Characterisation of the flavour of fresh bell peppers (*Capsicum annuum*) and its changes after hot-air drying; an instrumental and sensory evaluation

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ter verkrijging van de graad van doctor

in de landbouw- en milieuwetenschappen

op gezag van de rector magnificus,

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STELLINGEN

1. Het aroma van alle soorten paprika's bevat de karakteristieke paprika component 2-isobutyl-3-methoxypyrazine, en daarbij wisselende combinaties van aliphatische verbindingen met groene, groen fruitige en bloemige geureigenschappen.
   Dit Proefschrift.
   Govindarajan, V. S. Capsicum-Production, Technology, Chemistry, and Quality. Part III. Chemistry of the Color, Aroma, and Pungency Stimuli. *CRC Crit. Rev. Food Sci.Nutr.* **1986**, *24*, 245-355.

2. Het optellen van twee eerste-orde kinetische mechanismen leidt niet tot een tweede-orde mechanisme zoals gesuggereerd door Park et al. (1988).
   Dit proefschrift.
   Park, K.-H.; Kim, Y.-M.; Lee, C.-W. Thermal Inactivation Kinetics of Potato Tuber Lipoxygenase. *J. Agric. Food Chem.*, **1988**, *36*, 1012-1015.

3. In hun discussie omtrent de homologie tussen paprika en tomaat polygalacturonase (PG) zijn de auteurs vergeten te vermelden dat het tomaten PG een endo-knippend enzym is, terwijl de door hen gebruikte methode voor PG-activiteit in paprika geen onderscheidt maakt tussen endo en exo PG.
   Jen, J. J.; Robinson, M. L. Pectolytic Enzymes in Sweet Bell Peppers (*Capsicum annuum* L.). *J. Food Sci.* **1984**, *49*, 1085-1087.

4. Het schema voor de vorming van hexanal uit (*Z*)-3-hexenal, zoals voorgesteld door Stone et al. (1975) in tomaat, is niet waarschijnlijk.
   Dit Proefschrift.
   Stone E.J.; Hall, R. M.; Kazeniac, S. J. Formation of Aldehydes and Alcohols in Tomato Fruit from U-^{14} C-Labeled Linolenic and Linoleic acids. *J. Food Sci*, **1975**, *40*, 1138-1141.

5. De afbraak van chlorophyll en de vorming van carotenoiden tijdens rijping bepalen de kleur van paprika o.a., groen, rood, geel, oranje, wit, paars.
   Dit proefschrift.
   Daood, H. G.; Biacs, P. A.; Huszka, T.T.; Alcarza, C.F. International Conference Agri-Food Quality '95, IFR, June 1995, Norwich, UK.

6. De fysiologische en ecologische rol van secundaire metabolieten in hogere planten zou ook belangrijk kunnen zijn voor hun offensieve strategieën, naast de veelal onderzochte defensieve opslag of afweermechanismen.
   Rhodes, M. J. C. Physiological Roles for Secondary Metabolites in Plants: Some Progress, Many Understanding Problems. *Plant Mol. Biol.*, **1994**, *24*, 1-20.

7. Het aroma van paprika is als een pianospel, de combinatie van toetsen en de hardheid van de aanslag vormen de melodie.
   VPRO programma Noorderlicht november 1992.

8. Ondanks de computermatige verwerking van gaschromatogrammen blijft het gewoon monnikenwerk.

   Dit Proefschrift.

9. De emotiegerichte strategieën van mannen zijn anders dan van vrouwen. Mannen geven de voorkeur aan een oplossing waarbij ze het probleem buiten zichzelf plaatsen. Vrouwen zoeken juist de oorzaak van het probleem bij zichzelf.

   Intermediair 43, 28 oktober 1994

10. Een onderdeel van een personeelsadvertentie, zoals "Onderzoekers zijn vaak op hun best als het tijdelijk is" kan de overheidszorg voor de gezondheid duur komen te staan.

11. Het jaarlijks begeleiden van een DB-tje Don-Bosco kinderkamp op basis van vrijwilligheid, plaatst de werknemer/ster weer terug in het juiste perspectief van zijn/haar baan.

12. Regelmatig sporten houdt het lichaam gezond en verruimt de geest.

13. De luu, die 't hardste warkt, woont vaak in de kleinste hoessies.

Stellingen behorende bij het proefschrift:
"Characterisation of the flavour of fresh bell peppers (*Capsicum annuum*) and its changes after hot-air drying; an instrumental and sensory evaluation"
Pieternel Luning
Wageningen, 18 september 1995

Opgedragen aan diegenen die me dierbaar zijn

"Als ik een groenende twijg in mijn hart bewaar zal de zangvogel zeker komen"

"All things that is of good odor hath naturall heate though Camphir, Roses and things cold have sweet odors as authors have soules....

Also by odors this maie you learne,
Subtilness and groseness of matters to discerne.
A sweete-smelling thinge hath more puritie
and more of spirituall than stinking maie bee.
As colour changeth in your sight
So odours changeth the smelling by might"

(*Ordinall of Alkimy 1447: Observations on odours by Thomas Norton*)

Content

Chapter 1

General Introduction

Capsicum fruits appear to be one of the oldest spices used in the diet, dating from 5000-7000 B.C. in Mexico and Central and South America. Nowadays, *Capsicum* varieties are cultivated in most parts of the world (Govindarajan et al., 1987). The botanical classification of *Capsicum* members showed at least two major species i.e., *C. annuum* and *C. frutescens* (Bailey and Bailey, 1976). Varieties that belong to the first group are in general larger and less hot or even lack this characteristic property, while the other specie includes the smaller and hotter fruits. Usually trivial names are used like Sweet Chillis, Bell Peppers, Paprika, and Cayenne which belong to *C. annuum*; Hot Chili, Jalapenos, and Tabasco peppers are generally considered to belong to *C. frutescens* (Keller et al., 1981).

Fruits of different *Capsicum* varieties are consumed fresh or processed, as immature (i.e., green) or as mature fruit (i.e., red, yellow, white), as spice or as vegetable in the food, because of their distinct colours, pungency, and flavours (Govindarajan, 1986; Govindarajan et al., 1987). The acceptance of fruits and vegetables by consumers is generally determined by characteristics such as, the absence of external defects, size, shape, colour, and flavour. These characteristics are influenced by chemical and biochemical changes that occur during growth and maturation (Eskin, 1982). The maturation stage at which fruits and vegetables are harvested affects as well the quality of the fresh as the final quality of the processed fruits. Breeding programs of *Capsicum* varieties used to be mainly focused on disease resistance, crop yield, nutritional value like vitamin C content, pungency, and carotenoids levels (Somos 1984; Govindarajan, 1985; Minguez-Mosquera et al., 1994), which resulted in a wide assortment of different coloured *Capsicum* fruits. However, the growing "fresh" market and the increasing use of dried vegetables in convenience foods, shifted attention towards flavour as a quality parameter for fresh and dried vegetables (Feinberg 1973; Govindarajan et al., 1987; Koller, 1988; Zachariasse and Abrahamse 1993). The elucidation of factors controlling flavour quality of vegetables and fruits requires research on chemical and biochemical changes during maturation and processing (Freeman, 1979).

General aspects of flavour of fresh fruits and vegetables

What means flavour? The interaction of taste, odour, mouthfeel, sight, and sound when eating food provides an overall sensation, which is defined by the English expression *flavour. Sound, light, and mouthfeel are physical or indirect senses,* which influence taste and smell, and therefore influence food acceptance. The role of pectolytic enzymes, i.e., polygalacturonase (PG) and pectinesterase (PE), for the texture of tomatoes has been extensively studied; softening occurs upon advanced ripening of the fruits affecting negatively their firmness (Hobson, 1965; Sawamura et al., 1978; Tucker et al., 1980; Tucker and Grierson, 1982). The texture of bell peppers (*C. annuum*) is mainly characterized by its crispiness, which is also effected by these pectolytic enzymes. Jen and Robinson (1984) reported that, PG activity increased (maximal at the turning stage), while PE activity declined (maximal at the early immature green stage) during ripening. Activities of PG and PE in the turning and red stages were respectively 116- to 164-fold and 429- to 1900-fold lower than the corresponding tomato enzymes at the same ripening stages. The degradation of texture is therefore, a slow process in bell peppers, and maybe less important for the quality of freshly harvested fruits.

The remaining sensations are responses of the chemical senses and can globally be divided into two groups, i.e., compounds responsible for taste and compounds responsible for odour. Compounds belonging to the first group are mainly non-volatile at room temperature and interact with taste receptors located in taste buds on the tongue. Only four primary tastes are generally acknowledged i.e., bitter, salty, sweet, and sour. For the definition of these tastes, four chemical reference compounds have been established, i.e., quinine (bitter), sodium chloride (salty), sucrose (sweet), and hydrochloric acid (sour). However, all standard taste substances are capable of eliciting all primary tastes to a certain extent. Whether, this is due to impurities of the references or their ability to elicit other tastes is not yet clear (McBurney and Schick, 1971; Shallenberger, 1993). According to Amerine et al. (1965), typical bitter stimuli in plant foods are alkaloids like quinine, caffeine, and strychnine, but also the L-isomers of hydrophobic α-amino acids. Sodium chloride typifies the salty taste, while chlorides, bromides, iodides, nitrates, and sulphates of potassium and lithium give mixed tastes besides the salty taste. The sweet taste is generated by several alcohols, glycols, sugars, and sugar derivates. The sour taste is generally caused by inorganic and/or organic acids; not all acids are sour, i.e., amino acids are often sweet or bitter (Amerine et al., 1965). The taste intensity can be analysed in several ways such as, determination of the threshold concentration of a substance, paired, and multiple comparison techniques, and sensory magnitude scaling (Shallenberger, 1993). The recognition threshold is defined as the minimum concentration of a substance for which a particular taste can be recognized by a significant number of tasters; these thresholds can be applied as index of the magnitude of a compound's activity towards the taste receptor (Amerine et al., 1965; Shallenberger, 1993).

The major tastes of fresh fruits and vegetables are sweet and sour, which are mainly determined by sugars and organic acids. Additionally, tannins and (non)hydrolysable phenolic compounds also affect taste; some appeared to be responsible for astringency. In general, sourness and astringency decreased while sweetness increased during growth and ripening of fruits (Rhodes, 1980; Eskin, 1988). However, there are exceptions such as for bananas, in which the pH falls during ripening from 5.4 to 4.5 and the contents of the organic acids malate and citrate increased (Rhodes, 1980). Moreover, sugars and organic acids not only contribute to the sweet and sour taste, but can also influence the overall flavour intensity, e.g., in tomatoes (Stevens et al., 1979). A number of authors have demonstrated the presence of sugars, i.e., glucose, fructose, and sucrose in several *Capsicum* varieties, and it was suggested that the harvest time could have a considerable effect on the sweet taste (Polacsek-Rácz et al., 1981; Tarrach and Herrmann, 1986; Nielsen et al., 1991; Hubbard and Pharr, 1992). Also some organic acids were detected, but their implication for the sourness of *Capsicum* fruits was not determined (Rhaman et al., 1978; Tarrach and Herrmann, 1986; Biacs et al., 1992).

Non-specific or trigimental neural responses also provide important contributions to the flavour through detection of, for example cooling or pungency. The latter property is one of the major factors determining the quality of the hot *C. frutescens* varieties; the preference for particular types is even affected by the extent of pungency (Govindarajan, 1987). The pungency or "hot taste" of *Capsicum* fruits is determined by the composition of capsaicinoids and varies over a wide range (Govindarajan, 1986; Quinones-Seglie et al., 1989). Dutch trade varieties generally belong to the *C. annuum* species and lack the pungent principles. Although, Iwai et al. (1977) reported that sweet *C. annuum* L. var. *grossum* formed capsaicinoids during post-harvest ripening under continuous light.

Compounds belonging to the other group are volatile and are perceived by the odour receptor sides of the olfactory tissue of the nasal cavity. The lowest concentration of a compound that can be still detected and recognized by its odour, is defined as an odour threshold. The odour potency of a volatile compound is determined by its threshold value. This value depends on the vapour pressure, which is affected by temperature and medium, but also by the assay procedure and/or performance of the sensory panel (Belitz and Grosch, 1986). Compilations of literature on threshold concentrations, therefore, show frequent discrepancies in these values (Van Gemert and Nettenbrijer, 1977; Fazzalari, 1978). The odour potency, the concentration, and the composition of volatile compounds determine the aroma of the food. Particularly important are the so-called "character impact compounds" which bear the characteristic aroma of the food. Up to now, only a restricted number of these compounds have been identified, e.g., nootkatone (grapefruit), ethyl-2-methylbutyrate (apple), (*E,Z*)-2,6-nonadienal (cucumber), 1-octen-3-ol (mushroom), and 2-isobutyl-3-methoxypyrazine in bell peppers. Their occurrence in fruits and vegetables have been comprehensively described by several authors (Lindsey, 1985; Chang and Huang, 1989; Whitfield and Last, 1991). Belitz and Grosch (1986) divided food with regard to the presence of such key compounds, i.e., a) the aroma of the food is created by one impact compound in the presence of a few other compounds, which only round-off the characteristic aroma, b) several compounds are important, of which one is the major compound, c) a large number of volatile compounds, usually without a key compound, determine the aroma, which can be still satisfactorily reproduced, and d) the last group includes food, which consists of a large number of volatile compounds, lacking of a key compound and the aroma can not be reproduced. The aromas of fresh fruits and vegetables often belong to the first or second group, whereas aromas of thermally processed and/or fermented food are mostly classified in the last two groups. Moreover, the concentration of volatile compounds is important for the aroma of a food as well, e.g., aldehydes at low levels may provide the desired aroma, while at higher concentrations it may give an off-flavour (Grosch, 1987). Obviously, fruits and vegetables have many volatile compounds in common (Van Straten and Maarse, 1991), whereas their aromas are distinctively different, which suggests that the composition of these volatile compounds is also an important factor in determining the typical aromas of fruits and vegetables.

Volatile odour compounds originate from chemical and/or biochemical reactions. Several classifications of volatiles, on the basis of their origin, have been proposed like, primary and secondary compounds (Schreier, 1986), volatile compounds formed by enzymatic, thermal, and oxidative reaction mechanisms (Rothe, 1988), and volatile compounds originating from nonenzymatic and/or enzymatic reactions (Belitz and Grosch, 1986). Schreier (1986) suggested that basically two groups can be distinguished in fresh and processed fruits and vegetables, i.e, primary and secondary compounds. Primary volatile compounds are formed enzymatically from non-volatile precursors via intercellular pathways during growth and maturation. These primary volatile compounds are present in the raw product and are often unstable at high temperatures. Secondary volatile compounds can be divided in a) compounds which are enzymatically formed upon tissue disruption when substrate and enzyme match, and b) compounds formed upon heating; the latter process will be discussed in more detail in the drying part.

In spite of the great diversity of volatile compounds, a restricted number of basic biochemical pathways appeared to be responsible for the production of volatiles in fresh fruits and vegetables (Eriksson, 1979; Tressl and Albrecht, 1986). A scheme of principle pathways by which fruit volatiles can be formed has been supposed by Tressl and Albrecht (1986) as shown in Figure 1.

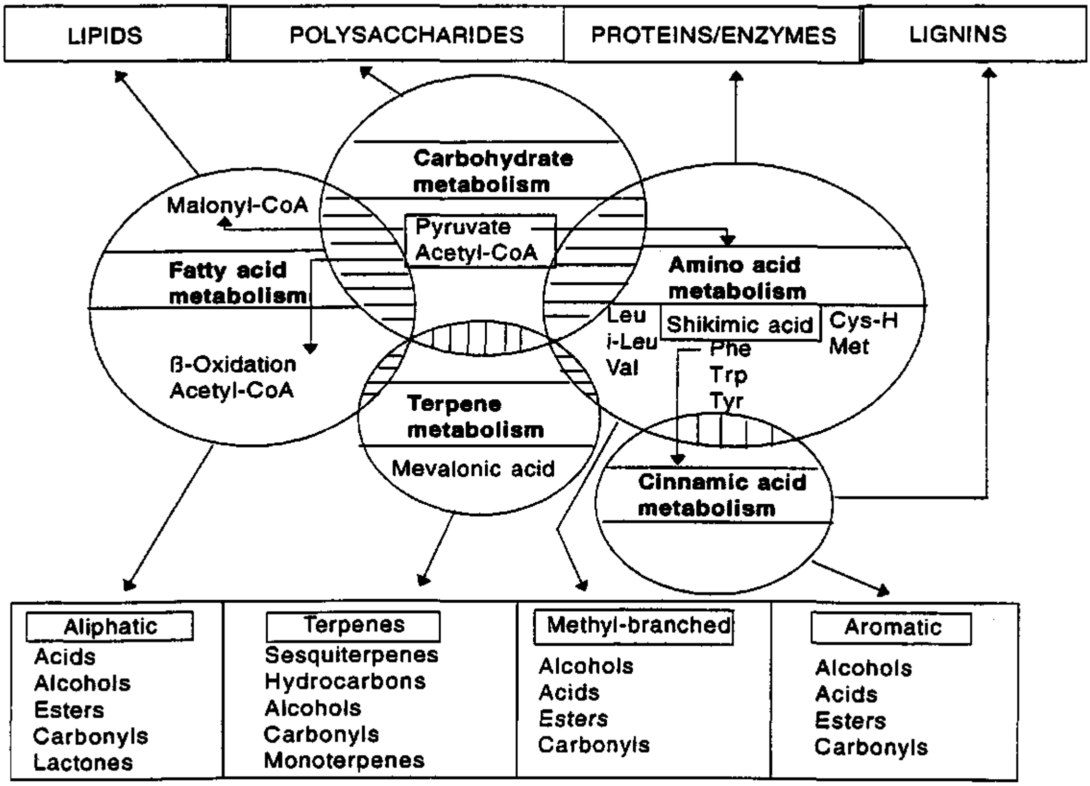

Figure 1. Formation of volatile compounds in fruits, metabolism of fatty acids, terpenes, amino acids, and carbohydrates (Tressl and Albrecht, 1986).

Carbohydrate degradation results only in the production of ethanal, while amino acids and fatty acids are precursors of a large number of volatile compounds (Belitz and Grosch, 1986). Many methyl branched alcohols, acids, esters, ketones, sulphur containing, and aromatic compounds are derived from amino acid metabolism (Fig. 1). For example, Tressl et al., (1977) proposed a pathway for the formation of asparagusic acid (1,2-dithiolane-4-carboxylic acid) with valine as precursor. Belitz and Grosch (1986) postulated a reaction in which 2-isobutylthiazole, an important odour compound of tomato, was formed as a product of the secondary metabolism of leucine and cysteine. Leucine was also assumed to be a precursor in the biosynthesis of 2-isobutyl-3-methoxypyrazine in *Capsicum* fruits as proposed by Murray et al. (1970) (Fig. 2).

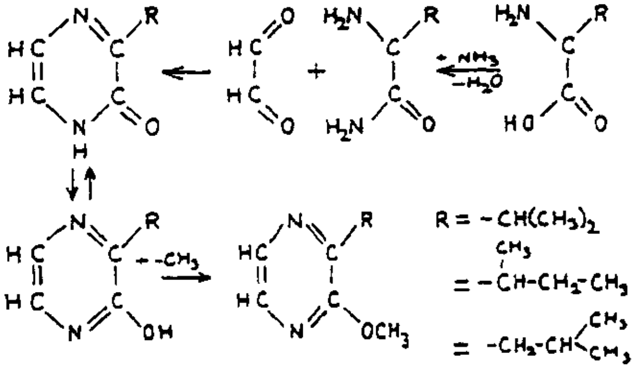

Figure 2. Proposed pathway for the biosynthesis of 2-methoxy-3-alkyl pyrazines (Murray, et al., 1970).

The ß-oxidation of fatty acids (Fig. 1) is an important source of odour compounds, which are basically formed in the intact cells i.e., primary volatiles. The biosynthesis of esters and alcohols from fatty acids by slices of fruit tissue of pears and strawberries have been demonstrated by several authors (Tressl and Drawert, 1973; Yamashita et al. 1977; Tressl and Albrecht, 1986). The production of these volatiles changed during ripening. Several esters have been reported in *Capsicum* varieties (Van Straaten en Maarse, 1991), but no biosynthetic pathways have been reported so far.

content decreases. Verifications for the existence of this theory have been comprehensively described by King (1988). The second theory includes the physical encapsulation of volatile compounds by macromolecules upon formation of microregions during the drying process. Several factors such as, initial content of solids, type of solids, sample thickness, initial volatile concentration, and drying rate, were considered to control the retention of volatiles (Chirife et al., 1973; Bartholomai et al., 1975a,b; Bangs and Reineccius, 1981; Rosenberg, et al., 1990).

Moreover, the loss of volatile compounds after drying may be due to inactivation of volatile forming enzymes and reduction of the precursors as well. Freeman and Whenham (1975) reported that alliinase activity of fresh onions decreased up to 90% after hot-air drying, while freeze-dried onions still showed 45% activity. Wijaya et al. (1991) and Leino (1992) reported that hot-air drying reduced markedly the amount of typical sulphur compounds, which were formed by alliinase activity.

Besides the loss of volatile compounds by physical and/or biochemical processes, chemical reactions can introduce new compounds and consequently influence the composition of volatile compounds too. Two major chemical reactions will be discussed, i.e., autoxidation and Maillard reaction. The flavour of food containing unsaturated fatty acids, can be drastically affected, if these lipids are in contact with air. Initiators such as light, metallic ions, and heat can initiate the autoxidation reaction, whereby unsaturated fatty acids [RH] form alkyl radicals [$R^\cdot$] and peroxyl radicals [$ROO^\cdot$]. The reaction propagates in the presence of oxygen by a free radical chain mechanism, as shown in Fig 3, to form the primary products of autoxidation, i.e., the hydroperoxides [ROOH]. The formation of the peroxy radicals [$ROO^\cdot$] in step 2 is extremely fast ($k = 10^9$ l. mole^{-1}. s^{-1}), while step 3 is the slowest reaction ($k = 10\text{-}60$ l. mole^{-1}. s^{-1}) and therefore controls the total autoxidation process (Uri, 1961, Ingold, 1963; Grosch, 1982). The decomposition of the hydroperoxides in step 5 increases the reaction rate (autocatalysis) and forms the starting point of the formation of volatile compounds (Grosch, 1982). Fragmentation of the hydroperoxides occurs by homolytic β- and heterolytic α-scission mechanisms, which affect the type of decomposition products formed (Frankel, 1982, 1990).

Initiation (1) RH -----> $R^{\cdot}$
Propagation (2) $R^{\cdot} + O_2$ -----> $RO_2^{\cdot}$
(3) $RO_2^{\cdot} + RH$ -----> $ROOH + R^{\cdot}$
(4) $RO^{\cdot} + RH$ -----> $ROH + R^{\cdot}$
Decomposition/fragmentation
(5) $ROOH$ ------> $RO^{\cdot} + {}^{\cdot}OH$
(6) $2ROOH$ ------> $ROO^{\cdot} + RO^{\cdot} + H_2O$

Termination (7) $2R^{\cdot}$ ------> stable end products
(8) $R^{\cdot} + RO^{\cdot}$ ------> stable end products
(9) $2RO_2^{\cdot}$ ------> stable end products

Figure 3. Free radical chain mechanism of autoxidation according to Farme et al. (1942) and Bolland (1949) obtained from Grosch (1982).

Grosch (1982) proposed a scheme, in which it was demonstrated that the chemical structures of the volatile compounds were influenced by the structure of the residual $R^{\cdot}$. The proposed breakdown pathways of peroxides to volatiles have been confirmed in model systems for several compounds e.g., pentane, 1-pentanol, pentanal, hexanal, 2-pentylfuran, non-2-enal, and 1-penten-3-one (Horvat et al., 1965; Smouse et al., 1965; Esterbauer and Schauenstein, 1967; Eriksson, 1970; Badings, 1970; Eriksson et al., 1973). Karel and Yong (1981) summarized the effects of water content on lipid autoxidation. They suggested that the water content is the major factor controlling lipid oxidation in dehydrated foods but its effects are rather complex. For example, in systems containing antioxidants and chelating agents, which can sequester metals, high water content solubilised these substances and thus decreased the rate of oxidation. On the other hand, high water contents (a_w = 0.5-0.6) may accelerate oxidation by solubilising catalysts or by inducing swelling of macromolecules to expose additional catalytic sides (Karel and Yong, 1981).

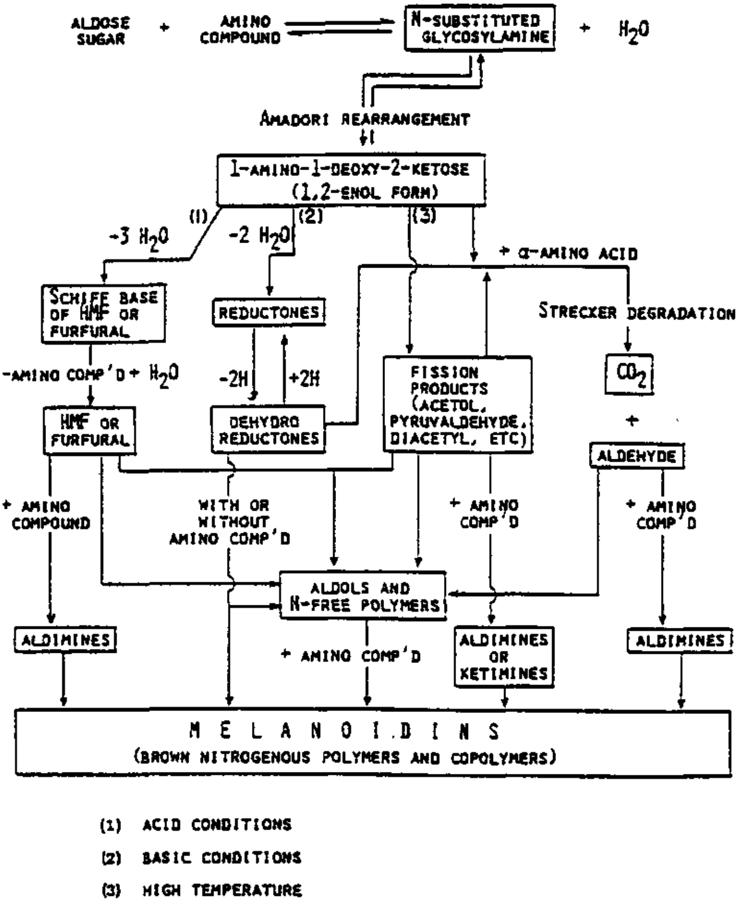

Figure 4. Maillard reaction pathways (Hodge, 1953).

The formation of volatile autoxidation products and the implication for the aroma have been extensively studied with pure fatty acid model systems and for lipid rich foods such as, vegetable oils and butter (Badings, 1970; Tressl, et al., 1981; Frankel, 1982; Grosch, 1982; Ullrich and Grosch, 1987, 1988; Frankel, 1990). However, to our information not much is known about the development of such volatile autoxidation products *in situ* during drying of low fat containing foods, such as fruits and vegetables.

The second important chemical process during drying is the Maillard reaction which affects colour, nutritional value, and flavour of the dried end product. Figure 4 shows that the reaction starts with the condensation between reducing sugars and free α-amino acid groups forming the Amadori or Heyns products. The second intermediate stage involves under acidic conditions the formation of (hydroxymethyl) furfural, under mild to basic conditions the formation of reductones and dehydroreductones, which can undergo Strecker degradation, and at high temperatures fragmentation products from the Amadori product are formed. Several products from this second stage can subsequently polymerize, which results in brown melanoidin pigments (Hodge, 1953; Labuza and Saltmarch, 1981; Eichner and Ciner-Doruk, 1981). A study on the effect of heat on carotenoids and colour appearance of *Capsicum* fruits demonstrated, that the colour change was due to a very large increase of brown compounds more than a reduction in carotenoids content (Ramakrishnan and Francis, 1973). Besides the effects on colour, the Maillard reaction has also a detrimental influence on the nutritional value. The incorporation of free α-amino groups in the formation of brown pigments and their incorporation in the Strecker degradation result in a considerable loss of essential amino acids and proteins after heating (Hodge, 1967; Chiou et al., 1991; Roe and Faulks, 1991; Tsai et al., 1991). Moreover, Feather (1994) suggested that ascorbic acid (vitamin C) easily decomposed under influence of oxygen giving carbonyl containing compounds, which subsequently interacted with amines giving Maillard reaction products. Ascorbic acid is one of the major acids of fresh *Capsicum* varieties (Rhaman and Buckle, 1978), and hot-air drying probably reduces the level of vitamin C, and therefore influences the nutritional value of the dried fruit.

The Maillard reaction is also an important source of volatile odour compounds, which can have a considerable effect on the aroma of the dried vegetable or fruit. Nursten (1980) has classified these volatiles into three groups based on their origin, i.e., sugar dehydration/fragmentation products, amino acid degradation products (Strecker degradation), and compounds produced by further interactions. Several volatile odour compounds which belong to the first and second class have been reported in processed *C. annuum* fruits (Wu et al., 1986; Wilkins, 1994; Van

Ruth and Roozen, 1994).

So far, there is a limited knowledge of the formation of volatile compounds by autoxidation and/or Maillard and Strecker degradation reaction during drying of bell pepper fruits, and the importance of these reactions for the changes in aroma of the fresh bell pepper fruits after drying.

Scope of the thesis

Objective of the study described in this thesis, was to evaluate the flavour of fresh bell peppers and to examine the changes in flavour due to hot-air drying, by using instrumental and sensory techniques. For this purpose, flavour of bell peppers was divided into taste versus non-volatile compounds and aroma versus volatile compounds, while textural properties (e.g., crispiness) were out of the scope of this thesis. Since, bell peppers are consumed both as immature (green) and ripe fruits (e.g., red, white, yellow etc.), studies on the evaluation of fresh bell peppers were centred on the development of flavour compounds during growth and ripening. The changes in the composition of non-volatile sugars and organic acids during growth and ripening are described in **Chapter 2**. Three distinct ripening stages are selected for further studies. In **Chapter 3**, the amounts of non-volatiles are analysed and the flavour, as perceived while eating, is evaluated by a taste panel. The relationships between several non-volatile compounds and some taste attributes are investigated by multivariate statistical analysis. The same ripening stages are subjected to evaluation of the volatile bell pepper compounds by gas chromatography (GC), combined GC-mass spectrometry and (GC)-sniffing port evaluation, in **Chapter 4**. The development and origin of enzymatic formed volatile compounds in fresh bell peppers are described in more detail in **Chapter 5**. The effects of hot-air drying on the composition of non-volatile and volatile flavour compounds of different bell pepper cultivars are studied in **Chapter 6**. In **Chapter 7**, the changes in fresh bell pepper aroma due to the drying process are investigated, and relationships between volatile odour compounds and sensory aroma attributes, as perceived while smelling, are examined. Finally, in **Chapter 8**, the complex behaviour of volatile bell pepper compounds during hot-air drying is described by four distinct kinetic mechanisms.

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Chapter 2

Changes in Contents of Sugars and Organic Acids During Growth and Ripening of Bell Peppers (*Capsicum annuum*).

Abstract

Sugars and organic acids and their changes during maturation of bell pepper cv. Mazurka and cv. Evident were quantified by high performance liquid chromatography. Colour pigments were determined spectrophotometrically. Glucose and fructose increased, while sucrose concentration dropped when bell pepper colour changed from green to red. The organic acids quantified were ascorbic, malic, citric, oxalic, fumaric, shikimic and pyroglutamic acid. Malic acid was predominant in the green bell peppers, and citric acid was highest in the mature red stage. Ascorbic acid increased sharply during the initial growth stages, followed by a slight increase when colour turns from green to red. Fumaric and oxalic acid declined during growth and maturation, whereas oxalic acid disappeared in the fully ripe stage.

Introduction

Capsicum fruits are used, both as spice or vegetable, in the diet for their distinct colour, pungency, and flavour. Two major species are well known i.e. *Capsicum annuum* and *C. frutescens*. Fruits of *C. annuum* generally are larger and less pungent than *C. frutescens*, which tends to include the smaller, hotter peppers (Smith and Heiser, 1951). Colour and pungency are important parameters for the quality of the fruits as comprehensively reviewed by Govindarajan et al. (1985, 1986, 1987).

However, with increasing consumption of vegetable *Capsicum*, such as green and red bell peppers, flavour becomes a more important quality parameter (Govindarajan, 1985). Flavour quality of fruits and vegetables depends on taste and aroma characteristics, which are affected by the chemical composition (Chang and Huang, 1989). Several studies included the determination of volatile flavour compounds in bell peppers (Buttery et al, 1969; Keller et al., 1981; Wilkens and Madsen, 1989). Luning et al. (1994 *a*, *c*) suggested that changes in the composition of volatile odour compounds are reflected in distinct aroma differences between ripening stages of bell peppers. Non-volatile flavour compounds of *Capsicum* fruits include pungent constituents, sugars, and organic acids (Somos, 1984). The capsaicinoids are well known to be responsible for the hot taste and vary between 0.35 to 0.85 % in *C. frutescens* and 0.05 to 0.33 % in *C. annuum* L. (Govindarajan et al.,1985). Glucose, fructose, and sucrose have been reported as main sugars in *C. annuum* varieties (Polacsek-Rácz et al., 1981; Tarrach and Herrmann, 1986). Ascorbic acid is best known of the organic acids in bell peppers. Considerable differences in ascorbic acid content existed between cultivars (Rhaman, 1978; Somos, 1984) and nowadays, the content of vitamin C is one of the selection criteria in breeding programs (Awasti and Singh, 1979). Less is known, however, about the development of other organic acids during growth and ripening of bell peppers. Tarrach and Herrmann (1984) reported differences in the composition of organic acids in tomatoes, cucumber, and sweet peppers at some maturation stages, using derivatisation and gas chromatographic analyses. Luning et al., (1994) proposed that changes in composition of sugars and acids may be responsible for distinct differences in taste of green and red bell peppers; the latter being more sweet and sour.

The object of this study was to investigate changes in the contents of sugars and organic acids during maturation of two Dutch commercial bell pepper cultivars, i.e., *C. annuum* cv. Mazurka and cv. Evident.

Material and methods

Bell pepper samples

Fruits of two Dutch trade cultivars, i.e., *C. annuum* cv. Mazurka and cv. Evident were grown on rock wool in a greenhouse with controlled temperature (20 ± 2°C) and humidity (75%). The first maturation stage was collected four weeks after fruit set and subsequently, six stages were harvested with one-week intervals (total seven stages). Sampling of fruits was further based on fresh weight and fruit colour appearance. Approximately 5 to 6 bell peppers were selected from different plants in early morning. The fruits were weighted, halved, deseeded, and stored at - 24°C. Frozen bell peppers were homogenised prior to analysis of colour pigments, dry matter content, sugars, and organic acids.

Dry matter analysis

Homogenised bell pepper samples of 1 g were dried for 3 h at 70°C and subsequently dried at 105°C for 16 h.

Pigment analysis

Extractions of chlorophyll *a* and carotenoids were carried out as described previously (Luning et al., 1994 *a*) with some minor modifications for carotenoids analysis. Aliquots of 5 g were homogenised with 60 mL of hexane, 40 mL of acetone and 0.1 g of $CaCO_3$ in a Waring blender for 2 min and vacuum filtered using a sintered glass funnel (G3). The residue was dispersed in a mixture of 25 mL of hexane and 25 mL of acetone and filtered to extract residual carotenoids. The filtrates were pooled and washed with 100 mL of water. The final upper layer was separated, mixed with 9 mL of acetone, and diluted with hexane to 100 mL. The carotenoid extracts were measured both at 436 and at 472 nm, which are the absorption maxima of respectively ß-carotene (97% Merck) and capsanthin (EG-Nr.

E160c, Roth, Karlsruhe) dissolved in hexane/acetone. Calibration curves were determined for both ß-carotene and capsanthin to calculate the specific absorption coefficients (*E*) in hexane/acetone.

Extraction and analysis of sugars

Sugars were extracted from homogenised frozen bell peppers as described previously (Luning et al., 1994 *b*). Samples of 5 g of homogenised frozen bell peppers were blended in 50 mL of Milli Q water with an Ultra Turrax (Ika Labortechnik, T25, Janke & Kunkel) and subsequently treated with 1 mL of carrez I and 1 mL of carrez II. Solution volume was made up to 100 mL with Milli Q water and filtered (Schleicher & Schull 595½). Aliquots of 1 mL of filtrate were diluted with 4 mL of CaEDTA (200 mg.L^{-1}) (Fluka) and the diluted filtrate was than pretreated with activated Sep-pak C_{18}; the first 4 mL was discarded and the last millilitre was used for HPLC. Sugars were analysed on a calcium loaded ion exchange column at 85°C (Waters Sugar-Pak), in combination with a sugar pak guard column, using a Waters HPLC system equipped with a Waters 510 pump and Waters U6K injector. Samples of 10 µL (three replicates) were injected at a flow rate of 0.5 mL.min^{-1}, using Milli Q as mobile phase. Components were detected with a differential refractometer (Waters 410).

Extraction and analysis of organic acids

Ascorbic acid. Ascorbic acid was determined, separately from the other organic acids, as previously described by Luning et al. (1994 *b*). Samples of 2.5 g of homogenised frozen bell peppers were mixed with 10 mL of methanol, 10 mL of 9.5% oxalic acid (Merck), and 30 mL of water, using an Ultra Turrax. Water was added to the slurry up to 100 mL, mixed, and filtered (Schleicher & Schull 595½). The total ascorbic acid content was determined by reduction of dehydroascorbic to ascorbic acid with 0.08% DL-homocysteine solution in the dark for 15 min. Ascorbic acid was analysed on a radially packed µBonda-Pak C_{18} column (5 µm) and a C_{18}

guard column (Waters) and effluents were monitored at 251 nm with a Waters 484 tunable detector. Samples of 10 µL (three replicates) were injected at a flow rate of 1.5 mL.min^{-1} using 2.5 g of tetrabutylammonium-hydrosulfate (Merck) in 55 mL of methanol.L^{-1} Milli Q as mobile phase.

Other organic acids. Extraction of organic acids was carried out as described previously (Luning et al., 1994 *b*). Samples of 5 g of homogenised frozen bell peppers were blended with 50 mL of Milli Q in an Ultra Turrax and filtered (Whatman GFC). Aliqouts of 1 mL of filtrate were diluted with 4 mL of milli Q water and subsequently pretreated with activated Sep-Pak C*18* cartridges; the first 4 mL was discarded and the last millilitre was used for HPLC analysis. Organic acids were analysed on a 60m Shodex KC811 ion-exchange column (Waters) at 65°C and combined with a fast fruit juice guard column (Waters). The same HPLC equipment was described for sugar analysis. Samples of 10 µL (three replicates) were injected at a flow rate of 0.7 mL.min^{-1} using 0.05 M H_3PO_4 as mobile phase. Column effluents were monitored at 210 nm with a Waters 484 tunable detector.

Identification and quantification

Organic acids and sugars were identified by comparison of retention times with those of authentic compounds prepared in the same way. Quantification of sugars and organic acids was related to peak areas of corresponding compounds.

Statistical analysis

All analyses were carried out in triplicate and the entire experiment was repeated two times. Least significant differences (LSD) for cultivars and ripening stages were calculated by ANOVA ($P < 0.05$), using genstat-5.

Results and discussion

Bell peppers of cv Mazurka and cv. Evident were harvested at seven stages of maturation starting four weeks after fruit set with one-week intervals. Fruits were further selected on fresh weight and colour appearance for analysis of colour pigments, sugars, and organic acids. During maturation, the weight of the individual bell pepper fruits increased rapidly in the initial growth stages between 4 and 6 weeks after fruit setting (Fig. 1). Correspondingly, Rhodes (1980) reported that growth patterns of *Capsicum* fruits followed a sigmoidal curve. Dry matter contents (Fig. 1) increased more gradually during maturation, i.e., from 6.0 to 8.4% for cv. Mazurka and from 5.9 to 9.0% for cv. Evident.

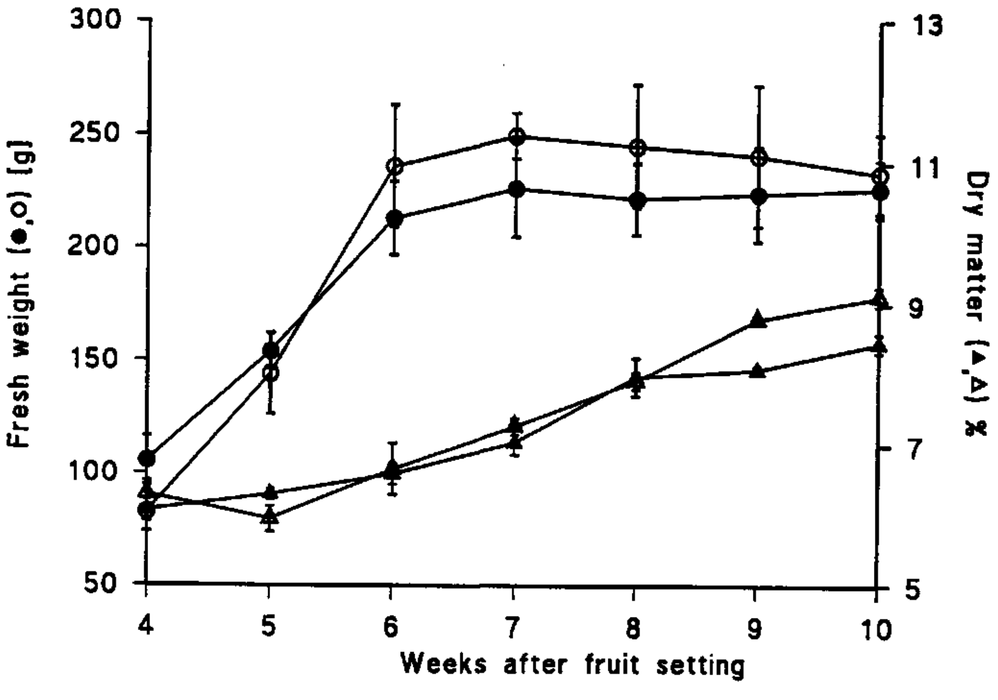

Figure 1. Changes in fresh weight (●, ○) and dry matter content (▲,△) during growth and maturation of cv. Mazurka (closed symbols) and cv. Evident (open symbols).

During growth and maturation the colour appearance of the bell pepper fruits of both cultivars changed drastically. The first stages (4-7 weeks) have a green appearance, which turned to green/brown (8 weeks), red with green spots (9 weeks), and completely red (10 weeks). Figures 2 A-C show the contents of chlorophyll [A] and of carotenoids, expressed as respectively ß-carotene [B] and capsanthin [C], at different harvest times. Only chlorophyll *a* was measured to determine the stage of ripening, since preliminary results revealed that chlorophyll *b* disappeared completely within 5 weeks. Figure 2 A shows that the chlorophyll *a* content slightly decreased in the immature green stages (4-5 weeks), and after a significant ($P < 0.05$) increase (5-7 weeks), it declined drastically at the turning ripening stage (8 weeks). Similar results were obtained by Rahman and Buckle (1980), who investigated the chlorophyll *a* and *b* contents at four maturation stages of several red and yellow varieties using thin-layer chromatography. The major orange-yellow and red carotenoids in green/red bell pepper varieties are respectively ß-carotene and capsanthin (Vinkler and Kiszel-Richter, 1970; Rahman and Buckle, 1980). Therefore, the absorption was measured at 436 and 472 nm, which are the absorption maxima for respectively ß-carotene and capsanthin in hexane/acetone. Obviously, the amounts of both carotenoids (Fig. 2 B-C) increased rapidly after the turning green/brown maturation stage (8 weeks). Likewise, Rhaman and Buckle (1980) observed a marked raise of ß-carotene and capsanthin from the half ripened to the fully ripe stage using high performance liquid chromatography. However, no capsanthin was detected in their immature and mature green ripening stages. In our study, capsanthin measurements were performed at 472 nm, at which other carotenoids, such as lutein and ß-carotene, can have their (sub)maximum absorption also (Vinkler et al., 1972). In fact, these compounds have been detected in immature and mature green stages by Rhaman and Buckle (1980). Therefore, the values at these green stages (4-6 weeks) may not reflect the capsanthin level but other carotenoids. However, the absorption values at 472 nm, which were obtained from the riper stages, i.e, from 7 to 10 weeks, presumably reflect the increase of capsanthin.

