessed (a) with and (b) without use of nose-clips. Beans were stored in absence of light and (D20,————) 20°C or (D40,.-.-.-.) 40°C or (L20,------) in presence of light and 20°C. [abc]Attribute that shows significant decrease in intensity score by use of nose-clips for D20, D40 and L20, respectively (Student's *t*-test, $P<0.05$).



(b)

Fig. 3. (continued)

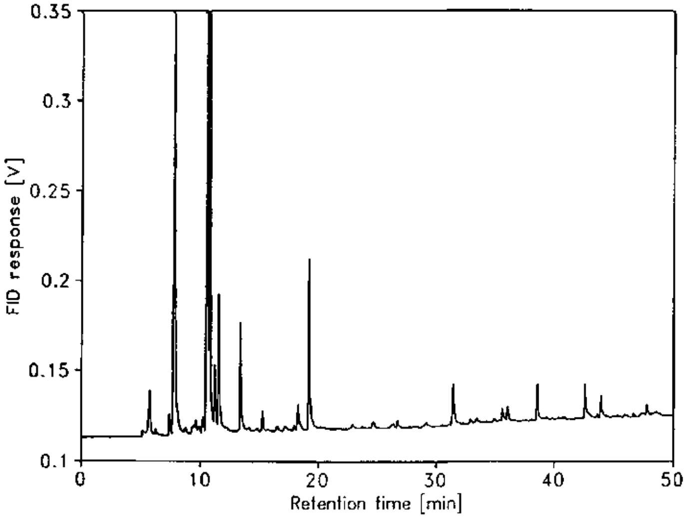
Table 3. Mean scores for sensory attributes of rehydrated French beans after storage at different conditions (*n*=21)

Code	Attribute	aw $0.1^{a,b}$					aw 0.3				aw 0.5				
		D40		D20		L20	D40		D20	L20	D40		D20		L20
A	Mealy	29^{1}		21^{I}	$<^{c}$	32^{x}	$37^{1,2}$		35^{II}	34^{x}	42^{2}	>	29^{II}		37^{x}
B	Fibrous	28^{1}		24^{I}		31^{x}	37^{2}		31^{II}	27^{x}	40^{2}	>	$25^{I,II}$		27^{x}
C	Bitter	14^{1}		17^{I}		20^{y}	$23^{1,2}$	>	15^{I}	12^{x}	29^{2}	>	14^{I}		14^{x}
D	Sour	11^{1}		14^{I}		18^{y}	10^{1}		11^{I}	10^{x}	12^{1}		9^{I}		8^{x}
E	Sweet	30^{2}		32^{II}		27^{x}	18^{1}	<	25^{I}	26^{x}	14^{1}	<	22^{I}		24^{x}
F	Crunchy	38^{2}		43^{I}	>	37^{x}	37^{2}		41^{I}	36^{x}	28^{1}	<	38^{I}		36^{x}
G	French bean	47^{2}	<	53^{I}	>	43^{x}	45^{2}	<	54^{I}	50^{x}	27^{1}	<	48^{I}		48^{x}
H	Cooked vegetables	46^{2}		44^{I}		41^{x}	42^{2}		43^{I}	45^{x}	31^{1}	<	48^{I}		47^{x}
I	Grassy	27^{1}		28^{I}		$25^{x,y}$	26^{1}	<	35^{II}	33^{y}	20^{1}		27^{I}		22^{x}
J	Spicy	15^{1}		18^{I}		15^{x}	22^{2}		20^{I}	20^{x}	15^{1}		18^{I}		18^{x}
K	Herbal	21^{1}		19^{I}		22^{x}	18^{1}		19^{I}	21^{x}	15^{1}		19^{I}		20^{x}
L	Mushroom	20^{1}		16^{I}		17^{x}	19^{1}		$19^{I,II}$	19^{x}	20^{1}		25^{II}		21^{x}
M	Chemical	10^{1}		10^{I}	<	17^{y}	$17^{1,2}$	>	8^{I}	9^{x}	20^{2}	>	9^{I}		$13^{x,y}$
N	Burned	16^{1}		14^{I}	<	20^{y}	$24^{1,2}$	>	15^{I}	11^{x}	32^{2}	>	12^{I}		13^{x}
O	Musty	26^{1}	>	19^{I}		24^{x}	36^{2}	>	27^{II}	30^{x}	36^{2}	>	$23^{I,II}$	<	31^{x}

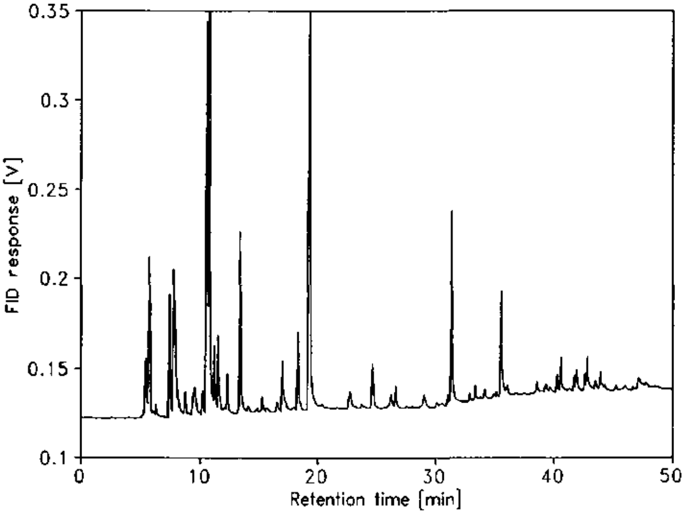
[a] For an explanation of codes see footnote to Table 2.

[b] 1-2; I-II; x-y, Values with different Latin numbers in superscripts (D40), respectively Roman numbers (D20) and letters (L20), within a row are significantly different (Student's t-test $P<0.05$).

[c] < and > indicate significant differences within aw (Student's *t*-test $P<0.05$).



(a)



(b)

Fig. 4. FID chromatograms of volatile compounds of dried French beans which were stored at water activity 0.3 and 20°C in (a) absence or (b) presence of light.

Table 4. Attributes of the first six principal components concerning differently stored French beans after rehydration, loadings in parentheses

First 29%[a]	Second 16%	Third 10%	Fourth 8%	Fifth 6%	Sixth 6%
Mealy (0.8)	Grassy (0.8)	Spicy (0.8)	Crunchy (0.8)	Chemical (0.8)	Fibrous (0.9)
Mushroom (0.8)	Herbal (0.8)	Bitter (0.6)	Cooked vegetables (0.7)	French bean (-0.5)	
Musty (0.8)	Sweet (0.8)	Sour (0.6)	French bean (0.6)		
		Burned (0.5)			

[a] Percentage of explained variance.