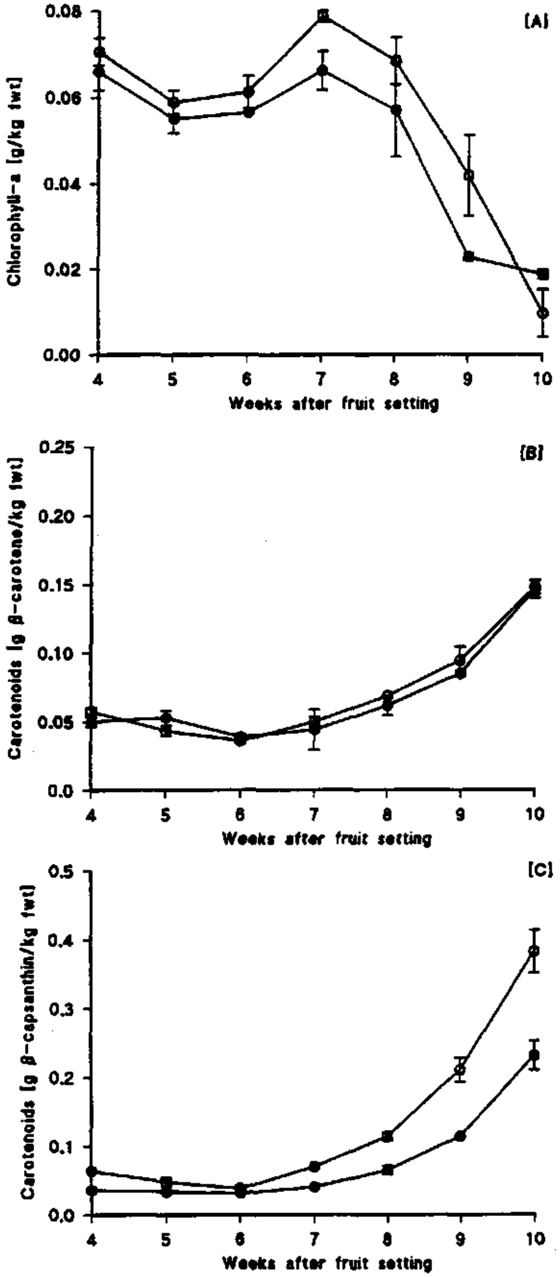

Figure 2. Development of chlorophyll *a* [A], and carotenoids expressed as β-carotene [B] (E = 185.9 L g^{-1}) and Capsanthin [C] (E = 90.7 L g^{-1}) respectively, during growth and maturation of cv. Mazurka (●) and cv. Evident (O).

In addition, no considerable differences were observed in developmental patterns of both cultivars for fresh weight, dry matter content, and colour. (Fig. 1-2). However, some significant ($P < 0.05$) quantitative differences were noticed at specific ripening stages. The fully ripe stage (10 weeks) of cv. Mazurka had a significantly ($P < 0.05$) lower dry matter content, a higher chlorophyll *a*, and a lower capsanthin content than cv. Evident.

The development of sugars during maturation of bell pepper cv. Mazurka is presented in Figure 3. Similarly, the contents of sugars changed in cv. Evident (not shown). Only some quantitative differences were observed, i.e., sucrose (0.40 g. 100 g^{-1} fresh weight), fructose (2.48 g.100 g^{-1} fwt), and glucose (2.55 g.100 g^{-1} fwt) contents in the fully red stage of cv. Evident were significantly ($P < 0.05$) higher than in the corresponding stage of cv. Mazurka. Figure 3 shows that the sucrose content increased up to a maximum value in the turning bell peppers (8 weeks) and than declined to less than 0.4 g.100 g^{-1} (fwt), whereas fructose and glucose levels increased significantly ($P < 0.05$) between 7 and 9 weeks. Nielsen et al. (1991) and Hubbard and Pharr (1992) suggested that, increased invertase activities during ripening of *Capsicum* fruits are responsible for the accumulation of hexoses.

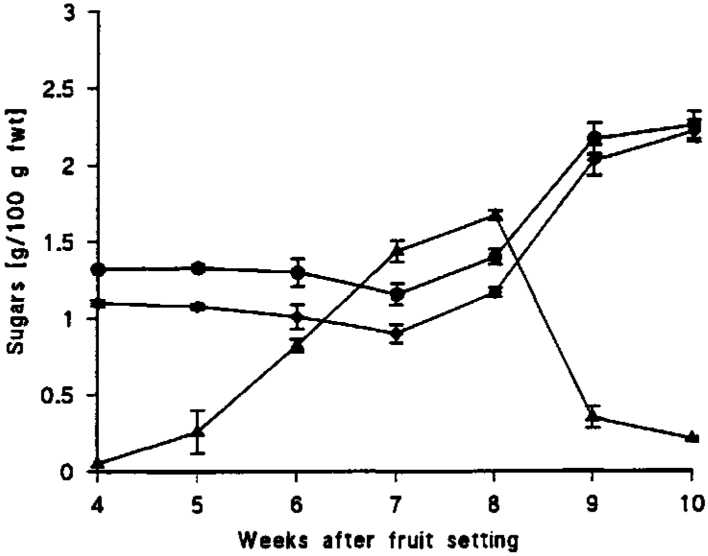

Figure 3. Development of sucrose (▲), fructose (◆), and glucose (●) in cv. Mazurka during growth and ripening.

Seven organic acids, i.e., ascorbic, citric, malic, oxalic, fumaric, pyroglutamic, and shikimic acid were identified and quantified in both bell pepper cultivars at different maturation stages. Ascorbic acid (Fig. 4) increased significantly ($P < 0.05$) during the first developmental stages (4-6 weeks), from ± 80 up to ± 180 mg.100 g^{-1} fwt, and than slowly increased to ± 200 mg.100 g^{-1} fwt in the red maturation stage. Correspondingly, Rhaman et al. (1978) demonstrated that ascorbic acid in immature fruits ranged from 82-145 mg.100 g^{-1} fwt and from 125-228 mg.100 g^{-1} fwt in the fully ripe fruits, within different red and yellow cultivars.

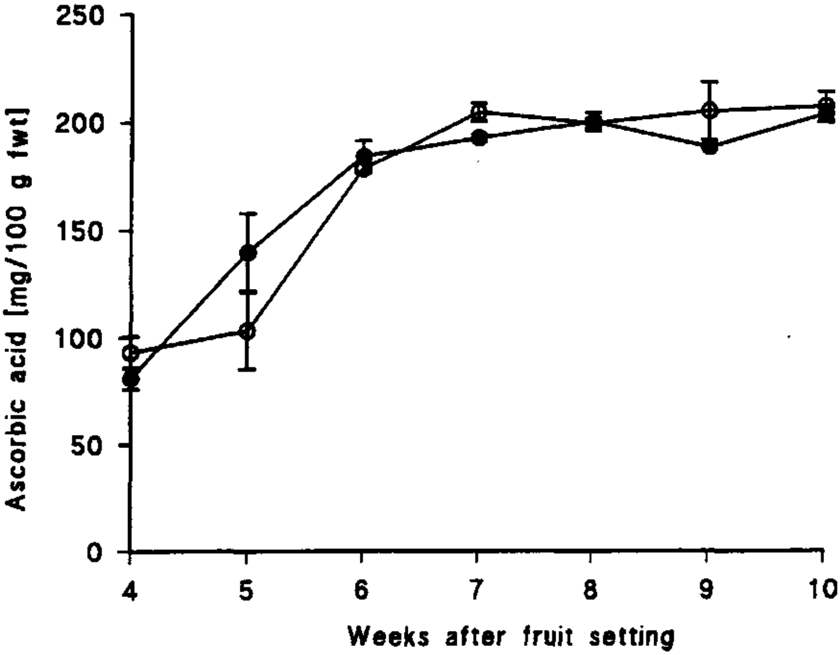

Figure 4. Changes in ascorbic acid content of bell pepper cv. Mazurka (●) and cv. Evident (O) during growth and ripening.

Other major acids (Fig. 5) were citric and malic acid, which are, together with oxalic and fumaric acid, known as intermediates of the tricarboxylic acid cycle (Biale and Young, 1981). Malic, oxalic, and fumaric acid decreased significantly ($P < 0.05$) during the initial growth stages between 4 and 6 weeks (Fig. 5 B-D), while citric acid (Fig. 5 A) accumulated during growth and ripening.

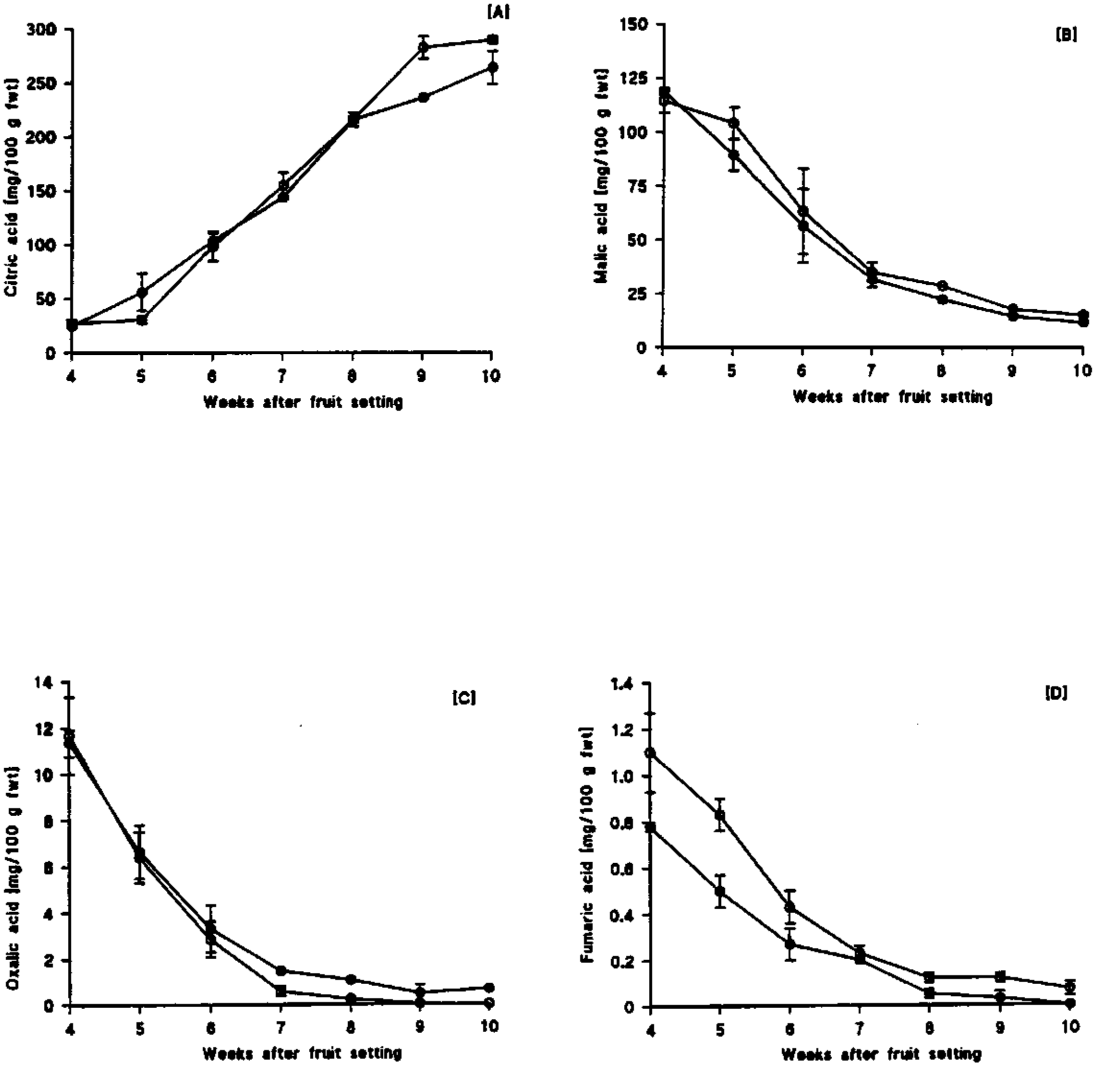

Figure 5. Changes in contents of citric [A], malic acid [B], oxalic [C], and fumaric acid [D] of cv. Mazurka (●) and cv. Evident (O) during growth and ripening.

Tarrach and Herrmann (1984) observed a comparable trend for citric and malic acid concentrations in green and red bell peppers. Furthermore, they reported succinic and fumaric acid at low levels, whereas no oxalic acid was detected. The tricarboxylic acid cycle encounters the metabolic energy requirement, it provides intermediates for biosyntheses and is strictly regulated by ATP, NADH, and succinyl CoA (Stryer, 1981). The observed changes in the composition of citric, malic, oxalic, and fumaric acid, probably resulted from an alternated requirement for energy and intermediates for biosyntheses, during growth and maturation of the bell peppers.

Pyroglutamic acid in bell peppers (Fig. 6) revealed maximal values in the beginning of the ripening (6-7 weeks) and declined after the turning stage (8 weeks). Pyroglutamic acid has not been determined before in bell peppers. However, it was found in other vegetables such as potatoes (Bushway et al, 1984) and mushrooms, in the latter it was formed as a side product in melanogenesis (Soulier, 1994).

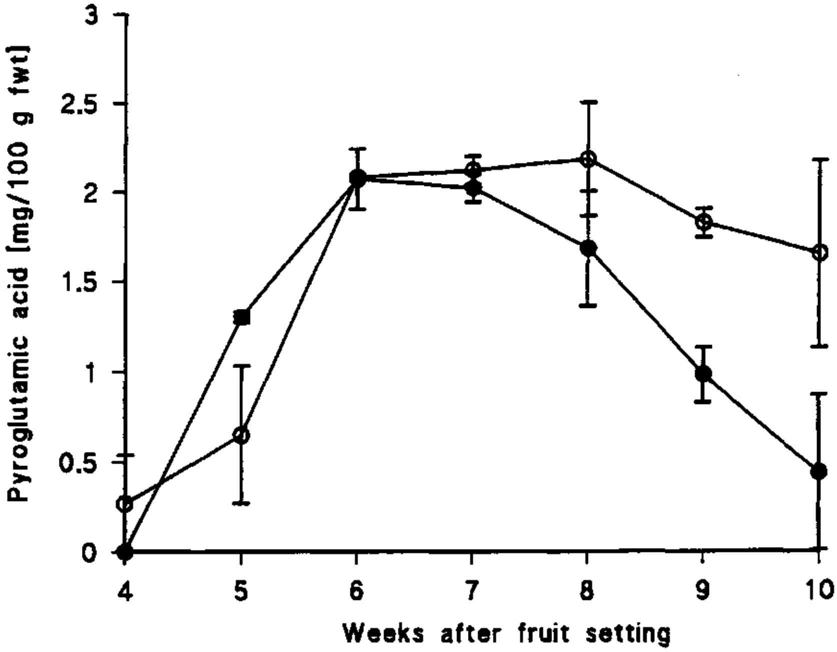

Figure 6. Changes in pyroglutamic acid content of cv. Mazurka (●) and cv. Evident (O) during growth and ripening.

Figure 7 shows that the level of shikimic acid dropped from the immature green (4 weeks) to the turning stage (7 weeks), and after a significant ($P < 0.05$) increase (7-8 weeks) the content declined to values of about 4 mg.100 g^{-1} fwt. Shikimic acid is well known as a precursor of phenylalanine, tyrosine, and tryptophan (Haslam, 1974). Tarrach and Herrmann (1986) also observed increased values at the intermediate turning ripening stage in the *C. annuum* cultivars Capri and Deltana. The reported concentrations of shikimic acid in their cultivars were, however, much lower (1.0-2.8 mg.100 g^{-1} fwt) than in cv. Mazurka and cv. Evident (3.8-6 mg.100^{-1} fwt).

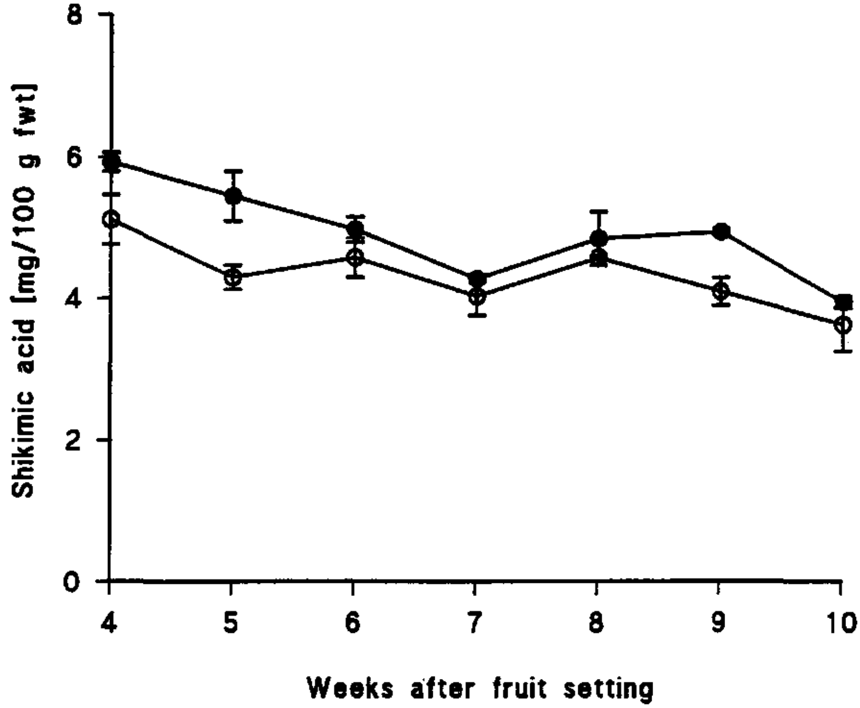

Figure 7. Changes in shikimic acid content of cv. Mazurka (●) and cv. Evident (O) during growth and ripening.

In addition, no differences were observed in developmental patterns of organic acids for both cultivars (Fig. 4-7). However, some significant ($P < 0.05$) quantitative differences were noticed at specific ripening stages. The level of citric acid (Fig. 5

A) was significantly ($P < 0.05$) lower in the ripe stages (9-10 weeks) of cv. Mazurka than in cv. Evident. Moreover, significant differences were observed, at all ripening stages, for the levels of fumaric and shikimic acid; fumaric acid (Fig. 5 D) was significantly ($P < 0.05$) higher, whereas shikimic acid (Fig. 7) was significantly ($P < 0.05$) lower in cv. Evident.

In conclusion, the composition of sugars and organic acids strongly depends on the stage of ripeness. The most drastic changes in sugar composition occur in the turning stages between 7 and 9 weeks after fruit set. Malic, fumaric, and oxalic acid decreased and ascorbic acid increased most rapidly in the initial growth stages between 4 and 6 weeks after fruit set. Moreover, only minor changes in sugars and organic acid contents were observed at the late ripening stages between 9 and 10 weeks.

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Chapter 3

Combined Instrumental and Sensory Evaluation of Flavour of Fresh Bell Peppers (*Capsicum annuum*) Harvested at Three Maturation Stages[1]

Abstract

Bell pepper *C. annuum* cv. Mazurka and cv. Evident, at three maturation stages, were evaluated sensorily on flavour attributes. Green bell peppers scored mainly on the attributes bitterness, grassy, cucumber, and green bell pepper aroma, whereas the attributes sweetness, sourness, and red bell pepper aroma were distinctive for the red ones. Sugars and organic acids were determined by high-performance liquid chromatography (HPLC), and concentrations of different ions of the acids were calculated from their dissociation equilibria. Principal component analysis (PCA) demonstrated that HPLC data of fructose, glucose, total sugar, and dry matter content were related to the attribute sweetness in the red maturation stage. HPLC concentrations of citric and ascorbic acid, as well as calculated concentrations of undissociated ascorbic and dissociated citric 1 and citric 2, showed close relationships with the attribute sourness. Moreover, pH and HPLC concentrations of malic, oxalic, fumaric, and pyroglutamic acid and calculated contents of dissociated malic 2, pyroglutamic 1, and oxalic 2 appeared to be negatively related with sourness.

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Introduction

The market for Dutch export bell peppers (*Capsicum annuum*) has increased in the last decade (Zachariasse and Abrahamse, 1993). Since consumers have become more critical, attention is shifting towards flavour as an important quality parameter for food products. The overall flavour of fruits and vegetables, as perceived during consumption, is influenced by the composition of volatile and non-volatile compounds, of which some (i.e., pungent principles) stimulate nonspecific or trigeminal neural responses (Chang and Huang, 1989).

A number of studies have dealt with the relations between instrumental data and sensory attributes of non-volatile flavour compounds and their contribution to total flavour quality in fruits and vegetables. Paterson et al. (1991) showed that in kiwifruits the fructose/glucose ratio was one of the important parameters in the prediction of sweetness of soft ripe fruits, whereas high sucrose contents correlated with unripeness. Furthermore, they observed that the sensory attribute tangy/acid was characteristic for the firm unripe kiwifruits, and that high attribute scores correlated with high citrate concentrations. Watada and Aulenbach (1979) suggested that for tomatoes sourness correlated with citric acid content. Dever et al. (1992) reported that fruitiness and sweetness of apple juice increased while sourness decreased with maturation of apples. Principal component analysis of their instrumental data suggested that, among others, malic acid and titratable acid were negatively related with glucose, fructose, soluble solids, and pH on the first component, representing changes during ripening. However, Watada et al. (1981) indicated that variation of sourness of several apple cultivars could only partly be attributed to titratable acids, soluble solids, and some volatile compounds. Shamaila et al. (1992) observed significant relationships between glucose and fructose concentrations and the sweetness of strawberry samples, but not for pH or titratable acidity. Whereas the concept of sweetness intensity and concentration of sugars has rather been well established (de Graaf, 1988), the relationship between sourness and organic acids has not yet been fully understood (Amerine et al., 1965; Ganzevles, 1987).

In this study, bell peppers were evaluated at three maturation stages by a sensory descriptive panel on flavour attributes perceived while eating. The composition of sugars and organic acids of the different samples was analysed by high-performance liquid chromatography, and the dissociation of organic acids was calculated at the pH of the samples. These parameters were used to study the relations between sugar concentrations and sweet perception, as well as contents of organic acids and perception of sourness by using joint principal component analysis and partial least squares regression analyses.

Materials and methods

Bell pepper samples

Fruits of two commercial Dutch cultivars, i.e., *C. annuum* cv. Mazurka and cv. Evident, were grown and harvested as previously described by Luning et al. (1994 *a*). Three ripening stages were collected at respectively 6, 8, and 10 weeks after fruit setting; bell peppers with similar colour appearance, i.e., green (6 weeks), turning (8 weeks), and red (10 weeks) respectively, were selected for experiments. Approximately 10-12 bell peppers were picked from different plants in early morning, and 5-6 fruits were stored at 13°C for sensory evaluation. The rest of the bell peppers collected were stored at - 24°C for analysis of sugars and organic acids.

Sensory analysis

Quantitative descriptive analysis (Stone et al., 1974; Stone and Sidel, 1993) was used to evaluate sensory attributes of bell pepper cv. Mazurka and cv. Evident at three maturation stages.

Training of panel. Fifteen judges, aged from 25 to 35 years, with ample sensory evaluation experience on other fruits and vegetables, generated a descriptive sensory profile for bell peppers (Stone and Sidel, 1993). An assortment of bell pepper varieties with different flavours were offered to the panel to provide a wide range of sensory notes that might be expected in the samples. Afterwards, the same assortment of bell pepper cultivars was used for intensity scaling of the perceived attributes over a period of 3 months. Attributes were then checked on their differentiating merits. Finally, the judges made suggestions and established flavour descriptions to characterize flavour attributes of fresh bell peppers, as perceived during eating. The flavour attributes generated were sweetness, sourness, bitterness, sharpness, grassy, cucumber, floral, and green and red bell pepper aroma.

Evaluation of bell peppers. Each bell pepper sample was evaluated twice by the panel, and the total procedure was repeated three times. Five fruits were evaluated per session. The judges indicated the intensity of each attribute by using an unstructured line scale anchored with 0 and 100 at the ends. Data were obtained by using the PSA system (OP&P, Utrecht, The Netherlands). Assessors for sensory evaluations were seated in individual air-conditioned booths.

Dry matter analysis

Homogenised bell pepper samples of 1 g were dried for 3 h at 70°C and subsequently dried at 105°C for 16 h.

Sugar analysis

Extraction of sugars was carried out as described for the enzymatic analysis of sugars by Boehringer (1989) with some modifications.

Extraction procedure. Samples of 5 g of homogenised frozen bell peppers were blended in 50 mL of Milli Q water with an Ultra Turrax (Ika Labortechnik, T25, Janke & Kunkel). The slurry was treated with 1 mL of potassium hexacyanoferrate (II) trihydrate (Carrez I) (106 g of $K_4[Fe(CN)_6]\cdot 3\ H_2O.L^{-1}$) and 1 mL of stannous acetate (Carrez II) (219.5 g of $Zn(CH_3COO)_2 \cdot 2\ H_2O.L^{-1}$) (Merck), respectively, to remove proteins. Solution volume was made up to 100 mL with Milli Q water and filtered (Schleicher & Schull 595½). Aliquots of 1 mL of filtrate were diluted with 4 mL of CaEDTA (200 $mg.L^{-1}$) (Fluka). Sep-Pak C_{18} cartridges were activated with 5 mL of ethanol and 10 mL of Milli Q. Aliqouts of 5 mL of diluted filtrate were pretreated with activated Sep-pak C_{18}; the first 4 mL was discarded and the last milliliter was used for HPLC. Analysis of sugars was carried out as recently described by Lázaro et al. (1989) with the following modifications.

HPLC-analysis. A Waters HPLC system (Milford, MA, USA) was used, which was equipped with a Waters 510 pump and a Waters U6K injector. Sugars were analysed on a calcium loaded ion exchange column at 85°C (Waters Sugar-Pak), in combination with a sugar pak guard column. Samples of 10 µL (four replicates) were injected at a flow rate of 0.5 $mL.min^{-1}$, using Milli Q as mobile phase. Components were detected with a differential refractometer (Waters 410).

Ascorbic acid analysis

Ascorbic acid was determined, separately from the other organic acids, as previously described by Keijbets and Ebbenhorst-Seller (1990) with some modifications.

Extraction procedure. Samples of 2.5 g of homogenised frozen bell peppers were mixed with 10 mL of methanol, 10 mL of 9.5% oxalic acid (Merck), and 30 mL of water, using an Ultra Turrax. Water was added to the slurry up to 100 mL, mixed, and filtered (Schleicher & Schull 595½). The total ascorbic acid content was determined by reduction of dehydroascorbic to ascorbic acid with homocysteine. The pH of 10 mL of filtrate was adjusted to pH 5 with 2 M KOH, and subsequently adjusted to pH 7 with 0.05 M Tris buffer. Volume was made up to 25 mL with Milli Q. Aliquots of 1 mL were treated for 15 min in the dark with 1 mL of 0.08% DL-*homocysteine solution (Fluka) and filtered (Millex 0.45 µm filter, Millipore).*

HPLC analysis. A radially packed µBonda-Pak C_{18} column (5 µm) and a C_{18} guard column (Waters) were used for the analysis of ascorbic acid. Effluents were monitored at 251 nm with a Waters 484 tunable detector. Samples of 10 µL (four replicates) were injected at a flow rate of 1.5 $mL.min^{-1}$ using 2.5 g of tetrabutylammoniumhydrosulfate (Merck) in 55 mL of $methanol.L^{-1}$ Milli Q as mobile phase.

Analysis of other organic acids

Extraction of organic acids was carried out as described for the enzymatic analysis of citric acid by Boehringer (1989) with the following modifications.

Extraction procedure. Samples of 5 g of homogenised frozen bell peppers were blended with 50 mL of Milli Q in an Ultra Turrax. The slurry was filtered (Whatman GFC) and 1 mL of filtrate was diluted with 4 mL of milli Q water. Diluted filtrate (5mL) was pretreated with activated Sep-Pak *C18* cartridges; the first 4 mL was discarded and the last milliliter was used for HPLC analysis.

HPLC analysis. Organic acids were analysed on a 60m Shodex KC811 ion-exchange column (Waters) at 65°C and combined with a fast fruit juice guard column (Waters). The same HPLC equipment was described for sugar analysis. Samples of 10 µL (four replicates) were injected at a flow rate of 0.7 $mL.min^{-1}$ using 0.05 M H_3PO_4 as mobile phase. Column effluents were monitored at 210 nm with a Waters 484 tunable detector.

Identification and quantification

Organic acids and sugars were identified by comparison of retention times with those of authentic compounds prepared in the same way. Quantification of sugars and organic acids was related to peak areas of corresponding compounds.

Measurement of pH.

Five-gram samples of bell peppers were homogenised in a Waring blender, and the slurry was vacuum filtered by using a sintered glass funnel (G3). The pH of the filtrate was measured with a pH meter (Metrohm Herisau, E603, Switzerland).

Calculation of dissociated organic acids.

The concentrations of organic acids at their different dissociation states were calculated as described by Chang (1981). The calculations were based upon dissociation constants of the individual organic acids (Table 1), the total concentration of the acid as determined by HPLC, and pH-values of the bell pepper filtrates. The dissociation constants of shikimic and pyroglutamic acid were determined by a titration method, whereas the others were available from the literature (Chang, 1981). Calculations of the different dissociation forms of an acid were carried out as described by Chang (1981).

Table 1. Dissociation Constants (at 25°C) of Organic Acids Present in Bell Peppers

Organic acid	K_1	K_2	K_3
Ascorbic acid[a]	8.0×10^{-5}	1.6×10^{-12}	
Citric acid[a]	8.7×10^{-4}	1.8×10^{-5}	4.0×10^{-6}
Malic acid[a]	4.1×10^{-4}	9.0×10^{-6}	
Oxalic acid[a]	6.5×10^{-2}	6.1×10^{-5}	
Fumaric acid[a]	9.3×10^{-4}	3.4×10^{-5}	
Pyroglutamic acid[b]	5.8×10^{-4}		
Shikimic acid[b]	6.2×10^{-5}		

[a] Data obtained from literature (Chang, 1981); [b] Dissociation constants determined by titration as described by Chang (1981).

Statistical analyses

Instrumental and sensory data sets were subjected to analysis of variance (ANOVA, Genstat 5) in order to determine least significant differences (lsd) among cultivars and ripening stages. All analyses were carried out four times, and the entire experiment was repeated three times with newly collected bell peppers.

Multivariate statistical analysis, using principal component analysis (PCA), was applied to study the interrelations between sensory data and the relations between instrumental and sensory data, as described by Piggot and Sharman (1986). PCA involves transformation of the original set of p variables (X_1, X_2,...X_p) from n observations into smaller sets of linear combinations that account for the maximum possible proportion of variance in the original data. In addition, combined data sets were subjected to partial least-squares regression (PLS) as previously described (Martens and Martens, 1986).

Results

Sensory analyses

Nine flavour attributes were used for sensory profiling of the bell pepper samples (Table 2). Statistical analysis of the mean score ratings of these attributes showed, that scores for sweetness, sourness, and red bell pepper aroma increased significantly ($P < 0.05$) during ripening from green to turning and/or red. On the other hand, scores for bitterness, grassy, green bell pepper, and cucumber aroma decreased significantly ($P < 0.05$). Cultivar differences were observed to a lesser extent. Table 2 shows that cv. Evident had significantly ($P < 0.05$) higher scores for sweetness in the turning and red stages, and for red bell pepper aroma in the red fruits. Green fruits had higher scores for sharp and cucumber aroma in comparison to cv. Mazurka.

Principal component analysis (PCA) was carried out on the mean scores of sensory attributes (Table 2) to study the interrelations of the different ripening stages and cultivars. PCA revealed two principal components (PC) explaining 92% of the variance. The sample scores diagram (Fig. 1) shows that the maturation stages green, turning, and red for both cultivars were separated along component 1. Figure 2 represents the loadings of the mean score ratings of sensory attributes on the first and second principal component. Obviously, bitterness and "green" flavour attributes such as grassy, green bell pepper, and cucumber aroma have negative loadings on component 1. These attributes are characteristic for the green bell peppers, since these samples also have negative loadings (Fig. 1) on PC1.

Table 2. Mean score ratings [a] of sensory attributes of bell pepper cultivars Mazurka (MAZ) and Evident (EVI) harvested at the maturation stages green, turning and red.

Sensory Attributes	Green		Turning		Red	
	MAZ	EVI	MAZ	EVI	MAZ	EVI
Sweet	24.6 *a*	24.0 *a*	38.5 *b*	46.4 *c*	42.6 *d*	52.1 *e*
Sour	13.0 *a*	11.2 *a*	25.9 *b*	25.5 *b*	28.6 *b*	30.6 *b*
Bitter	25.9 *a*	30.7 *a*	16.4 *b*	12.2 *b*	14.5 *b*	11.9 *b*
Sharp	12.7 *a*	17.2 *b*	13.2 *a*	13.0 *a*	12.5 *a*	12.9 *a*
Grassy	46.2 *a*	47.1 *a*	24.6 *b*	18.0 *b*	12.4 *c*	10.6 *c*
Green bell pepper	43.3 *a*	44.5 *a*	29.7 *b*	23.8 *b*	11.8 *c*	7.8 *c*
Floral	10.9 *a*	13.9 *a*	9.0 *a*	11.4 *a*	12.1 *a*	12.4 *a*
Cucumber	14.4 *a*	17.6 *b*	7.3 *c*	7.5 *c*	7.5 *c*	5.6 *c*
Red bell pepper	6.7 *a*	5.7 *a*	25.8 *b*	29.6 *b*	43.3 *c*	50.0 *d*

[a] Means of three experiments; mean values for each attribute are significantly ($P < 0.05$) different, for ripeness and/or variety, if followed by a different superscript letter.

On the positive side of PC1, the red and to a lesser extent the turning fruits have high loadings (Fig. 1). Correspondingly, sweet, sour, and red bell pepper aroma have positive loadings on the first component (Fig. 2) and therefore are typical for the flavour of ripening bell peppers.

On PC2, green maturation stages of cv. Evident and cv. Mazurka were differentiated; Evident has positive and Mazurka has negative loadings (Fig. 1). Figure 2 shows that floral aroma and to a lesser extent sharpness have high positive loadings on PC2 and therefore are characteristic for green cv. Evident.

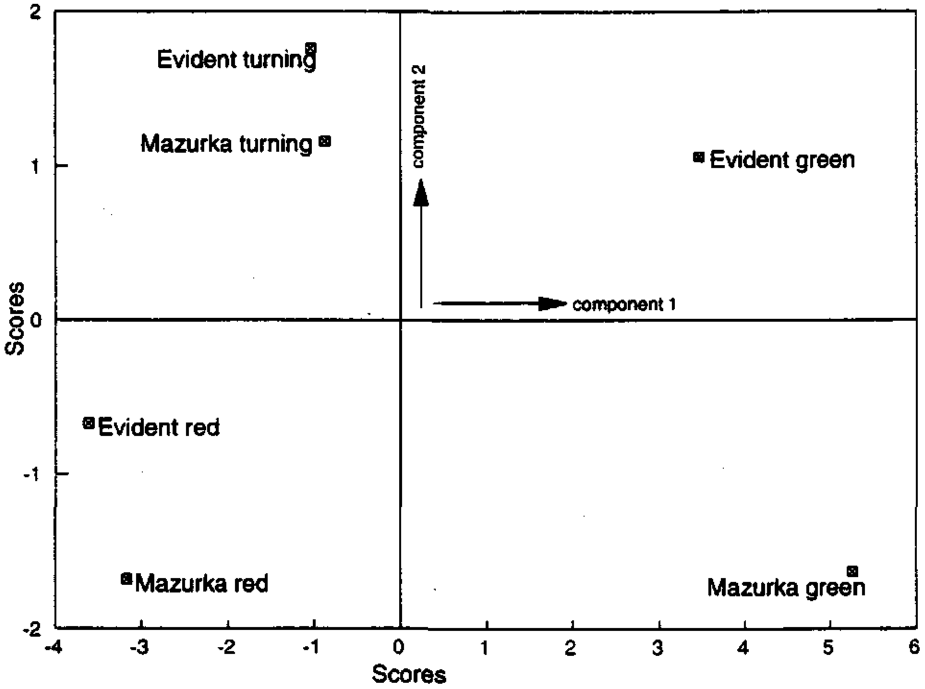

Figure 1. Sample scores of bell pepper cv. Mazurka and cv. Evident in the ripening stages green, turning, and red on the first and second principal component (sensory data set).

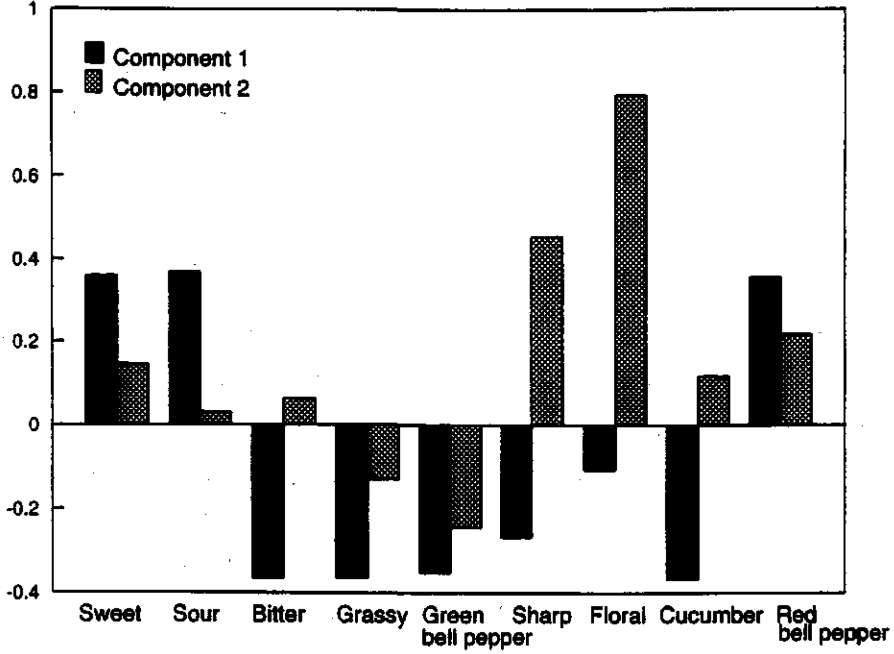

Figure 2. Loadings of mean score ratings of sensory attributes on PC 1 and 2.

Instrumental analyses

Dry matter contents in green, turning, and red fruits were 5.9, 7.3, and 8.4% in cv. Mazurka and 6.1, 7.4, and 9.0% in cv. Evident, respectively. Changes in sugar composition during maturation are shown in Table 3. Glucose and fructose contents increased significantly ($P < 0.05$) from stage green to turning, and subsequently to red, whereas no cultivar differences were observed. However, in the green stage the sucrose concentration of cv. Evident was significantly higher ($P < 0.05$) than in cv. Mazurka. Overall, the highest amounts of sucrose were noticed in the turning stages, whereas a significant ($P < 0.05$) decrease occurred upon further maturation to the red stage (Table 3).

Table 3. Sugar composition of bell pepper cultivars Mazurka (MAZ) and Evident (EVI) harvested at the maturation stages green, turning, and red.

Sugars [a]	Green		Turning		Red	
	MAZ	EVI	MAZ	EVI	MAZ	EVI
Fructose	1.18	1.06	1.79	1.76	2.58	2.58
Glucose	1.38	1.25	1.91	1.87	2.40	2.40
Sucrose	0.31	0.80	0.65	0.87	0.19	0.21

[a] Average of three experiments in g.100 g^{-1} fresh weight; mean coefficient of variation 9.3%.

Organic acids of bell peppers were determined by HPLC. Obviously, the composition of organic acids (underlined compounds in Table 4) of both cultivars changed markedly during ripening. From green to turning, and from turning to red ascorbic and citric acid contents increased significantly ($P < 0.05$); likewise malic, fumaric, oxalic, and pyroglutamic acid decreased significantly ($P < 0.05$).

Table 4. Composition of organic acids, their calculated dissociation states, and pH-values of bell pepper cv. Mazurka (MAZ) and cv. Evident (EVI) harvested at three ripening stages (average of three experiments) [a].

Acid	Green		Turning		Red	
	MAZ	EVI	MAZ	EVI	MAZ	EVI
pH	5.7	5.6	5.2	5.2	5.1	5.0
Ascorbic [b]	126.1	153.0	167.6	160.9	175.4	177.3
H_2Ascorbic [c]	3.4	5.3	13.6	13.2	20.2	19.1
Ascorbic-1 [d]	122.7	147.8	153.9	147.8	155.0	158.2
Ascorbic-2	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Citric	84.0	133.3	261.2	273.2	303.5	350.6
H_3Citric	<0.1	<0.1	0.4	0.4	0.9	0.9
Citric-1	3.2	8.0	47.6	50.7	80.7	86.0
Citric-2	27.3	51.3	131.8	138.2	154.9	179.9
Citric-3	53.5	74.1	81.5	83.9	67.0	83.8
Malic	87.7	65.7	31.7	40.0	28.1	33.4
H_2Malic	0.1	0.1	0.2	0.3	0.3	0.4
Malic-1	16.9	15.1	13.2	17.0	14.1	16.4
Malic-2	70.8	50.6	18.5	23.1	14.0	17.1
Oxalic	3.6	1.4	0.1	<0.1	<0.1	<0.1
H_2Oxalic	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Oxalic-1	0.1	0.1	<0.1	<0.1	<0.1	<0.1
Oxalic-2	3.5	1.3	0.1	<0.1	<0.1	<0.1
Fumaric	0.8	0.5	0.1	0.2	0.1	0.1
H_2Fumaric	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Fumaric-1	0.1	0.1	<0.1	<0.1	<0.1	<0.1
Fumaric-2	0.7	0.4	0.1	0.1	0.1	0.1
Pyroglutamic	3.6	2.8	2.7	2.2	2.4	2.0
HPyroglutamic	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Pyroglutamic-1	3.6	2.8	2.7	2.2	2.4	2.0
Shikimic	4.5	4.9	5.4	5.5	3.6	4.5
HShikimic	0.2	0.2	0.5	0.5	0.5	0.6
Shikimic-1	4.3	4.7	4.9	5.0	3.2	3.9

[a] Mean coefficient of variation of pH-data is 0.7% and of organic acids is 1.6%; [b] Underlined compounds are concentrations of organic acids determined by HPLC in mg.100 g^{-1} fresh weight; [c] Calculated concentrations of undissociated acids; [d] calculated concentrations of dissociated acids in their -1, -2, or -3.

Concentrations of shikimic acid dropped significantly from the turning to the red stage. Minor cultivar differences were only observed in the green stage. Malic, fumaric, and oxalic acid contents were significantly ($P < 0.05$) higher and ascorbic acid was significantly ($P < 0.05$) lower for cv. Mazurka compared to cv. Evident.

Organic acids are present in different dissociation states. The degree of dissociation is directly influenced by the pH as illustrated by the formulas in the section materials and methods, therefore, pH was measured to calculate dissociation states in the different maturation stages. Table 4 shows that the pH decreased upon maturation from green to red from 5.7 to 5.1 and 5.6 to 5.0 for cv. Mazurka and cv. Evident, respectively. Ascorbic, pyroglutamic, and shikimic acid are mainly present in the -1 dissociation state. Malic acid exists for a considerable part in the -1 and -2 forms, whereas oxalic and fumaric acid are fully dissociated. All three dissociation forms of citric acid are present in distinct amounts (Table 4). Remarkably, the HPLC concentration of malic acid decreased during ripening, whereas the calculated contents of undissociated malic acid increased significantly ($P < 0.05$). The calculated concentrations of undissociated ascorbic, citric, and shikimic acid also increased significantly ($P < 0.05$) from green to turning and/or red stages (Table 4).

Relationship between instrumental and sensory data

HPLC and sensory data were processed by PCA to study the relationship between sweetness and sugars, and between sourness and organic acids in their different states of dissociation. PCA on the first combined data set (sensory and HPLC data) gave two principal components, which explained 92% of variance. Loadings of sample scores show that bell peppers were differentiated on ripening stage along component 1 (Fig. 3). Red maturation stages have high negative loadings, green stages have high positive loadings, and turning bell peppers were in the middle of the first component. Figure 4 shows that sweetness, glucose, fructose, total sugar, and dry matter content have negative loadings on component 1. Sourness, the sum of acids, and ascorbic and citric acid have negative loadings too. Therefore, they are all characteristic for the also negatively loaded turning and red fruits (Fig. 3). In addition, the green maturation stages of both cultivars have positive loadings on component 1, while on component 2 cv. Mazurka has negative and cv. Evident has positive loadings (Fig. 3). The positive loadings on component 1 of pH, malic, fumaric, pyroglutamic, and oxalic acid (Fig. 4) suggest that they are more characteristic for the green fruits as compared to the ripe stages. However, fumaric, pyroglutamic, and oxalic acid also have relatively high negative loadings on component 2, which indicates that they are more characteristic for green fruits of cv. Mazurka than cv. Evident. Figure 4 shows that sucrose and total shikimic acid have positive loadings on component 2, which corresponds with the sample scores of the turning maturation stages of both cultivars, and the green stage of cv. Evident (Fig. 3).

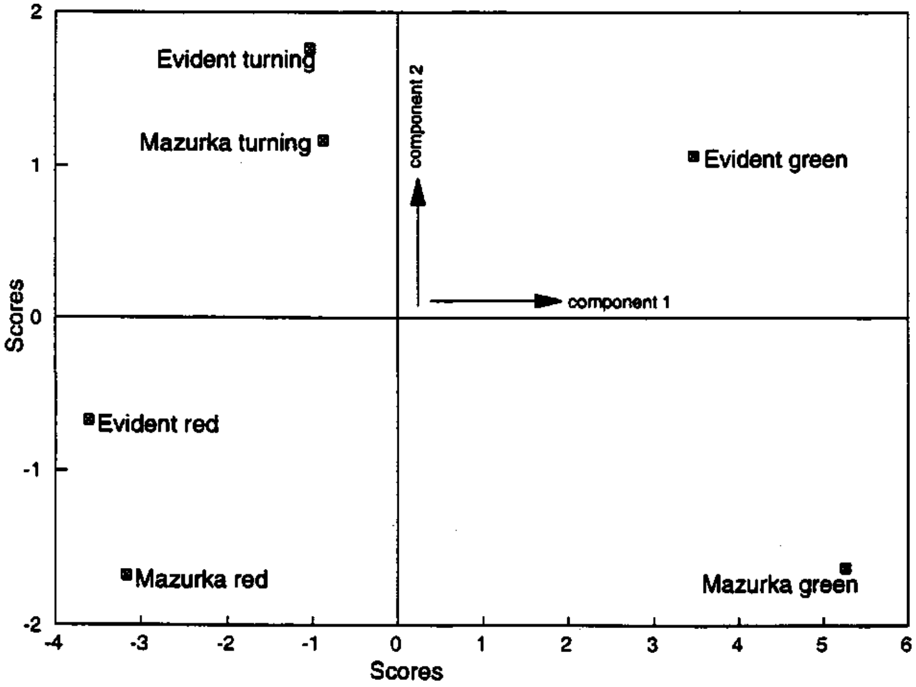

Figure 3. Sample scores of bell pepper cv. Mazurka and cv. Evident in the ripening stages green, turning, and red on PC 1 and 2 (combined HPLC & sensory data set).

Also, PCA was carried out on concentrations of different anions of the acids, calculated from their dissociation equilibria, and sensory data. Several of the amounts of acids calculated were far below known threshold values for corresponding organic acids, i.e., ± 0.0025 g.100 mL^{-1} (Amerine et al., 1965), and therefore not included in the data set. Two principal components were obtained, which explained 85% of variance of the data set. The loadings diagram of the bell pepper samples was similar to Figure 3. Loadings of calculated instrumental and sensory data are presented in Figure 5. Citric 1, citric 2, undissociated ascorbic acid, and the sum of undissociated and dissociated acids have high negative loadings on component 1, similar to citric acid, ascorbic acid, and sum of acids in Figure 4. However, citric 3 and ascorbic 1 shifted to relatively high positive values on the second component and therefore are more typical for the turning than the red stages (Fig. 5). Oxalic 2, pyroglutamic 1, and malic 2 have positive loadings on component 1 and negative loadings on component 2 (Fig. 5), which corresponds with loadings of green cv. Mazurka (Fig. 3). Malic 1, however, has low loadings on both principal components and is consequently not distinctive for any ripening stage or cultivar.

Partial least-squares regression (PLS) confirmed only principal component 1 in both data sets and showed similar relations for maturation stages as joint PCA. In the first set 75% of variance in the *X*-data explained 85% of variance in the *Y*-data, whereas 71% of variance in the *X*-data explained 86% of variance in the *Y*-data of the second data set.

Discussion

A complex of factors such as the composition of odour and taste compounds, and their interactions, might influence flavour as perceived during eating. In this study, distinctive sensory flavour attributes of bell peppers, at different maturation stages, were assessed by a trained descriptive panel, and some sensory attributes were related to the intrinsic composition of the samples.

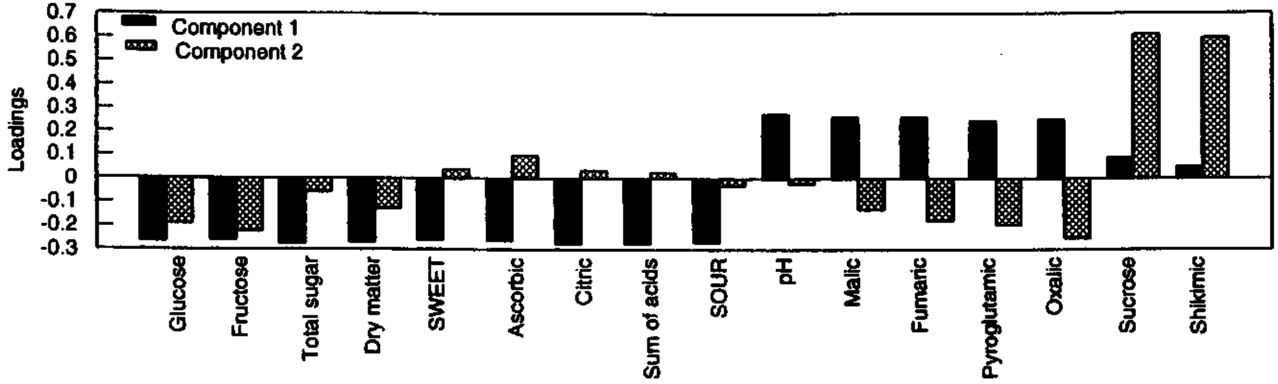

Figure 4. Loadings of sensory attributes and HPLC data of sugars and organic acids on PC 1 and 2.

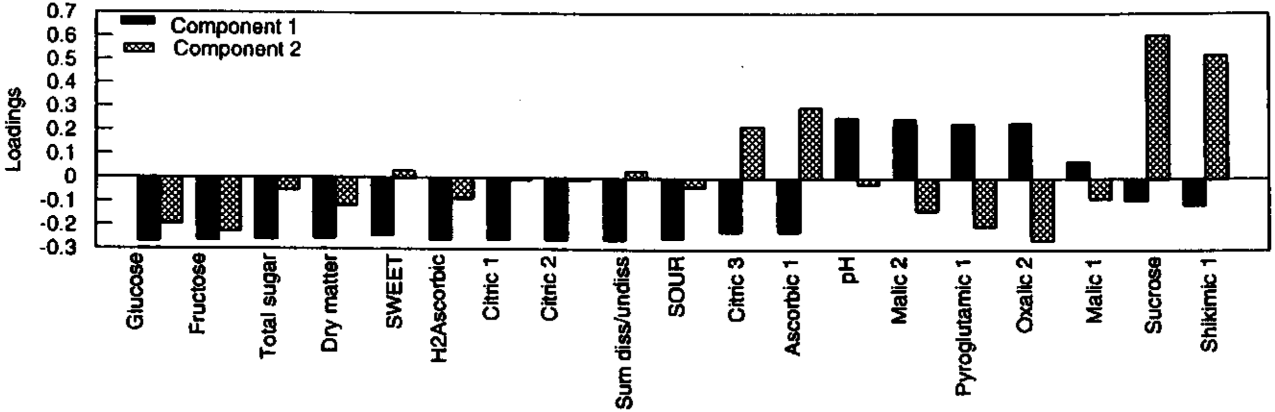

Figure 5. Loadings of sensory attributes, HPLC data of sugars, and calculated dissociated and undissociated acids on PC 1 and 2.

Green maturation stages were characterized by cucumber, grassy, and green bell pepper aroma for both cv. Mazurka and cv. Evident, whereas the ripe stages had a distinct red bell pepper aroma (Table 2; Fig. 1 and 2). Likewise, Chitwood et al. (1983) observed that "green" sensory attributes, such as grassy, fresh green bean, and garbanzo bean aroma, discriminated different cultivars of *Capsicum.* They suggested that volatile constituents such as 2-isobutyl-3-methoxypyrazine, 2-*sec*-butyl-3-methoxypyrazine, and (*Z*)-3-hexenol may be responsible for the "green" notes. Luning et al. (1994*a*) demonstrated by gas chromatography-sniffing port analysis that during ripening of bell peppers mainly volatile compounds with "green" aroma notes disappeared. Moreover, Luning et al. (1994*b*) suggested relationships between volatile compounds and sensory flavour attributes. Green peppers were apparently characterized by sensory aroma attributes such as, grassy, herbal/grassy, green bell pepper, and fruity/fresh and the presence of high levels of 1-penten-3-one, (*Z*)-3-hexenal, (*Z*)-3-hexenol, 2-isobutyl-3-methoxypyrazine, and linalool, which possess similar flavour notes, indicated that these compounds seem to be responsible for these green related attributes.

Apart from these aroma characteristics, other attributes appeared to be important for the flavour as perceived during eating. Green fruits of cv. Evident were perceived significantly ($P < 0.05$) sharper than green fruits of cv. Mazurka (Table 2). Sharpness of *Capsicum* varieties is caused by capsaicinoids, although sweet peppers in general contain very low amounts (Iwai et al., 1977; Govindarajan, 1985). Bitterness clearly discriminated the green stages from the turning and red ones (Fig. 1 and 2). To our knowledge, no specific bitter compounds have been reported in *Capsicum* varieties. Interestingly, some saponins, which can give a bitter perception, were identified in seeds of *C. annuum* fruits (Tschesche and Gutwinski, 1975). Their presence in other parts of the fruit and corresponding sensory characteristics have not been studied.

Sweetness appeared to be typical for ripe stages (Fig. 1 and 2) and closely related to glucose, fructose, total sugar, and dry matter content (Fig. 4). However, sucrose was not related to changes in sweetness during maturation (Fig. 4). Shamaila et al. (1992) observed a significant relation between glucose and fructose levels with the degree of sweetness in strawberry samples, and Dever et al. (1992) suggested that sweetness of apple juice was closely related to glucose, fructose and soluble solids. ANOVA results (Table 2) showed that cv. Evident was sweeter than cv. Mazurka in the turning and ripe stages. These significant differences were, nevertheless, not reflected in differences in sugar composition (Table 3). Probably interactions with other flavour compounds might influence perceived sweetness. Frank et al. (1989) suggested that strawberry flavour induced a significant increase in sweetness of sucrose solutions. Luning et al. (1994*b*) indicated that the volatile compositions of red fruits of cv. Mazurka and cv. Evident were different; the latter contained higher concentrations of 3-carene, 2-heptanone, 6-methyl-5-hepten-2-one, and dimethyl trisulphide. In addition, Schifferstein and Freijters (1990) demonstrated interactions between sucrose and citric acid on perceived sweet- and sourness. Unfortunately, the concentrations used in their model systems were much higher than in the investigated bell peppers. However, it might be possible, that differences in composition of organic acids and/or other flavour compounds of both cultivars influence the perceived sweetness.

Sourness appeared to be typical for turning and red stages of fresh bell peppers (Fig. 1 and 2). The perceived sourness increased significantly ($P < 0.05$) from the green to turning or red maturation stages but not from turning to red stages (Table 2). Citric acid and to a lesser extent ascorbic acid markedly increased during ripening (Table 4). Distinct amounts of citric and ascorbic acid are present in different dissociation states and they probably increased the H^+ concentration. Furthermore, the lowering of pH by 0.5 unit shifted the organic acids in favour of their undissociated states (Table 4). PCA illustrates clearly that total citric and to a lesser extent total ascorbic acid were closely related to sourness, while pH, total malic, fumaric, pyroglutamic, and total oxalic acid suggested a negative relationship (Fig. 4). Also, Watada and Aulenbach (1979) and Paterson et al. (1991) suggested correlations between perceived sourness and the citric acid content. PCA on calculated undissociated and dissociated acids revealed that mainly undissociated ascorbic acid and citric acid in the -1 and -2 form were closely related with sourness, while pH, malic 2, pyroglutamic 1, and oxalic 2 suggested a negative relationship (Fig. 5). Ganzevles and Kroeze (1987) studied the perceived sourness of several organic acids in model solutions. They suggested that in contrast to HCl, not only the H^+ concentration but also the undissociated acid appeared to be an important factor in eliciting sourness of organic acids. They implied that HCl sourness and the sourness of organic acids are caused by different receptor processes. According to their theory, undissociated ascorbic acid might be directly important for the sour perception, while the dissociated forms of citric acid influence the sourness by increasing the proton concentration.

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Chapter 4

Gas Chromatography, Mass Spectrometry and Sniffing Port Analyses of Volatile Compounds of Fresh Bell Peppers (*Capsicum annuum*) at Different Ripening Stages[1]

Abstract

Dynamic headspace gas chromatography, mass spectrometry, and sniffing port detection were used to analyze the volatile compounds of a Dutch commercial *Capsicum* cv. Mazurka at the ripening stages green, turning, and red. The samples were prepared by either cutting or blending of the fruits. The different bell pepper samples obtained had several odour compounds in common, i.e., 2,3-butanedione (caramel), 1-penten-3-one (chemical/pungent, spicy), hexanal (grassy), 3-carene (red bell pepper, rubbery), (*Z*)-ß-ocimene (rancid, sweaty), octanal (fruity), and 2-isobutyl-3-methoxypyrazine (green bell pepper). During bell pepper maturation the majority of volatile compounds, of which several had green-related odour notes, decreased or even disappeared. Only the levels of (*E*)-2-hexenal and (*E*)-2-hexenol, which have almond, fruity, sweet odours, were higher at the stages turning and red. Disruption of the cell structure favoured lipid oxidation and the formation of related alcohols, aldehydes, and ketones.

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Introduction

The two main species of *Capsicum*, i.e., *Capsicum annuum* var. *grossum* Sendt (c.q. sweet bell pepper) and *Capsicum frutescens*, are widely used for their colour, distinct taste, and aroma (Whitfield and Last, 1991). Bell peppers are consumed as immature (green) or mature (e.g., red, yellow) fruits due to their clear differences in flavour. The main quality parameters for *Capsicum* varieties are colour and pungency (Govindarajan, 1986; Govindarajan, et al., 1987). However, current research is also focusing on the flavour as an important parameter for the quality of fresh fruits and vegetables.

More than 125 volatile compounds have been identified (Van Straten and Maarse, 1991) in fresh and processed *Capsicum* fruits. The significance of these compounds for the aroma is not yet well-known, since the research on the odour evaluation of bell pepper volatile compounds has been very limited. Buttery et al. (1969) indicated that 2-methoxy-3-isobutylpyrazine, (*E*,*Z*)-2,6-nonadienal, and (*E*,*E*)-decadienal are important aroma compounds of bell peppers because of their low threshold values and distinct odours. Chitwood et al. (1983) suggested the relation between the volatile constituents 2-isobutyl-3-methoxypyrazine, 2-*sec*-butyl-3-methoxypyrazine, (*Z*)-3-hexenol and some sensory-perceived "green" aroma characteristics of three *Capsicum* cultivars grown in California. Wu and Liou (1986) indicated that tissue disruption increased the amount of volatile unsaturated C_6 aldehydes and alcohols. Some of these compounds have been sensorily described by green-related descriptors such as grassy and crushed leaves (Dravnieks, 1985). These aldehydes and alcohols might play an important role in determining the green bell pepper flavour. So far, the changes of volatile bell pepper constituents during maturation have not been investigated.

Several combinations of gas chromatography and column effluent sniffing techniques are in practice such as Charm analysis (Acree et al., 1984), aroma extraction dilution analysis (Schieberle and Grosch, 1987), the Osme method (Miranda-Lopez et al., 1992), and the sensory sniffing-port panel method (Linssen et al., 1993).

In the present study, the composition of volatile compounds of a Dutch bell pepper cultivar, Mazurka, at three ripening stages was studied by using gas chromatography (GC), mass spectrometry (MS), and sniffing port analysis (GC–sniffing). The latter technique enables a direct correlation between GC-separated volatile compounds and their sensory perception.

Materials and methods

Bell pepper fruits

C. annuum cv. Mazurka was grown on glass wool in a greenhouse with controlled temperature (± 20°C) and humidity (75%). The fruits were harvested at 6 (green stage), 8 (turning stage) and 10 (red stage) weeks after fruit setting, and individual weights of the whole bell peppers were measured. The fruits were subsequently stored at 13°C (maximal 3 days) until volatile compounds were isolated by a dynamic headspace technique.

Colour measurements

Chlorophyll content was determined as described by Moran and Porath (1980) with minor modifications. Aliquots of 0.5 g of frozen bell peppers were homogenised with 10 mL of *N,N*,-dimethylformamide (Merck, Amsterdam) and 10 mg of $CaCO_3$ in a Waring blender for 1 min at 4°C, and vacuum filtered using a sintered glass funnel (G3). The chlorophyll *a* concentration of the extracts was measured spectrophotometrically (Perkin-Elmer, UV–vis spectrometer Lambda 16) at 664.5 nm, using the specific absorption coefficient of chlorophyll *a* in *N,N*-dimethylformamide, 83.89 L g^{-1}.

Carotenoid extraction was performed with hexane/acetone mixtures according to the AOAC (1984) method with some minor modifications. Aliquots of 5 g were homogenised with 60 mL of hexane, 40 mL of acetone and 0.1 g of $CaCO_3$ in a

Waring blender for 2 min and vacuum filtered using a sintered glass funnel (G3). The residue was dispersed in a mixture of 25 mL of hexane and 25 mL of acetone and filtered to extract residual carotenoids. The filtrates were pooled and washed with 100 mL of water. The final upper layer was separated, mixed with 9 mL of acetone, and diluted with hexane to 100 mL. Standard curves were made with ß-carotene (97%, Merck), and the specific absorption coefficient was determined. The extracts were measured for carotenoids at 436 nm by using a spectrophotometer, and the absorbances were converted into grams of ß-carotene per kilogram of dry matter.

Isolation of volatiles

Bell pepper samples were prepared by slicing 250 g of sample or by homogenization of 45 g of sample with 50 mL of water in a Waring blender for 10 s. The samples obtained were transferred to 500-mL glass bottles for dynamic headspace isolation of volatile compounds as described by Macleod and Ames (1986), using Tenax TA as an adsorbent. The homogenised samples were flushed with 30 mL.min^{-1} purified nitrogen for 2 h at room temperature; the sliced samples were flushed for 6 hrs. The flushing times were arbitrarily chosen to obtain sufficient amounts of volatile components for flame ionization detection and sniffing port analysis. System control samples were made by using the same conditions as for the isolation procedures.

Gas chromatography analysis

Volatile compounds were desorbed from the Tenax tubes for 10 min at 200°C (Chrompack TCT injector 16200, Chrompack, Bergen op Zoom, The Netherlands) and injected in a capillary CP-Wax CB52 column (50 m x 0.32 mm i.d., 1.5-µm film thickness, Chrompack). The oven temperature was programmed from 40 to 150°C at 2°C.min^{-1} and subsequently heated to 250°C at 10°C.min^{-1}. The gas chromatograph (Carlo Erba GC vega 6000, Interscience, Breda, The Netherlands) was equipped with a flame ionization detector (FID) and two sniffing ports (SGE, Milton Keynes, Great Britain). FID response standard curves were made to calculate the quantitative amounts of bell pepper compounds such as hexanal, (*E*)-2-hexenal, octanal, (*Z*)-3-hexenol, (*E*)-2-hexenol, 2-isobutyl-3-methoxypyrazine, and linalool (Jansen Chimica, Tilburg, The Netherlands).

GC–MS analysis

Volatile compounds were identified by using a GC–MS (Carlo Erba, Mega 3600, QMD 8000, Interscience) which was equipped with a thermal desorption unit (Carlo Erba, Tekmar 5010, automatic desorber, Interscience). Thermal desorption (10 min at 200°C) and GC column conditions were identical to those for gas chromatography. Electron impact mass spectral (EI-MS) analysis was carried out at an ionization energy of 70 eV. The calculated Kovats indices (KI) and MS fragmentation patterns of each component were compared with those of the authentic compound as reported in the literature (Kawakami and Kobayashi, 1991; Umano et al., 1992; Vernin et al., 1987).

To compare differences in ripening stages and cultivars, the peak areas (Vs) of identified compounds (six replicates) were processed by analysis of variance (ANOVA).

Sniffing port evaluation of bell pepper volatiles

The GC–effluent was split in 20% for FID analysis, 40% for the left, and 40% for the right sniffing port. To prevent dehydration of the nasal membranes of the assessors, humidified air was added to the exit of the sniffing port.

An experienced GC–sniffing port panel was used for the evaluation of bell pepper odours. In five additional sessions, the assessors were trained to detect and describe the volatile compounds of bell peppers. A preliminary list of odour descriptors was created after the first session. Then, several reference samples such as crushed lettuce, cucumber, carrot, green and red bell pepper, and geranium (myrcene 97%, Jansen Chimica) were presented to the assessors to associate references with their odour descriptions. A list of 19 frequently used odour descriptors was compiled including almond, floral/soapy, mushroom, geranium-like, fruity, grassy/cut grass, chemical/pungent, cucumber/fresh green, caramel, musty, green bell pepper, red bell pepper, rubbery/burned rubber, lettuce, sweaty, spicy/herbal, rancid/oily, carrot and sweet. The term "unknown" was added to the list for detectable smells missing a suitable descriptor. The duration of detection and the description of volatile odour compounds were registered with portable computers, which were programmed as described by Linssen et al. (1993). The background noise of the sniffing port panel was determined by analyzing clean Tenax tubes (Linssen et al., 1993). The raw data sets of each assessor were transferred into a database (Foxpro) for summarizing the individual sniffing responses of the 12 assessors, calculating the sniffing responses above the noise level, and summarizing the odour descriptors given to the total sniffing responses.

Results and discussion

The composition of carbohydrates, organic acids, volatile flavour compounds, and colour pigments changes during the maturation of bell peppers (Govindarajan, 1985, 1986). Some of these parameters are used by growers to determine the harvest time of bell peppers cultivated in greenhouses (Govindarajan, 1985). Both green and red bell peppers (commercial products) and an intermediate turning stage were chosen for the analysis of volatile compounds. The three ripening stages were evaluated by determining fresh weight, contents of dry matter, and colour pigments. Table 1 presents the analytical data of green, turning, and red fruits from the cv. Mazurka. It appeared that fresh weight did not change during maturation while dry matter content increased gradually. The chlorophyll *a* content was maximal at the green stage and decreased during ripening. In contrast, the carotenoid content increased upon maturation.

Table 1. Fresh weight, dry matter, and colour pigment contents of bell pepper cv. Mazurka at the green, turning, and red stages.

Analyses[a]		Green			Turning			Red		
Fresh weight	(g)	181.2	±	15.6	199.2	±	17.0	211.0	±	26.9
Dry matter	(%)	6.1	±	0.4	7.5	±	0.2	8.7	±	0.6
Chlorophyll *a*	($g.kg^{-1}$ dm)	1.1	±	0.4	0.4	±	0.1	0.04	±	0.04
Carotenoids [b]	($g.kg^{-1}$ dm)	0.6	±	0.1	0.6	±	0.2	1.5	±	0.2

[a] Mean value of 12 replicates ± standard deviation; [b] Expressed as ß-carotene standard (E = 185,9 $L\ g^{-1}$).

GC and GC–MS analysis of volatile bell pepper compounds

Wu and Liou (1986) observed that disruption of the cell structure affected the composition of volatile compounds of bell peppers. In this study, two methods for sample preparation, i.e., homogenization and cutting, were used. Figures 1 and 2 represent the gas chromatograms of the cv. Mazurka in the maturation stages green, turning, and red. Comparison of these stages shows that the total peak areas decreased during maturation. Both in homogenised and in sliced samples, the volatile compounds (numbered peaks) decreased or disappeared and only a few compounds increased upon ripening. Comparison of Figures 1 and 2 illustrates that the FID patterns of homogenised and sliced bell peppers differ very much. In general, the intensity of the FID response of homogenised samples was higher than that of sliced bell peppers, which indicates that the concentrations of volatile compounds in the headspaces above blended bell peppers are higher. Similar gas chromatograms were obtained for another Dutch bell pepper cultivar, Evident (Luning et al., 1994). Identification of the different compounds from the bell pepper headspace concentrates was based upon mass spectral matching and comparison of calculated KI's with authentic samples.

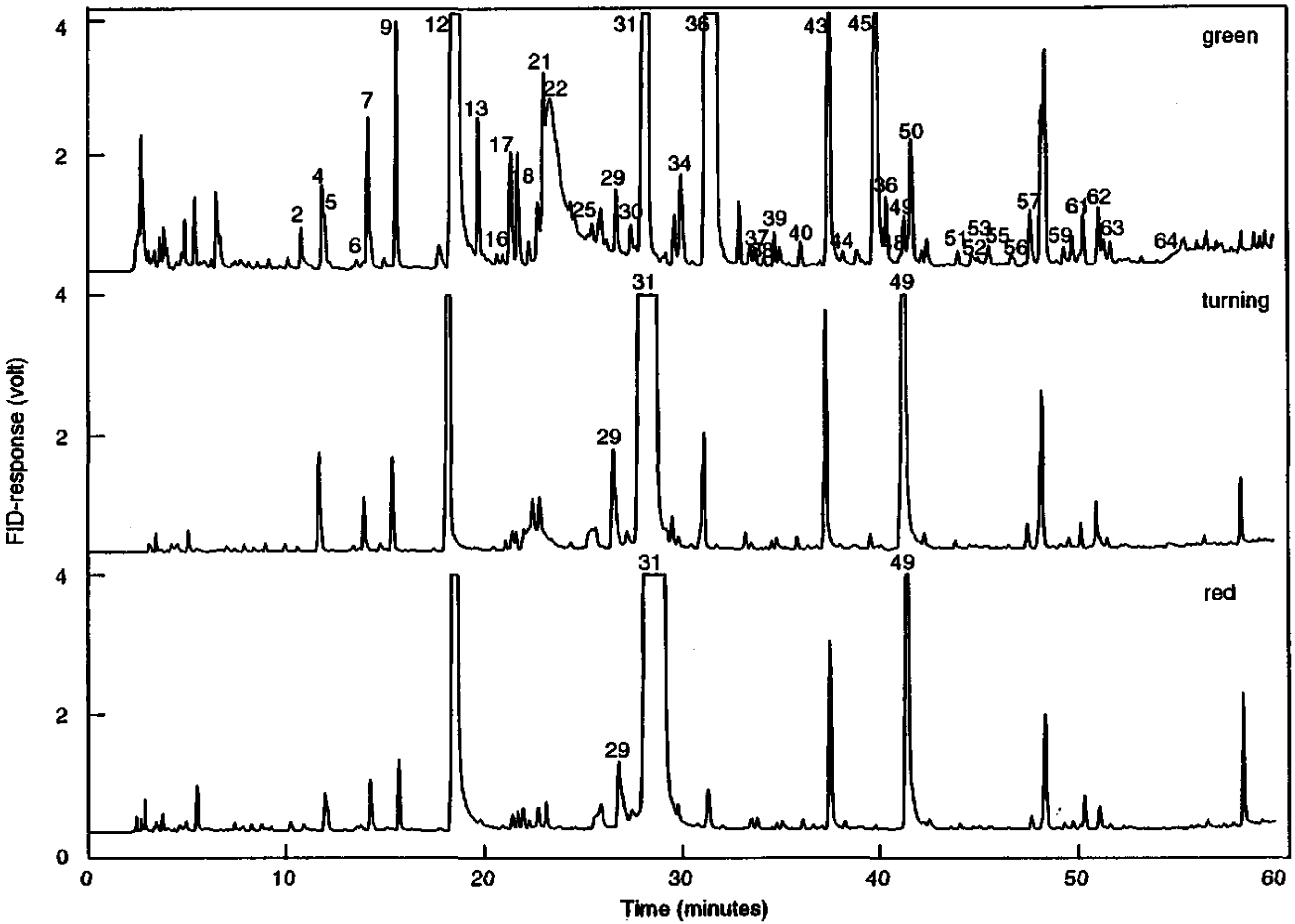

Figure 1. Dynamic headspace gas chromatograms of homogenised bell peppers of cv. Mazurka at the ripening stages green, turning, and red.

The identified volatile compounds are listed in Table 2 together with their average peak areas. Quantitative amounts (nanogram) were calculated using FID calibration curves of authentic compounds (Table 2). In total, 64 compounds were identified, of which 22 have not been detected before in *C. annuum* varieties, but they were often found in other fresh vegetables (Van Straten and Maarse, 1991).

The composition of volatile compounds of bell peppers differs clearly for the maturation stages and for the methods of sample preparation. Table 2 shows that the majority of volatile compounds from both homogenised and sliced samples decreased or even disappeared during ripening. The homogenised green bell peppers contain high levels of 1-penten-3-one (7), toluene (9), hexanal (12), (*Z*) and (*E*)-2-pentenal (13, 17), (*Z*)-3-hexenal (22), (*E*)-2-hexenal (31), (*E*)-ß-ocimene (36), 1-hexanol (43), (*Z*)-3-hexenol (45), and 6-methylheptyl 2-propenoate (58), of which 9, 36, 43, 45, 58, (*Z*)-ß-ocimene (34), and (*E,Z*)-3,4-dimethyl-2,4,6-octatriene (46) were also the major peaks from sliced samples. These compounds, except (*E*)-2-hexenal (31), decreased significantly ($P < 0.05$) during maturation. However, significantly ($P < 0.05$) higher average peak areas were observed in the homogenised samples at the turning and red stages for (*Z*)-2-hexenal (29), (*E*)-2-hexenal (31), and (*E*)-2-hexenol (49), of which 31 and 49 were also significantly ($P < 0.05$) higher at these stages of sliced bell peppers. Buttery et al. (1969) reported that (*E*)-ß-ocimene, methyl salicylate, limonene, (*Z*)-3-hexenol, linalool, (*E*)-2-none-4-one, 2-isobutyl-3-methoxypyrazine, (*E,Z*)-2,6-nonadienal, and (*E,E*)-2,4-decadienal were the major compounds isolated from fresh green California bell peppers. They used a vacuum Likens–Nickerson apparatus at ± 50°C to extract the volatile compounds. Our experiments were performed at room temperature using a dynamic headspace method, which might explain why volatile compounds with higher KI's such as methyl salicylate and (*E,E*)-2,4-decadienal were not released. In addition, Wilkins and Madsen (1989) observed that headspaces of 20 g of heated (40°C) Hungarian edelsüss paprika consisted mainly of hydrocarbons with limonene as the major component.

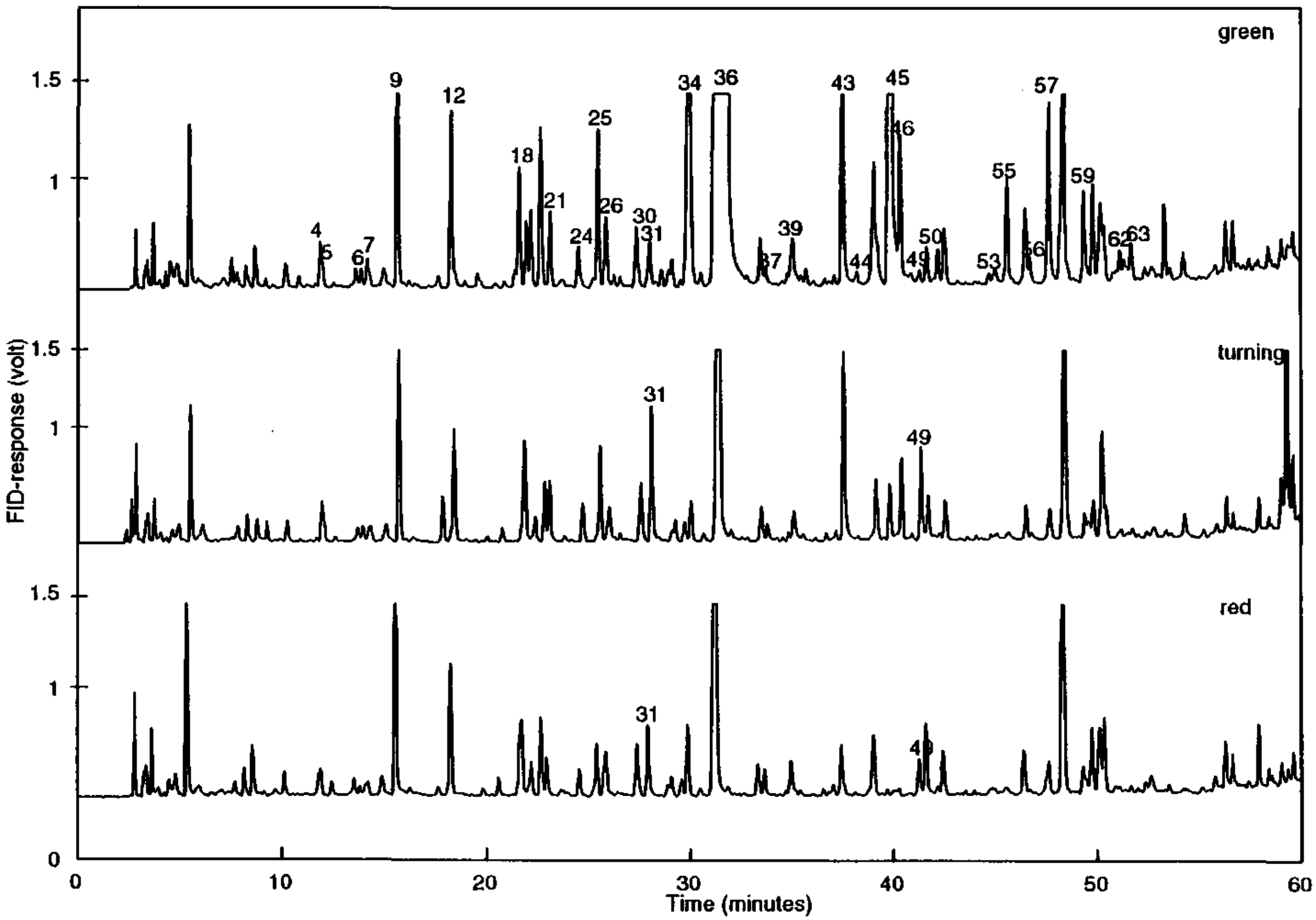

Figure 2. Dynamic headspace gas chromatograms of sliced bell peppers of *cv.* Mazurka at the *ripening stages green, turning, and red.*

Table 2. Identified compounds and their average GC peak areas of homogenised (HG) and cut (CT) Mazurka bell peppers at the ripening green, turning, and red stages.

			GC peak area [c]					
No.[a]	KI [b]	Compound	Green		Turning		Red	
			HG	CT	HG	CT	HG	CT
1	931	3-Buten-2-one [d]	1.04	1.59	0.90	1.00	0.89	0.96
2	944	2-Ethylfuran	3.38	*e*	0.53	*e*	0.81	*e*
3	962	2,3-Butanedione [d]	0.04	0.16	0.04	0.07	0.03	0.05
4	965	3-Pentanone [d]	8.49	1.91	7.02	1.62	4.41	1.28
5	967	Pentanal	3.61	1.02	2.46	0.80	2.08	0.87
6	1005	2-Butanol [d]	0.66	1.62	0.53	0.87	0.08	0.34
7	1009	1-Penten-3-one [d]	17.34	1.25	5.24	0.58	5.54	0.57
8	1021	α-Pinene	0.95	1.56	0.78	1.27	0.58	1.00
9	1032	Toluene	15.14	14.72	7.09	10.70	7.46	10.22
10	1047	2,3-Pentanedione [d]	*e*	0.17	*e*	0.17	*e*	0.14
11	1069	Dimethyl disulphide [d]	1.77	1.45	0.35	1.23	0.29	0.41
12	1076	Hexanal	**7.83** [f]	**0.28**	**4.88**	**0.17**	**5.31**	**0.19**
13	1095	(*Z*)-2-pentenal [d]	14.87	*e*	0.19	*e*	0.09	*e*
14	1108	1-Methoxy-2-propanol	1.01	0.86	0.74	0.54	0.86	0.63
15	1110	β-Pinene	0.05	0.06	0.06	0.05	0.05	0.03
16	1113	3-Penten-2-one [d]	1.69	*e*	0.07	*e*	0.05	*e*
17	1118	(*E*)-2-pentenal [d]	14.73	*e*	1.06	*e*	1.42	*e*
18	1125	1-Butanol [d]	7.94	4.41	5.29	4.55	3.12	2.18
19	1132	*p*-Xylene	2.14	2.19	2.33	2.36	1.97	1.54
20	1138	*m*-Xylene	1.72	4.84	3.00	5.22	2.73	3.39
21	1142	1-Penten-3-ol [d]	6.98	3.22	2.58	2.24	2.44	2.60
22	1147	(*Z*)-3-hexenal	219.43	*e*	8.54	*e*	6.73	*e*
23	1152	3-Carene	0.60	0.46	0.19	0.32	0.31	0.49
24	1167	Myrcene	1.39	1.98	0.25	1.43	0.19	0.73
25	1177	2-Heptanone	2.36	6.91	3.59	4.04	2.02	1.98
26	1181	Heptanal	1.18	2.62	1.73	2.30	1.35	2.30
27	1183	*o*-Xylene	2.42	2.17	2.50	4.82	2.60	2.53
28	1187	2,3-Dihydro-4-methylfuran [d]	0.85	0.57	0.46	0.13	0.34	0.17
29	1194	(*Z*)-2-hexenal	9.61	*e*	11.71	*e*	13.78	*e*
30	1204	Limonene	2.25	3.82	3.36	3.27	2.61	2.57
31	1212	(*E*)-2-hexenal	**5.76** [f]	**0.06**	**>13.9**	**0.21**	**>15.3**	**0.25**
32	1232	2-Pentylfuran	*g*	*g*	*g*	*g*	*g*	*g*
33	1233	1-Pentanol	*g*	*g*	*g*	*g*	*g*	*g*
34	1237	(*Z*)-β-ocimene	6.94	23.10	2.22	4.17	0.87	1.32
35	1253	3-Octanone [d]	*g*	*g*	*g*	*g*	*g*	*g*

(continue Table 2)

36	1254	(*E*)-β-ocimene	180.14	379.63	44.79	67.62	10.83	28.44
37	1283	Octanal	**0.07** [f]	**0.05**	**0.04**	**0.04**	**0.03**	**0.05**
38	1288	(*Z*)-2-pentenol [d]	0.89	0.10	0.05	0.06	0.06	0.04
39	1297	(*E*)-2-pentenol [d]	3.57	1.10	0.67	0.74	0.62	0.45
40	1313	2-Heptanal [d]	2.48	0.31	1.43	0.14	1.10	0.11
41	1322	4-Nonanone	0.18	0.27	0.47	0.33	0.47	0.22
42	1327	6-Methyl-5-hepten-2-one [d]	0.42	0.75	0.42	0.47	0.35	0.40
43	1332	1-Hexanol	40.69	11.42	29.82	10.66	23.72	4.87
44	1342	(*E*)-3-hexenol	2.13	0.68	0.54	0.17	0.83	0.16
45	1364	(*Z*)-3-hexenol	**2.88** [f]	**1.74**	**0.05**	**0.06**	**0.02**	**0.01**
46	1369	(*E*,*Z*)-3,4-dimethyl-2,4,6-octatriene	6.95	15.24	2.10	1.83	0.44	0.95
47	1377	Dimethyl trisulphide [d]	0.12	0.32	0.07	0.25	0.09	0.14
48	1379	(*E*,*E*)-2,4-hexadienal [d]	0.58	*e*	*e*	*e*	*e*	*e*
49	1383	(*E*)-2-hexenol	**0.11** [f]	**0.02**	**2.34**	**0.15**	**1.95**	**0.09**
50	1387	Nonanal [d]	11.28	3.35	0.73	2.67	0.32	2.72
51	1418	(*E*)-2-octenal	1.30	*e*	1.04	*e*	0.60	*e*
52	1430	1-Octen-3-ol	1.51	*e*	0.41	*e*	0.23	*e*
53	1435	1,2,4,5-Tetramethylbenzene	1.35	0.86	0.41	0.32	0.35	0.19
54	1441	2-Furancarboxyaldehyde	0.45	0.18	0.18	0.13	0.16	0.14
55	1442	1,3,8,-*p*-Menthatriene	1.12	4.33	0.44	0.63	0.30	0.27
56	1456	2-Pentylthiophene [d]	1.42	1.16	0.67	0.53	0.27	0.13
57	1470	2-Ethyl-1-hexanol [d]	4.87	5.88	2.52	1.76	2.24	1.46
58	1478	6-Methylheptyl 2-propenoate	21.80	18.81	16.54	20.83	16.56	18.17
59	1492	Decanal [d]	1.63	2.00	0.55	1.40	0.36	1.19
60	1500	2-*sec*-Butyl-3-methoxypyrazine	<0.01	<0.01	*e*	*e*	*e*	*e*
61	1506	Benzaldehyde	4.48	3.64	2.07	1.94	2.49	3.00
62	1510	2-Isobutyl-3-methoxypyrazine	**0.18** [f]	**0.03**	**0.13**	**0.01**	**0.07**	**0.01**
63	1527	Linalool	**0.15** [f]	**0.09**	**0.06**	**0.01**	**0.03**	**0.01**
64	1576	(*E*,*Z*)-2,6-nonadienal	<0.01	*e*	<0.01	*e*	*e*	*e*
		Total peak area	1234.6	608.9	992.8	190.2	985.9	123.7

[a] Numbers refer to Figures 1 and 2. [b] Calculated Kovats indices on CP-Wax CB52 column. [c] Average peak areas of six replicates (Vs). [d] Not identified before in *C. annuum* varieties. [e] Not detected. [f] (Bold print) amount (ng) calculated from FID standard curves. [g] Peaks were insufficiently separated from neighbouring compound.

Comparison of the volatile composition of homogenised and sliced bell peppers in Table 2 reveals that a number of volatile compounds such as 2-ethylfuran (2), (*Z*)- and (*E*)-2-pentenal (13, 17), 3-penten-2-one (16), (*Z*)-3-hexenal (22), (*Z*)-2-hexenal (29), (*E*,*E*)-2,4-hexadienal (48), (*E*)-2-octenal (51), 1-octen-3-ol (52), and (*E*,*Z*)-2,6-nonadienal (64) were only detectable in homogenised samples.

Furthermore, the average peak areas of 1-penten-3-one (7), hexanal (12), (*E*)-2-hexenal (31), hexanol (43), (*Z*)-3-hexenol (45), and (*E*)-2-hexenol (49) were significantly ($P < 0.05$) larger for the homogenised samples. These results confirm the findings of Wu and Liou (1986) that tissue disruption of green bell peppers favoured the formation of 2-ethylfuran, hexanal, (*Z*)-3-hexenal, (*E*)-2-hexenal, hexanol, (*Z*)-3-hexenol, and (*E*)-2-hexenol. They suggested the activity of oxidative and hydroperoxide-cleavage enzyme systems, because the addition of stannous chloride caused a significant reduction of these compounds. For tomatoes, lipoxygenase and specific cleavage enzymes yielded mainly hexanal and (*Z*)-3-hexenal (Galliard et al., 1977; Galliard and Matthew, 1977). These compounds were subsequently converted into their corresponding alcohols by an alcohol oxidoreductase (Schormüller and Grosch, 1965). Galliard and Matthew (1977) postulated that tomatoes have very low or no isomerase activity because the concentration of the more stable (*E*)-2-hexenal was relatively low. Table 2 shows that the levels of (*Z*)-3-hexenal (22) and (*Z*)-3-hexenol (45) decreased during bell pepper maturation while the levels of (*E*)-2-hexenal (31) and (*E*)-2-hexenol (49) increased. As stated by Galliard et al. (1977) for tomatoes, the activities of several enzymes seem to be changed during ripening of bell peppers, especially the ones involved in the formation of lipid degraded products.