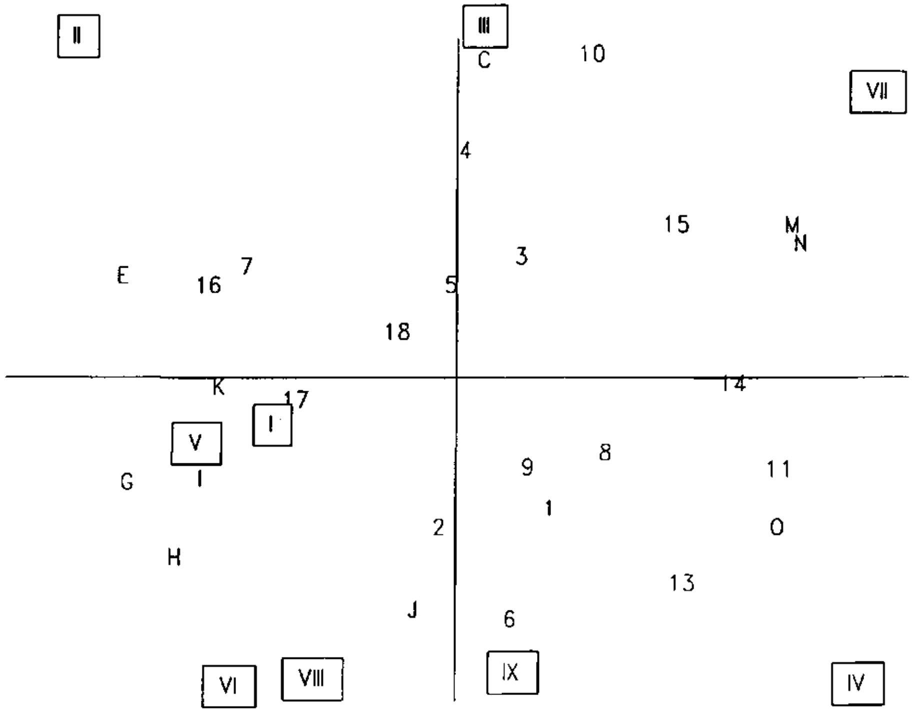


Fig. 5. Scores for differently stored dried French bean samples (I-IX) and loadings of volatile compounds and sensory attributes on the first (horizontal axis) and second (vertical axis) principal component axes. Numbers refer to volatile compounds in Table 2 and capitals to sensory attributes in Table 3. French bean samples (I-III, IV-VI, VII-IX) were stored at water activities 0.1, 0.3 or 0.5, in absence of light and (I, IV, VII) 40°C or (II, V, VIII) 20°C or (III, VI, IX) in presence of light and 20°C.

Table 5. Number of assessors with preference for differently stored French beans after rehydration in a hedonic paired comparison test

n[a]	Water activity level[b]			
	0.3			0.5
	D40	D20	L20	L20
53	14[c]	39[c]		
53		32	21	
51			27	24

[a] *n*=Number of assessors.

[b] For an explanation of codes see footnote to Table 2.

[c] Binomially significant ($P<0.05$).

and 'musty' loaded high on the first component, implicating that these attributes were strongly associated with each other. 'Grassy', 'herbal' and 'sweet' loaded high on the second component, as did 'spicy', 'bitter', 'sour' and 'burned' on the third.

PCA was also performed on combined GC/SP (number of assessors perceiving an odour active compound; Table 2) and QDA sensory data sets (intensities of sensory attributes; Table 3). In the PCA map obtained (Fig. 5), volatile compounds (Latin numbers), sensory attributes (capitals) and stored bean samples (Roman numbers) are mainly separated along the axis of the first principal component; from 'French bean' (G) and 'cooked vegetables' (H) with high negative loadings to 'chemical' (M), 'burned' (N) and 'musty' (O) with high positive loadings. Several volatile compounds of the common GC/SP profile show high negative loadings on the first component, i.e. 2,3-butanedione (7), 1-octen-3-one (16) and dimethyl trisulphide (17). As these compounds are present in the dried French beans without treatment, they are considered to contribute to the regular flavour of dried French beans. The sensory attributes 'French bean' (G), 'cooked vegetables' (H), 'grassy' (I), 'herbal' (K) and 'sweet' (E) have high negative loadings on the first component as well and correlate with French beans stored at aw 0.1 in absence of light at 40°C (I) or 20°C (II), at aw 0.3 and 20°C in absence (V) or presence of light (VI) and at aw 0.5 at 20°C in absence of light (VIII). None of these samples showed remarkable differences in intensity scores for sensory attributes and in the numbers of assessors perceiving an odour at the sniffing port.

'Chemical' (M), 'burned' (N) and 'musty' (O) and 2-methyl-2-butenal (11) and 2-octanone (14) show high positive loadings on the first component. The volatile compounds were described by the GC/SP panel as chemical, rotten, rancid and spicy. These highly positive loadings correlate well with the French beans which were stored in absence of light at 40°C and at aw 0.3 (IV) or 0.5 (VII). Between the mentioned extremes on the first component, there are several volatile compounds with chocolate odours, such as 2-methylpropanal (3), butanal (4), 2- and 3-methylbutanal (5, 6), as well as the sensory attribute 'bitter' (C). They correlate best with the French beans stored in presence of light at 20°C and at aw 0.1 (III) or 0.5 (IX). In these samples some changes were observed in intensity scores for sensory attributes and in GC/SP chromatograms, but less than in the beans stored at elevated temperature and aw 0.3 or 0.5.

In the three paired comparison tests, the hedonic panel compared French beans, which were stored respectively at aw 0.3 in absence of light at 20°C or 40°C, aw 0.3 at 20°C in absence or presence of light and at 20°C in presence of light at aw 0.3 or 0.5 (Table 5). The French beans stored in absence of light at 20°C were significantly more appreciated than the beans stored at 40°C. The latter two comparisons did not show significant differences. As shown before, they differed less in intensities for sensory attributes and in GC/SP patterns than the samples in the first comparison. By analogy, the difference in scores on the first principal component between French beans stored in absence of light at aw 0.3 and at 20°C or 40°C was considerably larger than between the other paired comparisons (Fig 5). For the beans stored at 40°C, QDA showed a significant decrease in scores for vegetable-like attributes ('French bean', 'grassy') and an increase for several less vegetable-like attributes ('bitter', 'chemical', 'burned', 'musty') in comparison with beans stored at 20°C and GC/SP revealed an increase in the numbers of assessors perceiving compounds with chemical and rotten odours. These volatile compounds correlated well with similar sensory attributes and are considered to be responsible for the difference in appreciation.

CONCLUSIONS

Relationships between sensory attributes and several volatile compounds of stored French beans showed a major contribution of volatile compounds to the change in flavour and appreciation of rehydrated French beans by storage conditions.

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CHAPTER 9

INSTRUMENTAL AND SENSORY ANALYSIS OF THE FLAVOUR OF FRENCH BEANS (*PHASEOLUS VULGARIS*) AFTER DIFFERENT REHYDRATION CONDITIONS

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ABSTRACT

The influence of rehydration conditions on the flavour of French beans (*Phaseolus vulgaris*) was evaluated. Dried French beans were rehydrated in closed and open flasks at 100°C for 0, 5, 10, 15, 30, 45 and 60 min. The odour active volatile compounds were released in a mouth model system and determined by gas chromatography/sniffing port analysis (GC/SP) and GC/mass spectrometry. Texture characteristics were analysed by Kramer shear press measurements. Moreover, the flavour of the beans was evaluated by descriptive sensory analysis. In GC/SP, extended rehydration resulted in increased intensities for 2-butenal, 2-methyl-2-butenal, 1-octen-3-one and one unknown compound. Intensities of several attributes, such as 'chemical', 'mealy', 'mushroom' and 'musty' increased with extended rehydration, while intensities decreased for 'grassy' and 'citrus'. Instrumental and sensory analysis of the texture of the beans revealed a gradual decrease with extended rehydration. In general, rehydration in open versus closed flasks decreased the odour intensities of volatile compounds in GC/SP, as well as the intensities of attributes in sensory analysis. Correlation of sensory attributes and volatile compounds showed major contribution of these compounds to the difference in flavour of French beans resulting from rehydration conditions.