Sniffing port evaluation of volatile bell pepper compounds

The volatile compounds of the GC column effluents were also detected by a GC–sniffing panel. Sniffing of blank Tenax revealed that detection of an odour by less than 6 of 12 assessors could be considered as background "noise" for our panel. Twenty odour descriptors were obtained from the training sessions and were used for odour evaluation of the volatile compounds by the GC–sniffing panel. The odour compounds were identified by comparing calculated KI's of sniffing responses with these indices of GC–MS-identified compounds. Moreover, the odour descriptors used by the panel were compared with descriptors reported in the literature (Dravnieks, 1985; Sheen et al., 1991). Table 3 shows the volatile odour compounds detected by the GC–sniffing panel of the cv. Mazurka and their major odour descriptions. In this table, 9 of 30 odour compounds could not be identified by GC–MS or detected by FID due to extremely low concentrations or masking by other (odourless) compounds. For instance, (*E*)-ß-ocimene (36) was associated with a mushroom, herbal odour (36a) because of their similar KI's. However, the mass spectrum revealed additional masses that do not belong to (*E*)-ß-ocimene, which is indicative of an inadequate GC separation. A minor compound probably contributed to the mushroom, herbal odour. Furthermore, the odour descriptors of the sniffing response at KI = 1364 changed from grassy, lettuce, green bell pepper (45) in the green maturation stage to geranium, spicy (45a) in the turning and red maturation stages. The KI value of (*Z*)-3-hexenol (45) corresponds with the KI value of the observed sniffing response (Table 3). (*Z*)-3-Hexenol (45), which is known as a green, grassy odour (Dravnieks, 1985), disappears upon maturation (Table 2). Since a sniffing response (45a) was still observed, another unidentified compound might be responsible for the detected geranium, spicy odour (45a).

Basic odour profile. Our GC–sniffing port results indicate that a kind of basic odour profile was present in each of the investigated bell pepper samples and that this profile was independent of the ripening stage and sample preparation method (Table 3). This basic odour profile consisted of 2,3-butanedione (caramel, sweet), 1-penten-3-one (chemical/pungent, spicy), hexanal (grassy, spicy, green bell pepper), 3-carene (red, green bell pepper, rubbery), (*Z*)-ß-ocimene (rancid, sweaty), octanal (fruity), 2-isobutyl-3-methoxypyrazine (green, red bell pepper, lettuce), and three unidentified compounds (12a, 13a, and 38a); all are listed as italicized compounds in Table 3. From these compounds, the characteristic green bell pepper odour of 2-isobutyl-3-methoxypyrazine is well-known (Buttery et al., 1969) and the alkylmethoxypyrazines are found in many vegetables (Murray and Whitfield, 1975). However, a volatile bell pepper compound with a red/green bell pepper and rubbery odour has not yet been reported. The mass spectrum, *m*/*z* (relative intensity) 93 (100), 91 (40), 92 (29), 79 (28), 77 (28), 41 (22), 136 (19), 121 (18), 105 (12), and KI value (1152) imply that this compound might be 3-carene.

Interestingly, Keller et al. (1981) detected the same compound (δ-3-carene) as one of the major monoterpene hydrocarbons (13.4%) in the neutral solvent fraction of mild red chili (*C. annuum*). This fraction had an odour threshold of 11 ppb in water.

Maturation odour profile. Table 3 demonstrates that differences in sniffing patterns were observed between the ripening stages and between sample preparation methods. In general, a larger number of volatile odour compounds was detected in the green bell peppers than in the turning and red ones. In fact, only one additional compound, peak 45a (geranium, spicy), was observed in the turning and red ripening stages of both sliced and homogenised samples. Likewise, (*E*)-2-hexenol (49), which has an almond, fruity, and spicy odour, was detected as an additional compound in the turning and red homogenised bell peppers. Table 3 shows also that several odour compounds associated with "green" descriptors, such as mushroom, herbal [unidentified (36a)], grassy, lettuce, cucumber [(*Z*)-3-hexenol (45)], mushroom, herbal [nonanal (50)], lettuce, green bell pepper, cucumber [unidentified (51a)], carrot, lettuce, grassy [2-*sec*-butyl-3-methoxypyrazine (60)], and floral, green bell pepper [linalool (63)] were detected mainly in the green bell peppers and disappeared or decreased upon maturation. Some green related compounds, such as (*Z*)-3-hexenal (22) (grassy, green bell pepper, fruity), 1-hexanol (43) (fruity, green bell pepper, herbal), and two unidentified compounds, (44a) (geranium, spicy) and (63a) (herbal, cucumber), were observed only in homogenised samples of green bell peppers. These results are in agreement with the study of Chitwood et al. (1983), who suggested that (*Z*)-3-hexenol, 2-*sec*-butyl-3-methoxypyrazine, and 2-isobutyl-3-methoxypyrazine might be responsible for the frequent use of green descriptors in the aroma descriptive analysis of three investigated *C. annuum* cultivars (Anaheim, Jalapeño, and Fresno). They reported also that other compounds with rose, floral, and apple odours may be important contributors to *Capsicum* aroma. Keller et al. (1981) observed that volatiles of fresh red Jalapeño pepper extracts had a pleasant floral aroma besides a characteristic bell pepper odour. Table 3 shows the presence of the volatile compounds linalool (63), (*E*)-2-hexenal (31), and (*E*)-2-hexenol (49), which have floral, almond, fruity, and sweet aroma notes.

Table 3. Odour compounds of Mazurka bell peppers detected and described by the sniffing port panel.

No.	KI [a]	Component	Odour Descriptors	Green		Turning		Red	
				HG [b]	CT [b]	HG	CT	HG	CT
1a	906	Unidentified	Chemical/pungent, rubbery	+ [c]	—	—	—	—	—
3	962	*2,3-Butanedione* [d]	Caramel, sweet	++	+++	++	++	++	++
7	1009	*1-Penten-3-one*	Chemical/pungent, spicy	+++	++	+++	++	+++	++
10	1047	2,3-Pentanedione	Fruity, sweet, caramel	—	—	—	+	—	+
12	1076	*Hexanal*	Grassy, herbal, green bell pepper(bp)	+++	++	+++	++	+++	++
12a	1092	*Unidentified*	Chemical/pungent, rubbery, spicy	++	++	++	++	++	++
13a	1104	*Unidentified*	Geranium, spicy, rotten	++	+++	+++	+++	++	+++
22	1147	(*Z*)-3-hexenal	Grassy, green bp, fruity	+++	—	—	—	—	—
23	1152	*3-Carene*	Red bp, rubbery, green bp	++	++	+++	+++	+++	+++
27	1183	*o*-Xylene	Geranium, rubbery, spicy	+	—	—	+	+	+
31	1212	(*E*)-2-hexenal	Fruity, almond, spicy, sweet	++	—	+++	—	+++	—
34	1237	*(Z)-β-ocimene*	Rancid, sweaty	++	++	++	++	++	+
36a	1255	Unidentified	Mushroom, herbal	++	++	—	++	—	+
37	1283	*Octanal*	Fruity	+++	+++	++	++	++	++

(continue Table 3)

38a	1294	*Unidentified*	Mushroom	+++	++	+++	++	++	++
43	1332	1-Hexanol	Fruity, green bp, herbal	+	—	—	—	—	—
44a	1351	Unidentified	Geranium, spicy	++	—	—	—	—	—
45	1364	(*Z*)-3-hexenol	Grassy, lettuce, cucumber	++	++	—	—	—	—
45a	1364	Unidentified	Geranium,spicy	—	—	+++	++	+++	++
47	1377	Dimethyl trisulphide	Rotten, musty	—	+++	++	+++	++	+++
49	1383	(*E*)-2-hexenol	Almond, fruity, spicy	—	—	+	—	+	—
50	1387	Nonanal	Mushroom, herbal	+	+	—	—	—	—
51	1418	(*E*)-2-octenal	Almond, sweet, herbal	++	—	+	—	—	—
51a	1427	Unidentified	Lettuce, green bp, cucumber	+++	+++	++	++	—	—
53	1435	1,2,4,5-Tetramethylbenzene	Rancid, sweet	++	++	+	—	—	—
60	1500	2-*sec*-Butyl-3-methoxypyrazine	Carrot, lettuce, grassy	+++	++	++	++	—	—
62	1510	*2-Isobutyl-3-methoxypyrazine*	Green bp, red bp, lettuce	+++	+++	+++	+++	+++	+++
63	1527	Linalool	Floral, green bp	++	++	++	++	—	—
63a	1554	Unidentified	Herbal, cucumber	++	—	++	—	—	—
64	1576	(*E,Z*)-2,6-nonadienal	Cucumber	++	—	++	—	+	—

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[a] Calculated Kovats index of sniffing response on CP-Wax CB52 column. [b] HG, homogenised; CT, cut bell pepper. [c] Number of assessors detecting a smell simultaneously; — ,< 6 assessors, below noise level; +, 6 assessors, above noise level; ++, 6 < assessors < 9, clear signal; +++, > 9 assessors, clear signal.[d] Italicized compounds belong to basic odour profile investigated.

Sampling odour profile. Comparison of the sniffing patterns of the two preparation methods demonstrates that a larger number of odour compounds were detected in the homogenised samples. In fact, (*Z*)-3-hexenal (22), (*E*)-2-hexenal (31), (*E*)-2-hexenol (49), (*E*)-2-octenal (51), and (*E,Z*)-2,6-nonadienal (64) were detected only in the homogenised bell peppers. These compounds have distinct odour characteristics such as grassy, almond, fruity, and cucumber. Therefore, it is likely that tissue disruption will influence the fresh bell pepper flavour drastically. Buttery et al. (1969) reported (*E,Z*)-2,6-nonadienal as an important contributor to fresh bell pepper flavour because of its relatively low threshold (0.01 ppb) and its typical cucumber smell.

Conclusion. The results of this study indicate that differences in flavour of fresh green and red bell peppers are mainly caused by a decrease of volatile compounds during maturation, of which several have green related odours. Furthermore, tissue disruption increased the number of odour active compounds.

Acknowledgments

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Chapter 5

Characterisation and Occurrence of Lipoxygenase in Bell Peppers at Different Ripening Stages in Relation to the Formation of Volatile Flavour Compounds[1]

Abstract

Extracts of green bell peppers (*Capsicum annuum* cv. Mazurka) were analysed for the presence of lipoxygenase (LOX). A pH optimum of 5.5-6.0, was obtained and enzyme activity was 100% and 51% inhibited by 10 mM *n*-propyl gallate and 340 μM 5,8,11,14-eicosatetraynoic acid (ETYA), respectively. When bell peppers ripened from green to completely red, LOX activity decreased 70%. Also, the composition of volatile breakdown products from unsaturated fatty acids changed drastically upon maturation. Four typical patterns for these volatile products could be distinguished during ripening; some changed similarly to LOX activity. In both green and red bell pepper homogenates, the addition of linoleic acid increased considerably the levels of hexanal and hexanol, whereas the levels of (*Z*)-3-hexenal and (*E*)-2-hexenal were markedly enhanced by the addition of linolenic acid. When ETYA was added to these systems, the enhancing effects of both substrates were drastically reduced. A biochemical pathway was proposed for the formation of volatile bell pepper compounds upon tissue disruption.

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Introduction

Physical disruption of plant tissue results in the production of volatile compounds from unsaturated fatty acids, which are responsible for characteristic taste and aroma notes of the plant material (Grosch, 1982). The involvement of lipoxygenase (EC 1.13.11.12; LOX) in the formation of these volatile flavour compounds has been reported in many plant food products such as bananas (Tressl and Drawert, 1973), apple (Schreier and Lorenz, 1982), cucumber (Grosch and Schwarz, 1971; Galliard and Phillips, 1976; Galliard et al., 1976), tomato (Schreier and Lorenz, 1982; Galliard and Matthew, 1977; Galliard et al., 1977), raw beans (De Lumen et al., 1978), mushrooms (Tressl et al., 1981; Grosch, 1987), and tea (Gonzales et al., 1972; Hatanaka and Harada, 1973). The enzymatic formation of volatile compounds from unsaturated fatty acids depends on a number of variables. Shiiba et al. (1991) examined substrate specificity of wheat germ LOX isoenzymes and demonstrated higher activity for linoleic acid compared to linolenic acid. Grosch (1982) and Gardner (1989) suggested that several LOX (iso)enzymes are specific for the production of either C-9 and/or C-13 hydroperoxides. Furthermore, hydroperoxide lyases can specifically cleave C-9 and/or C-13 hydroperoxides to form aldehyde fragments (Galliard and Phillips, 1976; Galliard et al., 1976, 1977; Galliard and Matthew, 1977; Sekiya et al., 1983). Other enzymes such as isomerases (Phillips et al., 1979) and alcohol dehydrogenases (Grosch and Schwencke, 1969; Stone et al, 1975) can modify the primary formed volatile compounds. Consequently, a broad variety of volatile compounds can be created by the primary action of LOX.

LOX activity has been previously observed in both green and red fruits of *Capsicum annuum* varieties (Pinsky et al., 1971; Minguez-Mosquera et al., 1993) and also in seeds of red bell pepper (Daood and Biacs, 1986). Wu and Liou (1986) proposed the involvement of LOX in volatile flavour formation in bell pepper, because addition of stannous chloride considerably reduced the amount of C_6 aldehydes and alcohols. Recently, Luning et al. (1994*a,b*) suggested that the composition and odour characteristics of C_6 aldehydes and alcohols may be partly responsible for the distinct aroma differences observed between green and red bell peppers. Additionally, Hatanaka et al. (1993) showed that olfactory characteristics of purified saturated and unsaturated C_6 aldehydes and alcohols were distinctively different. Fruity, sweet, and fresh odour notes were dominant for the 2-hexenals and 2-hexenols, while (*Z*)-3-hexenal and (*Z*)-3-hexenol have a strong spicy and grassy green odour.

To investigate the role of LOX in the formation of volatile flavour compounds of bell peppers, the enzyme was extracted from green fruits and partially characterised. Changes in LOX activity and composition of volatile compounds were monitored during maturation of bell peppers. The effects of specific LOX substrates (linoleic and linolenic acid) and a specific LOX inhibitor (5,8,11,14-eicosatetraynoic acid) on the volatile composition of homogenates of green and red bell peppers were analysed.

Materials and methods

Bell pepper samples

Capsicum annuum cv. Mazurka was cultivated as previously described (Luning et al., 1994*a*). Fruits were selected from different plants in early morning at different stages of maturity, depending on the type of experiment. Samples were collected at 6 weeks (mature green) after fruit set for partial characterisation of bell pepper LOX. The fruits were cut into four pieces, seeded, and then immediately immersed in liquid nitrogen and stored at - 80°C prior to enzyme extraction. Furthermore, to study LOX activity during maturation, fruits were collected at four ripening stages, i.e., 4 (immature green), 6 (mature green), 8 (turning), and 10 (completely red) weeks after fruit set and stored as described above. Analysis of volatile compounds was carried out with samples harvested at seven maturation stages, i.e., 4, 5, 6, 7, 8, 9, and 10 weeks after fruit set, of which stages 6 (mature green) and 10 (completely red) were also used for substrate/inhibitor addition experiments.

Collected fruits were stored at 13°C (maximal 3 days) prior to analysis of volatile compounds by a dynamic headspace technique.

Preparation of bell pepper LOX extracts

Two different samples were prepared, i.e., a crude (CR extract) and an ammonium sulfate precipitation extract (AS extract). Frozen bell peppers were homogenised, and 100 g of subsamples was mixed with 200 mL of 0.05 M phosphate buffer (pH 7) in a Waring blender at 4°C. The homogenate was stirred at 4°C for 30 min and then centrifuged at 20000*g* for 20 min at 4°C. The supernatant was used as CR extract. The AS extract was obtained by precipitation of the CR extract with ammonium sulfate between 25 and 75% saturation. Then, the pellet was sedimented at 20000g for 20 min and suspended in 20 mL of 0.05 M potassium phosphate buffer (pH 7). The suspension was dialyzed overnight at 4°C against 0.05 M potassium phosphate buffer (pH 7) with one change of buffer. Finally, the dialyzed ammonium sulfate fraction was centrifuged at 15000*g* for 5 min to obtain the AS extract.

Preparation of substrate

A modification of the method of Surrey (1964) was used for preparation of substrate. A stock solution of 16 mM of linoleic acid (Sigma, St. Louis, MO) and 1.5% (v/v) Tween 20 (Sigma) in sodium tetraborate buffer (pH 9) was prepared; N_2 was flushed continuously during preparation to maintain oxygen-free conditions. Substrate was stored under nitrogen at 4°C in the dark for a maximum of 4 days.

Enzyme assay

LOX activity was measured with a biological oxygen monitor system using the standard oxygen probe and batch assembly (YSI Inc., Yellow Springs, OH). Assays were performed at 30°C in aerated 0.2 M potassium phosphate buffer (pH 6) giving a final volume of 3 mL. Substrate was incubated at 30°C while all LOX extracts were stored on ice (< 4°C) prior to assay. Oxygen consumption by the enzyme extract was monitored for 5 min, and the reaction was initiated by the addition of 0.5 mL of substrate, giving a final concentration of 2.67 mM linoleic acid. O_2 consumption was followed for 5-10 min and calculated as percent decrease of oxygen in air saturation per minute. Enzyme activities were converted into rate of O_2 consumption, expressed as nanokatals per milliliter of sample assayed, assuming only LOX activity. (Katal is defined as 1 mol of substrate consumed or product formed by the enzyme per second under defined conditions.)

Calculation of Katals

Net LOX activity = [(O_2 uptake upon addition of substrate) - (O_2 uptake by LOX extract) -(water) controls]. The reaction cell contains 2 mL of aerated buffer, 0.5 mL of LOX extract, and 0.5 mL of substrate. Assuming that substrate is totally O_2 free and that dissolved O_2 concentration relative to air-saturated water at 30°C = 236 nmol of $O_2.mL^{-1}$ (Chappell, 1964). Then, 100% saturation = 236 nmol $O_2.mL^{-1}$ x 2 mL of buffer (in reaction cell) = 472 nmol of O_2 in reaction cell and the oxygen consumption rate ($nkat.mL^{-1}$ sample) = {[(236 x volume buffer) x (net % decrease air saturation/min) x 2]/60}; 2 is factor used to calculate per milliliter of sample and 60 is the factor to calculate per second.

LOX pH profile

Oxygen uptake was monitored at pH 5, 5.5, 6, 6.5, and 7 using 0.2 M potassium phosphate buffer and at pH 4, 4.5, 5, and 5.5 using 0.2 M sodium acetate/acetic acid buffer. In each experiment 0.50 mL of water and 2.25 mL of buffer were added to 0.25 mL of AS extract. Controls were run by omitting enzyme and/or substrate. The experiment was repeated twice.

LOX inhibition experiments

Stock solutions of 1.7, 3.4, and 6.8 mM 5,8,11,14-eicosatetraynoic acid (ETYA; Sigma) were prepared in 100% ethanol and diluted 10-fold with 0.2 M potassium phosphate buffer (pH 8) to lower the inhibitory effects of ethanol. The AS extracts (1 mL) were preincubated for 5 and 10 min with 1 mL of ETYA solution, giving final concentrations of 85, 170, and 340 μM ETYA. Activity was assayed using 0.5 μL of preincubated AS extract, and the reaction was initiated by the addition of 0.5 μL of substrate. Controls were run for buffer, ethanol, and inhibitor.

A stock solution of 1.5 M *n*-propyl gallate (Sigma) was prepared in ethanol and diluted 10 and 100 times. Each solution was further diluted 1:5 with water to reduce the inhibitory effect of ethanol, giving final solutions of 3, 30, and 300 mM, respectively. Amounts of 0.25 mL of AS extract and 0.1 mL of inhibitor solution were added to 2.15 mL of buffer (pH 6), giving final concentrations of 0.1, 1, and 10 mM *n*-propyl gallate, respectively, in the reaction cell. Controls were run with 0.1 mL of buffer instead of inhibitor. AS extracts were preincubated with the inhibitor for 5 min in the reaction cell. The assya was started upon the addition of 0.5 ml of substrate. Controls were run for buffer, ethanol, and inhibitor. All experiments were repeated twice.

Analysis of LOX activity during bell pepper maturation

Fruits of four ripening stages were collected at 4, 6, 8, and 10 weeks after fruit set, and CR extracts were prepared as described above. The reaction cell contained 2.0 mL of 0.2 M aerated potassium phosphate buffer (pH 6), 0.5 mL of CR extract, and 0.5 mL of 16 mM linoleic acid (triplicates). Proteins were measured according to the method of Bradford (1976) using BSA as standard, to calculate specific enzyme activity. Controls were run with extraction buffer instead of CR extract. The entire experiment was repeated three times.

Isolation of volatile bell pepper compounds

Five bell peppers were collected at seven ripening stages and cut into small pieces. Portions of 10 g (four replicates) were weighed and homogenised with 50 mL of water for analysis of volatile compounds. Volatiles were trapped on Tenax TA (100 mg, 35/60 mesh) as described previously (Luning et al., 1994*a*) with some minor modifications. Samples were flushed with 30 mL.min^{-1} purified nitrogen for 1 h at room temperature. The time series were repeated twice.

The effects of substrates and inhibitor on the composition of volatile compounds were analysed in green (collected 6 weeks after fruit set) and red bell peppers (10 weeks). Control bell pepper samples were prepared by blending of, respectively, 20 g of sliced green and 20 g of sliced red fruits with 50 mL of 0.05 M potassium phosphate buffer (pH 6). Linoleic or linolenic acid [0.5 mL 0.0134 mM; 1% (v/v) Tween 20] with or without ETYA (1 mL of 17 mM) were added to 0.05 M potassium phosphate buffer (total volume 50 mL) prior to homogenization of the sliced bell peppers. After homogenization of the samples, they were immediately transferred to closed flasks (500 mL) and gently stirred at 30°C for 30 min. Subsequently, the flasks were connected to the dynamic headspace isolation system and volatiles were trapped on Tenax TA (35/60 mesh). Samples were flushed with 30 mL.min^{-1} purified nitrogen for 30 min at room temperature. Control samples on artifacts were made of only 50 of mL buffer and of buffer with, respectively, 0.5 mL of linoleic acid, 0.5 mL of linolenic acid, and 1 mL of ETYA (total volume 50 mL). The artifact control samples were similarly treated as the bell pepper samples and analysed on the GC column to control for the presence of volatile compounds. Addition experiments were repeated four times.

Gas chromatography analysis

Volatile compounds were analysed as described previously (Luning et al., 1994*a*). The gas chromatograph (Carlo Erba GC vega 6000, Interscience, Breda, The Netherlands) was equipped with a flame ionization detector (FID). The oven temperature was programmed from 40 to 150°C at 2 °C.min^{-1} and subsequently heated to 250°C at 10 °C.min^{-1}. GC-mass spectrometry of volatile compounds was carried out as described previously (Luning et al., 1994*a*).

Statistical analysis

Data of volatile compounds were subjected to analysis of variance (ANOVA, Genstat 5) to determine least significant differences (lsd) among ripening stages and to determine lsd among the volatile composition of bell peppers with and without addition of substrate and/or inhibitor.

Results and discussion

Lipoxygenase activity in bell peppers

In this study the occurrence of LOX activity in fresh bell peppers (*C. annuum* cv. Mazurka) and its role in the formation of volatile flavour compounds were investigated.

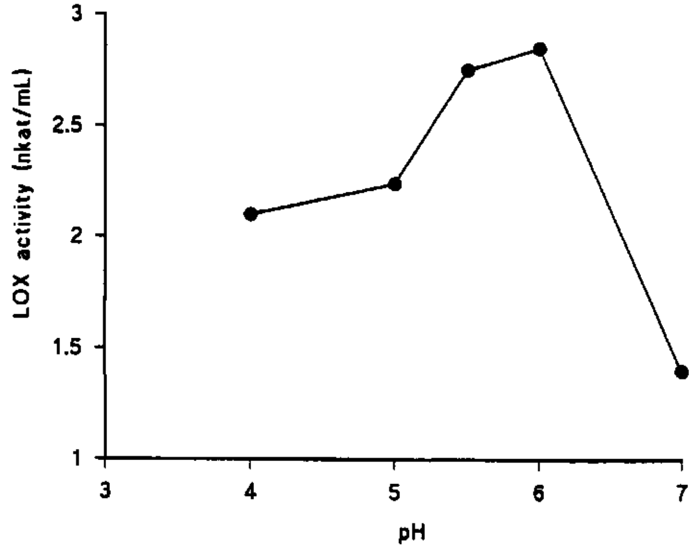

Figure 1. pH profile of bell pepper lipoxygenase (LOX) activity (cv. Mazurka) in ammonium sulfate precipitation extracts (AS extract) with linoleic acid as substrate.

AS extracts of green bell pepper fruits were used to study the pH profile and the effect of inhibitors. The AS extract showed activity with linoleic acid over a broad pH range, pH 4.0-7.0, and a maximum oxygen consumption at pH 5.5-6.0 (Figure 1). The observed activity optimum at pH 6.0 is typical for type-2 LOX and is comparable those of cucumber, pH 5.5 (Wardale and Lambert, 1980), pea, pH 6.0 (Yoon and Klein, 1979), and broad bean, pH 6.0 (Al-Obaidy and Siddiq, 1981). Minguez-Mosquera *et al.* (1993) reported a maximum activity at pH 6.5 for LOX isolated from bell pepper cv. Bola and cv. Agridulce, using the spectrophotometric assay. Furthermore, the activity of the AS extract was quite high at the lower pH end of the curve, approximately 75% of the maximal activity at pH 6.0, but it declined sharply at the higher pH end (Figure 1). Similar skewed pH profiles have been noticed for several other lipoxygenases (Al-Obaidy and Siddiqi, 1981; Ganthavorn and Powers, 1989; Boyes et al., 1992; Leonie et al., 1985).

Two known LOX inhibitors, i.e., *n*-propyl gallate and ETYA, were used to investigate their inhibitory effect on the AS extract of green bell peppers. The antioxidant, *n*-propyl gallate is a universal inhibitor of lipoxygenase activity (Schewe et al., 1986). Table 1 indicates that the percentage inhibition of enzyme activity was 54%, 82%, and 100% at concentrations of 100, 1000, and 10 000 µM *n*-propyl gallate, respectively. Al-Obaidy and Siddiqi (1981) reported 43% and 100% inhibition of broad bean LOX at 10 and 100 µM *n*-propyl gallate, respectively. Kristie and Thomson (1989) suggested that the effectiveness of *n*-propyl gallate as an inhibitor of tomato LOX in a crude system depended on the assay used. At a concentration of 1000 µM *n*-propyl gallate, the percentage inhibition was 100 % with the spectrophotometric method but only 60% with the polarographic assay. The acetylenic fatty acid ETYA is known to be a powerful inhibitor of lipoxygenases and is recommended as a selective criterium for true lipoxygenase activity (Schewe et al., 1986, 1987). Preliminary experiments with soybean LOX type-2 (Sigma) showed that 100 µM ETYA inhibited enzyme activity 85% after 5 min of incubation. However, ETYA was less effective in inhibiting LOX activity in the AS extract of green bell pepper (Table 1). There is no influence of ETYA on enzyme activity at a concentration of 85 µM; however, activity decreased 30% and 51% after preincubation for 30 min with 170 and 340 µM ETYA, respectively (Table 1). No total inhibition was achieved, which may be due to residual bell pepper LOX. It was not possible to carry out inhibition assays at ETYA concentrations above 340 µM, because of insolubility. In addition, other enzymatic oxidation reactions may be responsible for the residual oxygen uptake, since the AS extract is a crude protein preparation.

Table 1. Inhibitory Effects of *n*-propyl gallate and 5,8,11,14-eicosatetraynoic acid (ETYA) on lipoxygenase Activity in AS extract of Green Bell Pepper.

inhibitor	inhibitor concentration (µM)	preincubation time (min)	% Inhibition [a]
n-propyl gallate	100	5	54
	1000	5	82
	10000	5	100
ETYA	85	5	0
		30	0
	170	5	17
		30	30
	340	5	35
		30	51

[a] Average of two experiments; mean coefficient of variation of *n*-propyl gallate assay is 3.6% and of ETYA assay is 5.1%. [b] Maximal concentration of ethanol is 5% in the ETYA solutions.

CR extracts were prepared to monitor enzyme activity of cv. Mazurka at four different ripening stages. Figure 2 shows that LOX activity remained high at the immature (4 weeks after fruit set) and mature green (6 weeks) stages of development, and then it decreased significantly at the turning (8 weeks) and red (10 weeks) stages of maturation. Activities of the latter were approximately 45% and 30% of that of green bell peppers (4-6 weeks), respectively. Pinsky et al. (1971) observed that LOX activity in red bell pepper was approximately 50% of that of the green stage, and Minguez-Mosquera et al., (1993) noticed a decrease of more than 50% upon ripening from green to turning stage.

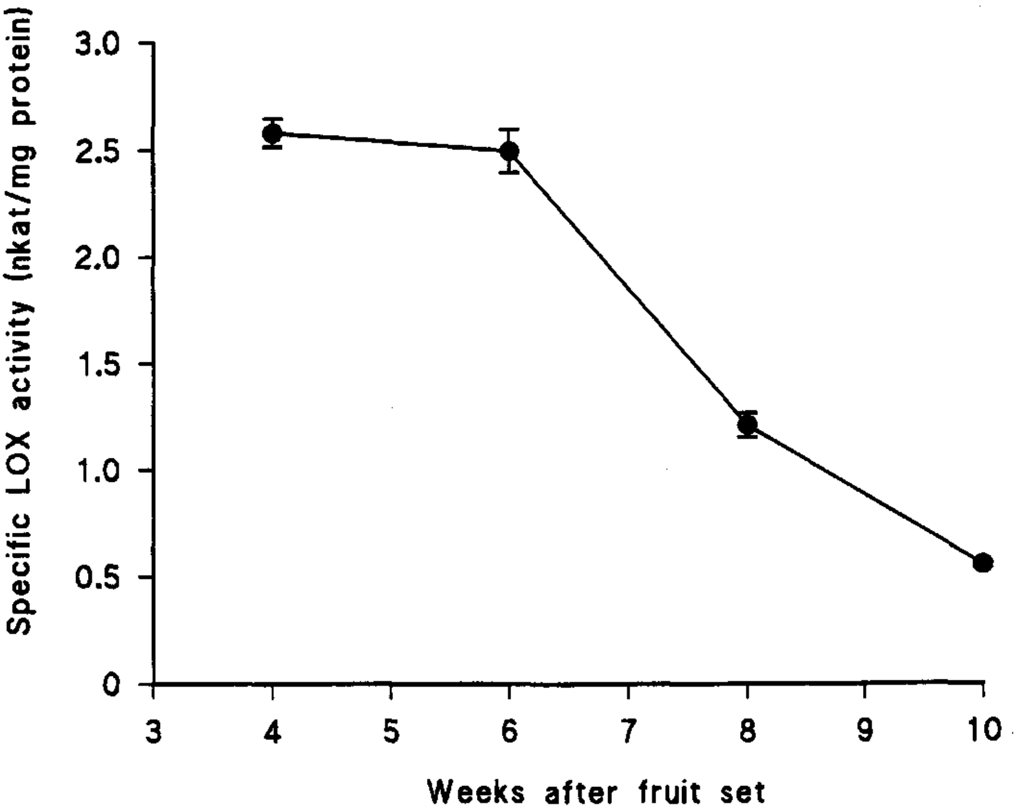

Figure 2. Specific lipoxygenase (LOX) activity in crude extracts (CR extract) at different stages of bell pepper maturation (cv. Mazurka). Bars represent standard deviation (*n* = 3).

Recently, Lomnitski et al. (1993) reported that soybean LOX type-2 activity towards linoleic acid was reduced 70% by 1 μM ß-carotene at pH 6.5. Luning et al. (1994*a*) showed that the content of carotenoids (expressed as ß-carotene) increased from 0.6 to 1.5 g.kg^{-1} dry matter when bell pepper colour turned from green to red. Probably, the increase in carotenoids influences bell pepper LOX activity as reviewed by Siedow (1991).

Table 2. Average GC Peak Areas [a] of Volatile Compounds obtained from Dynamic Headspace Gas Chromatography of Homogenates of Bell Peppers Collected at Different Maturation Stages.

		Harvest time [b]							
compound	KI [c]	4	5	6	7	8	9	10	Pattern [d]
hexanal	1074	194.2	123.9	81.8	72.9	62.6	35.4	12.9	A
1-hexanol	1330	10.8	8.4	4.2	4.6	5.7	4.2	2.1	A
(*Z*)-3-hexenal	1143	107.7	136.9	130.1	128.4	3.9	0.9	0.1	B
(*Z*)-3-hexenol	1361	17.0	24.9	19.1	17.4	2.2	0.5	0.3	B
(*Z*)-2-pentenal	1092	4.2	4.2	3.4	2.7	0.2	0.2	<0.1 [e]	B
(*Z*)-2-pentenol	1287	0.3	0.2	0.2	0.2	<0.1	<0.1	<0.1	B
(*E*)-2-pentenal	1116	0.1	0.1	0.1	0.1	<0.1	<0.1	<0.1	B
(*E*)-2-pentenol	1295	1.0	1.0	1.0	0.9	0.6	0.4	<0.1	B
1-penten-3-one	1008	5.9	7.4	7.0	6.8	2.3	1.8	1.0	B
(*E*)-2-hexenal	1210	53.6	44.0	34.8	105.9	301.2	279.9	170.9	C
(*Z*)-2-hexenal	1192	2.0	3.0	2.9	4.3	8.4	7.2	4.8	C
(*E*)-2-hexenol	1380	1.0	1.1	1.1	3.8	19.9	23.8	10.8	C
2-pentylfuran	1230	1.2	1.3	1.0	1.3	2.0	1.3	0.6	D
(*E*)-2-heptenal	1312	0.6	0.7	0.6	0.7	1.1	0.5	0.2	D
(*E*)-2-octenal	1416	0.5	0.5	0.4	0.5	0.6	0.3	0.2	D
(*E*)-2-nonenal	1525	0.4	0.4	0.3	0.2	0.5	0.2	0.1	D

[a] Average peak areas of two time series (Vs); mean coefficient of variation is 13%. [b] Weeks after fruit set. [c] Calculated Kovats indices on CP-wax CB52 column. [d] Codes correspond with patterns presented in Figure 3. [e] Mean peak area is below 0.1 (Vs), or compound is below detection limit.

Development of volatile compounds during bell pepper ripening

The enzymatic formation of volatile flavour compounds from unsaturated fatty acids upon tissue disruption has been demonstrated for numerous vegetables (Tressl, et al., 1981; Whitfield and Last, 1991). In this study, a selected number of volatile compounds, which may be derived from the breakdown of unsaturated fatty acids, were analysed in homogenates of bell peppers collected at seven maturation stages. Nine aldehydes, five alcohols, one ketone, and one furan were identified and quantified, of which hexanal, (*Z*)-3-hexenal, (*E*)-2-hexenal, (*Z*)-3-hexenol, (*E*)-2-hexenol, (*Z*)-2-hexenol, and hexanol were present in considerable amounts depending on the stage of maturation (Table 2). The composition of volatile compounds changed drastically during ripening and some particular patterns could be distinguished, as illustrated in Figure 3. Pattern A shows a significant ($P < 0.05$) decrease between 4 and 6 weeks (from immature to mature green); between 6 and 8 weeks no marked changes occur, but after 8 weeks the level dropped significantly ($P < 0.05$). This pattern is typical for the volatile compounds hexanal and hexanol (Table 2). Pattern B shows high levels in the green stages (4-6 weeks), which decreased significantly ($P < 0.05$) between 7 and 8 weeks, when bell pepper colour turned from green to red. This pattern represents the development of (*Z*)-3-hexenal, (*Z*)-3-hexenol, (*Z*)-2-pentenal, (*Z*)-2-pentenol, (*E*)-2-pentenal, (*E*)-2-pentenol, and 1-penten-3-one during bell pepper maturation (Table 2). In contrast, the third pattern (C) exhibits a significant ($P < 0.05$) increase between 7 and 8 weeks, followed by a significant ($P < 0.05$) decrease after 9 weeks, representing the changes of (*E*)-2-hexenal, (*E*)-2-hexenol, and (*Z*)-2-hexenal during ripening (Table 2). The remaining compounds, 2-pentylfuran, (*E*)-2-heptenal, (*E*)-2-octenal, and (*E*)-2-nonenal, showed a maximum level at 8 weeks (pattern D), which declined sharply thereafter. Comparing changes in LOX activity (Figure 2) with changes in volatile composition (Figure 3) during bell pepper maturation indicates that the volatile compounds belonging to pattern B showed a similar rapid decrease between 7 and 8 weeks as LOX activity, whereas volatile compounds belonging to pattern C showed a rapid increase.

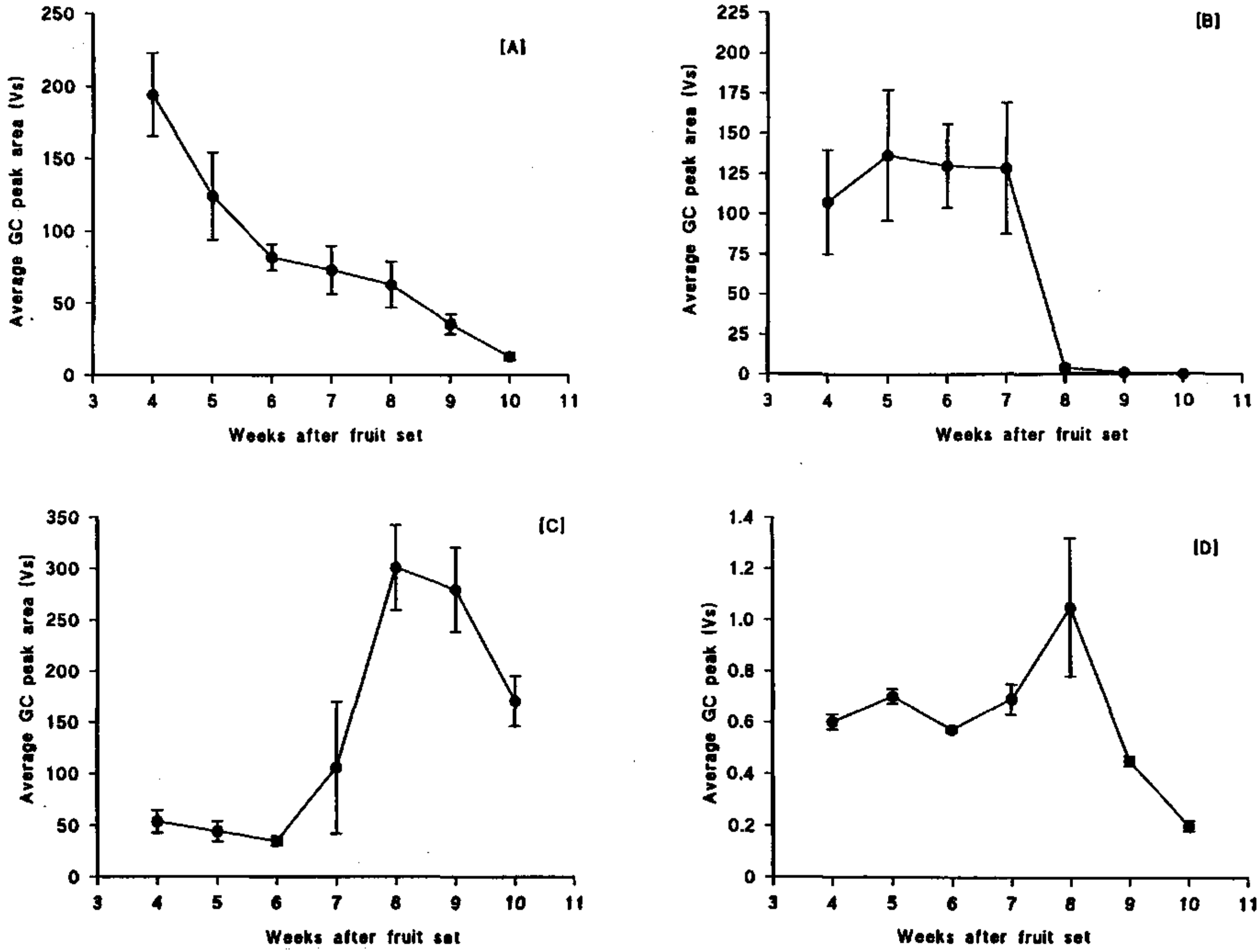

Figure 3. Four distinct ripening-related changes in average GC-peak areas, illustrated by the volatile compounds hexanal [A], (*Z*)-3-hexanal [B], (*E*)-2-hexenal [C], and (*E*)-2-heptenal [D], bars represent standard deviation (*n* =4); other compounds are classified in Table 2.

Influence of exogenous linoleic and linolenic acids on the composition of volatile compounds.

LOX substrates were added to bell pepper homogenates for determining the influence of LOX on the formation of volatile compounds. The effects of the addition of linoleic or linolenic acid on the composition of volatile compounds of mature green (collected 6 weeks after fruit set) and completely red (10 weeks) bell pepper homogenates are shown in Tables 3 and 4, respectively.

In green bell pepper homogenates, linoleic acid significantly ($P < 0.05$) increased the levels of hexanal, hexanol, 2-pentylfuran, (*E*)-2-heptenal, (*E*)-2-octenal, (*E*)-2-nonenal, and (*Z*)-3-hexenal (Table 3). The addition of linolenic acid to the same homogenates resulted in significantly ($P < 0.05$) higher levels of (*Z*)-3-hexenal, (*E*)-2-hexenal, (*Z*)-2-hexenal, (*Z*)-2-pentenal, (*E*)-2-pentenal, (*Z*)-2-pentenol, (*E*)-2-pentenol, and 1-penten-3-one. However, the alcohols (*Z*)-3-hexenol and (*E*)-2-hexenol did not change significantly (Table 3). Although the inherent volatile composition of green and red peppers is markedly different (Table 2), the effects of linoleic and linolenic acid on red pepper volatiles (Table 4) were quite similar to the ones reported for green fruits (Table 3). In red bell pepper homogenates, linoleic acid caused significant ($P < 0.05$) increases in hexanal and hexanol but was less effective in raising the levels of 2-pentylfuran, (*E*)-2-heptenal, (*E*)-2-octenal, and (*E*)-2-nonenal, as compared to in the green fruits (Tables 3 and 4). The addition of linolenic acid resulted in a larger increase of (*E*)-2-hexenal, (*Z*)-2-hexenal, and (*E*)-2-hexenol than recorded for green bell pepper. Headspace analysis of artifact control samples for buffer, ETYA, and linoleic and linolenic acids did not show detectable amounts of the volatile compounds, which increased or decreased upon addition of the inhibitor and substrates to green or red bell pepper homogenates (Tables 3 and 4).

The increase of hexanal and hexanol upon addition of linoleic acid and the increase of (*Z*)-3-hexenal and (*E*)-2-hexenal upon addition of linolenic acid have been reported for LOX in other vegetables such as tomato (Stone et al., 1975; Galliard and Matthew, 1977) and cucumber (Grosch and Schwarz, 1971; Galliard et

Table 3. Influence of Linoleic and Linolenic acids, with and without ETYA on the Average GC Peak Areas [a] of Volatile Compounds Obtained from Dynamic Headspace Gas Chromatography of Homogenates of Green Bell Peppers.

compounds	control [b]	linoleic acid added	linolenic acid added	linoleic acid + ETYA added	linolenic acid + ETYA added
hexanal	82.3 ± 14.4	270.1 ± 19.2	99.9 ± 12.0	158.0 ± 24.2	84.9 ± 12.0
hexanol	3.9 ± 0.2	8.3 ± 1.2	2.5 ± 0.4	3.8 ± 1.0	1.7 ± 0.1
2-pentylfuran	0.3 ± 0.1	2.5 ± 0.3	0.2 ± 0.0	3.2 ± 0.3	0.3 ± 0.1
(*E*)-2-heptenal	0.5 ± 0.2	1.3 ± 0.4	0.8 ± 0.1	2.2 ± 0.3	1.7 ± 0.2
(*E*)-2-octenal	0.3 ± 0.1	2.3 ± 0.5	0.5 ± 0.1	4.7 ± 0.2	2.8 ± 0.3
(*E*)-2-nonenal	0.2 ± 0.1	4.0 ± 0.8	0.8 ± 0.1	3.5 ± 0.9	0.6 ± 0.2
(*Z*)-3-hexenal	78.7 ± 13.6	122.4 ± 20.7	179.8 ± 26.6	69.7 ± 6.9	68.2 ± 25.6
(*E*)-2-hexenal	67.2 ± 12.5	75.7 ± 11.6	122.3 ± 2.8	49.7 ± 13.0	73.5 ± 11.1
(*Z*)-2-hexenal	2.1 ± 0.6	2.3 ± 0.2	3.2 ± 0.1	1.8 ± 0.3	1.8 ± 0.4
(*Z*)-2-pentenal	1.1 ± 0.3	1.8 ± 0.5	2.9 ± 0.5	1.3 ± 0.4	1.2 ± 0.0
(*E*)-2-pentenal	0.9 ± 0.2	1.3 ± 0.3	1.6 ± 0.3	0.6 ± 0.1	0.8 ± 0.3
(*Z*)-2-pentenol	0.2 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.1 ± 0.0	0.2 ± 0.0
(*E*)-2-pentenol	0.6 ± 0.2	0.7 ± 0.2	1.8 ± 0.5	0.1 ± 0.0	0.4 ± 0.1
1-penten-3-one	2.8 ± 0.8	4.0 ± 0.7	7.2 ± 1.9	4.0 ± 0.4	6.9 ± 0.2
(*Z*)-3-hexenol	12.2 ± 1.0	10.3 ± 1.5	13.6 ± 2.7	9.2 ± 1.5	10.1 ± 1.4
(*E*)-2-hexenol	0.8 ± 0.3	0.6 ± 0.2	0.8 ± 0.1	0.5 ± 0.1	0.8 ± 0.1

[a] Average peak areas (Vs) of four replicates ± standard deviation. [b] Control of 20 g of sliced green fruits homogenised with 50 mL of 0.05 M potassium phosphapte buffer, (pH 6).

al. 1976, 1977; Tressl et al., 1981). The increase of (*Z*)-3-hexenal and (*E*)-2-hexenal upon addition of linoleic acid has been demonstrated also for fresh green beans by Tressl et al. (1981). However, the significant increase ($P < 0.05$) of (*Z*)-3-hexenal in green bell pepper (Table 3) and of (*E*)-2-hexenal in red (Table 4) bell pepper homogenates upon addition of linoleic acid does not confirm the breakdown pathways of this unsaturated acid as proposed by Galliard et al. (1976, 1977), Galliard and Matthew (1977), and Stone et al. (1975). Possibly, some linoleic acid is enzymatically converted to linolenic acid during headspace isolation.