ZUSAMMENFASSUNG

Der Einfluß der Rehydration auf dem Geschmack von getrockneten grünen Bohnen (*Phaseolus vulgaris*) wurde untersucht. Getrocknete grüne Bohnen wurden 0, 5, 10, 15, 30, 45 und 60 Minuten in geschlossenen und offenen Gefäßen bei 100°C erhitzt. Die in einem Mund-Modell-System frei gesetzten flüchtigen Komponenten, wurden mit Hilfe der Gaschromatographie/Schnüffeltechnik (GC/SP) und des Massenspektrometers analysiert. Die Textur wurde mit einem Scherkraft-Meßgerät (Kramer Shear Press) bestimmt. Außerdem wurden Geschmack durch die beschreibende sensorische Analyse bewertet. Bei steigender Rehydrationszeit nahmen in GC/SP die Intensitäten von 2-Butenal, 2-Methyl-2-butenal, 1-Octen-3-on und einer unbekannten Verbindung zu. Die Intensitäten der sensorischen Eigenschaften, wie 'chemisch', 'mehlig', 'pilzig' und 'muffig' nahmen mit zunehmender Rehydrationszeit zu, während die Intensitäten anderer Eigenschaften, wie 'grasig' und 'zitrusähnlich' abnahmen. Meßwerte und sensorische Analyse der Textur bestätigten eine allmähliche Abnahme bei verlängerter Rehydration. Bei der Rehydration in offenen Gefäßen nahm allgemein die Intensität der flüchtigen Verbindungen (GC/SP), sowie die Intensität der sensorischen Eigenschaften ab, im Vergleich zu geschlossenen Gefäßen. Das Verhältnis zwischen den sensorischen Eigenschaften und den Meßwerten der flüchtigen Verbindungen weist darauf hin, daß bei den grünen Bohnen die flüchtigen Verbindungen in hohen Maßen zu Geschmacksveränderungen beitragen.

INTRODUCTION

The nose is capable of discerning hundreds of different odours, whereas taste involves four major stimuli. Therefore, it is not surprising that a major part of flavour research has dealt with analysis of volatile compounds (Heath, 1981). Besides aroma, food texture is an important property of processed foods (Sakamoto *et al.*, 1989). Sensory perception of texture depends on the deformation of a food as a result of the application of pressure. It depends also on surface properties such as roughness, smoothness or stickiness, which are estimated by the sense of touch (Yeatman, 1972). The physical processing of foods usually alters one or more of their physical properties and thereby may alter both flavour release and texture characteristics (McNulty, 1987). A limiting characteristic for most dehydrated products is loss of desirable flavour. Some flavour compounds are lost in dehydration and additional losses may occur during storage (Villota *et al.*, 1980). Flavour defects in dehydrated products are, however, not solely due to volatile losses. Chemical reactions and in particular oxidation and nonenzymatic browning, greatly contribute to flavour deterioration (Buttery *et al.*, 1975; Kermasha & Metche, 1987; Van Ruth *et al.*, 1995a). Besides aroma and taste characteristics, texture characteristics depend on the method of drying, pretreatment, handling and storage conditions too. Many commercially dried vegetables exhibit a dense structure, in which most of the interior capillaries are collapsed, or greatly shrunken. This kind of structure does not rehydrate well, affecting both texture quality and flavour release from the vegetables. Final moisture contents below 2 % are likely to result in problems at the rehydration stage (Villota *et al.*, 1980).
During drying of vegetables, flavour retention is a desirable objective. However, it is in contrary with rapid

flavour release, which is normally a desirable quality parameter during food consumption (McNulty, 1987). The physico-chemical environment changes markedly during the eating process. It affects the profile of volatile compounds and the perception of aroma (Linforth & Taylor, 1993). The breakdown of the food matrix through mastication enhances flavour release (Haring, 1990; Van Ruth *et al.*, 1994). Substances secreted in saliva can affect flavour release as well, such as amylase, which functions in the initial digestion of starch, (McBurney & Pfaffmann, 1963; Van Ruth *et al.*, 1995b).

Recently the authors reported a dynamic headspace model system with mastication device (DHM) for isolation of volatile compounds from vegetables (Van Ruth *et al.*, 1994). This model system enabled the study of flavour release under mouth conditions, such as volume, temperature, salivation and mastication. Flavour release from rehydrated French beans, bell peppers and leeks did not differ significantly from their release in the mouth of 12 assessors (Van Ruth *et al.*, 1995c). The present study deals with the instrumental and sensory evaluation of the flavour of rehydrated French beans after different rehydration conditions.

MATERIALS AND METHODS

Plant material and rehydration

Commercially air-dried French beans (*Phaseolus vulgaris*), were supplied in pieces by Top Foods b.v. (Elburg, The Netherlands). The beans were blanched before drying. The beans were stored in glass jars at 4°C in absence of light until sampling.

After storage, the dried beans (1.2 g for flavour analysis and 11 g for texture measurements) were rehydrated prior to analysis. Distilled water (10 ml for flavour analysis and

90 ml for texture measurements) was added and the beans were heated in flasks in a waterbath at 100°C for 0, 5, 10, 15, 30, 45 and 60 min, respectively, with (closed mode) and without caps (open mode). A small hole for pressure relieve was present in both closed and open flasks. The beans were cooled down in a waterbath at 25°C for 4 min.

Instrumental aroma analysis

The rehydrated vegetables were transferred into the sample flask of the mouth model system with mastication device

Table 1. Comparison of the descriptors of the gas chromatography/sniffing port panel (GC/SP) and the attributes of the analytical sensory panel

Descriptors (GC/SP panel)	Attributes (sensory panel)
Burned	Burned
Chemical	Chemical
Citrus	Citrus
French bean	French bean
Grassy	Grassy
Mushrooms	Mushrooms
Musty	Musty
Rotten	Rotten
Sour	Sour
Spicy	Spicy
Sweet	Sweet
Caramel	Bitter
Chocolate	Cooked vegetables
Fruity	Crunchy
Metal	Fibrous
Rancid	Herbal
Soapy	Mealy
	Wood

(Van Ruth *et al.*, 1994). Artificial saliva (4 ml) was added and the headspace flushed with purified nitrogen gas in order to trap volatile compounds in Tenax TA, as described previously (Van Ruth *et al.*, 1995c).

In preliminary gas chromatography/sniffing port analysis (GC/SP) experiments 10 assessors (aged 20-53) generated odour descriptors for the volatile compounds of rehydrated French beans, which were clustered during group sessions of the panel. Completed with 'other/I do not know', one of these descriptors (Table 1) had to be chosen for each compound detected by the assessors at the sniffing port. In separated sessions, the assessors rated perceived intensities of the eluting compounds at the sniffing port on a 9-points intensity interval scale (1=extremely weak; 9=extremely strong). Tenax tubes without adsorbed volatile compounds were used as dummy samples for determining the signal-to-noise level of the group of assessors.

The volatile compounds trapped in Tenax TA were identified by combined gas chromatography/mass spectrometry (GC/MS) as described previously (Van Ruth *et al.*, 1995d).

Texture measurements

The texture characteristics of the rehydrated French beans were determined in duplicate shear press experiments (Kramer shear press, serial no S157, model no SP12, Allo Precision Metals Engineering Inc., Rockville, MD, USA), in which maximum forces were measured during compression-extrusion strokes.

Sensory evaluation

For quantitative descriptive analysis (QDA) a panel of 20 assessors (aged 17-53) was selected and trained as described previously (Van Ruth & Roozen, 1994). The dried French beans were rehydrated as described above. They were cooled down and stored in a waterbath at 50°C for 4 min, subsequently served to the panel and assessed for flavour evaluation. Flavour attributes, including aroma, taste and mouthfeel attributes, were generated during training sessions, resulting in a list of 18 attributes (Table 1). Perceived intensities were scored on a 70 mm visual analogue scale on a portable computer screen and data were processed as reported previously (Van Ruth & Roozen, 1994).

Statistical analysis

Spearman's ranked correlation tests were performed on the GC/SP data, concerning the number of assessors perceiving an odour active compound and the average odour intensity scores. Analysis of variance was conducted on the instrumental texture data. Instrumental and sensory texture data were subjected to Pearson's product-moment correlation coefficient tests. Principal component analysis (PCA) was conducted on the sensory data sets as well as on the combined GC/SP and sensory data sets, using the SPSS/PC+ program (SPSS Inc., Chicago, IL, USA). A significance level of $P<0.05$ was used throughout the study.

RESULTS AND DISCUSSION

Volatile compounds of diced French beans were isolated in the mouth model system after 0, 5, 10, 15, 30, 45 and 60 min rehydration in closed and open flasks and analysed by GC/SP. Fig. 1 represents the GC/SP chromatogram of French beans rehydrated for 5 min in the closed mode. This rehydration condition exhibited most sniffing peaks. The odour active compounds were identified by GC/MS, their retention times, FID peak areas and the odours described by the assessors at the sniffing port. The compounds, the number of assessors perceiving an odour active compound and the mean odour intensity scores are presented in Table 2.

In general, the odour descriptions of volatile compounds were similar for the different rehydration conditions. GC/SP of dummy samples showed that detection of an odour at the sniffing port by two or less out of 10 assessors can be considered as 'noise'. GC/SP revealed that 14 volatile compounds isolated from the French beans possessed detectable odours. This odour profile is similar to the common odour profile of rehydrated French beans, bell peppers and leeks reported previously for closed flask rehydration during 10 min (Van Ruth *et al.*, 1995d).

Both the number of assessors perceiving an odour active compound and the mean odour intensity scores increased substantially when rehydration was performed (0 min rehydration vs. all other conditions). This is probably due to hindered flavour release during isolation of the volatiles, which is caused by the resistance to mass transfer of volatile compounds from the dense structure of the non-rehydrated French beans into the saliva. Besides partitioning of volatile compounds (Bakker *et al.*, 1994), mass transfer was reported to be an important factor in flavour release (Karel, 1975; De Roos & Wolswinkel, 1994). Moreover, studies of Pangborn & Szczesniak (1974), Godshall (1988), Godshall & Solms (1992) and Castelain *et al.* (1994) showed that mass transfer affected flavour intensity perception. High percentage losses of volatiles occur during dehydration processing, unless mass transfer of the compounds becomes sufficiently rate-limiting to retard the losses (King, 1983). The resistance to diffusion of flavour volatiles within the food matrix rises dramatically when dehydration nears completion (Thijssen, 1971). Thijssen proposed that volatile compounds are retained in food matrices by a 'selective diffusion entrapment' mechanism, whereas Flink & Karel (1970) proposed a 'micro-region entrapment' theory. In the latter case small pools of volatiles have been entrapped and sealed in localised areas throughout the matrices of dried model systems. The

Table 2. **Odour active compounds of diced French beans after 0, 5, 10, 15, 30, 45 and 60 minutes rehydration in closed and open flasks, odour descriptions, number of assessors with an odour perception and in parentheses average odour intensity as perceived by the assessors at the sniffing port[a]**

Peak no	Compound	Odour description	Closed							Open					
			0[b]	5	10	15	30	45	60	5	10	15	30	45	60
1	Propanal	Citrus, chemical, sour	BD	5 (1.7)	4 (1.8)	4 (1.1)	5 (1.2)	6 (1.5)	5 (1.6)	BD	BD	BD	BD	BD	BD
2	Acetone	Chemical	BD	3 (1.1)	BD	3 (1.0)	5 (1.6)	3 (0.7)	BD	3 (0.8)	BD	BD	BD	BD	BD
3	2-Methylpropanal	Chocolate	5 (2.7)	5 (3.9)	5 (3.4)	5 (3.1)	5 (2.9)	5 (2.2)	4 (2.1)	5 (2.2)	6 (2.8)	6 (3.1)	5 (2.7)	4 (2.5)	BD
4	2-/3-Methylbutanal	Chocolate	8 (5.8)	10 (6.0)	8 (5.9)	8 (5.9)	7 (6.3)	8 (5.5)	8 (5.6)	8 (5.4)	8 (5.9)	8 (4.4)	8 (4.7)	8 (4.2)	6 (3.3)
5	2,3-Butanedione	Caramel	6 (4.8)	9 (6.5)	8 (6.3)	8 (6.3)	7 (5.6)	9 (5.4)	8 (6.8)	6 (4.0)	8 (6.5)	8 (5.0)	8 (4.1)	8 (5.0)	8 (3.7)
6	4-Methyl-2-pentanone	Soapy, chemical, chocolate, musty	3 (0.9)	4 (3.2)	BD	BD	BD	BD	BD	4 (1.4)	4 (1.7)	BD	BD	BD	BD
7	1-Penten-3-one	Sweet, chemical	3 (1.7)	5 (2.8)	BD	BD	BD	5 (1.9)	BD	4 (1.5)	BD	BD	BD	3 (1.5)	3 (1.7)
8	2-Butenal	Chemical, rotten	BD	BD	BD	BD	BD	4 (1.4)	3 (1.3)	BD	BD	3 (0.5)	BD	5 (1.2)	BD
9	Hexanal	Grassy	5 (2.7)	6 (4.3)	5 (3.2)	6 (3.2)	10 (3.8)	5 (2.3)	6 (2.8)	4 (1.9)	7 (3.6)	6 (2.6)	6 (2.6)	BD	3 (1.0)
10	2-Methyl-2-butenal	Chemical	BD	3 (2.4)	BD	5 (1.2)	5 (2.6)	3 (1.8)	8 (3.8)	3 (1.1)	5 (2.4)	4 (1.7)	5 (2.2)	BD	BD
11	Unknown	Chemical, rancid, grassy	BD	4 (2.8)	4 (3.1)	BD	6 (2.7)	3 (3.0)	6 (3.7)	5 (2.5)	3 (2.9)	4 (2.4)	4 (1.9)	3 (1.5)	3 (0.6)
12	Octanal	Citrus	3 (1.3)	3 (0.2)	4 (1.4)	BD	BD	BD	BD	3 (0.7)	BD	BD	3 (0.5)	BD	BD
13	1-Octen-3-one	Mushrooms	7 (3.5)	6 (4.1)	8 (3.5)	5 (2.3)	6 (3.8)	6 (4.1)	7 (4.6)	7 (4.4)	6 (4.1)	9 (3.9)	7 (3.5)	5 (2.5)	7 (3.3)
14	Dimethyl trisulphide	Rotten, spicy	6 (5.2)	9 (5.6)	7 (4.3)	7 (4.8)	7 (5.7)	7 (4.5)	8 (4.9)	7 (5.1)	8 (4.6)	7 (4.1)	9 (5.1)	6 (3.9)	8 (3.7)

[a] BD, below detection.