The increase of 2-pentylfuran and (*E*)-2-nonenal upon addition of linoleic acid has been reported previously for cucumber homogenates by Tressl et al. (1981), who suggested that labile (*Z*)-3-nonenal was enzymatically converted into 2-pentylfuran and (*E*)-2-nonenal. Gardner et al. (1991) showed that in soybeans seeds (*E*)-2-nonenal derived from the 9-hydroperoxide of linoleic acid, and proposed that it was converted from (*Z*)-3-nonenal. In our experiments, no (*Z*)-3-nonenal was detected in any of the bell pepper homogenates and possibly this compound was converted into (*E*)-2-nonenal and 2-pentylfuran. Additionally, pea and soybean lipoxygenases generated amongst others, 2-pentylfuran on pure linoleic acid (Sessa, 1979).

The effect of linolenic acid on the level of (*E*)-2-pentenal has been demonstrated by Grosch and Laskawy (1975), who suggested that (*E*)-2-pentenal was generated from 13-hydroperoxides of linolenic acid by soybean LOX. Mattick and Hand (1969) implied that 1-penten-3-one derived from 13-hydroperoxides of linolenic acid in soybeans.

Influence of ETYA combined with linoleic or linolenic acid on the composition of volatile compounds

The levels of hexanal, hexanol, and (*Z*)-3-hexanal decreased when ETYA was added to homogenates containing linoleic acid, whereas levels of (*Z*)-3-hexenal, (*E*)-2-hexenal, (*Z*)-2-hexenal, (*E*)-2-hexenol, (*Z*)-2-pentenal, (*E*)-2-pentenal, (*Z*)-2-pentenol, and (*E*)-2-pentenol decreased when ETYA was added to green and/or

Table 4. Influence of Linoleic and Linolenic acids, with and without ETYA on the Average GC Peak Areas [a] of Volatile Compounds Obtained from Dynamic Headspace Gas Chromatography of Homogenates of Red Bell Peppers.

compounds	control	linoleic acid added	linolenic acid added	linoleic acid + ETYA added	linolenic acid + ETYA added
hexanal	3.9 ± 1.2	21.6 ± 4.0	6.2 ± 1.2	3.8 ± 0.7	3.2 ± 0.5
hexanol	0.9 ± 0.4	3.0 ± 0.5	1.1 ± 0.3	1.9 ± 0.1	0.7 ± 0.1
2-pentylfuran	0.1 ± 0.0	0.5 ± 0.1	0.2 ± 0.0	1.0 ± 0.1	0.1 ± 0.1
(*E*)-2-heptenal	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.0
(*E*)-2-octenal	0.1 ± 0.0	0.3 ± 0.1	0.2 ± 0.0	0.3 ± 0.1	0.1 ± 0.0
(*E*)-2-nonenal	<0.1 [b]	0.3 ± 0.1	<0.1	0.3 ± 0.1	<0.1
(*Z*)-3-hexenal	0.8 ± 0.2	2.0 ± 1.2	2.9 ± 0.9	0.2 ± 0.1	1.6 ± 0.2
(*E*)-2-hexenal	22.8 ± 5.9	58.5 ± 15.6	157.5 ± 35.8	17.4 ± 6.7	69.5 ± 17.0
(*Z*)-2-hexenal	0.8 ± 0.2	1.0 ± 0.2	2.8 ± 1.2	0.6 ± 0.2	1.0 ± 0.0
(*Z*)-2-pentenal	<0.1	<0.1	<0.1 <0.1	<0.1	
(*E*)-2-pentenal	<0.1	<0.1	<0.1 <0.1	<0.1	
(*Z*)-2-pentenol	<0.1	<0.1	<0.1 <0.1	<0.1	
(*E*)-2-pentenol	<0.1	<0.1	<0.1 <0.1	<0.1	
1-penten-3-one	0.5 ± 0.2	0.6 ± 0.2	1.3 ± 0.3	0.5 ± 0.1	1.4 ± 0.2
(*Z*)-3-hexenol	0.1 ± 0.0	0.1 ± 0.0	0.4 ± 0.1	0.1 ± 0.1	0.3 ± 0.1
(*E*)-2-hexenol	2.4 ± 0.6	2.5 ± 0.5	7.6 ± 3.1	2.3 ± 0.4	4.2 ± 0.5

[a] Average peak areas (Vs) of four replicates ± standard deviation. [b] Mean peak area is below 0.1 (Vs), or compound is not detected.

red bell pepper homogenates containing linolenic acid (Tables 3 and 4). However, some volatiles such as 1-penten-3-one, 2-pentylfuran, (*E*)-2-heptenal, (*E*)-2-octenal, and (*E*)-2-nonenal were unaffected or even significantly ($P < 0.05$) increased by the addition of ETYA (Tables 3 and 4). The effect of LOX inhibitors on the formation of volatile compounds has been reported by Wu and Liou (1986). They suggested that stannous chloride reduced the enzymatically formed C_6 aldehydes and alcohols in disrupted bell peppers. In fresh green beans, De Lumen et al. (1978) showed that 20 mM cyanide decreased (*E*)-2-hexenal and hexanal to one-sixth of the control, while practically no volatiles were formed at 100 mM.

Formation of volatile bell pepper compounds from unsaturated fatty acids

A biochemical pathway has been proposed for the formation of volatile bell pepper compounds from unsaturated fatty acids, based on our results and available literature (Figure 4). Grosch (1982) showed that bell pepper LOX specifically produced 9-hydroperoxides with linoleic acid as substrate. However, the majority of volatile compounds detected in bell peppers were probably formed from 13-hydroperoxides of linoleic and linolenic acid (Tables 3 and 4; Fig. 4). Galliard et al. (1977) and Galliard and Matthew (1977) demonstrated that tomato LOX produces mainly 9-hydroperoxides, whereas C_6 aldehydes were only formed from the 13-hydroperoxides. They suggested that a hydroperoxide lyase (HPOL) in tomato specifically cleaved the 13-hydroperoxides. Likewise, bell peppers seem to contain an HPOL cleaving mainly the 13-hydroperoxides to volatile compounds (Fig. 4).

Figure 4 shows that only the minor compounds 2-pentylfuran and (*E*)-2-nonenal can be derived from 9-hydroperoxides of linoleic acid as reported earlier (Grosch and Laskawy, 1975; Tressl et al., 1981; Gardner et al., 1991). The major compound, hexanal, is formed from the 13-hydroperoxides of linoleic acid and is converted by an alcohol dehydrogenase to hexanol as demonstrated for tomatoes (Galliard and Matthew, 1977; Galliard et al., 1977). The minor compounds (*E*)-2-octenal and (*E*)-2-heptenal appeared to be formed from, respectively, the 10- and 12-hydroperoxides of linoleic acid (Tressl et al., 1981).

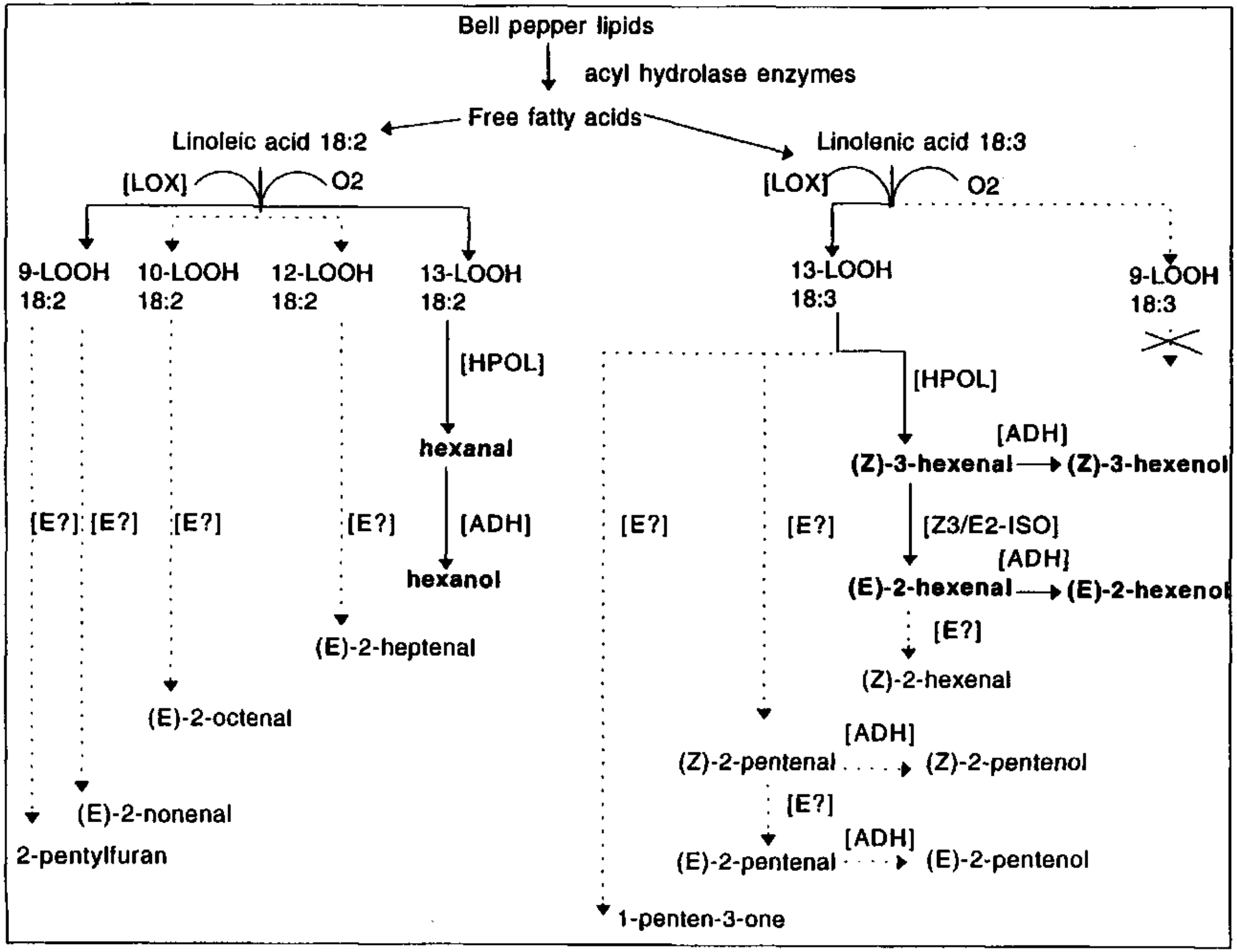

Figure 4. Biochemical pathways proposed for the formation of volatile compounds from linoleic and linolenic acids in fresh bell peppers. Abbreviations: LOOH: hydroperoxide; [LOX]: bell pepper lipoxygenase; [HPOL]: hydroperoxide lyase; [ADH]: alcohol dehydrogenase; [Z3/E2-ISO]; *cis*-3/*trans*-2-isomerase; [E?]: possible enzymatic transformation.

Chan et al. (1979) proposed a mechanism for the rearrangement of linoleic acid hydroperoxides leading to interisomerization, which affect the distribution of hydroperoxide isomers obtained and therefore the type of volatile compounds formed. Figure 4 shows that the volatile compounds which increased upon the addition of linolenic acid are all derived from the 13-hydroperoxides. The formation of (*Z*)-3-hexenal from 13-hydroperoxides of linolenic acid and the conversion to (*E*)-2-hexenal by a *cis*-3/*trans*-2 isomerase have been demonstrated for cucumber fruits (Galliard et al., 1976; Phillips and Galliard, 1978; Phillips et al., 1979); (*Z*)-2-hexenal is possibly formed from its *trans*-form chemically or by another isomerase enzyme. The conversion of 1-penten-3-one from 13-hydroperoxides of linolenic acid has been proposed by Mattick and Hand (1969). Additionally, (*Z*)-2-pentenal could be formed according to the same pathway prior to 1-penten-3-one formation. Other enzymes such as a *cis*/*trans* isomerase probably convert (*Z*)-2-pentenal to (*E*)-2-pentenal. All aldehydes seemed to be converted to their corresponding alcohols by an alcohol dehydrogenase, as reported for several aldehydes in tomatoes (Stone et al., 1975). Obviously, the aldehydes derived from the 9-, 10-, and 12-hydroperoxides of linoleic acid such as (*E*)-2-heptenal, (*E*)-2-octenal, and (*E*)-2-nonenal were not converted to their corresponding alcohols.

In conclusion, changes in the composition of volatile compounds during ripening of bell peppers and different quantitative effects of LOX substrates on the composition of green and red bell peppers indicated that several changes in the pathways of breakdown of fatty acids might occur. Our results demonstrated that LOX activity decreased rapidly when colour turned from green to red. Similarly, major compounds (*Z*)-3-hexenal and (*Z*)-3-hexenol decreased, whereas (*E*)-2-hexenal and (*E*)-2-hexenol rapidly increased up to 8 weeks after fruit set. Moreover, the addition of linolenic acid increased (*Z*)-3-hexenal most in the green fruits, whereas in the red fruits the increase of (*E*)-2-hexenal was highest, suggesting an increased *cis*-3/*trans*-2 isomerase activity during ripening. In the completely red fruits (10 weeks), levels of all volatile compounds decreased or disappeared, which may be due to the decreased level of hydroperoxides formed by the lower bell pepper LOX activity.

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Chapter 6

Effect of Hot-air Drying on Flavour Compounds of Fresh Bell Peppers (*Capsicum annuum*)[1]

Abstract

The influence of hot-air drying on the composition of both volatile and non-volatile flavour compounds of different Dutch bell pepper cultivars was investigated. Contents of glucose, fructose, ascorbic, citric, and oxalic acid decreased significantly after drying, while the levels of sucrose, malic, fumaric, and *cis*-aconitic acid increased. Glucose, fructose and ascorbic acid probably participate in Maillard reactions during heating. Different effects of drying could be distinguished on the composition of volatile compounds. The majority of these compounds evaporated during drying. Some compounds were partly retained in the dried bell peppers, whereas others disappeared completely. Gas chromatography–sniffing port analysis revealed that compounds with mainly "fresh" odour notes like lettuce/grassy/green bell pepper ((*Z*)-3-hexenal), fruity/almond ((*E*)-2-hexenal), fruity (octanal), lettuce/green bell pepper ((*Z*)-3-hexenol), and grassy/green bell pepper ((*Z*)-2-hexenal) decreased or disappeared after drying. Another group of compounds increased or were formed during drying, like 4-octen-3-one, (*E*)-2-heptenal, (*E*)-2-octenal, (*E,Z*) and (*E,E*)-2,4-heptadienal, decanal, and (*E*)-2-nonenal; most are known as autoxidation products of unsaturated fatty acids. The increased levels of 2-methylpropionic and 2- and 3-methylbutyric acid, 2-methylpropanal, and 2- and 3-methylbutanal seemed to be due to Strecker degradation. Gas chromatography sniffing port analysis showed that the latter three compounds had distinct cacao, sweaty, and spicy odour characteristics.

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Introduction

Capsicum annuum and *C. frutescens* varieties are extensively used for their colour, pungency, distinct taste, and aroma. Bell peppers (*C. annuum*) are consumed as immature (green) or mature (e.g., red, yellow, white, orange) fruits, as fresh or as cooked vegetables, or as spices in foods due to their evident differences in flavour (Whitfield and Last, 1991; Govindarajan et al., 1987). The primary processing of *Capsicum* is drying to conserve the perishable fruits, to reduce storage volume, and to decrease transport costs; the major drying methods are traditional sun drying and hot-air drying (Govindarajan, 1985). Selection of *Capsicum* cultivars for commercial drying is traditionally based on the desired combination of intensity of colour, degree of pungency, and economic yield (Govindarajan, 1985; Govindarajan et al., 1987). However, the increasing use of dried vegetables in convenience foods, dry salad dressing mixes, dehydrated soups, and flavoured rice makes higher demands on the flavour quality of the dried product (Feinberg, 1973; Govindarajan et al., 1987). Properties of dried vegetables are influenced by chemical and physical changes. The first affects sensory properties like colour, taste, and aroma, whereas the latter influences handling properties such as swelling capacity and cooking time.

Drying of *Capsicum* fruits causes changes in pungency (Lease and Lease, 1962; Govindarajan, 1985) and colour pigments, i.e, chlorophyll (Luhadiya and Kulkarni, 1978) and carotenoids (Ramakrishnan and Francis, 1973; Minguez-Mosquera et al., 1994), but it probably also affects the composition of flavour compounds. The contributions of volatile and non-volatile components to the flavour of fresh *C. annuum* cultivars have been reported previously. Sugars such as glucose and fructose seem to contribute to the sweet taste, and ascorbic and citric acid appear to be relevant for the sourness perception of red bell peppers (Luning et al., 1994b). Several volatile compounds such as, 2-methoxy-3-isobutyl-pyrazine, (*E,Z*)-2,6-nonadienal, deca-2,4-dienals, limonene, methylsalicylate, 2,3-butanedione, 1-penten-3-one, hexanal, 3-carene, and octanal have been considered to be important for the aroma of fresh green and/or red bell peppers of different cultivars

(Buttery et al., 1969; Chitwood et al., 1983; Keller, 1981; Luning et al., 1994a). Also characteristic for the aroma of fresh bell peppers are the C_6-aldehydes and alcohols such as, hexanal, (*Z*)-3-hexenal, (*Z*)-3-hexenol, (*E*)-2-hexenal, and (*E*)-2-hexenol (Wu and Liou, 1986; Luning et al., 1994 a,c). Recently, Van Ruth and Roozen (1994) reported differences in the composition of volatile odour compounds of commercially dried red Hungarian, Turkish, and Chilean bell peppers. However, to our knowledge, not much is known about the influence of hot-air drying on the composition of flavour compounds of fresh bell peppers with different aromas.

In this study the effects of hot air-drying on the compositions of flavour compounds of several Dutch bell pepper cultivars with different aromas indicated by different colours, were investigated. Sugars and organic acids were determined by high performance liquid chromatography. Volatile compounds were analysed by headspace gas chromatography (GC), identified by combined gas chromatography-mass spectrometry (GC–MS), and their odour characteristics were evaluated by a sniffing port panel (GC–sniffing).

Materials and methods

Plant material

Commercial green and red bell peppers of cv. Mazurka and white and yellow bell peppers of respectively cv. Blondy and cv. Kelvin were obtained from two different green houses (Zaltbommel and Berkel-Rodenrijs, The Netherlands). The unripe green fruits were harvested approximately 6 weeks after fruit set, while the ripe red, yellow, and white fruits were collected about 10 weeks after fruit set. Bell peppers were picked from different plants in early morning and individual weights of the whole bell peppers were measured (Table 1). In total, 12-18 fruits were prepared for analysis of sugars and organic acids of fresh bell peppers by halving, deseeding and storage in closed boxes at - 24°C (maximal 6 months). For analysis of volatile compounds of fresh bell peppers, about 25 to 30 fruits were stored at 13°C (maximal 3 days).

Dried bell pepper samples were prepared by drying approximately 6-7 kg bell peppers of each colour in a pilot-scale hot-air tray dryer (Bronswerk Heat Transfer B.V., Nijkerk, The Netherlands). Fruits were washed, deseeded, and cut into pieces of 1 cm^2 (cell wall thickness ± 0.5 cm) and subsequently dried at 65°C until weight decreased less than 0.1 g min^{-1}. Initial and final moisture contents are shown in Table 1. Dried samples were stored in the dark, under vacuum at 4°C until analysis of volatile compounds (maximal 8 weeks storage) and analysis of non-volatile compounds (maximal 6 months).

Standards of volatile compounds were obtained from Jansen Chimica (Tilburg, The Netherlands).

Table 1. Fresh weight of collected bell peppers, dry matter and moisture contents before and after hot-air drying at 65°C.

Analysis[a]	Green	White	Red	Yellow
Fresh weight (g)	216	137	216	191
Dry matter fresh (%)	6.4	5.9	8.4	8.5
Dry matter dried (%)	92.7	92.5	90.5	91.5
Moisture fresh (kg $H_2O.kg^{-1}$ dm)	14.8	16.0	10.9	10.8
Moisture dried (kg H_2O/kg^{-1} dm)	0.1	0.1	0.1	0.1

[a] Analyses are means of five replicates, except for fresh weight, i.e., average of 25-30 bell peppers; percentages of coefficient of variation are 11, 4, 1, 4, and 6 % for respectively fresh weight, dry matter (dm) of fresh and dried, and moisture of fresh and dried bell peppers.

Analysis of sugars and organic acids

The pieces of 2-3 frozen fresh fruits of each colour were homogenised and portions of 5 g were extracted and analysed for sugars and organic acids as reported previously (Luning et al., 1994b). Dry matter contents of fresh and dried bell peppers were determined prior to analysis of sugars and organic acids to calculate the data on a dry matter basis (Luning et al., 1994b). Dried bell peppers were rehydrated and homogenised and portions of 1 g were similarly extracted and analysed for sugars and organic acids as described for the frozen fresh samples. Extractions of fresh and dried samples were carried out six times.

Isolation of volatile compounds

Five to 6 fresh bell peppers of each colour were sliced into small pieces and portions of 45 g were homogenised with 50 ml water in a Waring blender. Portions of the dried bell pepper samples of each colour corresponded with the dry matter content of 45 g of the fresh samples (Table 1). Dried samples were rehydrated with water at 80°C for 15 min in closed flasks, cooled on ice to room temperature, and subsequently homogenised with 50 ml water in a Waring blender. The amount of rehydration water corresponded to the amount of water evaporated from 45 g fresh sample. Volatile compounds were isolated from homogenised fresh and rehydrated samples respectively, by flushing with 30 ml min^{-1} purified nitrogen for 2 h at room temperature in a dynamic headspace system as described by Luning et al., (1994a). The extractions of volatile compounds of fresh and dried bell peppers were repeated five times.

Gas chromatography (GC) and sniffing port analysis

Volatile compounds were desorbed from the Tenax tubes as reported previously (Luning et al., 1994a) with some minor modifications. A capillary Supelcowax 10 column (60 m x 0.25 mm i.d., 0.25μm film thickness, Supelco inc., Bellafonte PA) was used and the oven temperature was programmed from 40 to 150°C at 2°C min^{-1} and subsequently heated to 250°C at 10°C min^{-1}. The gas chromatograph (Carlo Erba GC vega 6000, Interscience, Breda, The Netherlands) was equipped with a flame ionization detector (FID) and two sniffing ports (SGE, Milton Keynes, Great Britain). FID response standard curves were constructed to calculate the amounts of selected bell pepper compounds such as hexanal, (*E*)-2-hexenal, (*Z*)-3-hexenol, (*E*)-2-hexenol, octanal, linalool, and 2-isobutyl-3-methoxypyrazine.

Volatile compounds were sniffed at the GC-effluent by a sniffing port panel as described by Linssen et al., (1993). An experienced GC–sniffing port panel of 10 assessors was trained to detect and to describe volatile compounds of both fresh and dried bell peppers. In two preliminary training sessions headspaces of 25 ml bottles, containing vegetable products, such as crushed lettuce, carrots, cucumber, green, yellow, and red bell peppers, and chemicals, such as (*E*)-2-pentenal, myrcene, dimethyldisulfide, 3-methylbutanal, 3-buten-2-one, 2,3-pentanedione, hexanal, (*E*)-2-hexenal, 2-octanone, octanal, (*Z*)-3-hexenol, (*E*)-2-hexenol, 1-octen-3-ol, linalool, and 2-isobutyl-3-methoxypyrazine were smelled and described to train the odour recognition of the assessors. The latter 12 volatile compounds were also sniffed twice at the GC–effluent, and characterised by the assessors using an existing list of odour descriptors (Luning et al., 1994a). During four final sessions, volatile compounds of fresh and dried green and red bell peppers were evaluated by GC–sniffing and a list of 19 frequently used odour descriptors was compiled including almond, floral/soapy, mushroom/fungi, cacao, geranium-like, fruity, grassy/cut grass, chemical/pungent, cucumber, caramel, rotten/cooked vegetable, green bell pepper, red bell pepper, rubbery/plastic, lettuce, sweaty, spicy/herbal, rancid/oily, carrot, and sweet. Determination of the background noise of the sniffing port panel, data registration, and data processing were carried out as described previously (Linssen et al., 1993; Luning et al., 1994a).

Odour compounds were identified by comparing calculated KI of sniffing responses with these indices of GC–MS identified compounds. Furthermore, odour descriptors used by the panel were compared with descriptors of authentic compounds as reported in literature (Dravnieks, 1985; Ullrich and Grosch, 1987, 1988; Holscher and Steinhart, 1994).

GC-mass spectrometry (GC–MS)

Identification of the volatile compounds was carried out by GC–MS as reported in our previous study (Luning et al., 1994a). Thermal desorption and GC column conditions were identical to those for gas chromatography. Electron impact mass spectral (EI–MS) analysis was carried out at an ionization energy of 70 eV. MS fragmentation patterns of each component were compared with those of the authentic compound using the MIST/EPA/NIH database (National Institute of Standards Technology, Wilmington, USA). Kovats indices (KI) were calculated and compared with KI values of identical compounds as reported in the literature (cf. Table 3).

Statistical analysis

GC–peak areas of identified compounds and quantified HPLC data of sugars and organic acids were both subjected to analysis of variance (ANOVA, Genstat–5) to calculate least significant differences (LSD) among the different coloured bell peppers and between the fresh and dried samples of each colour.

Results and discussion

Influence of hot-air drying on the composition of sugars and organic acids.

The influence of hot-air drying on the major non-volatile flavour compounds of sweet bell peppers was studied. Table 2 shows that hot-air drying affects the amounts of both sugars and organic acids of the unripe fruits of cv. Mazurka (green) and the ripe fruits of cv. Mazurka (red), cv. Blondy (white), and cv. Kelvin (yellow). The sucrose contents of all dried samples increased significantly ($P <$ 0.05) compared to the fresh fruits; the increase was highest in the dried unripe green bell peppers.

Table 2. Composition of sugars (g.100 g^{-1} dry matter) and organic acids (mg. 100 g^{-1} dry matter) in fresh and hot-air dried bell peppers.

	Fresh				Dried			
Analyses [a]	Green	White	Red	Yellow	Green	White	Red	Yellow
Sucrose	3.8	2.1	1.7	2.1	7.9	3.4	2.5	2.8
Glucose	24.0	27.0	29.5	32.6	18.3	20.9	22.0	24.7
Fructose	20.2	23.9	32.0	35.5	16.8	21.1	28.1	29.7
Ascorbic acid	1987	1751	2131	2148	1481	1350	1390	1511
Oxalic	61.8	69.0	9.2	8.7	41.8	59.7	n.d.[b]	n.d.
Cis-aconitic acid	n.d.	n.d.	n.d.	n.d.	17.4	10.1	15.3	13.3
Citric acid	1384	998	3456	3107	1093	593	2452	2082
Malic acid	1134	1505	276	278	1384	2191	1041	1050
Fumaric acid	6.2	8.8	n.d.	n.d.	21.9	57.5	2.8	3.0
Shikimic acid	72.2	46.1	49.7	15.9	57.9	39.7	37.2	11.4
Pyroglutamic acid	34.5	42.4	22.8	22.3	34.4	48.1	28.7	25.5

[a] Mean values of six replicates; mean percentage coefficient of variation for sugars is 6%, and for organic acids is 11 and 6% in respectively fresh and dried bell peppers. [b] n.d.: not detected

Nielsen et al., (1991) and Hubbard and Pharr (1992) reported that the sucrose content during ripening of C. *annuum* varieties is controlled by invertase and sucrose phosphate synthase activities. The sucrose content increased to a maximum level when colour changed from green to turning and than dropped when bell peppers ripened to red (Hubbard and Pharr, 1992; Luning et al., 1994a). Possibly, during the early stages of hot air-drying, changes in enzyme activities resulted in increased levels of sucrose. The glucose and fructose contents decreased significantly ($P < 0.05$) after drying, which can not be explained by sucrose formation only. Both reducing sugars may react with amino compounds in early stages of Maillard reaction forming Amadori compounds (Feather, 1994).

Hot-air drying also affected the levels of organic acids. Table 2 shows that, in all fruits, ascorbic, citric, and oxalic acid decreased, whereas malic and fumaric acid increased significantly ($P < 0.05$); shikimic acid was only significantly ($P < 0.05$) lower in green and red fruits of cv. Mazurka. Moreover, the decrease of citric and the increase of malic acid were highest in the dried ripe red and yellow bell peppers as compared to the green and white fruits. Apparently, the quantitative decrease of citric acid is in the same order as the increase of malic acid (Table 2). Possibly, citric acid is converted to malic acid during the drying process. Moreover, *cis*-aconitic acid was only detected in the dried fruits; this acid can be formed by dehydration of citric acid (Streyer, 1981). The decrease of ascorbic acid after drying may be due to Maillard reaction. Feather (1994) reported that this acid serves as an active participant, degradation products of ascorbic acid in the presence of oxygen may provide carbonyl-containing compounds, which are capable to react with amines giving Maillard reaction products. The decrease of glucose, fructose, ascorbic, and citric acid, which play an important role in the sweet and sourness perception of fresh bell peppers (Luning et al., 1994b), possibly influences the taste quality of the dried fruits.

Influence of hot-air drying on the composition of volatile compounds

The quantitative and qualitative composition of bell pepper volatile compounds changed drastically upon hot-air drying. Figure 1 represents the gas chromatograms (GC–FID) of fresh and dried green fruits of cv. Mazurka. Basically, the latter contained much lower levels of volatile compounds, but some appeared to be increased or formed during drying. Table 3 lists the volatile compounds identified in the headspace concentrates of both fresh and dried fruits of green and red cv. Mazurka, white cv. Blondy, and yellow cv. Kelvin. In total 76 compounds were identified, of which the majority of compounds have been detected previously in fresh and/or processed fruits of other cultivars of C. *annuum* and/or C. *frutescens.*

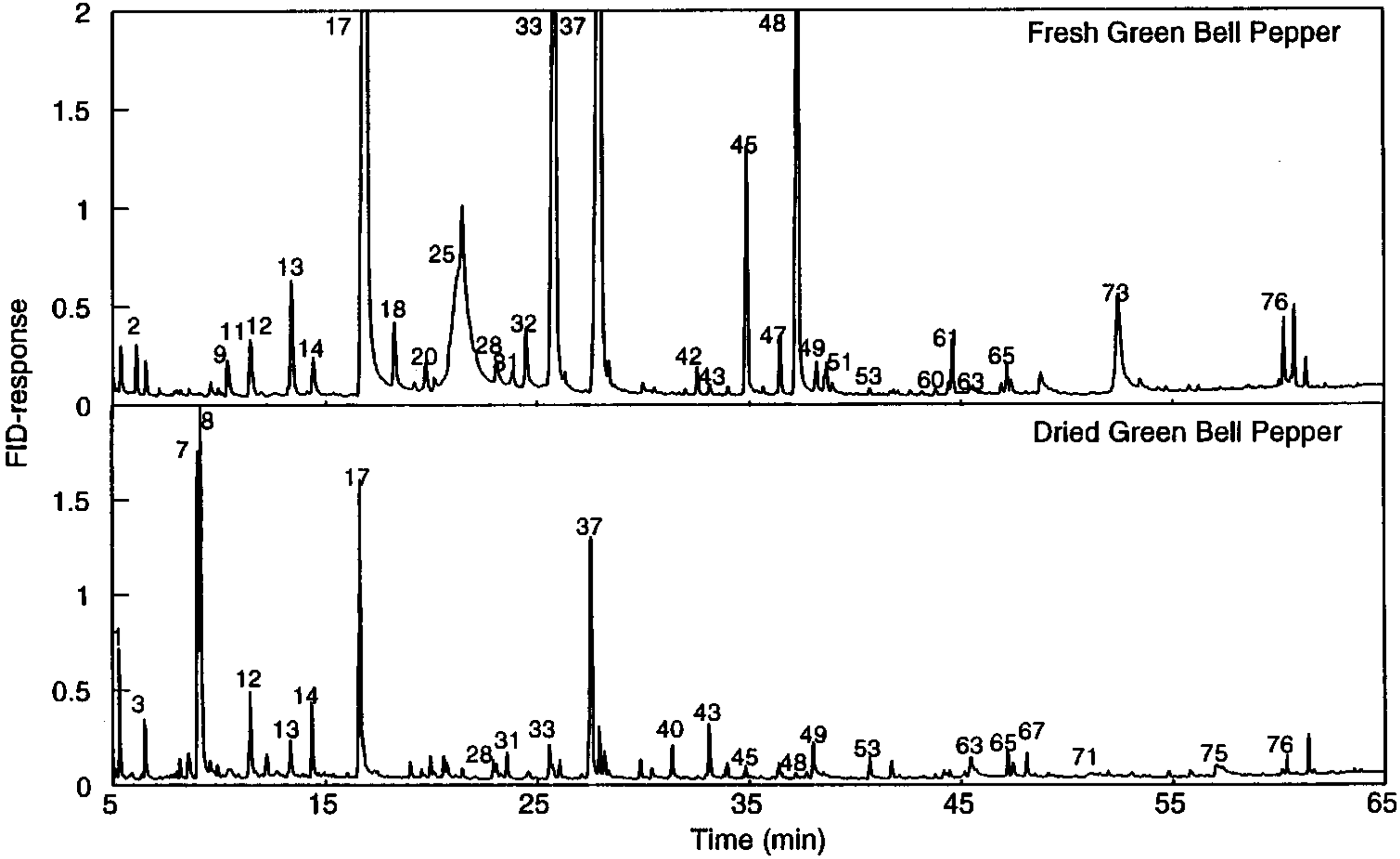

Figure 1. Gas chromatograms of fresh (top) and dried (bottom) green fruits of cv. Mazurka. Peak numbers correspond with compounds in Tables 3 and 4.

Only thiobismethane (1), 4-octen-3-one (40), (*E,Z*)-2,4-hexadienal (52), (*E*)-2-nonenal (67), (*Z*)-2-nonenal (70), 5-ethyl-2(5H)-furanone (73), and 1-phenylethanone (74) were not reported before. Quantitative amounts of some bell pepper volatile compounds were calculated by using FID calibration curves of authentic compounds, and these compounds were present in the range of 0.01-5.8 ng (Table 3).

In order to study the effect of hot-air drying on the composition of volatile compounds, the average GC–peak area units (Volt.sec) were processed by ANOVA to analyze differences between fresh and dried samples and differences among the fruits. The effects of hot-air drying on volatile bell pepper compounds could be distinguished in three groups, i.e., (D) compound decreased significantly ($P < 0.05$) or disappeared, (I) compound increased significantly ($P < 0.05$) or was formed, and (N) no clear trend was observed for all bell pepper samples.

Group (D) represents the majority of volatile compounds, which are apparently evaporated during drying (Table 3). Most of these compounds were still detectable in the dried product, although, the individual volatile compounds were not retained at the same level for the different bell peppers (Table 3). Several theories have been postulated on the retention of volatile compounds during drying processes, such as selective diffusion of water versus volatile compounds (Thijssen and Rulkens, 1968) and formation of microregions in which volatiles are trapped (Flink and Karel, 1970). The major factors considered were drying temperature, solid concentrations, nature and concentration of volatile compounds (Bartholomai et al., 1975; Mazza and LeMaguer, 1979; Rosenberg et al., 1990). High solid concentrations generally increased flavour retention, whereas high initial concentrations of the volatile compounds adversely affected the retention. Likewise, in our study these compounds were apparently less well retained in the bell pepper samples having the higher concentrations of volatile compounds. For instance, the decrease of the relatively high levels of (*E*)-ß-ocimene (37), hexanol (45), (*Z*)-3-hexenol (48), and 6-methylheptyl-2-propenoate (61) in green cv. Mazurka, is bigger than the decrease of the relatively lower levels of corresponding compounds in yellow bell pepper (Table 3).

Table 3. Composition of volatile compounds isolated by dynamic headspace analysis from fresh and hot-air dried bell peppers (GC-peak areas[a]).

No	Compound	KI [b]	KI [c]	Ref [d]	Drying effect [e]	Fresh				Dried			
						Green	White	Red	Yellow	Green	White	Red	Yellow
1	Thiobismethane	743	-	c	I	n.d.	n.d.	n.d.	n.d.	2.34	0.22	2.16	2.06
2	Propanal	791	788[1]	a,b	D	1.25	n.d.	0.38	n.d.	n.d.	n.d.	n.d.	n.d.
3	2-Methylpropanal	817	801[2]	b	I	n.d.	n.d.	n.d.	n.d.	2.20	2.46	1.30	0.99
4	2-Methylfuran	870	876[1]	b	I	n.d.	n.d.	n.d.	n.d.	0.11	0.10	0.17	0.12
5	Butanal	874	864[1]	a,b	N	0.16	0.09	0.08	0.07	0.11	0.14	0.14	0.08
6	Ethylacetate	890	882[1]	a,b	I	0.20	0.37	0.40	0.29	0.57	0.44	1.06	1.05
7	2-Methylbutanal	917	896[2]	a,b	I	0.05	0.11	0.09	0.04	11.45	18.30	11.77	8.11
8	3-Methylbutanal	921	924[3]	a,b	I	0.08	0.07	0.07	0.05	13.80	21.49	24.56	15.96
9	2-Ethylfuran	953	960[2]	a,b	D	1.28	0.38	0.43	n.d.	n.d.	n.d.	n.d.	n.d.
10	2,3-Butanedione	975	971[2]	a,b	N	0.09	0.36	0.08	0.19	0.51	0.28	0.43	0.29
11	3-Pentanone	978	971[2]	a	D	1.16	n.d.	0.43	n.d.	n.d.	n.d.	n.d.	n.d.
12	Pentanal	983	987[3]	a,b	I,N	1.88	1.67	1.53	1.19	3.47	6.03	1.51	1.21
13	1-Penten-3-one	1024	1025[4]	a,b	D,I	4.60	1.09	1.01	0.60	1.26	0.52	4.11	1.11
14	Toluene	1046	1042[2]	a,b	N	2.50	4.94	5.13	4.12	2.73	2.17	2.59	2.22
15	2,3-Pentanedione	1068	1062[2]	a,b	I	n.d.	n.d.	n.d.	n.d.	0.08	0.06	0.07	0.06
16	Dimethyldisulfide	1084	1078[2]	a,b	D	0.04	0.07	0.22	0.07	0.02	0.03	0.11	0.03
17	Hexanal	1096	1090[3]	a,b	D,N	88.87	19.10	14.24	8.45	14.34	16.05	9.79	10.05
						5.79[f]	**1.24**	**0.93**	**0.55**	**0.93**	**1.05**	**0.64**	**0.65**
18	(*Z*)-2-Pentenal	1119	1115[3]	a	D	2.96	0.24	0.06	n.d.	n.d.	n.d.	n.d.	n.d.
19	Ethylbenzene	1134	1130[4]	a,b	N	0.96	1.54	1.64	1.45	0.23	0.38	0.46	1.34
20	(*E*)-2-Pentenal	1139	1136[3]	a	D	1.50	0.09	n.d.	0.06	n.d.	n.d.	n.d.	n.d.
21	*p*-Xylene	1141	1137[4]	a,b	N	0.41	0.66	0.71	0.51	0.25	0.20	0.90	0.52
22	*m*-Xylene	1149	1144[4]	a	D	1.08	1.68	1.50	1.25	0.71	0.37	1.06	1.16
23	3-Carene	1155	1152[5]	a,b	D	0.31	0.53	0.44	0.40	0.23	0.24	0.39	0.21
24	1-Butanol	1157	1161[9]	a,b	D	1.93	1.01	1.24	1.95	0.65	0.40	0.68	0.61
25	(*Z*)-3-Hexenal	1160	1152[3]	a,b	D	38.84	0.96	0.25	n.d.	n.d.	n.d.	n.d.	n.d.