[b] Rehydration [min].

increase observed in the release of volatiles with extended rehydration could result from retarded release of the latter entrapped volatile compounds.

During extended rehydration in the closed mode (>15 min), both the number of assessors perceiving an odour active compound and the intensity scores decreased to some extent for several compounds, e.g. 2-methylpropanal, 2-/3-methylbutanal, 4-methyl-2-pentanone, 1-penten-3-one, hexanal and octanal. This could be due to volatilisation losses through the pressure relieve hole. Kermasha *et al.* (1988) showed similar decreases for several saturated aldehydes in French beans by thermal treatments. 2-Butenal, 2-methyl-2-butenal, peak no 11 and 1-octen-3-one increased in intensity. These compounds can originate from lipid oxidation reactions, which might be enforced during extended rehydration at an elevated temperature.

The mode of rehydration (closed, open) influenced the flavour profile of French beans as well. The number of assessors perceiving odour active compounds and odour intensity scores diminished in the open mode. This distortion of the profile is probably due to volatilisation losses. Distortion of the volatile composition of a product can alter the flavour as perceived during consumption (Dirinck & De Winne, 1994).

In GC/SP, the number of assessors perceiving an odour correlated significantly with odour intensity scores (Spearman's ranked correlation test, $P<0.05$), indicating that the number of assessors is a sufficient measure for odour intensity. This confirms previous results, in which volatile compounds (number of assessors perceiving an odour active compound in GC/SP) and sensory attributes (intensity scores) correlated quite well (Van Ruth *et al.*, 1995a & d).

French beans were subjected to texture measurements after 0, 5, 10, 15, 30, 45 and 60 min rehydration in closed and open flasks. A decrease in maximum forces in the shear press measurements was shown with extended rehydration

(Fig. 2). Analysis of variance of the data revealed that maximum forces were significantly influenced ($P<0.05$) by rehydration mode (open, closed) and rehydration time (5, 10, 30, 45, 60 min). No interaction was observed.

A QDA sensory panel of 20 judges evaluated the diced French beans after 0, 5, 10, 15, 30, 45 and 60 min rehydration in closed and open flasks. Although individual assessors responded differently, the panel was consistent across replications. Most of the attributes generated by the sensory panel were similar to the descriptors of the GC/SP panel (Table 1). Apparently, volatile compounds contributed to the flavour of rehydrated French beans. Mean scores for the sensory attributes are presented in Table 3. The flavour of rehydrated French beans is in particular characterised by the attributes 'crunchy', 'fibrous', 'French bean', 'cooked vegetables', 'grassy' and 'herbal', indicated by high intensity scores (>30) for French beans after 10 min rehydration in a closed flask (standard rehydration procedure as advised by the supplier of the vegetables).

Scores for texture attributes such as 'crunchy' and 'fibrous' decreased gradually with extended rehydration for both the closed and open mode. These results correlated well with the instrumental texture data. However, the latter data had a smaller linear correlation coefficient with the intensity scores for 'crunchy' in sensory analysis (closed mode, 0.81; open mode, 0.85) than the log transferred ones (closed mode, 0.90; open mode, 0.93). Similar linear relationships between the log stimulus and the perceived intensity were previously shown for taste (Pangborn, 1981), odour (Rothe *et al.*, 1994) and loudness (Galanter & Messick, 1961). The closed and open mode did not differ significantly in intensity scores for texture attributes, which could be due to the log relationship mentioned above. As the instrumental data showed rather small differences between the modes, this difference is not likely to cause a significant difference in intensity scores for sensory attributes.

The closed mode, as well as the open mode to a lesser extent, revealed a general increase in intensity scores for the attributes 'musty', 'rotten', 'mushrooms' and 'chemical' with extended rehydration. This could be due to partially increased or decreased release of odour active volatile compounds resulting in distortion of the flavour profile, as was shown in GC/SP. In the open mode, loss of volatile compounds diminished probably the effect of increased release with extended rehydration.

Sensory data were subjected to PCA, the first four principal components of which are shown in Table 4, together with the sample scores of all rehydration conditions. PCA shows relationships between attributes, e.g. 'sour', 'bitter', 'chemical', 'spicy' and 'citrus' loaded high on the first component, indicating that these attributes were associated with each other. Sample scores on the second component (attributes 'mealy', 'rotten', 'musty' and 'mushroom') increased with extended rehydration for both the closed and open mode. Sample scores on the fourth component ('fibrous' and 'crunchy') decreased gradually with extended rehydration.

PCA was conducted on combined GC/SP (intensity of odour active compounds) and QDA sensory data sets (intensity of sensory attributes). In general, sample scores on the first principal component increased with extended rehydration (Table 5). The sensory attributes 'crunchy', 'fibrous', 'grassy', 'citrus' and the compounds 2-methylpropanal, 2-/3-methylbutanal, 4-methyl-2-pentanone, 1-penten-3-one and octanal showed high negative loadings on the first component and correlated with shortly rehydrated beans. 'Sour', 'bitter', 'mealy', 'spicy', 'mushroom', 'chemical', 'burned', 'musty', 'rotten', 'wood' and 2-butenal and 2-methyl-2-butenal showed high positive loadings on the first principal component and correlated with extended

Table 3. Scores for sensory attributes and coefficients of variance (CV) of diced French beans after 0, 5, 10, 15, 30, 45 and 60 min rehydration in closed and open flasks (mean, $n=20$)

Code	Attribute	Closed							Open					
		0[a]	5	10	15	30	45	60	5	10	15	30	45	60
A	Sweet	31.5	25.9	26.1	26.9	22.0	29.8	30.6	32.8	31.2	32.4	26.5	33.8	33.9
B	Sour	13.7	10.4	15.3	19.0	20.2	19.4	20.8	15.8	19.0	17.4	20.2	15.3	16.9
C	Bitter	18.3	15.4	21.3	18.0	21.1	22.9	25.2	13.8	17.0	18.2	17.2	14.0	18.2
D	Crunchy	63.9	44.1	41.3	37.7	30.5	20.2	17.6	43.5	36.7	36.4	24.0	21.6	18.6
E	Fibrous	52.4	44.8	40.8	38.2	33.3	25.9	19.5	44.3	36.5	34.1	22.3	20.1	19.0
F	Mealy	17.4	26.2	24.8	32.0	31.8	38.4	37.1	23.3	30.9	28.4	32.2	34.7	31.3
G	French bean	24.1	37.5	31.5	36.0	33.7	31.9	34.2	38.3	38.3	40.2	39.4	41.0	40.9
H	Cooked vegetables	15.4	33.9	39.1	39.6	41.4	42.2	38.4	31.3	36.3	36.7	41.0	42.5	40.7
I	Grassy	37.8	39.2	39.2	40.9	32.0	32.1	31.0	32.3	37.9	36.5	29.5	35.3	35.1
J	Spicy	26.1	25.6	25.7	28.9	31.4	32.4	30.7	21.3	27.7	27.4	27.0	27.7	25.5
K	Herbal	28.7	30.9	34.8	32.7	30.0	29.2	27.6	29.4	28.5	30.7	24.5	30.3	24.5
L	Mushrooms	13.3	19.2	21.8	24.3	25.3	30.4	25.7	17.7	21.0	20.5	23.4	25.4	25.6
M	Chemical	12.7	9.0	15.4	16.6	22.0	19.3	20.4	10.7	12.2	12.0	12.4	17.3	18.8
N	Burned	15.6	12.6	19.1	17.5	15.6	21.7	21.3	13.9	15.3	15.8	17.4	16.1	16.7
O	Musty	23.8	25.8	25.6	26.4	30.9	39.4	36.5	19.7	22.6	22.6	27.8	29.4	30.2
P	Citrus	12.0	15.0	12.2	12.0	12.5	11.6	11.3	11.3	14.7	11.9	10.2	11.5	12.5
Q	Rotten	10.6	10.1	16.4	17.8	22.1	25.7	23.4	14.1	14.6	14.7	17.9	17.5	20.1
R	Wood	30.5	27.5	27.1	29.0	22.4	22.1	25.6	25.4	29.3	27.5	25.6	25.1	19.8
	CV [%]	32	33	30	25	27	30	24	27	28	29	29	27	34

[a] Rehydration [min].

Table 4. Scores of French bean samples (0, 5, 10, 15, 30, 45 and 60 min rehydration in closed and open flasks) on the first four principal components of the sensory data, attributes and loadings of attributes in parentheses

Principal Component	Attributes	Closed							Open					
		0[a]	5	10	15	30	45	60	5	10	15	30	45	60
1	Sour (0.8), Bitter (0.7), Chemical (0.7), Spicy (0.6), Citrus (0.6)	0.05	-0.31	0.04	0.01	0.20	0.15	0.28	-0.19	-0.02	-0.05	-0.04	-0.12	0.00
2	Mealy (0.7), Rotten (0.7), Musty (0.7), Mushrooms (0.6)	-0.60	-0.34	-0.15	0.06	0.18	0.73	0.54	-0.48	-0.18	-0.23	0.10	0.21	0.16
3	French bean (0.7), Cooked vegetables (0.7), Grassy (0.7), Herbal (0.6)	-0.51	0.18	0.12	0.22	-0.18	-0.16	-0.25	-0.01	0.17	0.24	-0.09	0.23	0.05
4	Fibrous (0.9), Crunchy (0.8)	1.63	0.68	0.46	0.29	-0.27	-0.66	-0.82	0.54	0.22	0.12	-0.66	-0.67	-0.87

[a] Rehydration [min].

Table 5. Scores of French bean samples (0, 5, 10, 15, 30, 45 and 60 min rehydration in closed and open flasks) on the first principal component of the combined sensory and gas chromatography/sniffing port data

Rehydration [min]	Closed	Open
0	-1.71	-1.71
5	-1.25	-1.08
10	-0.30	-0.38
15	0.01	-0.25
30	0.80	0.31
45	1.62	0.22
60	1.57	0.44

rehydration. Beans rehydrated in open flasks showed lower scores on the first principal component than their equivalents in the closed flasks. During extended rehydration the flavour seems to change from more ('grassy', 'citrus') to less vegetable-like attributes ('chemical', 'musty', 'rotten'), however, to a lesser extent in the open mode than in the closed mode.

Relationships between QDA sensory attributes and GC/SP volatile compounds showed a major contribution of volatile compounds to the differences in flavour of French beans resulting from rehydration conditions. Highest odour intensity scores in GC/SP and highest scores for sensory attributes were obtained in French beans rehydrated for 5 min in a closed flask.

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CHAPTER 10

GENERAL DISCUSSION

The present thesis dealt with flavour release from dried vegetables in three types of a mouth model system. This experimental design allowed the investigation of

(1) factors influencing flavour release under mouth conditions (Chapter 2, 3 and 4);
(2) suitability of the systems for simulation of flavour release in the mouth (Chapter 5, 6, 8 and 9);
(3) factors/conditions affecting the flavours of dried vegetables (Chapter 6, 7, 8 and 9).

In this chapter these three aspects will be subsequently discussed.

FLAVOUR RELEASE IN THE MOUTH

In the present study, flavour release under mouth conditions was shown to be influenced by saliva composition, by saliva volume, as well as by mastication. Saliva is secreted into the mouth by three major glands. Secretion of saliva from the salivary glands is generally elicited only in reponse to stimulation of the autonomic innervation to the glands or in response to drugs (Schneyer *et al.*, 1972). Changes in the intensity and duration of gland stimulation cause salivary flow rates to vary widely. Concomitant with the change in flow rate, they cause changes in the salivary composition too (Dawes, 1969 & 1974; Young & Schneyer, 1981). Saliva composition and flow rate are affected by the degree of hydration, body position, exposure to light, olfaction, smoking, previous stimulation, climatological circumstances, circadian and circannual rhythms (Shannon, 1966; Dawes & Ong, 1973; Dawes, 1987; Wisniewski *et al.*, 1992). Both saliva composition and volume can affect flavour release in the mouth. Flavour compounds could interact with saliva components. The combined volume of product and saliva was reported as variable in models for flavour release (McNulty, 1987; De Roos & Wolswinkel, 1994).

In the present work, salts in saliva did not affect flavour release, but mucin resulted generally in a decreased release of volatile compounds from rehydrated bell peppers (Chapter 3), as well as from French beans (Chapter 4). Similar effects of proteins were shown previously in studies of Kim & Min (1988), Kinsella (1988) and O'Keefe *et al.* (1991). α-Amylase showed a similar protein effect in bell peppers, which had very low starch contents. In contrary, flavour release in French beans (high starch content) was increased by α-amylase, probably by degradation of starch inclusion complexes formed during dehydration. Dilution by saliva decreased flavour release in French beans markedly, which agrees with flavour release models as presented by McNulty (1987) and De Roos & Wolswinkel (1994).

As gas chromatography/sniffing port (GC/SP) patterns were hardly influenced by saliva composition and volume (Chapter 4), and GC/SP and sensory analysis correlated quite well (Chapter 6, 8 and 9), only slight effects on flavour perception of rehydrated French beans can be expected by saliva composition and volume.

Mastication increased flavour release from rehydrated bell peppers, French beans and leeks substantially (Chapter 2, 3, 4 and 5). In conjunction, the GC/SP patterns of volatile compounds of bell peppers isolated in the dynamic headspace system with (DHM) and without mastication (DH) demonstrated a remarkable difference (Chapter 3). Therefore, mastication is expected to affect flavour perception. This is in agreement with sensory evaluations of Burdach & Doty (1987), who reported different flavour scores of assessors by various mouth movements.