26	1-Penten-3-ol	1167	1175^{3}	a,b	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
27	ß-Myrcene	1168	1167^{5}	a,b	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
28	2-Heptanone	1191	1195^{4}	a,b	D	1.08	1.69	2.03	2.66	0.49	0.76	0.50	0.95
29	*o*-Xylene	1192	1189^{4}	a	N	0.43	1.27	0.90	0.96	0.49	0.55	0.83	0.65
30	Heptanal	1194	1194^{3}	a,b	D	0.65	0.52	0.64	0.49	0.52	0.17	0.25	0.28
31	Limonene	1205	1204^{5}	a,b	D	0.96	1.50	2.19	1.34	0.88	0.90	0.83	0.74
32	(*Z*)-2-Hexenal	1212	1212^{3}	a	D	3.03	0.32	0.42	0.23	0.12	0.09	0.13	0.13
33	(*E*)-2-Hexenal	1230	1227^{3}	a,b	D	39.09	16.43	44.39	1.25	1.27	0.30	1.52	0.47
						2.06	**0.87**	**2.34**	**0.07**	**0.07**	**0.02**	**0.08**	**0.03**
34	2-Pentylfuran	1239	1232^{5}	a,b	D	0.99	0.57	0.92	0.50	0.49	0.49	0.47	0.33
35	(*Z*)-ß-Ocimene	1242	1237^{5}	a	D	0.55	0.50	0.39	0.16	0.42	0.19	0.21	0.04
36	1-Pentanol	1257	1258^{4}	a,b	-	n.s.	n.s.	0.53	n.s.	1.77	1.02	0.33	0.27
37	(*E*)-ß-Ocimene	1258	1254^{5}	a,b	D	57.52	88.65	8.96	20.96	7.99	3.26	0.31	1.86
38	3-Octanone	1261	1259^{4}	a	D	2.41	1.50	0.14	0.25	1.59	1.06	0.09	0.09
39	Octanal	1296	1296^{3}	a,b	D	0.61	0.98	1.07	0.88	0.43	0.36	0.40	0.35
						0.05	**0.08**	**0.08**	**0.07**	**0.03**	**0.03**	**0.03**	**0.03**
40	4-Octen-3-one	1309	-	c	I	0.08	0.15	0.15	0.18	1.38	0.76	0.74	0.74
41	(*Z*)-2-Pentenol	1316	1317^{6}	a	D	0.24	0.06	0.07	0.05	n.d.	n.d.	n.d.	n.d.
42	(*E*)-2-Pentenol	1324	1319^{4}	a	D	0.97	0.06	0.09	0.04	n.d.	n.d.	n.d.	n.d.
43	(*E*)-2-Heptenal	1332	1334^{3}	a,b	I	0.48	0.38	0.36	0.33	2.11	1.66	0.68	0.42
44	6-Methyl-5-hepten-2-one	1344	1349^{3}	a,b	N	0.45	1.07	1.04	0.63	0.28	0.33	0.66	0.59
45	Hexanol	1362	1369^{3}	a,b	D	7.90	3.25	1.98	0.63	0.40	0.23	0.19	0.06
46	(*E*)-3-Hexenol	1367	-	a	D	0.24	0.24	0.15	n.d.	n.d.	n.d.	n.d.	n.d.
47	(*E*,*Z*)-3,4-Dimethyl-2,4,6-octatriene	1378	1369^{5}	a	D	2.41	4.13	0.70	1.42	0.48	0.20	0.07	0.18
48	(*Z*)-3-Hexenol	1395	1399^{3}	a,b	D	22.22	6.09	0.09	0.07	0.20	0.07	n.d.	n.d.
						0.93	**0.26**			**0.01**			
49	Nonanal	1405	1398^{4}	a,b	D	3.34	4.58	2.77	2.62	1.70	1.17	2.08	1.41
50	(*E*,*Z*)-2,4-Hexadienal	1407	-	c	D	0.62	1.37	0.37	0.37	n.d.	n.d.	n.d.	n.d.
51	(E)-2-Hexenol	1410	1404^{6}	a,b	D	0.90	1.07	8.74	0.40	0.16	0.15	0.19	0.22
						0.04	**0.05**	**0.38**	**0.02**	**0.01**	**0.01**	**0.01**	**0.01**
52	(*E*,*E*)-2,4-Hexadienal	1412	1414^{3}	a	D	4.47	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
53	(*E*)-2-Octenal	1437	1441^{3}	a,b	I	0.30	0.35	0.32	0.26	0.94	0.79	0.71	0.54
54	1-Octene-3-ol	1453	1451^{7}	a,b	N	0.54	0.49	0.42	0.19	0.64	0.38	0.23	0.22
55	Heptanol	1457	1456^{7}	c	D	0.31	0.58	0.54	0.46	0.11	0.12	0.13	0.08

56	2-Pentylthiophene	1465	1456[5]	a	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
57	Acetic acid	1467	1464[3]	a,b	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
58	(*E*,*Z*)-2,4-Heptadienal	1472	1478[3]	b	I	n.d.	n.d.	n.d.	n.d.	0.12	0.05	0.68	0.23
59	2-Furancarboxyaldehyde	1473	1471[3]	a	D	0.17	0.33	0.28	0.19	0.05	0.03	0.10	0.08
60	2-Ethyl-1-hexanol	1490	1491[7]	a	D	1.39	1.54	1.73	1.73	0.19	0.17	0.18	0.19
61	6-Methylheptyl-2-propenoate	1493	1478[5]	a	D	3.60	1.07	2.01	1.67	0.29	0.23	0.25	0.33
62	(*E*,*E*)-2,4-Heptadienal	1503	1500[3]	b	I	n.d.	n.d.	n.d.	n.d.	0.23	0.20	0.23	0.14
63	Decanal	1506	1492[5]	a,b	I	0.65	0.97	0.55	0.35	1.20	1.28	1.05	0.79
64	3-*Sec*-butyl-3-methoxypyrazine	1520	1500[5]	a	-	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
65	2-Isobutyl-3-methoxypyrazine	1531	1510[5]	a,b	N	1.15	0.88	0.66	0.55	0.82	0.84	0.42	0.45
						0.08	**0.06**	**0.04**	**0.04**	**0.06**	**0.06**	**0.03**	**0.03**
66	Benzaldehyde	1534	1536[3]	a,b	D	1.85	2.89	2.33	2.34	0.42	0.47	0.50	0.34
67	(*E*)-2-Nonenal	1545	1542[3]	c	I,D	0.16	2.46	0.20	0.16	0.99	1.65	0.32	0.61
68	Linalool	1550	1549[7]	a,b	D	0.05	0.46	0.09	0.07	n.d.	0.07	n.d.	n.d.
							0.03						
69	1-Octanol	1560	1562[4]	a	D	0.40	0.43	0.66	0.43	0.17	0.11	0.11	0.10
70	(Z)-2-Nonenal	1565	-	c	D	0.24	0.34	0.35	0.30	0.12	0.06	0.10	0.11
71	2-Methylpropionic acid	1578	1579[4]	a	I	n.d.	n.d.	n.d.	n.d.	0.64	1.15	0.45	0.52
72	(*E*,*Z*)-2,6-Nonadienal	1595	1596[4]	a,b	N	0.09	0.20	0.14	0.16	0.17	0.23	0.10	0.13
73	5-Ethyl-2(5H)-furanone	1609	-	c	D	10.25	2.30	0.60	0.40	n.d.	n.d.	n.d.	n.d.
74	1-Phenyl-ethanone	1667	-	c	D	0.42	0.36	0.68	0.55	0.20	0.17	0.13	0.15
75	2/3-Methylbutyric acid	1670	1665[7]	a	I	n.d.	n.d.	n.d.	n.d.	1.10	2.44	2.24	1.37
76	1-Decanol	1743	1730[1]	a,b	D	2.90	2.43	2.64	2.87	0.71	0.61	0.72	0.48
Total Peak area (Vs)						320.5	189.4	122.6	73.3	86.1	93.6	82.0	63.4

[a] Average peak areas of five replicates (Vs); the mean coefficient of variation is 15%; [b] Calculated Kovats indices on Supelcowax 10 column; [c] Literature KI's were obtained from [1] Kawakami and Kobayashi 1991; [2] Shimoda and Shibamoto 1990; [3] Young et al., 1992; [4] Tanchotikul and Hsieh 1989; [5] Luning et al., 1994a; [6] Binder et al., 1990; [7] Umano et al., 1992; [d] Volatile compounds were previously reported in (a) fresh bell peppers: Buttery et al., 1969; Luning et al., 1994a; Wu and Liou 1986; Keller et al., 1981; Maarse and Visscher 1989, or (b) in processed bell peppers: Wu et al., 1986; Wilkins 1994; Van Ruth and Roozen 1994, or (c) compounds were not reported before in bell peppers. [e] Drying effect for all bell pepper samples: (D) volatile compound decreased significantly ($P < 0.05$) or disappeared, (I) compound increased significantly ($P < 0.05$) or was formed, or (N) no significant changes for all bell peppers were observed; n.d. Not detected or peak area below 0.01 (Vs); n.s. Peaks were insufficiently separated from neighbouring compound; [f] Bold print is amount (ng) calculated from FID standard curves.

Several compounds of group D, such as (*Z*)-2-pentenal (18), (*E*)-2-pentenal (20), (*Z*)-3-hexenal (25), (*Z*)-2-pentenol (41), (*E*)-2-pentenol (42), (*E,E*)-2,4-hexadienal (50), (*E,Z*)-2,4-hexadienal (52), and 5-ethyl-2(5H)-furanone (73) disappeared completely after drying (Table 3). Several of these compounds, i.e., (18), (20), (25), (41), and (42) could be formed from hydroperoxides of unsaturated fatty acids by the action of bell pepper lipoxygenase and other enzymes upon tissue disruption of green and red bell peppers (Luning et al., 1994a). Possibly, hot-air drying at 65°C affects the activities of these enzymes and consequently compounds could not be formed in the dried bell peppers. Minguez-Mosquera et al. (1993) demonstrated that lipoxygenase activity decreased when red bell peppers were dried at 30°C for 12 days in a recycled-air dryer.

Group (I) of Table 3 contained the increased levels of 2-methylpropanal (3), 2- and 3-methylbutanal (7,8), which may be due to Strecker degradation of the α-amino acids valine, leucine and isoleucine respectively (Vernin and Parkanyi, 1982). The corresponding short chain fatty acids, 2-methylpropionic (71) and 2- and 3-methylbutyric acid (75) are probably formed upon further oxidation during drying. The volatile compounds 2-methylfuran (4) and 2,3-pentanedione (15) were only detected in the dried product. These compounds have been previously reported in model Maillard reactions with sugars and α-amino acids (Baltes, 1987; Apriyantono and Ames, 1993). No other volatiles related to the Maillard reaction, such as pyrazines and pyrroles, were detected in the dried bell peppers, which may be due to the relatively low processing temperature (65°C). Some of the compounds mentioned in Table 3 originated probably from autoxidation of unsaturated fatty acids, i.e., 4-octen-3-one (40), (*E*)-2-heptenal (43), (*E*)-2-octenal (53), (*E,Z*) and (*E,E*)-2,4-heptadienal (58, 62), and decanal (63) (Badings, 1970). In addition, the amounts of volatile compounds, such as pentanal (12), 1-penten-3-one (13), hexanal (17), and (*E*)-2-nonenal (67) may be the net effect of different processes, such as evaporation and/or inactivation of enzymes and autoxidation of unsaturated fatty acids. Also, 2,3-butanedione may be evaporated as well as formed in the Maillard reaction (Baltes, 1987; Apriyantono and Ames, 1993).

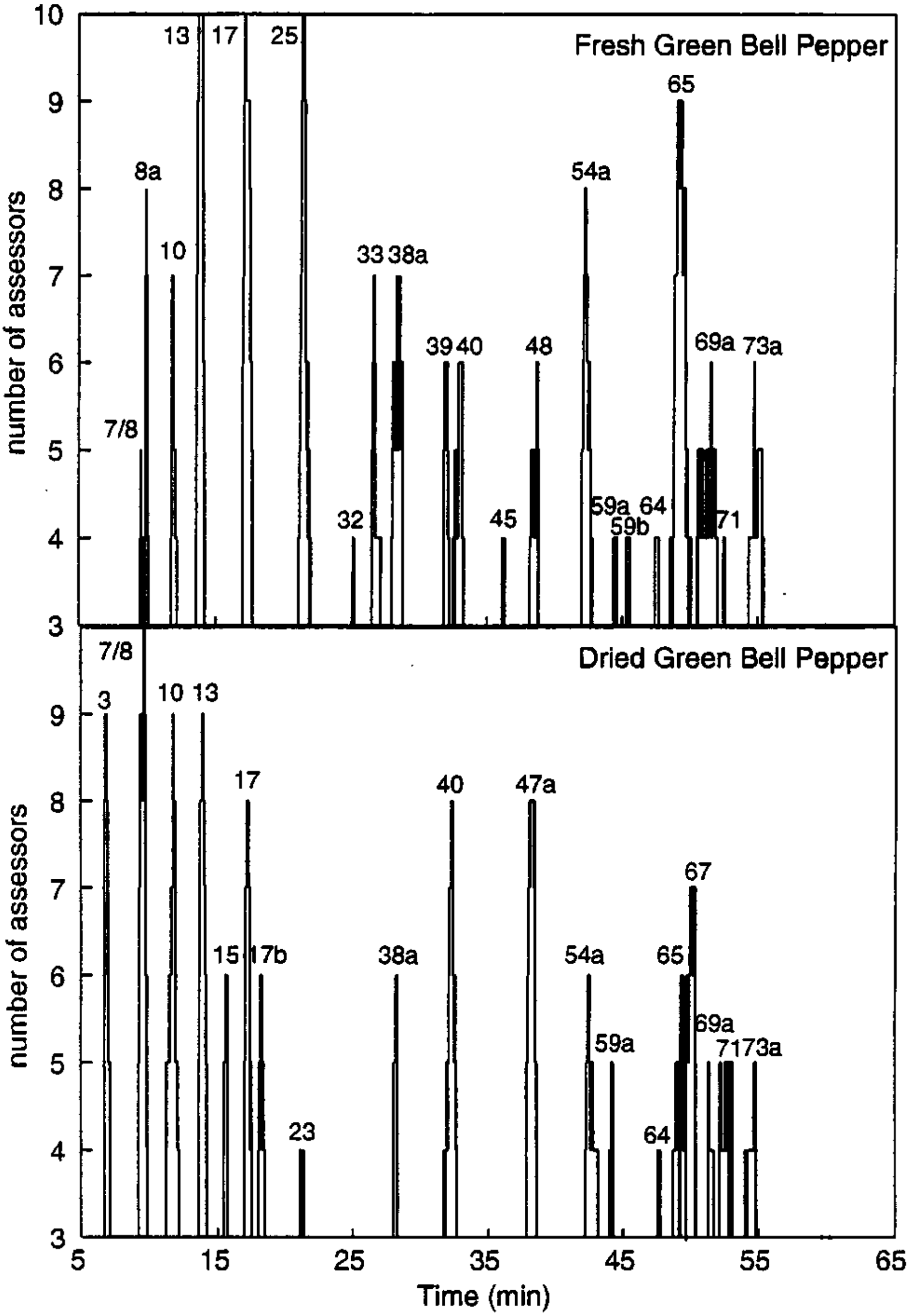

Figure 2. GC–sniffing patterns of fresh (top) and dried (bottom) green fruits of cv. Mazurka. Peak numbers correspond with compounds in Tables 3 and 4.

Influence of hot-air drying on the GC–sniffing profiles

The samples used for GC–FID chromatograms were also analysed by GC–sniffing. Figure 2 shows that changes occurring in the GC–sniffing patterns are less drastic than in the FID patterns (Fig 1) of fresh and dried green fruits of the cv. Mazurka. Table 4 presents the odour compounds of the different fresh and dried bell pepper samples, which were detected and described by the GC–sniffing panel. Thirty odour active constituents were registered simultaneously by 4 or more assessors (i.e., above noise level), of which 19 compounds were identified by comparing KI values and odour characteristics of sniffing responses with KI values and odour notes of identified volatile compounds.

Drying effect on GC–sniffing profiles. Comparing the GC–sniffing results of fresh and dried bell peppers in Table 4 reveals that the disappearance and/or decrease of sniffing responses in fresh bell peppers are typical for each individual fruit. In green bell peppers (Table 4), mainly compounds with "green" odour notes disappeared like chemical/pungent, spicy/herbal (8a), grassy, green bell pepper, lettuce ((*Z*)-3-hexenal, 25), lettuce, spicy (hexanol, 45), and lettuce, green bell pepper, spicy ((*Z*)-3-hexenol, 48). The latter disappeared also in the white fruits. The almond/fruity odour of (*E*)-2-hexenal (33) is typical for both fresh green and red fruits of cv. Mazurka and is not observed in the corresponding dried samples. In red and yellow bell peppers, the unidentified compound (17b) with characteristic red bell pepper, rubbery and geranium odour notes disappeared after drying. Similarly, the spicy, red bell pepper, sweet notes of 2-heptanone were not detected in the dried yellow bell peppers. Besides these specific changes after drying, Table 4 shows that the GC–sniffing responses for (*Z*)-2-hexenal (31a) and octanal (39), which have "fresh" odour notes, like lettuce, grassy, green bell pepper and fruity, floral, disappeared in all samples.

The changes in "fresh" odour notes during drying are typical for each bell pepper, while the additions or increase of GC–sniffing responses are quite similar for all dried samples.

Table 4. Odour compounds of green, red, white, and yellow bell peppers detected and described by the sniffing port panel.

				Fresh				Dried			
No[a]	KI[b]	Compound	Odour description	Green	White	Red	Yellow	Green	White	Red	Yellow
3	822	2-Methylpropanal	Cacao, spicy, sweaty	-[c]	-	-	-	+++	+++	+++	+++
7/8	923	2/3-Methylbutanal	Cacao, spicy, sweaty, rotten/cv [d]	+	+	+	-	+++	+++	+++	+++
8a	935	Unidentified	Chemical/pungent, spicy/herbal	+++	-	-	-	-	-	-	-
10	981	2,3-Butanedione	Caramel, sweet, fruity	++	+++	+++	+++	+++	+++	+++	+++
13	1028	1-Penten-3-one	Chemical/pungent, spicy/herbal	+++	++	++	++	+++	+	+++	+++
15	1073	2,3-Pentanedione	Fruity, sweet, caramel	-	-	-	-	++	++	++	++
17	1101	Hexanal	Grassy, lettuce, fruity, green bp [e]	+++	+++	++	++	++	++	++	++
17a	1114	Unidentified	Red bp, rubbery, geranium	-	-	++	++	-	-	-	-
17b	1118	Unidentified	Chemical/pungent, rubbery, green bp	-	+++	-	-	++	++	++	++
23	1160	3-Carene	Red bp, green bp, rubbery	-	+++	+++	+++	+	++	++	++
25	1166	(*Z*)-3-Hexenal	Lettuce, grassy, green bp	+++	-	-	-	-	-	-	-
28	1197	2-Heptanone	Spicy, red bp	-	-	-	++	-	-	-	-
32	1218	(*Z*)-2-hexenal	Grassy, green bp, lettuce	+	+	+	+	-	-	-	-
33	1237	(*E*)-2-Hexenal	Almond, fruity	++	-	++	-	-	-	-	-
38a	1262	Unidentified	Rancid/oily, rotten/cooked vegetable	++	+	-	-	++	++	+++	++
39	1311	Octanal	Fruity, floral	++	+	+	+	-	-	-	-

(continue (Table 4)

40	1316	4-Octen-3-one	Mushroom, fruity	++	++	++	++	+++	+++	+++	+++
45	1369	1-Hexanol	Lettuce, spicy/herbal, rotten/cv.	+	-	-	-	-	-	-	-
47a	1401	Unidentified	Geranium, spicy, rubbery	-	-	++	+	+++	+++	+++	+++
48	1405	(*Z*)-3-Hexenol	Lettuce, green bp, spicy/herbal	++	+	-	-	-	-	-	-
51	1416	(*E*)-2-Hexenol	Spicy, geranium, red bp	-	-	+	-	-	-	-	-
54a	1458	Unidentified	Green bp, lettuce	+++	++	-	-	++	+	-	-
59a	1483	Unidentified	Spicy, rubbery, rancid/oily	+	+	++	+	++	++	-	++
59b	1503	Unidentified	Sweaty, rubbery	+	+	-	-	-	-	-	-
64	1529	2-*Sec*-butyl-3-mp[f]	Carrot, green, red bp	+	+	++	++	+	+	+	+
65	1540	2-Isobutyl-3-mp[f]	Green bp, red bp, floral	+++	+++	+++	+++	++	++	+++	+++
67	1554	(*E*)-2-Nonenal	Cucumber, green bp, carrot	-	++	-	-	++	++	-	-
69a	1579	Unidentified	Cucumber, carrot, rancid/oily	++	++	++	++	++	+	-	++
71	1605	(*E*,*Z*)-2,6-Nonadienal	Lettuce, cucumber	+	+	+	++	++	+	+	++
73a	1641	Unidentified	Grassy, lettuce, cucumber	++	-	++	+	++	+	+	++

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[a] Numbers refer to Table 3.

[b] Mean calculated KI of sniffing responses on supelcowax 10 column.

[c] Maximal number of assessors detecting the smell simultaneously; — ,< 4 assessors, i.e., below noise level; +, 4 assessors; ++, 4 < assessors ≤ 7; +++, > 7 assessors out of 10.

[d] cv= cooked vegetable, [e] bp= bell pepper, [f] mp= methoxy pyrazine.

New odour compounds were detected in all dried samples, i.e., 2-methylpropanal (3, cacao, spicy, sweaty) and 2,3-pentanedione (15, fruity, sweet, caramel). Moreover, GC–sniffing responses of other odour compounds i.e., 2 and 3-methylbutanal (7/8), 1-octen-3-one (40), and unidentified compounds (17b), (38a), and (47a) increased in all dried samples (Table 4). The odour descriptions obtained for these compounds were cacao, spicy, sweaty, rotten/cooked vegetable (7/8), chemical/pungent, rubbery (17b), rancid, rotten/cooked vegetable (38a), mushroom, fruity (40), and geranium, spicy (47a). In fact, these odour characteristics are basically different from the "fresh" and "green" odour notes in the fresh bell pepper samples.

Retained GC–sniffing profile. Although drying affects the GC–sniffing pattern of the fresh bell peppers, a number of odour active compounds were retained in the dried samples (Table 4). For instance, the unidentified compound 54a with the typical odour notes, green bell pepper and lettuce, was still detected in the dried green and white samples after drying. Moreover, 2,3-butanedione (10), 1-penten-3-one (13), hexanal (17), 3-carene (23), 2-*sec*-isobutyl-3-methoxypyrazine (64), 2-isobutyl-3-methoxypyrazine (65), and (*E*,*Z*)-2,6-nonadienal (71) were still detected after drying of green and red cv. Mazurka, white cv. Blondy, and yellow cv. Kelvin. Interestingly, the compounds (10), (13), (17), (23), and (65) have been previously reported as constituents belonging to a basic GC–sniffing odour profile of fresh green and red bell peppers of cv. Mazurka (Luning et al., 1994a). Apparently, these compounds are important for the basic flavour of both fresh and dried bell peppers of the different cultivars investigated.

Conclusions

Hot-air drying modifies the quantitative and qualitative composition of volatile and non-volatile flavour compounds of bell peppers. Probably, browning reactions affect the levels of glucose, fructose, and ascorbic acid. Drying affects volatile compounds in three ways, i.e., 1) evaporation and partial retention of volatiles, 2) inactivation of flavour forming enzymes, and 3) formation or increase of volatile compounds by Maillard and Strecker degradation reactions or by autoxidation of unsaturated fatty acids. The decrease of odour active compounds is typical for the different coloured fruits, whereas the formation of new compounds is quite similar in all fruits. Since a number of these volatile compounds have typical odour characteristics, it is likely, that the overall flavour of the different fresh bell peppers will be markedly changed after the drying treatment.

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Chapter 7

Conversion of the Aroma of Fresh Bell Peppers (*Capsicum annuum*) due to Hot-air Drying[1]

Abstract

The aroma of fresh and hot-air dried bell peppers (*Capsicum annuum*) was evaluated by sensory and instrumental methods. Hot-air drying decreased levels of the odour compounds (*Z*)-3-hexenal, 2-heptanone, (*Z*)-2-hexenal, (*E*)-2-hexenal, hexanol, (*Z*)-3-hexanol, (*E*)-2-hexenol, and linalool, which possess green, vegetable-like, fruity, and floral notes, while intensity scores of corresponding sensory aroma attributes decreased also. The aroma of rehydrated dried samples was mainly characterised as savoury, rancid/sweaty, sweet/sickly, hay-like, cacao, caramel, and nutty. Drying increased the levels of 2-methylpropanal, 2 and 3-methylbutanal, which possess cacao, spicy, and rancid/sweaty odour notes; these volatiles may be correlated with the corresponding aroma attributes in the dried fruits. Principal component analysis demonstrated relationships between instrumental and sensory data for fresh samples, whereas it was more complex for dried fruits.

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Introduction

Capsicum annuum belongs to the family of Solanaceae. The fruits are used fresh or processed in the diet for their colour, their pungent character (i.e., capsaicinoids), and their typical aroma and taste (Somos, 1984; Govindarajan, 1985). The latter two characteristics have been considered a less important quality parameter for fresh and processed *Capsicum* fruits than colour and pungency (Govindarajan et al., 1987). Breeding programs are mainly focused on disease resistance, crop yield, carotenoids levels, and nutritional value like vitamin C content (Somos, 1984; Minguez-Mosquera et al., 1994). However, the growing "fresh" market and the increasing use of dried vegetables in convenience foods, shifts attention towards aroma as a quality parameter (Feinberg, 1973; Govindarajan et al., 1987; Zachariasse and Abrahamse, 1993).

Several studies have dealt with the flavour evaluation of different fresh and processed *Capsicum* varieties. Chitwood et al. (1983) observed that "green" aroma attributes discriminated different cultivars of fresh California *Capsicums*. Luning et al. (1994b) studied the flavour evaluation of fresh bell peppers at different ripening stages, perceived while eating. Sweetness, sourness, and red bell pepper aroma were characteristic attributes for the ripe red stages, while bitterness and "green" flavour notes, such as grassy, herbal, cucumber, and green bell pepper aroma, characterised the immature green stages. The aroma profiles of commercial dried Hungarian Edelsüss and Spanish *Capsicums* were studied by Wilkins (1994). The Spanish *Capsicum* scored relatively high for off-aroma, rubbery, and sharp, while the Hungarian samples generally scored higher for tomato, hay, and sweet aroma. Van Ruth and Roozen (1994) showed that rehydrated dried Chilean, Turkish, and Hungarian bell peppers were more different in taste attributes like sour, bitter, and pungency than in aroma attributes. Drying of fresh bell peppers drastically changed the composition of volatile compounds; the majority of volatiles evaporated, whereas new volatile odour compounds were formed by chemical reactions (Luning et al., 1994a; Van Ruth and Roozen, 1994). These changes in volatile compounds might effect the aroma of fresh peppers after drying.

There is a limited knowledge of changes in aroma of fresh bell peppers by hot-air drying as related to changes in composition of volatile odour compounds. The aim of this study was to characterise the aroma of both fresh and hot-air dried bell peppers of four different colours (i.e., green, red, white, and yellow), and second to evaluate the differences due to drying. Their composition of odour active volatile compounds was analysed by gas chromatography (GC) and GC-sniffing. Their aromas, as perceived by smelling, were evaluated by sensory descriptive analysis. Combined sets of instrumental and sensory variables of fresh and of dried samples were subjected to principal component analysis (PCA), to examine relationships between chemical composition and perceived sensory properties of the differently coloured bell peppers.

Materials and methods

Plant material

Bell peppers of three commercial Dutch cultivars i.e., cv. Mazurka, cv. Blondy, and cv. Kelvin were grown under greenhouse conditions as reported by Luning et al. (1994a). Immature green fruits of cv. Mazurka were harvested at approximately 6 weeks after fruit set. The ripe red fruits of cv. Mazurka, the ripe white and yellow fruits of cv. Blondy and cv. Kelvin respectively were harvested at about 10 weeks after fruit set. Forty fruits of each colour were collected in the early morning and stored at 13°C for a maximum of 5 days. Twenty bell peppers of each colour were used for isolation of volatile compounds, and the remaining ones were used for sensory evaluation of the fresh fruits.

Dried bell pepper samples were prepared by drying approximately 40 fruits of each colour in a pilot-scale hot-air tray dryer (Bronswerk Heat Transfer B.V., Nijkerk, The Netherlands). Bell peppers were washed, deseeded, and cut into pieces of 1 cm^2. Subsequently, the bell pepper samples were dried at 65°C until a final moisture content of 0.1 kg $H_2O.kg^{-1}$ dry matter was obtained. Dried samples were stored in the dark, under vacuum at 4°C until analysis of volatile compounds and sensory evaluation (up to 2 months).

All chemicals were obtained from Jansen Chimica (Tilburg, The Netherlands).

Sensory analysis

Sensory descriptive analysis was used to evaluate aroma attributes of fresh and hot-air dried bell peppers as described by Stone and Sidel (1993).

Sample preparation and presentation. Fresh bell peppers of each colour were cleaned, then the top and bottom parts and seed lobes were removed. The parts were ground in a household mixer (Quick Foodmaster, Tefal) and 30 g portions of the crushed samples were immediately transferred into glass jars and closed with odourless click-on caps. Hot-air dried bell peppers were rehydrated with water at 80°C for 10 minutes and cooled to room temperature. Preliminary experiments revealed that up to 60% of the total amount of evaporated water was absorbed. Three-gram portions of dried bell pepper were crushed in a mortar and subsequently rehydrated with water at 80°C in the glass jars.

Training of the panel. Fifteen assessors, aged from 25 to 35 years, and with extensive sensory evaluation experience on other fruits and vegetables, were selected for aroma evaluation of fresh and hot-air dried bell peppers. Panel training was executed in different phases. In the first phase, twenty chemicals dissolved in water were sniffed and described (Table 1). Successively, the same chemicals were evaluated using a checklist including the compiled descriptors, to train the odour recognition of the assessors. In the next sessions, an assortment of fresh bell pepper varieties with different aromas were offered as a homogenate to the panel to provide a wide range of perceived sensory properties, to be expected in the fresh samples.

Table 1. List of descriptors and reference chemicals used for panel training.

Reference chemical	Amount in 10 mL water	Major descriptor
Linalool	1.5 mL	Floral, fragrant
Dimethylsulfide	30.0 μL	Sulfury, gassy
α-Limonene	2.4 mL	Fruity, citric
α-Pinene	0.7 mL	Woody/pine-like
Myrcene	5.0 μL	Geranium leaves
(*E*)-2-hexenal	10.0 μL	Almond-like
(*Z*)-3-hexenol	10.0 μL	Grassy, herbal
Onion oil	10.0 μL	Onion, garlic
Formic acid	0.3 mL	Pungent, sharp
Soap/water solution	0.1 mL	Soapy
Trimethylamine	30.0 μL	Fishy
Butyl acetate	10.0 μL	Fruity (ester-like)
Ethyl octanoate	10.0 μL	Fruity (other)
1-Octene-3-ol	10.0 μL	Mushroom
Vanillin	0.1 μg	Sweet, caramel
Phenol	10.0 μL	Rubbery
Hexanal	1.0 μL	Grassy, green vegetable
2-Isobutyl-3-methoxypyrazine	1.0 μL	Green bell pepper
Butyric acid	0.6 mL	Rancid, sweaty
Methyl salicylate	10.0 μL	Winter green-, menthol-like
2-Methylbutanal	20.0 μL	Cacao, sweaty

These varieties were also offered as freeze-dried and hot-air dried samples to generate sensory attributes for the processed fruits. The same assortment of fresh and processed bell pepper cultivars were used for training the intensity scaling of the perceived attributes over a period of 1 month. Finally, the assessors made suggestions and selected attributes for the panel, to describe the aroma characteristics of the fresh and dried bell peppers; two lists of consensus attributes were obtained (cf. Tables 3 and 4)

Sensory evaluation. For assessment of aroma, all samples were presented in coded, aluminum foil covered jars, at room temperature in isolated air-conditioned booths with slight positive pressure. Fresh and hot-air dried samples of each colour (green, red, yellow, and white) were evaluated three times by the panel. Per session, the aromas of four fresh or four dried bell peppers were evaluated.

Samples were assesed in the morning and afternoon, using a complete block design. The judges indicated the intensity of each attribute by using an unstructured line scale anchored with 0 and 100 at the ends. Data were acquired by using the computerized PSA-SYSTEM (OP&P, Utrecht, The Netherlands).

Gas chromatography

Details of the isolation procedure of volatile bell pepper compounds, of the analysis and quantification by gas chromatography (GC), and of the identification by GC-mass spectrometry (GC-MS) were described previously (Luning et al., 1994a). Five fresh fruits of each colour were sliced into small pieces and 45 g portions were homogenised with 50 mL water in a Waring blender. Weights of the dried samples were taken relative to the dry matter contents of the 45 g portions of fresh bell peppers. Dried samples were rehydrated with 80°C water for 15 min in closed flasks and cooled on ice to room temperature prior to homogenization with 50 mL water in a Waring blender. Volatile compounds were isolated from homogenised fresh and rehydrated samples, by flushing with 30 $mL.min^{-1}$ purified nitrogen for 2 h at room temperature (three replicates). Detection and description of odour active compounds in the GC-effluent were carried out by an experienced sniffing port panel as reported previously (Linssen et al., 1993; Luning et al., 1994a). Peak areas of identified odour active compounds were expressed as percentage of total extracted peak area (Volt x sec).

Data handling

GC peak areas (expressed as percentage of total peak area) of the odour compounds and mean scores of each sensory attribute were subjected to analysis of variance (ANOVA, Genstat-5), to determine least significant differences (LSD) among colours (one-way ANOVA) and between fresh and rehydrated hot-air dried samples (two-way ANOVA). Principal component analysis (PCA) was applied to the combined data set of percentage peak areas and mean score ratings, to study the relationships between instrumental and sensory data of respectively fresh and of hot-air dried bell peppers, as described by Piggot and Sharman (1986). PCA was carried with the UNSCRAMBLER analytical software (OP&P, Utrecht, The Netherlands). In addition, combined data sets were subjected to partial least-squares regression (PLS) as previously described Martens and Martens, 1986)

Results and discussion

Sensory evaluation of fresh and hot-air dried bell peppers

Table 2 shows the results for analysis of variance of the descriptive analysis data for each attribute for fresh and dried samples. There were highly significant differences among fresh samples for all attributes, while less attributes were significantly different among dried samples. Although inconsistent assessors were eliminated during selection, the assessors were still an important source of variation in all attributes studied, which is due to the subjective nature of sensory evaluation and individual differences between assessors. There were only a few significant assessor-by-replicate and sample-by-replicate interactions.

Drying of green and red cv. Mazurka, white cv. Blondy, and yellow cv. Kelvin bell peppers produced completely different aromas as compared to the fresh fruits. Generation of descriptors for the aroma evaluation resulted in different lists for fresh and dried samples (respectively Tables 3 and 4). The aroma perception of both fresh unripe green and ripe white bell peppers was dominated by fresh green aroma attributes; the intensity scores for herbal, grassy, cucumber, and fruity/fresh were significantly higher ($P < 0.05$) than for the ripe red and yellow bell peppers. In addition, the white fruits had highest scores for floral and cucumber aroma, and the green fruits for herbal, grassy, and green bell pepper. The aroma of ripe red and yellow fruits was mainly characterised by significantly higher scores for rubbery,

Table 2. Analysis of variance of attribute ratings in fresh and dried samples

	F ratios													
Attribute	Sample(S)		Assessor(A)		Replicate(R)		SxA		AxR		SxR		MSE[b]	
	F[a]	D	F	D	F	D	F	D	F	D	F	D	F	D
Herbal	20.8***	1.1	16.6***	17.9***	1.6	1.9	3.8***	0.9	0.7	1.9	1.0	1.0	55.8	77.0
Green bell pepper	79.6***	1.1	11.7***	23.2***	1.7	0.5	2.3***	1.0	1.2	1.1	1.9	0.8	117.1	43.3
Grassy	52.7***	1.1	9.3***	19.7***	6.4**	4.1	1.5*	1.5	0.7	1.2	3.1**	0.7	199.8	35.9
Cucumber	17.9***	5.9***	16.6***	39.9***	3.7*	10.9*	2.6***	1.5*	2.0**	4.3**	1.0	0.7	94.6	30.8
Floral	12.9***	4.1**	10.6***	27.9***	0.6	2.9	1.9**	1.6*	1.6	1.2	1.8	0.6	124.5	34.4
Fruity/fresh	24.4***	4.2*	12.0***	9.1***	0.1	0.6	2.9***	2.2*	1.3	1.4	4.5**	0.9	204.4	84.0
Resinous	4.6**	1.1	32.0***	13.9***	0.3	2.1	1.9**	1.1	1.4	1.6	1.2	1.2	28.6	26.2
Rubbery	33.3***	9.9***	34.1***	6.9***	2.7	1.0	3.2***	2.7***	3.1**	0.8	6.2**	0.2	108.6	52.5
Rancid/sweaty	44.4***	1.1	23.2***	14.3***	8.4**	1.6	3.7***	0.9	1.3	1.4	6.1**	0.5	45.1	134.1
Sweet/sickly	8.6***	0.6	9.7***	23.6***	1.1	0.3	1.8**	0.7	1.1	0.9	1.8	1.9	110.4	170.0
Fruity/chemical	10.9***	3.3*	29.2***	6.9***	1.0	30.0**	3.9***	1.5*	1.2	1.3	0.9	2.4*	98.9	72.3
Musty/earthy	9.7***	0.6	22.6***	11.9***	0.5	6.0	2.5***	1.4	0.9	2.0	3.1**	0.6	23.6	77.8
Sharp	9.5***	1.8*	44.5***	14.8***	2.9	0.6	4.0***	1.8**	2.6**	1.7*	1.1	1.7	81.5	111.1
Spicy	3.0**	0.7	17.1***	31.3***	1.7	2.0	2.2**	1.6	1.1	1.1	1.9	1.1	91.6	58.2
Hay-like	5.0**	2.2**	37.1***	19.4***	1.2	1.3	4.6***	2.0**	1.5	1.1	0.5	0.5	13.8	152.4
Cacao	n.e.[c]	17.1***	n.e.	6.0***	n.e.	0.5	n.e.	2.8***	n.e.	1.3	n.e.	0.4	n.e.	51.3
Caramel	n.e.	12.7***	n.e.	19.5***	n.e.	1.7	n.e.	3.8***	n.e.	1.1	n.e.	1.7	n.e.	45.9
Nutty	n.e.	12.6***	n.e.	21.2***	n.e.	0.7	n.e.	4.9***	n.e.	0.9	n.e.	2.6*	n.e.	38.3
Savoury	n.e.	2.7*	n.e.	20.4***	n.e.	0.1	n.e.	1.7**	n.e.	0.9	n.e.	0.9	n.e.	116.9
Dried tomato	n.e.	6.9***	n.e.	13.0***	n.e.	1.3	n.e.	3.1***	n.e.	0.8	n.e.	0.8	n.e.	51.0

[a] Fresh (F) and hot-air dried (D) sample. [b] MSE: mean squares error. ***,**,* Significantly different at $P < 0.001$, $P < 0.01$ and $P < 0.05$ respectively. [c] n.e. not evaluated.

Table 3. Mean score ratings [a] of sensory attributes of fresh green and red cv. Mazurka (GM, RM), white cv. Blondy (WB), and yellow cv. Kelvin (YK)

Attribute	Code	GM	WB	RM	YK
Herbal	HER	33.3 *a*	19.2 *b*	12.0 *c*	10.5 *c*
Green bell pepper	GBP	32.4 *a*	14.9 *b*	9.8 *b*	10.4 *b*
Grassy	GRA	13.8 *a*	9.9 *b*	5.5 *c*	4.7 *c*
Cucumber	CUC	14.6 *a*	22.5 *b*	7.6 *c*	9.5 *c*
Floral	FLO	13.5 *a*	23.5 *b*	11.7 *a*	9.6 *a*
Fruity/fresh	FRF	30.8 *a*	40.0 *b*	19.2 *c*	17.8 *c*
Resinous	RES	5.6 *a*	4.2 *a*	3.8 *ab*	2.2 *b*
Rubbery	RUB	13.0 *a*	10.2 *a*	33.9 *b*	27.6 *b*
Rancid/sweaty	RAN	5.7 *a*	4.2 *a*	17.8 *b*	9.6 *c*
Sweet/sickly	SIC	10.4 *a*	9.2 *a*	18.7 *b*	15.8 *b*
Fruity/chemical	FRC	6.5 *a*	13.4 *b*	19.5 *c*	14.5 *b*
Musty/earthy	MUS	3.6 *a*	3.3 *a*	11.4 *b*	6.9 *a*
Sharp	SHA	18.6 *a*	10.3 *b*	17.7 *ac*	13.8 *bc*
Spicy	SPI	11.0 *ab*	7.3 *b*	13.3 *a*	11.8 *ab*
Hay-like	HAY	5.7 *a*	2.9 *b*	4.8 *ac*	3.8 *bc*

[a] Mean scores (n=3) in rows with different letters are significantly different ($P < 0.05$).

rancid/sweaty, and sweet/sickly; the red bell peppers had also high scores for musty. The fruity/chemical aroma appeared to be characteristic for all ripe fruits (Table 3). Similarly, Chitwood et al. (1983) reported "green" aroma characteristics, like grassy, green and garbanzo bean to describe the aroma of different *Capsicum* cultivars. Moreover, floral, rose, and apple appeared to be also important contributors to the aroma of these *Capsicums.* However, they reported no aroma attributes like rancid/sweaty, sweet/sickly and/or rubbery.

Table 4. Mean score ratings [a] of sensory attributes of hot-air dried green and red cv. Mazurka (GM, RM), white cv. Blondy (WB), and yellow cv. Kelvin (YK)

Attribute	Code	GM	WB	RM	YK
Cacao	CAC	11.6 *a*	7.9 *b*	3.0 *c*	3.2 *c*
Caramel	CAR	8.0 *a*	7.2 *a*	4.3 *b*	3.4 *b*
Nutty	NUT	9.3 *a*	13.4 *a*	3.7 *b*	4.7 *b*
Savoury	SAV	15.6 *a*	15.3 *a*	11.9 *ab*	9.3 *b*
Dried tomato	TOD	7.8 *a*	4.8 *b*	11.1 *c*	6.6 *ab*
Herbal	HER	6.5 *a*	5.8 *ab*	5.1 *ab*	4.0 *b*
Green bell pepper	GBP	7.7	7.2	6.2	6.1
Grassy	GRA	8.7 *a*	6.4 *ab*	5.5 *b*	6.2 *ab*
Cucumber	CUC	6.5 *a*	11.1 *b*	7.5 *ab*	9.1 *ab*
Floral	FLO	4.4 *a*	4.9 *a*	6.2 *ab*	8.6 *b*
Fruity/fresh	FRF	6.1 *a*	6.1 *a*	9.4 *b*	12.3 *c*
Resinous	RES	6.7 *ab*	8.5 *a*	5.1 *b*	4.4 *b*
Rubbery	RUB	4.5 *a*	4.5 *a*	12.3 *b*	6.4 *a*
Rancid/sweaty	RAN	10.3	10.6	10.2	12.4
Sweet/sickly	SIC	16.6	17.4	20.0	18.7
Fruity/chemical	FRC	6.2 *a*	9.6 *a*	10.0 *ab*	14.1 *b*
Musty/earthy	MUS	6.9	8.3	7.2	4.3
Sharp	SHA	9.0 *ab*	9.6 *ab*	12.2 *a*	7.5 *b*
Spicy	SPI	8.4	7.3	10.5	7.9
Hay-like	HAY	16.0 *a*	20.1 *a*	10.2 *b*	8.7 b

[a] Mean scores (n=3) in rows with different letters are significantly different ($P < 0.05$).

After drying, five extra attributes, i.e., cacao, caramel, nutty, savoury, and dried tomato, were necessary to describe the aroma of the bell pepper samples; these were not relevant in the fresh samples (Table 4). Obviously, all dried samples were characterised by relatively high scores for savoury, rancid/sweaty, sweet/sickly, and hay-like (Table 4). Furthermore, the intensity scores for cacao; caramel, and nutty were significantly ($P < 0.05$) higher in the green and white fruits, whereas dried tomato and rubbery aroma scored highest in the red, and fruity/chemical and fruity/fresh aroma rated highest in the yellow fruits. Wilkins (1994) reported similar odour attributes like rubber, tomato, and hay for the evaluation of dried Hungarian and Spanish *Capsicum* varieties.

Drying changed not only the main aroma attributes of the fresh fruits, but also the perceived aroma intensity. Tables 3 and 4 show that overall the intensity scores were lower in the dried than in the fresh samples. Mean scores of the corresponding attributes for fresh and dried fruits were subjected to ANOVA. Figure 1 shows the attributes which changed significantly ($P < 0.05$) upon drying. Obviously, in all samples the scores for hay-like aroma increased while herbal aroma decreased. Figures 1 [A] and [B] show the intensity scores of all "green" attributes were lower in the dried green and white samples, whereas, scores for sweet/sickly and rancid aroma increased; musty aroma increased only in the white fruits (Fig. 1 B). The intensity of fruity/fresh and floral aroma notes decreased in the green, white, and red but not in the yellow samples. The dried green, red, and yellow fruits (Fig. 1 A, C, and D) scored lower on sharp aroma, whereas rubbery aroma scored lower in the dried red and yellow fruits only.

Volatile odour active compounds of fresh and hot-air dried bell peppers

The volatile compounds were extracted from homogenised fresh and rehydrated dried bell peppers by a dynamic headspace isolation, trapped on Tenax, and thermally desorbed. The odour active compounds were detected and characterised previously by an experienced GC-sniffing port panel and identified by GC/MS (Luning et al., 1994a,c). Table 5 lists the percentage peak areas of 26 identified odour active compounds, the total peak area of extracted volatile compounds, and the sum of percentage peak areas of odour active volatiles. Obviously, the proportional contribution of odour active compounds to the total extracted amount of volatiles was higher in the fresh green and red than fresh yellow and white bell

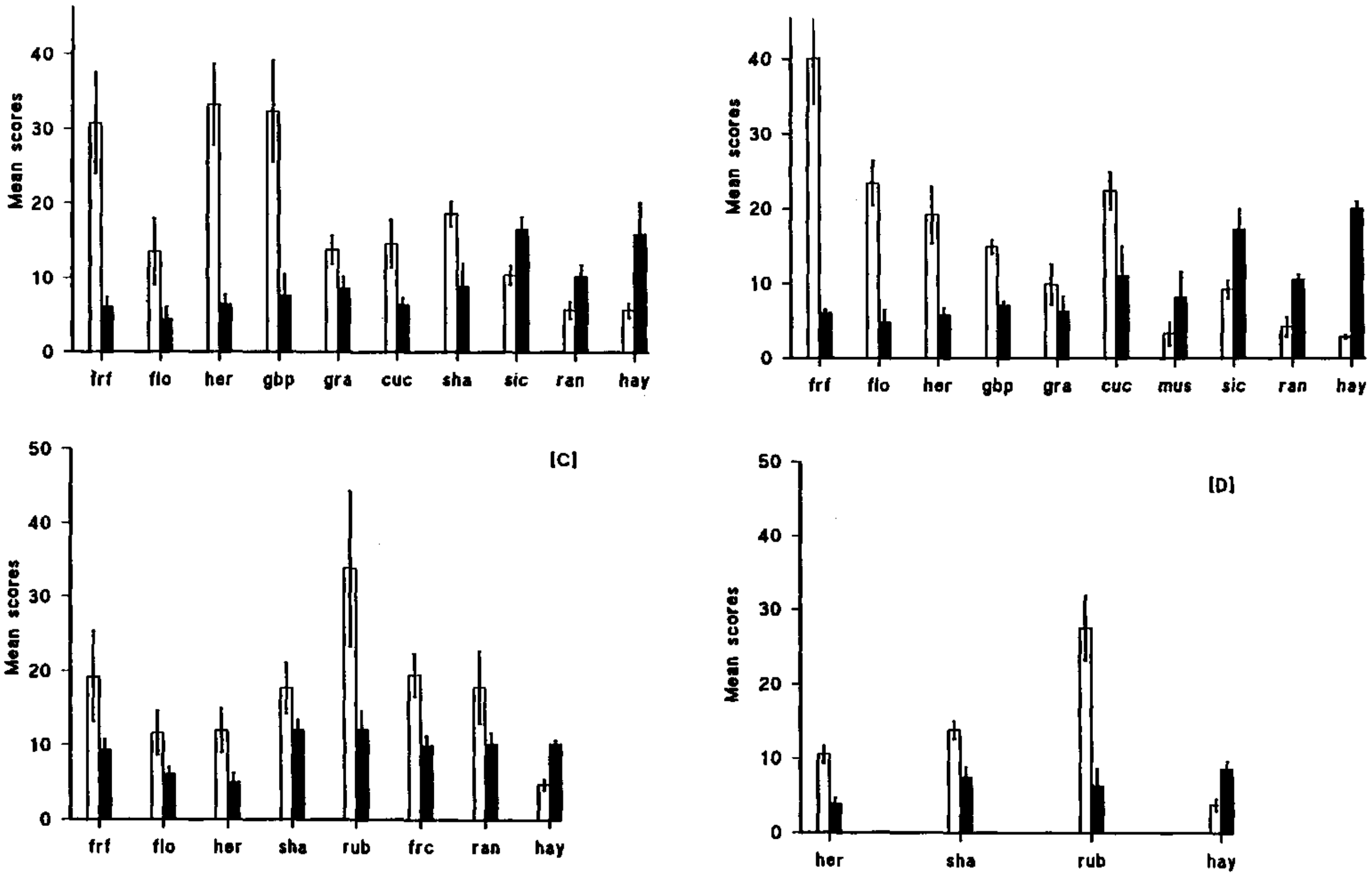

Figure 1. Significant changes ($P < 0.05$) in intensity scores of aroma attributes due to drying of fresh (open bars) green [A], white [B], red [C], and yellow [D] bell peppers to dried fruits (filled bars); codes are explained in Tables 2 and 3.

peppers, whereas it was approximately the same in all dried samples (Table 5). Hot-air drying decreased not only the total amount of extracted volatile compounds, but it also changed significantly ($P < 0.05$) the proportions between the volatile compounds (Table 5). The percentage peak areas of (*Z*)-3-hexenal (9), 2-heptanone (10), (*Z*)-2-hexenal (12), (*E*)-2-hexenal (13), hexanol (18), (*Z*)-3-hexanol (19), and (*E*)-2-hexenol (21), and linalool (25), which possessed green, vegetable-like, fruity, and floral characteristics, decreased significantly ($P < 0.05$) during drying in most of the bell peppers studied. Probably, these changes were responsible for the decreased intensity scores of corresponding green, fruity, and floral sensory aroma attributes in the dried samples (Fig. 1 A-D). In all dried samples the major odour active compounds were 2-methyl-propanal (1), 2 and 3-methylbutanal (2,3), hexanal (7), and nonanal (20). The first three volatiles had typical cacao, spicy, sweaty, and rotten/cooked vegetable odour notes, while the latter had more spicy and herbal characteristics (Table 5). Moreover, the percentage peak areas of 2,3-butanedione (4, caramel, sweet), 2,3-pentanedione (6, fruity, sweet, caramel), 4-octene-3-one (16, mushroom), (*E*)-2-octenal (22, almond, sweet), and (*E*)-2-nonenal (24, cucumber, green bell pepper) were significantly ($P < 0.05$) higher in the dried bell peppers. Several of these compounds had cacao, spicy, sweaty/rancid, and sweet odour characteristics and may contribute to sensory aroma attributes like savoury (i.e, spicy, salty), sweet/sickly, rancid/sweaty, cacao, and caramel, which were characteristic for all dried samples (Table 4) as compared to the fresh fruits (Table 3). In addition, although hay-like was a typical sensory aroma attribute for all dried samples, no odour compounds with hay-like notes were observed by the GC-sniffing port panel. Leino (1992) reported that the large relative proportions of carbonyl compounds like 2-methyl-2-pentenal, hexanal, 2-methyl-2-butenal, nonanal, and 2-hexenal, and high intensity scores for hay-like aroma were characteristic for hot air-dried chives (40°C) as compared to the fresh products. Moreover, Wilkins (1994) demonstrated that hexanal correlated highly with hay, tomato and off-aroma of dried Hungarian *Capsicums*, but suggested that these aroma notes may be the result of extensive interaction between odour compounds.

Table 5. Percentage peak areas [a] of volatile odour compounds isolated by dynamic headspace analysis from fresh and hot-air dried green and red cv. Mazurka (GM, RM), white cv. Blondy (WB), and yellow cv. Kelvin (YK)

				Fresh				Dried			
No	Compound	Odour descriptions [b]	Sign [c]	GM	WB	RM	YK	GM	WB	RM	YK
1	2-Methylpropanal	Cacao, spicy, sweaty	*y,z*	0.00	0.00	0.00	0.00	2.62	2.60	1.59	1.50
2	2-Methylbutanal	Cacao, spicy, sweaty, rotten/cv[d]	*x,y,z*	0.04	0.06	0.08	0.06	13.03	19.44	14.24	12.51
3	3-Methylbutanal	Cacao, spicy, sweaty, rotten/cv	*x,y,z*	0.04	0.04	0.06	0.08	16.00	23.01	29.92	25.28
4	2,3-Butanedione	Caramel, sweet	*x,y,z*	0.04	0.19	0.06	0.28	0.60	0.30	0.53	0.43
5	1-Penten-3-one	Chemical/pungent, spicy	*x,y,z*	1.35	0.63	0.81	0.76	1.45	0.58	4.94	1.83
6	2,3-Pentanedione	Fruity, sweet, caramel	*z*	0.00	0.00	0.00	0.00	0.09	0.06	0.10	0.08
7	Hexanal	Grassy, lettuce, green bp[e]	*x,y,z*	29.44	10.93	11.73	11.57	16.27	17.07	11.89	15.55
8	3-Carene	Red bp, rubbery, green bp	*x,z*	0.09	0.30	0.37	0.48	0.27	0.26	0.45	0.34
9	(*Z*)-3-hexenal	Lettuce, grassy, green bp, fruity	*x,z*	10.73	0.51	0.20	0.00	0.00	0.00	0.00	0.00
10	2-Heptanone	Fruity, Spicy, red bp	*x,y,z*	0.32	0.95	1.66	3.62	0.60	0.82	0.63	1.51
11	*o*-Xylene	Rubbery, spicy, geranium	*x,y,z*	0.16	0.69	0.75	1.26	0.58	0.59	0.99	1.06
12	(*Z*)-2-hexenal	Grassy, lettuce, green bp	*x,z*	0.86	0.16	0.35	0.35	0.13	0.10	0.14	0.20
13	(*E*)-2-hexenal	Almond, fruity, sweet	*x,y,z*	12.48	8.07	36.38	1.80	1.46	0.35	1.84	0.74
14	(*Z*)-ß-Ocimene	Rancid, sweaty	*x,y,z*	0.14	0.27	0.33	0.22	0.52	0.22	0.28	0.06
15	Octanal	Fruity	*x,z*	0.15	0.57	0.87	1.16	0.49	0.39	0.50	0.58

(continue Table 4)

16	4-Octen-3-one	Mushroom	*x,y,z*	0.02	0.08	0.12	0.26	1.57	0.81	0.95	1.19
17	6-Methyl-5-hepten-2-one	Sweaty, musty	*x,y,z*	0.14	0.64	0.85	0.86	0.57	0.35	0.86	0.98
18	Hexanol	Fruity, lettuce, rotten/cv	*x,y,z*	2.61	1.86	1.66	0.97	0.46	0.25	0.22	0.10
19	(*Z*)-3-Hexenol	Grassy, lettuce, cucumber	*x,y,z*	6.72	3.68	0.07	0.11	0.23	0.08	0.00	0.00
20	Nonanal	Mushroom, herbal	*x,z*	1.07	2.61	2.39	4.01	2.00	1.30	2.32	2.21
21	(*E*)-2-Hexenol	Almond, spicy, geranium, red bp	*x,y,z*	0.27	0.55	7.50	0.59	0.21	0.16	0.24	0.33
22	(*E*)-2-Octenal	Almond, sweet, herbal	*x,y,z*	0.10	0.20	0.26	0.35	1.08	0.84	0.85	0.85
23	2-Isobutyl-3-methoxypyrazine	Green bp, red bp, lettuce	*x,y,z*	0.34	0.51	0.53	0.76	0.96	0.90	0.51	0.69
24	(*E*)-2-Nonenal	Cucumber, green bp, carrot	*x,y,z*	0.05	1.36	0.16	0.23	1.12	1.75	0.40	0.96
25	Linalool	Floral	*x,y,z*	0.10	0.27	0.07	0.10	0.12	0.07	0.00	0.00
26	(*E,Z*)-2,6-Nonadienal	Cucumber, lettuce	*x,y,z*	0.02	0.13	0.11	0.23	0.19	0.25	0.12	0.20
	Sum percentage peak areas			67.2	35.3	67.4	30.1	62.6	72.5	74.5	69.5
	Total Peak area of extracted volatile compounds			324.8	184.2	125.8	71.4	85.1	94.2	80.8	62.1

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[a] Average percentage peak areas of three experiments; mean coefficient of variation is 15%.

[b] Odour descriptions obtained previously by GC-sniffing port analysis (Luning et al. 1994 a,c).

[c] Significance of differences at $P < 0.05$, x : significant differences within fresh samples, y : within dried samples and z : between fresh and dried samples of the same colour.

[d] cv= abbreviation for cooked vegetable.

[e] bp= abbreviation for bell pepper.

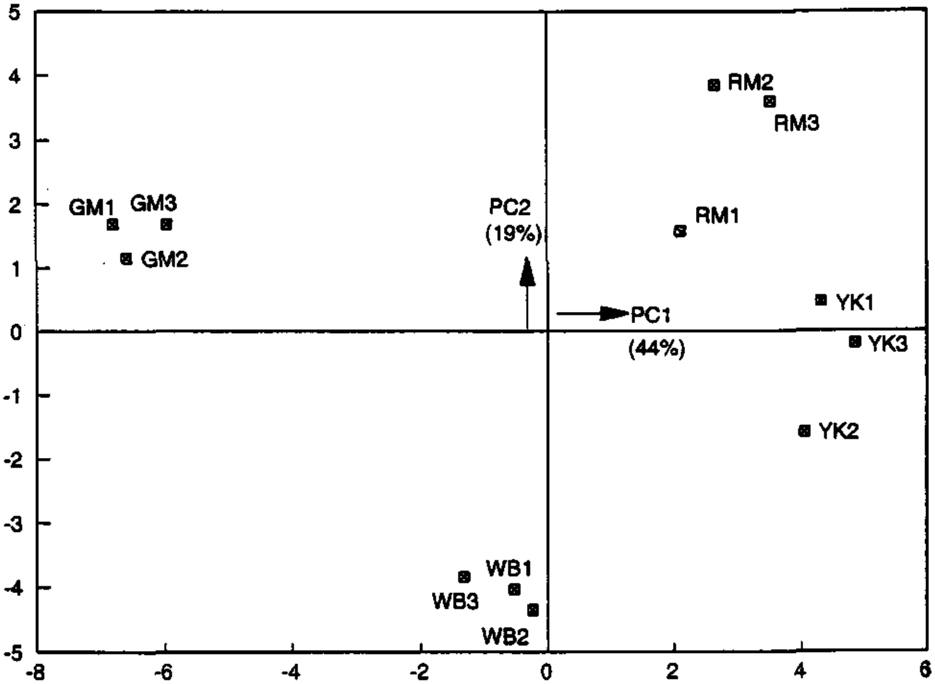

Figure 2. PCA sample scores of fresh green and red cv. Mazurka, white cv. Blondy and yellow cv. Kelvin; codes explained in Table 2.

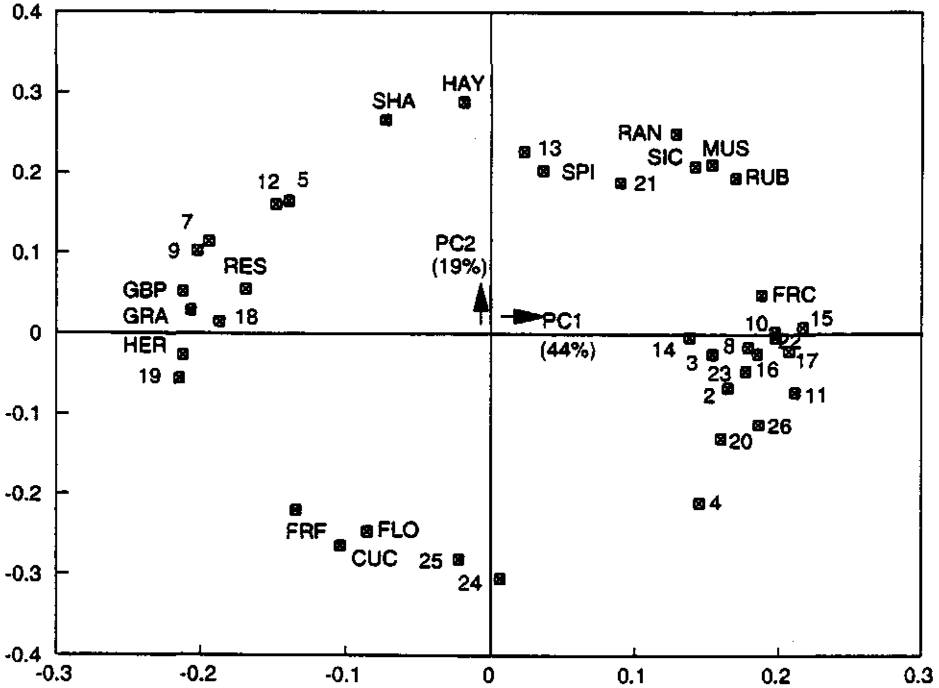

Figure 3. PCA loadings of aroma attributes and percentage peak areas of odour compounds of fresh bell peppers; codes explained in Tables 2 and 4.

Relationships between odour compounds and aroma attributes of fresh bell peppers

Relationships between the composition of volatile odour compounds and the evaluated aroma attributes of the different coloured fresh and processed bell pepper samples were studied by principal component analysis (PCA). Odour compounds and aroma attributes, which differed significantly between two or more colours were included in the data set. PCA on the data of the fresh fruits revealed three principal components (PC) accounting for 74% of variation. Sample scores on the first two PC's (Fig. 2) show that the unripe green bell peppers were separated from the ripe yellow and ripe red fruits on PC1, whereas the white fruits were separated from the others on PC2. The loadings plot (Fig. 3) demonstrates that on PC1 and PC2 several sensory aroma attributes and volatile odour compounds were related to each other.

On the negative side of PC1 the aroma attributes herbal, grassy, green bell pepper, and resinous, and the volatile odour compounds (*Z*)-3-hexenol (19), hexanol (18), (*Z*)-3-hexenal (9), and hexanal (7) had relatively high loadings. The sniffing port panel characterised these compounds as grassy, green bell pepper, spicy, herbal, and lettuce like (Table 5). Probably, these volatiles were responsible for the corresponding "green" aroma attributes, which are typical for the green fruits (Fig 2). Similar relationships were observed previously for green fruits of cv. Evident (Luning et al., 1994c).

On the positive side of PC1 the aroma attribute fruity/chemical and several volatile odour compounds had high loadings. The volatiles 2-heptanone (10) and octanal (15) closely related to this aroma attribute, and were described as fruity by the sniffing panel (Table 5). Possibly, they contributed to the perceived fruity/chemical aroma, which was typical for both fresh red and yellow fruits (Fig 2).

Figure 2 shows that on PC2, red bell peppers had high positive scores. Figure 3 shows that the volatile compounds 13 and 21 and the aroma attributes spicy, rancid, sweet/sickly, musty, rubbery, sharp and hay had high positive loadings on PC2 as well, and therefore are typical for the red fruits. The odour compounds (*E*)-

2-hexenal (13) and (*E*)-2-hexenol (21) and the aroma attributes spicy and sweet/sickly are relatively closely located to each other and thus showed a relationship. The spicy, sweet, and almond odour notes of these volatiles may be important for the corresponding aroma attributes. Additionally, several volatile compounds, which were located on the positive side of PC1, like 3-carene (8), *o*-xylene (11), (*Z*)-ß-ocimene (14), 4-octene-3-one (16), and 6-methyl-5-hepten-2-one (17) had musty, earthy, sweaty, rancid, and rubbery odour characteristics. However, they showed no relationships with the corresponding aroma attributes rubbery, musty and rancid/sweaty which had higher loadings on PC2 than on PC1 (Fig. 3).

On the negative side of PC 2 (Fig. 3), the aroma attributes floral, cucumber, and fruity/fresh, and the odour compounds (*E*)-2-nonenal (24) and linalool (25) had high loadings, and are typical for the white fruits (Fig. 2). Moreover, the cucumber and floral notes of the volatiles (Table 5) were similar to the closely located attributes. Examination of the third PC, which accounted for 11% of variance, revealed no further grouping of instrumental and sensory data. It appeared only that the red and yellow fruits were separated from each other on the presence of (*E*)-2-hexenol and (*E*)-2-hexenal; the compounds were typical for the red fruits.

Partial least-squares regression (PLS) confirmed the three PC's obtained by PCA and showed similar relationships. PLS revealed that 70.3% of variance in the X-data (instrumental) explained 66.8% of variance in the Y-data (sensory).

Relationships between odour compounds and aroma attributes of rehydrated hot-air dried bell peppers

PCA on the data set of the rehydrated dried fruits revealed three principal components (PC) accounting for 73% of variation. Sample scores on the first two PC's (Fig. 4) show that the green and white peppers were separated from the red and yellow fruits on PC1, whereas on PC2 the green and white, and the red and yellow samples were differentiated from each other. Figure 5, however, shows that distribution of aroma attributes and volatile odour compounds on PC1 and PC2 was more diffuse as compared to the fresh samples.

On the negative side of PC1 the aroma attributes rubbery, fruity/fresh, and floral, and the odour compounds 6-methyl-5-hepten-2-one (17), *o*-xylene (11), (*E*)-2-hexenol (21), and 3-methylbutanal (3) had high loadings and are therefore more typical for the red and yellow than for the white and green fruits. The volatile compound *o*-xylene (11) had a rubbery odour but it showed no strong relationship with the aroma attribute rubbery (Fig. 5). In fact, *o*-xylene (11) and 3-methylbutanal (3) were nearly located to the aroma attribute fruity/fresh but their odour notes, as characterised by the sniffing panel, were completely different. However, Holscher and Steinhart (1994) described 3-methylbutanal as fruity and pungent in their study. Possibly, this compound contributed to the fruity impression of the dried yellow and red fruits.

On the positive side of PC1 the aroma attributes savoury, caramel, cacao, hay, and nutty, and the volatile odour compounds 2-methylpropanal (1), (*Z*)-3-hexenol (19), linalool (25), and 2-isobutyl-3-methoxypyrazine (23) had relatively high positive loadings. Therefore, they were more typical for the dried green and white fruits than for the yellow and red bell peppers. The green, spicy characteristics of volatiles 19, and 23, and the cacao, rancid/sweaty odour of 2-methylpropanal (1) may support the corresponding aroma attributes.

On PC2, only the aroma attribute fruity/chemical and the fruity smelling compound 2-heptanone (10) were relatively closely located to each other (Fig. 5). They were typical for the dried yellow samples which have had relatively high negative loadings on PC 2 as well (Fig. 4). The dried tomato aroma was more typical for the red samples since it was positively located on PC2, but no odour compounds related to this aroma attribute. Some volatile compounds like hexanal (7), (*E*)-2-nonenal (24), and (*E*,*Z*)-2,6-nonadienal (26) had as well high positive loadings on PC1 as high negative loadings on PC2.

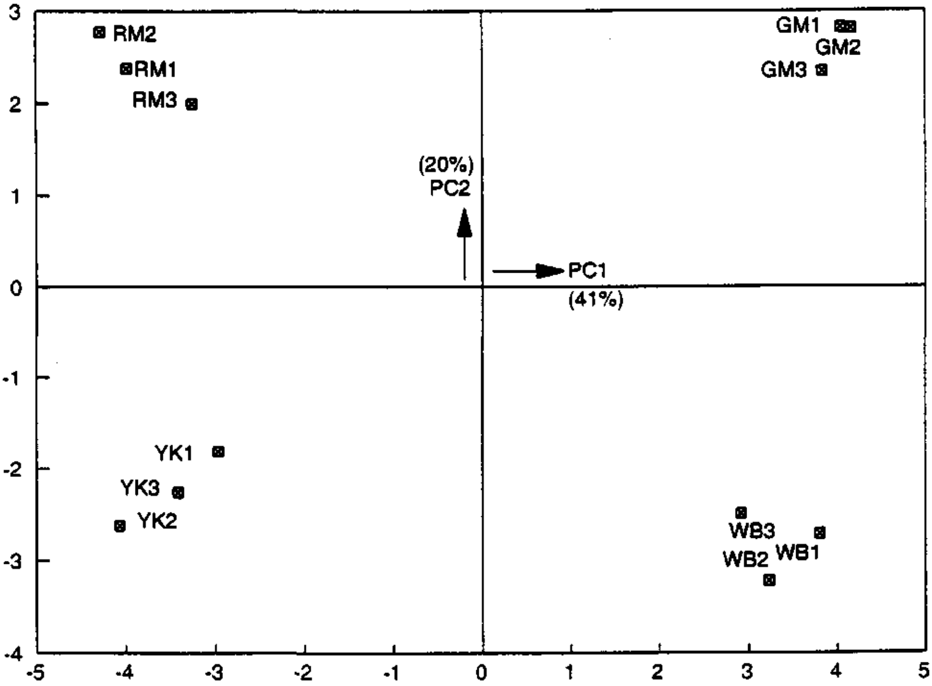

Figure 4. PCA sample scores of hot-air dried green and red cv. Mazurka, white cv. Blondy and yellow cv. Kelvin; codes explained in Table 2.

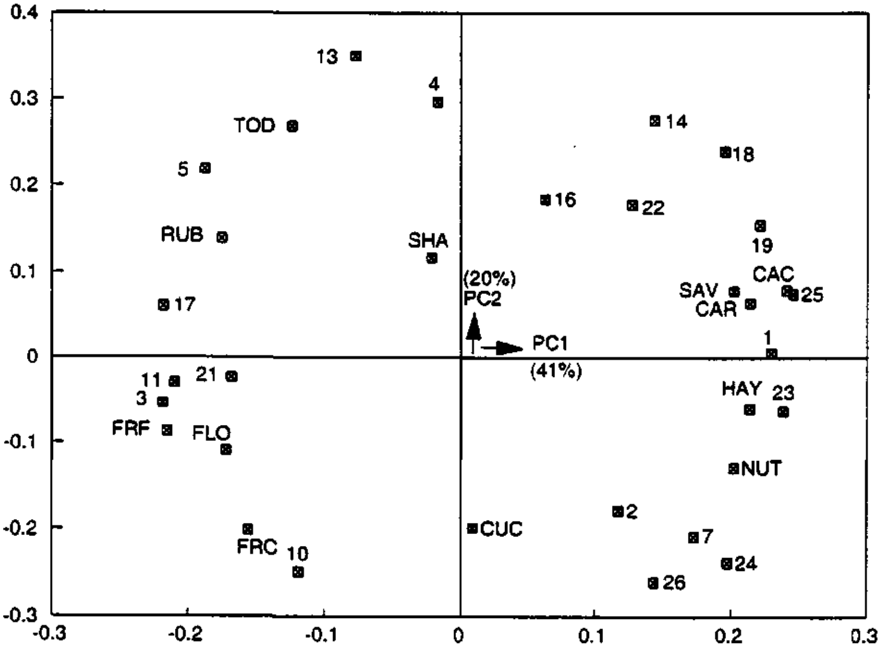

Figure 5. PCA loadings of aroma attributes and percentage peak areas of odour compounds of hot-air dried bell peppers; codes explained in Tables 3 and 4.

Therefore, these odour compounds were typical for both the dried green, white, and yellow samples as compared to the red fruits (Fig. 5). However, there were no aroma attributes found to be closely related to these odour compounds, indicating that they were less important for the differences in perceived aroma of dried fruits. On the third PC, which accounted for 12% of variance, no further grouping of instrumental and sensory data was observed.

PLS confirmed only the first two PC's obtained by PCA and showed similar relationships. PLS revealed that 68.3% of variance in the X-data explained 46.2% of variance in the Y-data.

In our study, the relationships between aroma attributes and volatile odour compounds were less distinct in the dried than in the fresh fruits. As a matter of fact, drying reduced the amount of odour compounds, and on the other hand it levelled down the differences between the colours in the composition of volatiles (Tables 3 and 4). Consequently, it appeared to be more difficult to differentiate between the aromas of the different coloured dried samples. This confirmed the conclusion of Van Ruth and Roozen (1994), who suggested that commercial Chilean, Turkish, and Hungarian dried bell peppers were more different in taste than in aroma attributes.

Conclusions

Hot-air drying changes the composition of fresh bell peppers dramatically. The decrease of volatile compounds with fruity, green, vegetable-like, and floral odours seems to be reflected in the decreased intensity of the corresponding aroma attributes after drying. The increase of volatile compounds with cacao, spicy, sweaty, rancid and sweet, caramel-like odour notes during drying, may be correlated with some of the typical aroma attributes of dried bell peppers like savoury, rancid/sweaty, and sweet/sickly, and hay-like aroma.

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Chapter 8

Modelling of Lipid Oxidation, Strecker Degradation, and Maillard Reaction Products during Hot-Air Drying of Bell Peppers (*Capsicum annuum*)[1]

Abstract

Green fruits of *Capsicum annuum* cv. Mazurka were dried during different times and at different temperature conditions. The complex behaviour of several lipid oxidation, Strecker degradation, and Maillard reaction products during drying could be described by four distinct kinetic mechanisms. The model "First Order Reaction at constant Temperature" (FORT) explained over 90% of the time and temperature dependence of Maillard reaction products, loss of ninhydrine reactive substances, and formation of Strecker degradation volatiles. The time and temperature dependence of the disappearance of several enzymatically formed volatiles could be described with the model "Dislocated Exponential Enzyme Decay" (DEED), which was based on first-order kinetics of enzyme inactivation during heating and on first-order kinetics of enzymatic formation of volatiles upon tissue disruption. The model "COnsecutive First-order Reaction" (COFR) was based on first-order formation and subsequent first-order decrease of volatiles. This model explained only a part of the time and temperature dependence of volatile compounds derived from autoxidation of lipids. The combination model COFR-FORT, explained more than 80% of the time and temperature dependence of several processes which determined, for example the amounts of 1-penten-3-one and hexanal during drying.

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[1] Part of this chapter will be submitted in *J. Sci. Food Agric.* Authors: P.A. Luning, L.M. M. Tijskens, S. Sakin, and J.P. Roozen.

Introduction

A wide variety of dehydrated vegetables are used as ingredients for dried salad dressings, convenience foods, instant soups, and flavoured pasta and rice (Feinberg, 1973). Because of their distinct colour, pungency, and flavour, fruits of *Capsicum annuum* and *C. frutescence* are among the most widely used vegetables in the preservation industry (Govindarajan, 1985; Govindarajan et al., 1987). Chemical and enzymatic reactions during hot-air drying affect quality parameters such as colour, nutritional value, taste, and aroma.

One of the major processes influencing these parameters is the Maillard reaction. The change in colour of *Capsicum* fruits during drying is due to brown compounds formed by the Maillard reaction (Ramakrishnan and Francis, 1973). The loss of essential amino acids and proteins after heating is ascribed to the participation of free α-amino groups in the Maillard reaction and Strecker degradation (Hodge, 1967; Vernin and Parkanyi, 1982; Chiou et al., 1991; Roe and Faulks, 1991; Tsai et al., 1991). Volatile compounds are affected also by the Maillard reaction and Strecker degradation. Some of these volatile compounds have been detected in dried fruits of several *Capsicum* varieties (Wu et al., 1986; Van Ruth and Roozen, 1994; Wilkins, 1994; Luning et al., 1995b).

The autoxidation of unsaturated fatty acids changes probably the amounts of volatiles during heating as well (Grosch, 1982; Frankel 1991). Luning et al. (1995b) suggested that this process could be responsible for the increase of several unsaturated aldehydes after hot-air drying of bell peppers.

Moreover, drying can have a considerable effect on the activity of enzymes involved in the formation of volatile compounds. The loss of sulphur compounds in *Allium* species during heat treatments is ascribed to the inactivation of the enzyme alliinase (Freeman and Whenham, 1975; Wijaya et al., 1991; Leino, 1992). Several volatile compounds were assumed to be formed by lipoxygenase, hydroperoxide lyases, and other enzymes upon tissue disruption of bell peppers (Wu and Liou, 1986; Luning et al., 1995a). The lower amounts of some volatile compounds after hot-air drying might be due to inactivation of these enzymes (Luning et al., 1995b).

The increased consumption of dehydrated vegetables stimulated not only the economical optimization of the drying process, but the optimization of quality parameters such as nutritional value, colour, taste, and aroma as well. The control of quality parameters requires more knowledge about the effects of drying conditions on those compounds that determine these parameters.

The aim of this study was to examine the effects of drying time and temperature on the changes of several lipid oxidation and strecker degradation volatiles, the loss of Ninhydrine Reactive Substances (NRS), and the formation of Maillard Reaction Products (MRP) in green bell peppers. The dependence of these compounds on drying time and temperature was modelled, which was based on possible kinetic mechanisms involved.

Materials and methods

Bell pepper fruits

Bell peppers of *C. annuum* cv. Mazurka were grown on glass wool in a greenhouse at controlled conditions of temperature (about 20°C) and of relative humidity (75%). The fruits were harvested in the green stage 6 weeks after fruit set. The bell peppers were stored at 13°C for 7 days at the most.

Preparation of dried bell peppers

Six fresh green bell peppers were cleaned. Top and bottom parts, and seed lobes were removed. The fruits were cut into pieces of 1 cm^2 (about 0.5 cm thickness). A portion of 400 g of sliced bell peppers were stacked in a 3 cm layer in a cylindrical hot-air pass-through dryer (Retsch BV Ochten, The Netherlands). To obtain a uniform distribution of drying air, the bottom of the dryer was covered with a 2 cm layer of glass beads. The loss of weight was continuously measured with a balance (Mettler HK160) connected to the dryer. The temperature of the drying air and of the product were measured with temperature sensors. Temperatures and loss of weight were recorded automatically with a data logger. Drying experiments were carried out at temperatures 60, 80, and 100°C. The drying process was terminated at 8 different times. Each combination of time and temperature started with a new portion of 400 g of fresh sliced bell peppers. The experiments were conducted at random. Each time and temperature condition was carried out twice.

Immediately after drying, the dried samples were precooled at -20°C for 10 minutes and then stored at 4°C until analysis of volatile compounds. The remaining parts were stored at -20°C (1 month at the most) for the analysis of MRP and NRS.

Isolation of volatile bell pepper compounds

Volatile compounds were isolated by dynamic headspace analysis using Tenax TA (100 mg 35/60 mesh) as adsorbent (Luning et al., 1994).

Isolation of volatiles of fresh bell peppers. Six fresh bell peppers were cleaned and sliced as described for the drying process. Portions of 20 g were homogenised with 50 mL of water in a Waring blender. The headspaces of the bell pepper slurries were flushed with purified nitrogen (30 $mL.min^{-1}$), for 1 h while the volatile compounds were trapped on Tenax TA (3 replicates).

Isolation of volatiles of dried bell peppers. The masses of the dried samples used in these experiments were calculated in order to contain the same dry matter content as for the fresh product. The samples were then rehydrated with water (10 min 80°C) to obtain the same total weight as for fresh product (20 g). The rehydrated samples were cooled on ice to room temperature and homogenised with 50 mL of water in a Waring blender. Volatiles were isolated as described for the fresh samples (3 replicates).

Gas chromatography (GC) and GC-mass spectrometry

Volatile compounds were desorbed thermally from the Tenax tubes for 10 min at 200°C and injected (Chrompack TCT injector 16200, Chrompack, Bergen op Zoom, The Netherlands) on a capillary CP-Wax CB52 column (50 m x 0.32 mm i.d., 1.5

μm film thickness, Chrompack). The oven temperature increased with 2°C.min^{-1} between 40 and 150°C, and with 10 °C.min^{-1} between 150 and 250°C. Volatile compounds were identified by using a GC–MS (Carlo Erba, Mega 3600, QMD 8000, Interscience) equipped with a thermal desorption unit (Carlo Erba, Tekmar 5010, automatic desorber, Interscience) as described previously (Luning et al., 1994). Thermal desorption (10 min at 200°C) and GC column conditions were identical to those for gas chromatography. Electron impact mass spectral (EI-MS) analysis was carried out at an ionization energy of 70 eV.

Analysis of Maillard Reaction Products (MRP)

Extraction of fresh bell peppers. A portion of 5 g of fresh green bell pepper (2 replicates) was homogenised with 10 mL of sodium citrate buffer (pH 5.2) for 2 min using an Ultra Turrax (Ika Labortechnik, T25, Janke & Kunkel) (2 replicates). The slurry was centrifuged (Survey instruments) at 20000 rpm for 15 min and the clear supernatant was used for analysis. The absorbance of an aliquot of 1 mL was measured at 400 nm (Uvikon 810P, Kontron Instruments). Controls were run with 1 mL of water.

Extraction of dried bell peppers. Dry matter of the thawed dried bell peppers was determined (Luning et al., 1994). Weight of the dried samples used for extraction was calculated to contain the same dry matter content as for the fresh product. Samples were rehydrated with water (10 min 80°C) to obtain the same total weight as for fresh product (5 g). Extraction and analysis of brown pigments was conducted as described for the fresh samples (2 replicates).

Analysis of Ninhydrine Reactive Substances (NRS)

The same extracts, as prepared for MRP, were analysed for NRS using the ninhydrin colour test. The ninhydrin reagents were prepared as described by Colowick (1955). For each test 0.2 mL of clear supernatant of the bell pepper extract was mixed with 2 mL of sodium citrate (pH 5.2) and 0.2 mL of ninhydrin reagent in a glass tube. The tubes were covered with aluminum foil and heated for 5 min in boiling water. The absorbance at 570 nm was measured after cooling of the extracts on ice for 15 min (Uvikon 810P, Kontron Instruments). Control samples were prepared without bell pepper extracts added.

Description of the models

During hot-air drying of bell peppers, compounds are formed and/or disappear due to chemical reactions and/or inactivation of enzymes forming volatile compounds (Luning et al., 1995b). Four models were developed based on general mechanisms to describe the complex processes during hot-air drying.

Temperature dependence of reaction rates

In each model discussed, the reaction rate (k_d, k_f, $k_{d,1}$, $k_{d,2}$) is assumed to depend on temperature according to Arrhenius' law:

$$k_i = k_{i,ref}\, e^{\frac{E_i}{R_{gas}} \cdot \left(\frac{1}{T_{ref}} - \frac{1}{T_{abs}}\right)} \tag{1}$$

where i = index for any reaction, k_{ref} = the reaction rate constant at reference temperature (T_{ref} fixed at 353 K), E represents the energy of activation, R_{gas} = the gas constant, and T_{abs} = the absolute temperature. Units for parameters in each equation can be found in list of symbols.

Model FORT

The model FORT (First Order Reaction at constant Temperature) is based on a general first order mechanism, where C_d is the degradation product of compound C and k is the reaction rate, as shown in eq. 2:

$$C \xrightarrow{k} C_d \qquad (2)$$

A general analytical solution at constant temperature assuming first order kinetics can be deduced, which resulted in our model FORT; eq. 3:

$$C(t) = C_{fix} + C_{var}\, e^{(-k\, t)} \qquad (3)$$

in which C(t) = the amount of compound C at any drying time t, C_{fix} = the invariable part of compound C, and C_{var} = the variable part of compound C, k = the reaction rate constant. In eq. 3, a positive k represents a degradation (k_d) and a negative k represents a formation (k_f) reaction.

Model DEED

The model DEED (Dislocated Exponential Enzyme Decay) is based on the hypothesis that some volatile bell pepper compounds are formed by enzymes upon tissue disruption (Wu and Liou, 1986; Luning et al., 1994, 1995a). The decrease of the amounts of volatile compounds detected may be due to inactivation of these enzymes during drying. The enzymatic formation of a volatile compound from a precursor upon tissue disruption, and the inactivation of an enzyme during drying can be represented as follows:

$$Enz + C_p \xrightarrow{k_f} C + Enz \qquad (4)$$

$$Enz \xrightarrow{k_d} Enz_{na} \qquad (5)$$

where Enz is the active enzyme, C_p is the precursor for compound C, k_f is the reaction rate of the formation of volatile compound C upon tissue disruption, k_d is the reaction rate of enzyme degradation, and Enz_{na} is the inactivated enzyme.

Though Michaelis-Menten kinetics may be more appropriate to describe the enzymatic formation of volatiles, the specific conditions and simplifications tend to converge a cascade of the linear part of Michaelis-Menten kinetics into kinetics that can be adequately described by first-order kinetics (Tijskens et al., 1994). Therefore, first-order kinetics were applied to deduce the differential equations (eqs. 6 and 7).

$$\frac{dC(t_i)}{dt_i} = S(t_i)\ k_f\ Enz(t) \tag{6}$$

$$\frac{dS(t_i)}{dt_i} = -S(t_i)\ k_f\ Enz(t) \tag{7}$$

where t_i = time of isolation of volatiles (60 min), $C(t_i)$ = the amount of volatile compound C at time t_i, $S(t_i)$ = the amount of substrate at time t_i, k_f = the reaction rate of the enzymatic formation of volatile compound C, t = time of drying, and Enz(t) = the amount of enzyme at time t.

The thermal inactivation of lipoxygenase has been previously described by first-order kinetics (Svensson and Eriksson, 1972, 1974; Park et al., 1988). Hence enzyme inactivation during hot-air drying can be described by the following differential equation (eq. 8).

$$\frac{dEnz(t)}{dt} = -k_d\ Enz(t) \tag{8}$$

where t = time of drying, Enz(t) = the amount of enzyme at time t, and

k_d = the reaction rate of enzyme inactivation.

Analytical integration of eq. 8 at constant temperature between time 0 and time t yields:

$$Enz(t) = Enz_0\ e^{(-k_d\ t)} \tag{9}$$

where Enz_0 = the initial enzyme concentration at drying time t=0.

Substitution of eq. (9) in eq. (7), considering that the amount of enzyme at the start of isolation of volatiles is determined by the amount of active enzyme remaining after drying (Enz(t)) results in:

$$\frac{dS(t_l)}{dt_l} = - Enz_0\, S(t_l)\, k_f\, e^{(-k_d\, t)} \qquad (10)$$

Analytical integration of eq (10) at constant temperature between time 0 and time t results in:

$$S(t_l) = e^{\left(-k_f\, e^{(-k_d\, t)}\, Enz_0\, t_l\right)}\, S_0 \qquad (11)$$

As the total mass of $C(t_l)$ and $S(t_l)$ at any time is equal to $S_0 + C_0$, the solution for $C(t_l)$ at constant temperature yields our model DEED:

$$C(t_l) = S_0 \left(1 - e^{\left(-Enz_0\, k_f\, e^{(-k_d\, t)}\, t_l\right)}\right) + C_0 \qquad (12)$$

This is the well known Gompertz curve, which is often empirically used in modelling of various types of microbial growth processes (France and Thornley, 1984).

Model COFR

The COnsecutive First-order Reaction (COFR) model is based on the observation that during hot-air drying several processes may occur simultaneously (Luning et al., 1995b). Volatile compounds can be formed by one process, whereas they may be simultaneously degrade by another process. This combined action can be presented in a chemical reaction scheme as follows:

$$C_p \xrightarrow{k_f} C \xrightarrow{k_d} C_d \qquad (13)$$

From the basic laws of chemical kinetics the differential equations for $C_p(t)$ and $C(t)$ can be obtained:

$$\frac{dC_p(t)}{dt} = -k_f\, C_p(t) \tag{14}$$

$$\frac{dC(t)}{dt} = -k_d\, C(t) + k_f\, C_p(t) \tag{15}$$

where t = time of drying, $C_p(t)$ = the amount of precursor of volatile compound C at any time t, k_f = the reaction rate of formation of volatile compound C, C(t) = the amount of volatile compound C at any time t, and k_d = the reaction rate of degradation.

Analytical integration of eqs. 14 and 15 at constant temperature between time 0 and time t yields eq. 16:

$$C_p(t) = C_{p,0}\, e^{(-k_f\, t)} \tag{16}$$

and our model COFR eq 17:

$$C(t) = C_0\, e^{(-k_d\, t)} + C_{p,0}\, k_f\, \frac{\left(e^{(-k_f\, t)} - e^{(-k_d\, t)}\right)}{k_d - k_f} \tag{17}$$

Model COFR-FORT

This model is a combination of the models COFR and FORT to describe the nett outcome of volatile compounds, which can be formed and/or degraded by different processes:

Degradation process 1

$$C_1 \xrightarrow{k_{d,1}} C_{d,1} \tag{18}$$

Formation process and degradation process 2

$$C_{p,2} \xrightarrow{k_f} C_2 \xrightarrow{k_{d,2}} C_{d,2} \tag{19}$$

where $k_{d,1}$ = the reaction rate of degradation process 1, $C_{d,1}$ = the reaction product of volatile compound C_1, $C_{p,2}$ = the precursor of volatile compound C_2, k_f = the reaction rate of formation of volatile compound C_2, $C_{d,2}$ = reaction product of volatile compound C_2, $k_{d,2}$ = reaction rate of degradation process 2. The total amount of volatile compound C experimentally measured consists than of both the amounts of C_1 and C_2.

Using fundamental kinetics, the two reactions (eqs. 18 and 19) can be converted in a set of differential equations assuming first-order kinetics for all reactions:

$$\frac{dC_1(t)}{dt} = -k_{d,1} C_1(t) \tag{20}$$

$$\frac{dC_2(t)}{dt} = k_f\, C_{p,2}(t) - k_{d,2}\, C_2(t) \tag{21}$$

$$\frac{dC_{p,2}(t)}{dt} = -k_f\, C_{p,2}(t) \tag{22}$$

The analytical integration between time is 0 and t at constant temperature, and subsequent substitution, whereby $C_1(t) + C_2(t) = C(t)$, yields the model COFR-FORT; eq 23:

$$C(t) = C_{1,0}\, e^{(-k_{d,1}\, t)} + C_{2,0}\, e^{(-k_{d,2}\, t)} + \frac{C_{p,2,0}\, k_f \left(e^{(-k_f\, t)} - e^{(-k_{d,2}\, t)}\right)}{k_{d,2} - k_f} \tag{23}$$

which is the exact additive combination of both the models COFR and FORT.