SUITABILITY OF THE MOUTH MODEL SYSTEMS

Representative isolation of volatile compounds and selection of aroma contributing compounds out of the range of volatile compounds are important aspects in instrumental flavour analysis. These aspects are crucial to obtain relationships between instrumental procedures and sensory analysis (Dirinck & De Winne, 1994). The present thesis showed that flavour release from three rehydrated vegetables in the DHM mouth model system did not differ significantly from flavour release in the mouth (Chapter 5). Headspace techniques without mastication are frequently performed in instrumental flavour analysis of vegetables (Freeman & Whenham, 1974; Fischer & Grosch, 1987; Shinohara *et al.*, 1991; Pino, 1992; Luning *et al.*, 1994). However, flavour release in the DH as well as in the purge-and-trap model system differed significantly from release in the DHM model and in oral vapour, in the present work. These results indicated the necessity of mechanical treatment in headspace techniques for simulation of flavour release in the mouth.

Correlations of instrumental and sensory flavour data implicates that:

(1) isolation and selection of volatile compounds were performed in a sufficient way;

(2) volatile compounds contributed to the flavour of the product.

In the present study volatile compounds were correlated with sensory attributes of vegetable samples, which were compared on cultivar, as well as on storage and rehydration conditions. Results confirmed the suitability of mouth model system DHM in combination with GC/SP (Chapter 6, 8 and 9). In Chapter 7 and 8 samples differed markedly in flame ionisation detection (FID) patterns obtained, although GC/SP patterns and scores for sensory attributes hardly differed. These results implicated that GC/SP data are more suitable for correlation with sensory data than GC/FID data. This is probably due to the fact that sensory intensity is not linear related to the physical concentration of a volatile compound. Galanter & Messick

(1961), Pangborn (1981) and Moskowitz (1983) showed that sensory data are often linear related to the log of the stimulus intensity, as described by Fechner's law (Meilgaard *et al.*, 1991). In similarity, Rothe *et al.* (1994) reported positive correlations between the odour intensity of a volatile compound perceived at the sniffing port and the log physical concentration.

Besides 'odour' aspects, 'taste' aspects are also important in flavour evaluation (Chapter 7). The present thesis was mainly focused on 'odour' aspects. Factors influencing flavour release in the mouth (e.g. saliva composition and volume) and storage and rehydration conditions were expected to result in particular in 'odour' alterations.

FACTORS/CONDITIONS AFFECTING THE FLAVOURS OF DRIED VEGETABLES

The cultivar of the vegetables (Chapter 6), as well as the origin (Chapter 7) were shown to determine the flavours of the dried vegetables. Although differences between three vegetables were obvious, similarities were found too; 12 common odour active compounds were determined in rehydrated bell peppers, French beans and leeks. Three origins of bell peppers hardly differed in GC/SP patterns and in scores for 'odour' attributes in sensory analysis. They differed in 'taste' attributes only, these attributes were therefore assumed to be responsible for differences in preference of Turkish and Chilean bell peppers. Differences in origin of bell peppers included possibly differences in cultivation, handling, transport and drying conditions. These individual aspects were not included within the scope of the present thesis, but were studied by Luning (1995).

Alterations in the flavour of rehydrated French beans by storage and rehydration conditions were described in Chapter 8 and 9, respectively. Substantial alterations in the flavour of French beans by storage conditions (water activity, light and temperature) resulted in changes in GC/SP patterns, in scores for flavour attributes in sensory analysis, as well as in preferences. However, the flavour of French beans was stable during storage over two months in absence of light, at 20°C and at water activity 0.3.

Rehydration of French beans led to increased release of volatile compounds, which was probably due to impeded flavour release in the non-rehydrated beans. During isolation, the dense structure of the latter French beans caused resistance to mass transfer of volatile compounds into the saliva mixture. Extended rehydration effectuated a distortion of the volatile composition and an increase in scores for less vegetable-like attributes of French beans in sensory analysis. Best results were obtained by short rehydration (5-10 min).

Finally, the present thesis revealed that flavour release from rehydrated vegetables was influenced by both saliva composition and volume. Mouth model system DHM simulated sufficiently flavour release in the mouth. The flavours of dried vegetables were shown to be influenced by cultivar, origin, as well as by storage and rehydration conditions.

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SUMMARY

The research described in this thesis was focused on the development of an *in vitro* model system for isolation of volatile compounds from dried vegetables under mouth conditions, such as volume of the mouth, temperature, salivation and mastication. Instrumental analysis of these volatile compounds by gas chromatography (GC), combined with mass spectrometry, flame ionisation detection (FID) and sniffing port detection (SP) was correlated with descriptive sensory analysis. Dried vegetables were subjected to these techniques in order to study three aspects. (1) Factors influencing flavour release from dried vegetables under mouth conditions. (2) Suitability of three types of the model system for simulation of flavour release in the mouth. (3) Characterisation of the flavours of rehydrated vegetables, as influenced by cultivar, origin, storage and rehydration conditions.

Flavour perception, physical aspects of flavour release, flavour analysis and factors affecting the flavour of dried vegetables, including literature concerned were discussed briefly in **Chapter 1**. The thesis was outlined at the end of this chapter.

Three types of the mouth model system were introduced in **Chapter 2** and compared for flavour release from rehydrated bell peppers. Release was largest in the 'purge-and-trap' system, followed by the 'dynamic headspace and mastication' (DHM) and 'dynamic headspace' system, respectively.

Flavour release influenced by the composition of artificial saliva was studied for bell peppers in **Chapter 3** and for French beans in **Chapter 4**. Influence of saliva volume on the flavour release from French beans was studied in **Chapter 4** as well. Saliva component mucin mainly decreased flavour release in both vegetables, because of flavour-protein interactions, a similar effect showed α-amylase in bell peppers. Release in French beans was generally increased by α-amylase, probably due to degradation of inclusion complexes of starch. Decrease in flavour release

from French beans by saliva volume was evaluated in a model study. GC/SP patterns of odour active compounds were hardly influenced by both saliva composition and volume.

Chapter 5 revealed that flavour release from rehydrated French beans, red bell peppers and leeks in the DHM model system did not differ significantly from their release in the mouth of 12 assessors. These assessors released volatile compounds with different efficiencies, but they showed a statistically consistent efficiency in flavour release across the vegetables.

The flavours of the three rehydrated vegetables mentioned above were characterised by GC/SP of volatile compounds released in the DHM model system and by descriptive sensory analysis (**Chapter 6**). A common odour profile was shown in the vegetables, comprising each of the odour active compounds present in French beans. Correlation of rehydrated vegetables with sensory attributes and volatile compounds showed considerable contribution of volatile compounds to the flavour of rehydrated vegetables.

The flavours of commercially dried bell peppers (origins Chile, Hungary and Turkey) were evaluated by GC/SP and descriptive and hedonic panels (**Chapter 7**). Although the three origins differed substantially in FID patterns, GC/SP patterns and scores for 'odour' attributes in sensory analysis were hardly different. Differences in appreciation between Chilean and Turkish bell peppers are therefore expected to be due to differences in 'taste' attributes (sour, bitter, sharp and pungent).

The influence of storage conditions (water activity (aw), temperature and light) on the flavour of dried French beans was studied in **Chapter 8**. Elevated temperature at aw 0.3 and 0.5 resulted in an increase in the number of assessors perceiving chemical, rotten odours in GC/SP and in an increase in scores for 'chemical', 'burned', 'musty' and 'bitter' attributes in sensory analysis. Light exposure at aw 0.1 resulted in an increase in the number of assessors perceiving chemical odours at the sniffing port and in increased scores for 'chemical' and 'burned' attributes in sensory analysis. French beans stored at 20°C were more appreciated by the hedonic panel than those stored at 40°C (absence of light, aw 0.3). Relationships between differently stored French beans, sensory attributes and volatile compounds indicated the latter compounds to be responsible for the difference in flavour and appreciation of dried French beans by storage conditions.

The flavour of French beans influenced by rehydration conditions was described in **Chapter 9**. Extended rehydration resulted in GC/SP in increased intensity for 2-butenal, 2-methyl-2-butenal, 1-octen-3-one and one unknown compound and in increased intensities for 'chemical', 'mealy', 'mushroom' and 'musty' attributes in sensory analysis. Both instrumental and sensory analysis of the texture revealed a gradual decrease with extended rehydration. Relationships between sensory attributes and volatile compounds showed major contribution of volatile compounds to the difference in flavour of French beans resulting from rehydration conditions.

It can be concluded (**Chapter 10**) that flavour release from rehydrated vegetables was influenced by both saliva composition and volume. Mouth model system DHM simulated flavour release in the mouth quite well. The flavours of dried vegetables were shown to be influenced by cultivar, origin, storage and rehydration conditions.

SAMENVATTING

In dit proefschrift wordt een onderzoek beschreven dat gericht was op de ontwikkeling van een *in vitro* modelsysteem ten behoeve van de isolatie van geurhoudende verbindingen uit gedroogde groenten. Tijdens de isolatie werden de omstandigheden van de mond nagebootst, zoals volume van de mond, temperatuur, aanwezigheid van speeksel en mechanische verkleining. De instrumentele analyses van deze vluchtige verbindingen door middel van gaschromatografie (GC), gecombineerd met massaspectrometrie, vlamionisatiedetectie (FID) en snuffelpoortdetectie (SP), werden gecorreleerd met beschrijvende sensorische analyses. Met behulp van de bovenstaande technieken werden drie aspekten bestudeerd. (1) Faktoren die het vrijkomen van vluchtige verbindingen beïnvloeden onder de omstandigheden van de mond. (2) Mate van geschiktheid van drie uitvoeringen van het modelsysteem voor de simulatie van het vrijkomen van vluchtige verbindingen in de mond. (3) De smaak van gedroogde groente, zoals die wordt beïnvloed door soort, herkomst en omstandigheden tijdens bewaren en rehydratatie (opkoken).

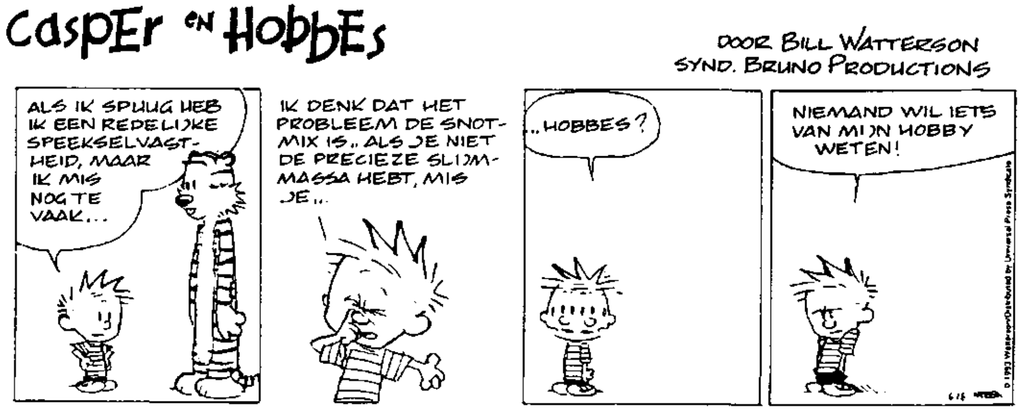
In **Hoofdstuk 1** werden een viertal onderwerpen tesamen met de hierop betrekking hebbende literatuur besproken: smaakgewaarwording, fysische aspekten van het vrijkomen van vluchtige verbindingen, instrumentele en sensorische analyse van smaak en faktoren die de smaak van gedroogde groente beïnvloeden.

De drie uitvoeringen van het modelsysteem voor de mond zijn beschreven in **Hoofdstuk 2**. Deze uitvoeringen werden vergeleken voor wat betreft het vrijkomen van vluchtige verbindingen uit gerehydrateerde stukjes gedroogde paprika. De grootste hoeveelheden kwamen vrij in het 'purge-and-trap' systeem, gevolgd door respectievelijk het 'dynamic headspace and mastication' (DHM) en 'dynamic headspace' systeem.

De invloed van de samenstelling van kunstspeeksel op het vrijkomen van vluchtige verbindingen uit paprika's werd intensiteiten van de attributen 'chemisch', 'melig', 'paddestoel' en 'muf' toe bij de sensorische analyses. Zowel instrumentele als sensorische analyses van de textuur lieten een geleidelijke afname zien bij langere rehydratatie. Correlaties van sensorische attributen en vluchtige verbindingen duidden op een belangrijke bijdrage van de vluchtige verbindingen aan de smaakverschillen van sperziebonen ten gevolge van de omstandigheden tijdens rehydratatie.

Samenvattend werd geconstateerd (**Hoofdstuk 10**) dat het vrijkomen van vluchtige verbindingen uit gerehydrateerde groenten wordt beïnvloed door zowel de samenstelling als het volume van speeksel en dat het vrijkomen van vluchtige verbindingen in de mond goed nagebootst wordt door het DHM modelsysteem. Er werd tevens geconstateerd dat de smaak van gedroogde groenten beïnvloed wordt door soort, herkomst en omstandigheden tijdens bewaren en rehydratatie.

DANKWOORD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WATTERSON

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Saskia

CURRICULUM VITAE

Saskia Marieke van Ruth werd op 15 juli 1965 te Mijdrecht geboren. Na het behalen van het diploma Voorbereidend Wetenschappelijk Onderwijs aan de Rijksscholengemeenschap te Meppel in 1984, begon zij aan haar studie Levensmiddelentechnologie aan de toenmalige Landbouwhogeschool. Van mei tot augustus 1989 voerde zij een stage uit op het gebied van de levensmiddelenchemie aan de University of Nebraska-Lincoln (USA). Het doctoraal diploma werd haar uitgereikt op 26 maart 1990, met als afstudeervakken Levensmiddelenchemie, Voedingsleer en Voedingsmiddelen van Dierlijke Oorsprong. Vanaf 1 februari 1990 was zij achtereenvolgens als onderzoeker werkzaam bij de sectie Levensmiddelenchemie en -microbiologie, vakgroep Levensmiddelentechnologie van de Landbouwuniversiteit (Wageningen), Handelsmij A. Smit & Zn (Amersfoort), vakgroep Instrumentele Analyse aan de Technische Universiteit (Eindhoven) en Plukon (Wezep).

Het in dit proefschrift beschreven onderzoek werd vanaf 1 januari 1992 uitgevoerd binnen de sectie Levensmiddelenchemie en -microbiologie, vakgroep Levensmiddelentechnologie van de Landbouwuniversiteit te Wageningen, waar zij als Assistent in Opleiding was aangesteld.