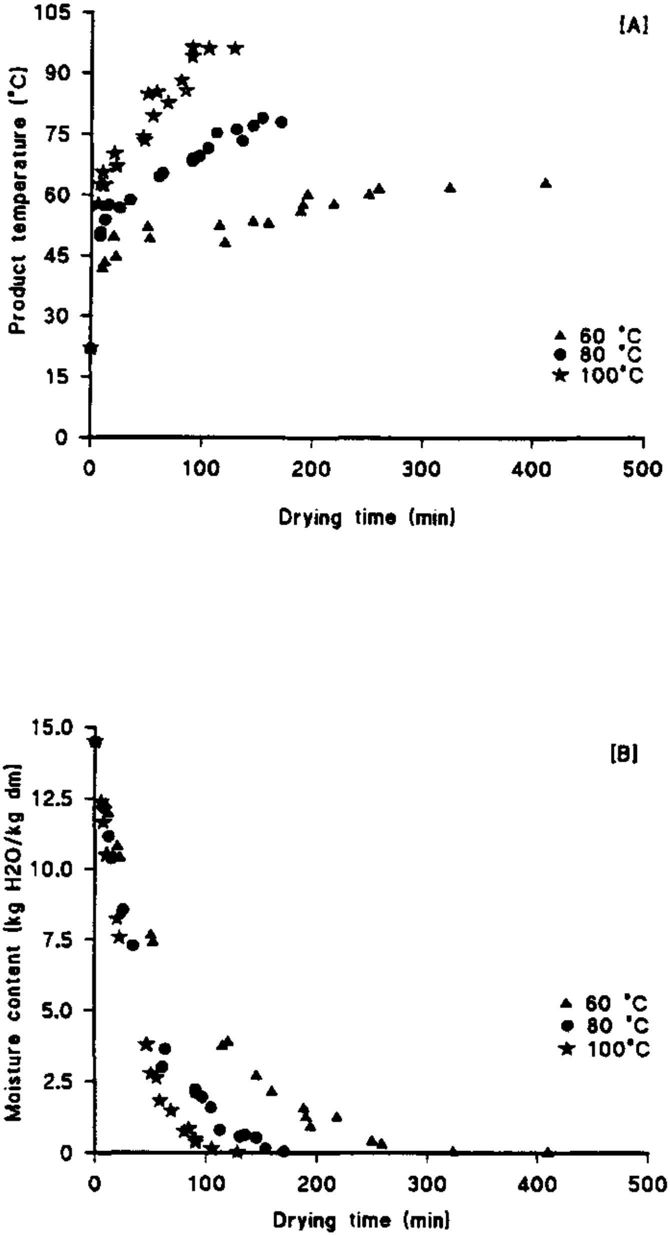

Figure 1. Changes in profiles of product temperature [A] and of moisture content [B] during hot-air drying at different temperatures. For product temperature [A], each point is the mean value of two replicates [A] and for the moisture content [B] it is one value.

Statistical Analysis

Using nonlinear regression (GENSTAT statistical package, Rothamstead, UK) the measured data of all combinations of drying time and temperature were analysed in a integral set for each of the models mentioned. No transformations were carried out on the data, in order to calculate the correct deviation by the statistical procedure (Ross, 1990; Tijskens, 1993). In model DEED the parameters Enz_0 and k_f occur always together and in the statistical analyses they appear as one parameter. Therefore, the value of Enz_0 is fixed to 1 and k_f contains the information of the combined parameters.

Results and discussion

Raw Data

Green bell peppers were dried at different time and temperature conditions. At the end of each experiment, temperature and weight of the samples were measured. MRP, NRS, Strecker degradation volatiles, and several lipid oxidation volatiles were determined in the samples, after adjusting of the dry matter contents as found for the fresh samples.

Temperature and moisture content. Figure 1 A shows the increase of product temperature of green bell peppers as a function of time at different temperatures. The loss of moisture as function of time at different drying air temperatures are shown in Figure 1 B. The temperature and moisture profiles are in agreement with the theories of heat and moisture transfer during heat treatments (Carslaw and Jeager, 1959; Luikov, 1968; Rizvi, 1986). The latter author distinguished two major periods, which are typical for air-drying, i.e., the initial constant rate period, in which drying occurs as if pure water is being evaporated, and the falling rate period, in which moisture content is controlled by internal resistance. These periods are reflected in the steep decrease of the curves at the initial stage of bell pepper drying (constant rate) and the more gradual decrease and flattening at the end of the drying curves (falling rate).

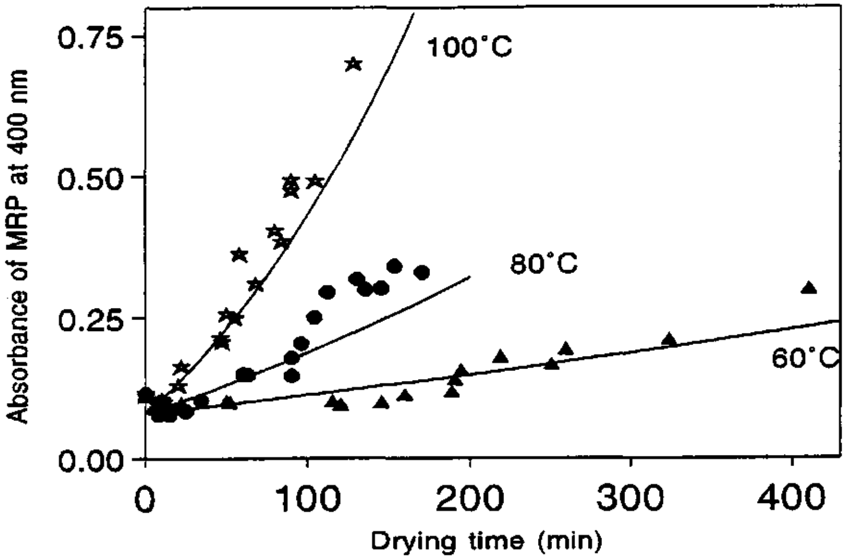

Figure 2. Increase of MRP during hot-air drying at different temperatures. Each point is the mean value of two replicates. Symbols are measured values and lines are simulated data according to the model FORT (eq. 3).

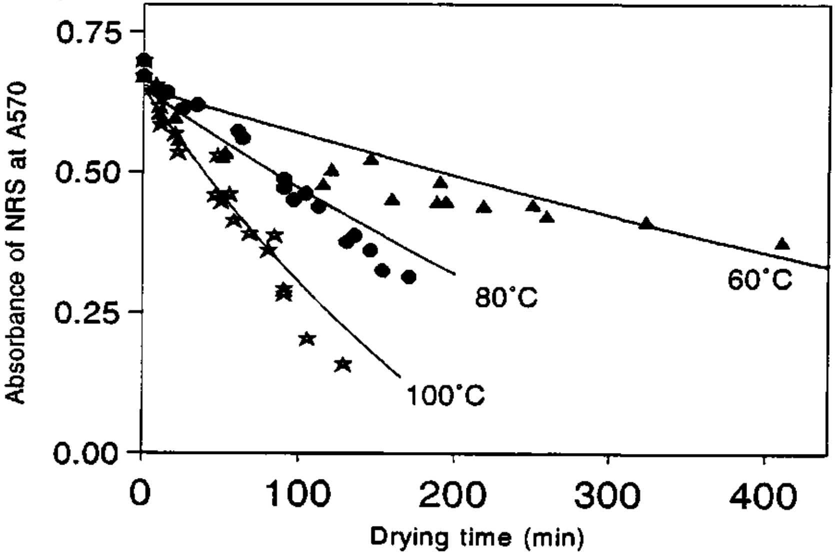

Figure 3. Decrease of NRS during hot-air drying at different temperatures. Each point is the mean value of a two replicates. Symbols are measured values and lines are simulated data according to the model FORT (eq. 3).

Maillard Reaction Products and Ninhydrine Reactive Substances. Figure 2 shows the increase of MRP as a function of drying time at different temperatures. The fresh samples showed absorbance at 400 nm, which might be due to the presence of low amounts of carotenoids. Ramakrishnan and Francis (1973) expressed the increase of the absorbance at 400 nm by MRP in red bell peppers as a ratio of the initial absorbance. They reported that the absorbance was 3 times higher after drying the bell peppers at 125°C for 60 min. In our study, the absorbance at 400 nm of the green bell pepper samples was 5 times higher after 120 minutes drying at the 100°C.

Figure 3 shows the decrease of NRS as function of drying time at different drying air temperatures. Warren and Labuza (1977) reported similar profiles for the loss of lysine in model systems. The loss of NRS was ascribed to their reaction with reducing sugars for the formation of brown pigments, and their reaction with α-dicarbonyl compounds in the Strecker degradation reaction (Hodge, 1967; Vernin and Parkanyi, 1982; Chiou et al., 1991).

Strecker Degradation Volatiles. Vernin and Parkanyi (1982) reported that 2- and 3-methylbutanal can be formed by Strecker degradation of isoleucine and leucine respectively. Luning et al. (1995b) demonstrated that these compounds had distinct odour notes, like cacao, sweaty, and rancid. They suggested that the formation of these compounds might have a considerable effect on the aroma of dried bell peppers. The increase of 3-methylbutanal as function of drying time at different temperatures is presented in Figure 4; a similar profile was obtained for 2-methylbutanal (not shown). Baltes and Song (1994) demonstrated that the amount of 3-methylbutanal increased sharply at the end of the baking period of white bread. Figure 4 shows that the formation of volatiles in the Strecker degradation started after approximately 75, 100, and 200 min drying at temperatures of 100, 80, and 60°C, respectively. Interestingly, the moisture content at these drying times was about 1.25 (kg $H_2O.kg^{-1}$ dm) for all drying temperatures (Fig. 1 B). Although, the Strecker degradation seemed to start at a certain low moisture content, it can not be deduced from these data whether the rapid increase was caused by the lower moisture content or it was merely the expression of a parallel process.

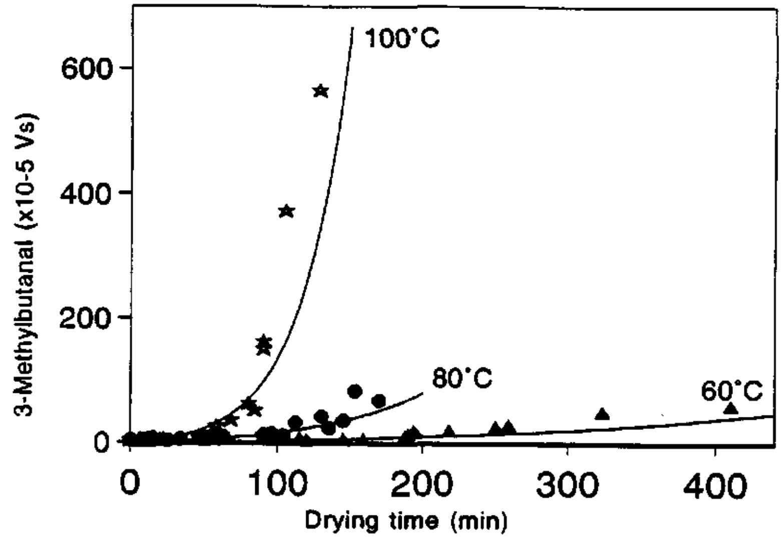

Figure 4. Increase of 3-methylbutanal during hot-air drying at different temperatures. Each point is the mean value of a two replicates. Symbols are measured values and lines are simulated data according to the model FORT (eq. 3).

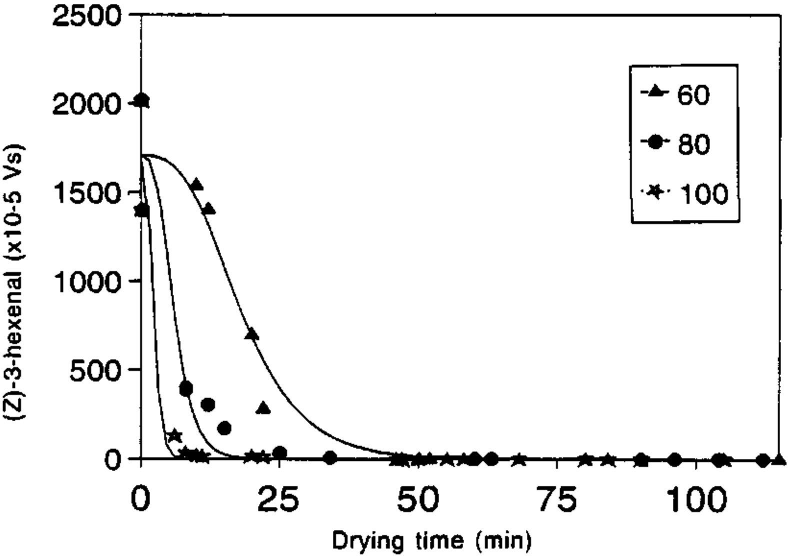

Figure 5. Decrease of (*Z*)-3-hexenal during hot-air drying at different temperatures. Each point is the mean of three replicates. Symbols are experimental values and lines are simulated data according to model DEED (eq. 12).

Volatile compounds from unsaturated fatty acids. Several volatile compounds of fresh bell peppers originated from oxidation of unsaturated fatty acids. Luning et al. (1995a) reported that for example (*Z*)-3-hexenal was enzymatically formed from linolenic acid upon tissue disruption. (*Z*)-3-hexenal had characteristic "green" odour notes and was assumed to be an important compound of fresh green bell peppers (Luning et al., 1994). Figure 5 shows the decrease of (*Z*)-3-hexenal as function of drying time at different temperatures. Obviously, the amount of (*Z*)-3-hexenal decreased to zero within 50 minutes of drying, depending on the drying air temperature. Similar drying profiles were obtained for other volatiles, which are enzymatically formed such as, (*E*)-2-pentenal, (*Z*)-2-pentenal, (*Z*)-3-hexenol, and hexanol (not shown). The latter two compounds have typical "green" odour notes (Luning et al., 1994). Presumably, the decrease of these compounds is not due to evaporation but to inactivation of enzymes, as these compounds are formed upon tissue disruption of the bell pepper samples after drying.

However, not all volatile compounds, which were assumed to be formed from unsaturated fatty acids, decreased during drying. Figure 6 indicates the changes of 1-pentanol as function of drying time at different temperatures. Initially, the amount of 1-pentanol increased markedly but decreased gradually to the end of the drying process. Similar patterns were observed for 2-pentylfuran, (*E*)-2-octenal, and (*E*)-2-heptenal (not shown). The latter three compounds were ascribed to the enzymatic oxidation of linoleic acid in fresh bell peppers (Luning et al., 1995a). However, Eriksson et al., (1973) and Badings (1970) reported that 1-pentanol, 2-pentylfuran, (*E*)-2-heptenal, and (*E*)-2-octenal, amongst others, could be derived from autoxidized linoleic acid as well. Therefore, the increase of the amounts of these compounds could be caused by autoxidation during drying of the bell pepper samples. The subsequent decrease may be due to evaporation and/or degradation reactions (Fig. 6). Grosch (1982) discussed several breakdown reactions of lipid derived volatiles. *A major path is the reaction of aldehydes with free amino groups* to Schiff's bases, which can be subsequently transformed into brown pigments.

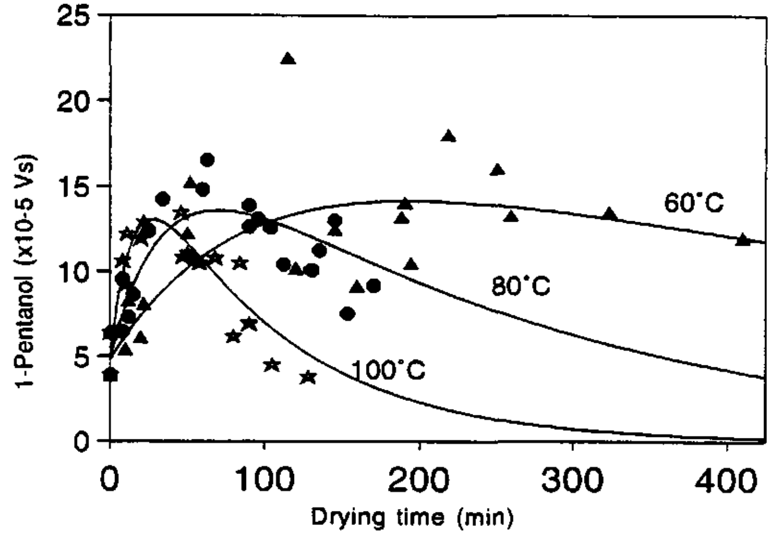

Figure 6. Changes of 1-pentanol during hot-air drying at different temperatures. Each point is the mean of three replicates. Symbols are experimental values and lines are simulated data according to model COFR (eq. 17).

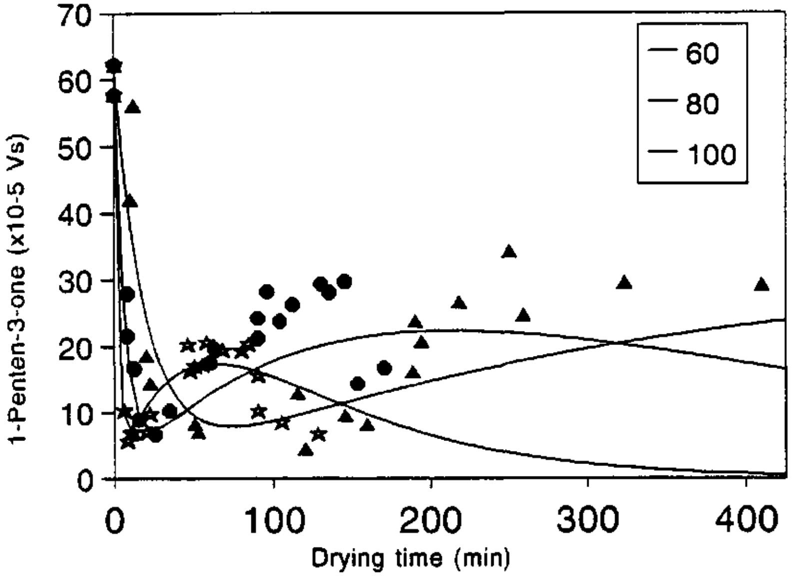

Figure 7. Changes of 1-penten-3-one during hot-air drying at different temperatures. Each point is the mean value of a two replicates. Symbols are experimental values and lines are simulated data according to model COFR-FORT (eq. 23).

As shown in Figure 7, some other lipid derived volatiles, such as 1-penten-3-one, exhibited a more complex behaviour during drying at different temperatures. Similar results were obtained for hexanal (not shown). Luning et al. (1995b) suggested that the final amount of these compounds after hot-air drying may be due to the net effect of different processes. The formation of hexanal and 1-penten-3-one in bell peppers was ascribed to the enzymatic breakdown of linoleic and linolenic acid, respectively (Luning et al., 1995a). The same volatiles, however, have been detected in autoxidation experiments as well (Badings, 1970; Grosch, 1982). Therefore, the initial rapid decrease was possibly caused by enzyme inactivation (Fig. 7), while the consecutive increase may be due to increased autoxidation of unsaturated fatty acids followed by degradation as discussed above (Fig. 6).

Statistical data analysis based on four kinetic models

The formation of MRP and the loss of NRS have already been modelled frequently, as reviewed by Labuza and Saltmarch (1981). Studies with regard to the effects of drying on volatile compounds were only scarcely published and mainly focused on evaporation and retention of volatiles in model systems containing only a few compounds (Flink and Karel, 1970; Chirife et al., 1973; Bangs and Reineccius, 1981; King, 1988). Green bell peppers were used in our study, which are much more complex, as a food, than the model systems mentioned. Moreover, it was focused on the effects of (bio)chemical changes on the composition of volatiles due to drying, rather than on the physical effects of evaporation.

The raw data were analysed statistically, and based on four kinetic models developed. The integral set of data (each combination of time and temperature) was subjected to nonlinear regression, which explained simultaneously several variables related to drying time and temperature. The temperature of the product should be used for exact analysis of the data. However, this temperature increased over a large range in the initial stage of drying (Fig. 1 A).

Table 1. Parameter estimates[a] obtained with model FORT for NRS, MRP, and Strecker degradation volatiles 2 and 3-methylbutanal.

	Estimates				
Component	C_{fix}[b]	C_{var}	k_{ref}	E	R^2_{adj}
NRS	-0.53	1.18	0.00164	41.1	90.7
MRP	-0.40	0.48	-0.00205	54.1	94.7
2-Methylbutanal	-9.35	4.72	-0.01372	48.0	92.6
3-Methylbutanal	-8.25	6.66	-0.01294	47.5	92.5

	Standard error of estimates			
Component	C_{fix}	C_{var}	k_{ref}	E
NRS	0.69	0.69	0.00113	2.2
MRP	0.18	0.18	0.000613	2.2
2-Methylbutanal	4.99	1.44	0.00134	2.6
3-Methylbutanal	5.63	1.94	0.00124	2.3

[a] Number of data points for estimation of parameters *n*= 60; each point of NRS and MRP was based on 2 replicates, each point of the Strecker degradation volatiles was based on 3 replicates.
[b] Abbreviations are explained in the list of symbols.

The experimental design did not allow to use the correct procedures, which included this dynamic behaviour of product temperature. The eqs. 2, 12, 17, and 23 may only be applied to data assuming constant temperature. Therefore, the measured temperature of drying air was used throughout the analyses to get a proper impression of the validity of the models and the estimated parameters. In fact, the values for the energy of activation (E_i) will be the same, whereas only the values for the reaction rates (k_i) might be affected.

Maillard Reaction Products and Ninhydrine Reactive Substances. The loss of NRS is generally assumed to follow first-order kinetics. The formation of MRP is often described with zero-order kinetics, assuming no induction period (Labuza and Saltmarch, 1981; Stamp and Labuza, 1983). Figure 2, however, shows a kind of induction period in the formation of brown pigments. Therefore, an exponential increase seemed more relevant than linear zero-order kinetics to describe the observed behaviour. The model FORT (eq. 2), based on first-order kinetics, was applied to the data of both NRS and MRP. The parameters estimated are shown in Table 1. The percentage variance accounted for (R^2_{adj}) was 94.7% for the MRP and 90.7% for the NRS. In Table 1, the k_{ref} represents the rate of reaction at the chosen reference temperature (353 K). The negative value for C_{fix} is caused by the combination of the estimated value for C_{fix} and its confidence interval. Therefore, C_{fix} is approximately zero in all cases. The magnitude of activation energy reflects directly the temperature susceptibility of the reaction.

Strecker degradation volatiles. The kinetics of some Strecker degradation volatiles have been described by zero-order kinetics (Hall et al., 1985). However, an induction period seemed to be present for the formation of Strecker degradation volatiles during drying of bell peppers at different temperatures (Fig. 4), similar as the formation of brown pigments (Fig. 2). The parameters estimated by model FORT are shown in Table 1. The percentage variance accounted for (R^2_{adj}) was 92.6% for 2-methylbutanal and 92.5% for 3-methylbutanal. The values for the respective activation energies in Table 1 are well within the range normally encountered for chemical reactions (Thompson, 1982; Stamp and Labuza, 1983; Tsai et al., 1991).

Volatile compounds from unsaturated fatty acids. The formation of volatile compounds from oxidation of unsaturated fatty acids upon tissue disruption includes at least two enzymes i.e., lipoxygenase and a hydroperoxide lyase (Galliard and Matthew, 1977). The decrease of the enzymatically formed volatile compounds during drying of bell peppers has been ascribed to inactivation of these enzymes (Luning et al., 1995b). Modelling of these volatiles should therefore include the inactivation of these enzymes.

Table 2. Parameter estimates [a] obtained with the model DEED of volatile bell pepper compounds derived from enzymatic oxidation of unsaturated fatty acids.

			Estimates			
Component	S_0[b]	$k_{f,ref}$	$k_{d,ref}$	E_d	C_{fix}	R^2_{adj}
(*Z*)-3-Hexenal	1709.8	0.1440	0.4060	55.0	0.0	93.1
(*Z*)-3-Hexenol	137.8	0.0237	0.1957	49.9	1.9	97.9
Hexanol	27.0	0.0299	0.1706	68.0	4.2	94.2
(*Z*)-2-pentenal	30.24	0.1000	0.3277	43.8	0.0	94.2
(*E*)-2-pentenal	22.76	0.0301	0.2410	52.6	0.0	93.2

			Standard error of estimates		
Component	S_0	$k_{f,ref}$	$k_{d,ref}$	E_d	C_{fix}
(*Z*)-3-Hexenal	80.0	0.1020	0.0882	1.7	*
(*Z*)-3-Hexenol	32.0	0.0115	0.0321	0.9	2.9
Hexanol	6.3	0.0184	0.0402	1.7	0.5
(*Z*)-2-pentenal	1.3	0.0547	0.0654	1.4	*
(*E*)-2-pentenal	9.2	0.0330	0.0787	2.4	*

[a] The data with very low values (after about 100 min drying) were excluded from the analysis to avoid excessive effects of these data on the percentage of variance explained. Each data point is the mean of 3 replicates.

[b] Abbreviations are explained in the list of symbols.

Park et al. (1988) reported that the thermal inactivation of two LOX isoenzymes in potato tubers followed first-order kinetics. Each of the isoenzymes had different inactivation rate constants. They assumed that the apparent inactivation of the crude extract and of both reconstituted enzymes could be described by second-order kinetics. However, a combination of two first-order kinetic mechanisms never results in second-order kinetics but in a parallel mechanism. In our opinion, the rate limiting reaction during drying finally determined the apparent mechanism. So, the decrease of enzymatically formed volatiles can be described by a mechanism comprising only one enzyme. In the model DEED (eq. 12), the number of enzymes determining the overall reaction was, therefore, limited to one. The parameters estimated for several enzymatically formed bell pepper volatiles are presented in Table 2. The data with very low values (after about 100 min drying) were excluded from the analysis to avoid excessive effects of these data on the percentage of variance explained, and to restrict the data to the stage were changes occurred. The percentage of variance accounted for (R^2_{adj}) ranged from 93.1 to 97.9 % for the different compounds (Table 2). The majority of volatiles had similar E_d values, which implied a similar temperature dependence. Hexanol seemed to be more susceptible to the drying temperature. Moreover, the reaction rates of enzyme degradation (k_d) varied only by a factor of two. The reaction rates of enzymatic formation of the volatiles (k_f) varied more over the different volatile compounds (Table 2). These results indicate that during drying the same rate limiting factor, e.g., inactivation of LOX, determines the production of the volatile compounds studied.

The volatile compounds, which were probably produced by autoxidation of unsaturated fatty acids, were also analysed. It is generally accepted that the autoxidation of unsaturated fatty acids is based on a free radical mechanism (Labuza, 1971; Grosch, 1982; Nawar, 1985). The autoxidation has been divided into an initiation, a propagation and a termination phase. Kinetics of lipid oxidation in the initial and propagation phase have been described by mechanistic models, and by statistical linear and exponential curve fitting (Labuza, 1971; Hall et al., 1985). Recently, Koelsch et al. (1991) described a mechanistic model for hexanal formation as function of oxygen concentration. They limited their model to the initiation and propagation phase and assumed that the production of hexanal exceeded drastic its degradation by e.g., the Maillard reaction. Figure 6 shows that the decrease of autoxidation volatiles, such as 1-pentanol, appeared to be relevant during drying of bell peppers, whereas the reaction rates of the initiation phase seemed to be negligible. First-order kinetics (model COFR eq. 17) were applied to describe the increase of autoxidation volatiles in the propagation phase and to describe their subsequent degradation. The percentage of variance accounted for (R^2_{adj}) was 74.0, 69.4, 68.2, and 60.8% for 1-pentanol, (*E*)-2-heptenal, 2-pentylfuran, and (*E*)-2-octenal, respectively. These low percentages stresses that the model explained only a part of the behaviour of these compounds and was consequently not adequate. The large variation among data points, however, did not permit the deduction and calibration of a more appropriate model. Moreover, a small difference in the concentration of metal ions in the fresh bell peppers can have a considerable effect on the amount of autoxidation volatiles formed during drying (Frankel, 1991).

The behaviour of 1-penten-3-one and hexanal appeared to be a combination of several processes like enzyme inactivation, autoxidative formation and subsequent degradation. Therefore, models were combined to describe this complex behaviour. Preliminary studies revealed that the model FORT fairly described the rapid decrease of enzymatically formed compounds, whereas the model COFR would describe the consecutive formation and degradation processes. Both models were combined into the model COFR-FORT (eq. 23) and the estimates for the parameters are presented in Table 3. The percentage of variance accounted for (R^2_{adj}) was 80.3% for 1-penten-3-one and 93.0% for hexanal. Table 3 shows that the estimated parameters were very similar for both compounds tested. Only the initial levels of the precurors $C_{1,0}$ and $C_{2,p,0}$ were not the same for both compounds. In fact, the differences in the ratio between $C_{1,0}$ and $C_{2,p,0}$ implicated a marked difference in the relative importance of the formation and degradation process 1 of the respective compounds. In addition, the second degradation process ($E_{d,2}$) was more susceptible to temperature than the first degradation ($E_{d,1}$) and subsequent formation process (E_f). Consequently, the final amount of the volatile odour compounds 1-penten-3-one and hexanal will be higher at lower drying temperatures as illustrated in Figure 7 for 1-penten-3-one.

Conclusions

This study attempted to describe the complex behaviour of lipid oxidation and Maillard related compounds during drying at different time and temperature conditions with four kinetic mechanisms. The model FORT explained over 90% of the time and temperature dependence of browning, loss of free amino groups, and of the formation of Strecker degradation volatiles. The model DEED explained over 90% of the time and temperature dependence of the decrease of several enzymatically formed volatiles. The model COFR explained only part of the time and temperature dependence of volatile compounds generated by autoxidation of lipids, probably due to the large variation among data points. The combination model COFR-FORT, explained more than 80% of the time and temperature dependence of the processes which determined the amounts of 1-penten-3-one and hexanal during drying.

List of symbols

Variable

C : concentration of compound (10^{-5} Vs)
E : energy of activation ($KJ.mol^{-1}$)
k : reaction rate (min^{-1})
t : time (min)
R : gas constant (8.314 $J.mol^{-1}.K^{-1}$)
T : temperature (K)
S : concentration of substrate (arbitrary)
Enz : concentration of enzyme (arbitrary)
dm : dry matter (%)

Indices

var : variable part
fix : invariable part
ref : reference
f : formation
d : degradation
p : precursor
1,2 : process number
i : during headspace isolation
na : not active
0 : initial value at time t = 0

Table 3. Parameter estimates obtained with the combination model COFR-FORT for temperature dependence of bell pepper compounds, formed and degraded by several processes.

					Estimates					
Compound	$C_{1,0}$[a]	$C_{2,0}$	$C_{p,2,0}$	$k_{d,1,ref}$	$E_{d,1}$	$k_{f,ref}$	E_f	$k_{d,2,ref}$	$E_{d,2}$	R^2_{adj}
1-Penten-3-one	61.8	0.0	63.7	0.1574	55.8	0.0045	53.9	0.0049	78.6	80.3
Hexanal	1202.0	0.0	252.4	0.1431	57.0	0.0066	51.8	0.0054	72.4	93.0

[a] Number of data points for estimation of parameters $n = 60$; each point was based on 3 replicates. No standard error of estimates could be obtained from the statistical procedure.

[b] Abbreviations are explained in the list of symbols.

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Chapter 9

General Discussion

Fruits of *Capsicum annuum* and *C. frutescens* are often used in the diet because of their typical colour, pungency, taste, and distinct aroma. The fruits are eaten fresh or processed, as unripe (green) or ripe (e.g., red, yellow, orange, white) peppers. The acceptance of fruits and vegetables by consumers is determined by properties such as, the absence of external defects, size, shape, colour, and flavour. These characteristics are influenced by chemical and biochemical changes that occur during growth and maturation (Eskin, 1982); the maturation stage at which fruits and vegetables are harvested affects as well the quality of the fresh as the final quality of the processed fruits. Up to now breeding programs of *Capsicum* varieties were mainly focused on disease resistance, crop yield, nutritional value like vitamin C content, pungency, and carotenoids levels (Somos 1984; Govindarajan, 1985; Minguez-Mosquera *et al* 1994). However, the growing "fresh" market and the increasing use of dried vegetables in convenience foods, shifted attention towards flavour as a quality parameter for fresh and dried vegetables (Feinberg 1973; Govindarajan et al., 1987; Koller, 1988; Zachariasse and Abrahamse 1993). The elucidation of factors controlling flavour quality of vegetables and fruits requires research on chemical and biochemical changes during maturation and processing. In this thesis the flavour profiles of fresh and dried bell peppers of cv. Mazurka were evaluated and relationships between instrumental and sensory data were examined. Furthermore, the effects of biochemical changes during bell pepper development and the effects of (bio)chemical changes during hot-air drying of bell peppers on the composition of flavour compounds, were investigated.

Flavour of Fresh Green and Red Bell Peppers of cv. Mazurka

Figure 1 presents the flavour profiles of fresh green and red fruits of cv. Mazurka evaluated by the eating panel [A] and smelling panel [B] respectively. Only those attributes, which were significantly different ($P < 0.05$) between the green and red fruits, were included (Chapter 3 and 7). Apparently, bitterness was an important taste aspect of the green fruits, whereas sweetness and sourness were typical for the red bell peppers (Chapter 3). Relationships between bitterness and bitter compounds were not investigated in this thesis. However, some saponins, which can give a bitter perception, have been identified in seeds of *Capsicum annuum* fruits by Tschesche and Gutwinski (1975), and it would be of interest to study their presence in other parts of the fruit and their sensory characteristics.

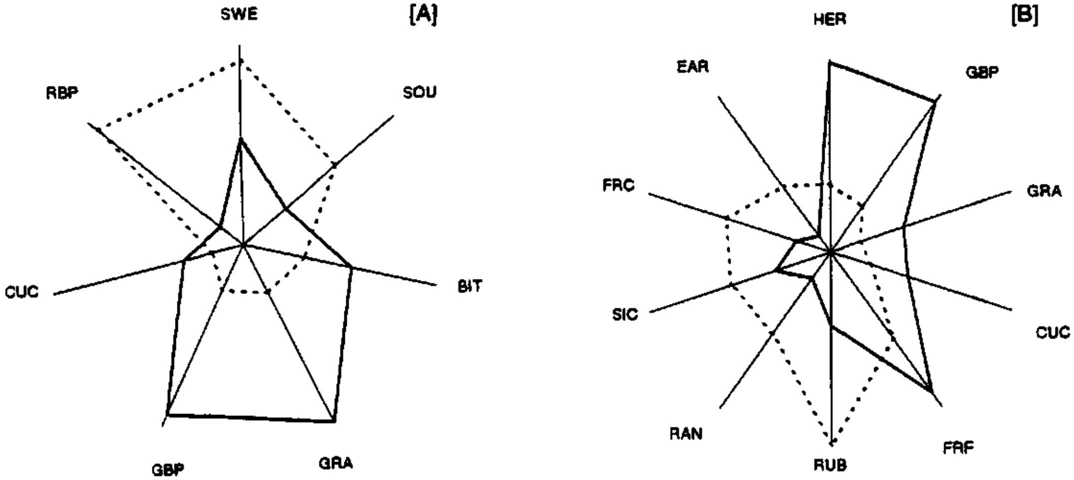

Figure 1. Flavour profiles of fresh green (solid line) and red fruits (interrupted line) of cv. Mazurka evaluated by the eating panel [A] and smelling panel [B]; abbreviations explained in Chapter 7.

Sweetness appeared to be related with the levels of glucose, fructose, and total sugar (Chapter 3), which was in agreement with the concept of sweetness intensity and concentration of sugars as observed in several studies (Amerine *et al.*, 1963; De Graaf, 1988; Shamaila *et al.*, 1992; Dever *et al.*, 1992). Sourness seemed to be related with the total amounts of citric acid and to a lesser extent ascorbic acid. Accordingly, Watada and Aulenbach (1979) and Paterson *et al.* (1991) suggested correlations between perceived sourness and the citric acid content. However, considering the dissociation states of the acids, it appeared that sourness correlated with undissociated ascorbic acid and dissociated citric acid in the -1 and -2 state. Ganzevles and Kroeze (1987) proposed that HCl-sourness (i.e., level of H^+ ions) and the sourness of organic acids (i.e., undissociated state) were caused by different receptor processes. According to their theory, undissociated ascorbic acid might be directly important for the sour perception, while the dissociated forms of citric acid influenced the sourness by increasing the proton concentration. Therefore, not only the total amounts of acids but also their dissociation forms should be considered when studying the effect of organic acids on the sour perception of bell peppers.

Sensory panel evaluation by mouth cq. by nose revealed that green bell peppers had corresponding "green" aroma notes like grassy, green bell pepper, and cucumber (Figure 1 A and B). However, the red bell peppers had no aroma notes in common when evaluated by both panels. Red bell pepper aroma appeared to be typical for the oral perception of the red fruits (Chapter 3), while aroma notes like rubbery, rancid/sweaty, sweet/sickly, fruity/chemical, and musty/earthy dominated the nasal perception of the red fruits (Chapter 7). Comparing the GC-sniffing profiles of green and red bell peppers revealed that mainly volatile compounds with "green" odour notes disappeared upon ripening, whereas only the levels of (*E*)-2-hexenal and (*E*)-2-hexenal, having sweet/almond notes, increased (Chapter 4). Moreover, the PCA results suggested that in the green fruits, the aroma attributes herbal, grassy, green bell pepper, and resinous, correlated with the volatile odour compounds (*Z*)-3-hexenol, hexanol, (*Z*)-3-hexenal, and hexanal having similar odour notes (Chapter 7). For the red fruits only the aroma attribute fruity/chemical seemed to be related with the levels of octanal and 2-heptanone having fruity notes. No clear relationships were observed for the other typical aroma attributes of red bell peppers such as, rubbery, rancid/sweaty, sweet/sickly and musty. Possibly, these aroma attributes were caused by a combination of volatiles at low levels, while in the green fruits the high amounts of volatiles with green notes dominated the flavour perception.

Besides the clear differences between the green and red ripening stages, the different bell pepper samples investigated (three ripening stages, two preparation methods) had several odour compounds in common, i.e., (*Z*)-ß-ocimene (rancid, sweaty), 2,3-butanedione (caramel), 1-penten-3-one (chemical/pungent, spicy), hexanal (grassy), 3-carene (red bell pepper, rubbery), octanal (fruity), and 2-isobutyl-3-methoxypyrazine (green bell pepper) (Chapter 4). Interestingly, all these compounds, except for (*Z*)-ß-ocimene have been detected in the yellow fruits of cv. Kelvin and white fruits of cv. Blondy as well (Chapter 7). Possibly, these compounds are important for the basic flavour of a bell pepper, while additional differences in composition of non-volatile and volatile compounds differentiated the ripening stages and cultivars (Chapters 3, 7, and 8). Belitz and Grosch (1986) divided aroma of food with regard to the presence of character impact compounds (Chapter 1). According to their classification the fresh bell pepper fruits belong to the second group with 3-isobutyl-3-methoxypyrazine as the impact flavour compound supported by several other odour compounds.

As mentioned in Chapter 1, volatile compounds can be formed during ripening (primary volatiles) and/or formed upon tissue disruption (secondary volatiles) in fresh fruit/vegetables. Tissue disruption of bell peppers increased the total amount of volatiles released and it changed the composition of volatile compounds (Chapter 4). Moreover, several of these compounds had distinct odour notes and their composition appeared markedly different for the green and red fruits. The compounds formed upon tissue disruption were ascribed to degradation of unsaturated fatty acids by lipoxygenase and other enzymes (Wu and Liou, 1986). Examination of LOX activity during ripening revealed that the activity decreased markedly between 6 and 8 weeks after fruit set, when colour turned from green to red (Chapter 5). Accordingly, Pinsky et al. (1971) and Minguez-Mosquera et al. (1993) observed that LOX activity in red bell pepper was about 50% lower than in green fruits. The lipid oxidation related volatile compounds investigated in our study could be distinguished in four typical ripening related patterns i.e., gradual decrease, sharp decrease between 6 and 8 weeks, sharp increase (6-8 weeks) and subsequent decrease, and maximum level at 8 weeks (Chapter 5). Addition of specific LOX substrates to homogenates of green and red bell peppers demonstrated the origin of the volatile compounds formed, but also indicated that the activities of the enzymes were different in green and red bell peppers. Possibly, the ripening related patterns of the volatiles were due to the decreased levels of hydroperoxides formed by the lowering in bell pepper LOX activity on one hand, and the changes in activities of other enzymes such as cis/trans isomerase on the other hand. In conclusion, the combined action of enzymes seemed to have a considerable effect on the differences in aromas of green and red bell peppers (Chapters 4, 5, and 7).

Influence of Hot-air Drying on the Aroma Profile of Fresh Green and Red Bell Peppers of cv. Mazurka.

Figure 2 shows the effects of hot-air drying on the aroma profiles of fresh green [A] and red [B] bell peppers (Chapter 7). It is obvious that drying decreased the intensity scores of characteristic fresh aroma attributes of green bell peppers such as, herbal, green bell pepper, grassy, cucumber, and fruity/fresh. In red bell peppers, the means scores of the typical fresh aroma attributes fruity/chemical, rancid/sweaty, and rubbery decreased significantly. GC-sniffing port analysis revealed that in green bell peppers mainly compounds with "green" odour notes disappeared like, unidentified compound 8a (chemical/pungent, spicy/herbal), (*Z*)-3-hexenal (grassy, green bell pepper, lettuce), hexanol (lettuce, spicy), and (*Z*)-3-hexenol (lettuce, green bell pepper, spicy), whereas in red peppers the unidentified compound (17a) with typical red bell pepper, rubbery and geranium odour notes disappeared (Chapter 6).

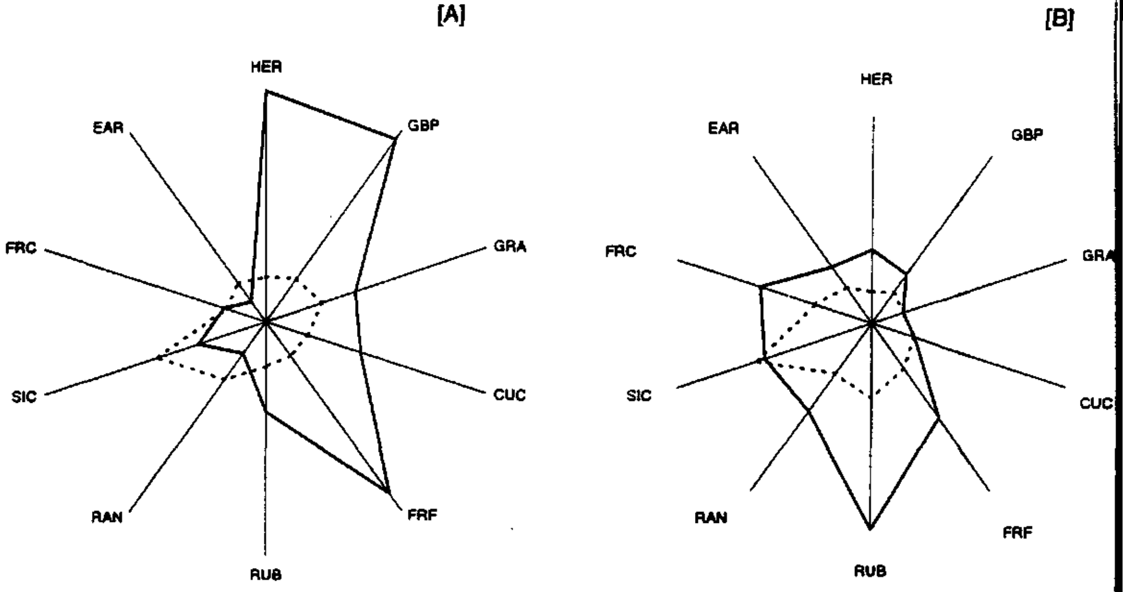

Figure 2. Effects of hot-air drying on the aroma profiles of fresh green [A] and red [B] bell peppers (solid lines=fresh; interrupted lines=dried); abbreviations expleined in Chapter 7.

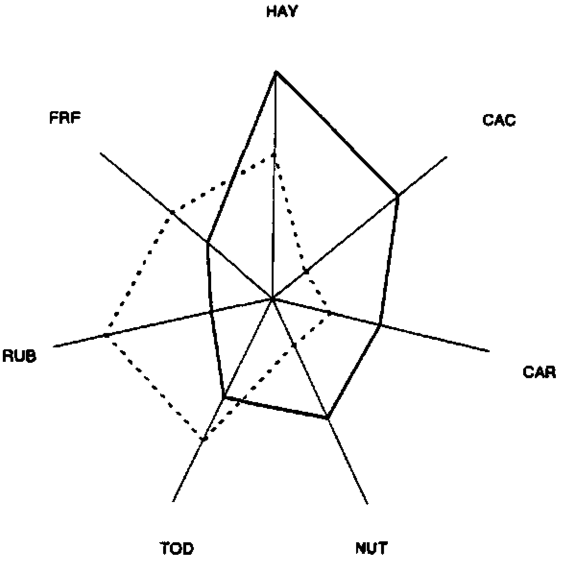

Figure 3. Aroma profiles of dried green (solid line) and red bell peppers (interrupted line) including the significantly different aroma attributes; abbreviations explianed in Chapter 7.

The levels of (*E*)-2-hexenal and octanal having fruity notes decreased in both green and red fruits. However, hot-air drying not only effected the aroma profiles by decreasing the levels of volatile compounds with typical odour notes, but it also changed the characteristic aroma profiles of green and red fruits. Figure 3 shows the aroma profiles of dried green and red bell peppers including only the aroma attributes, which were significantly different between the dried fruits (Chapter 7). Comparison with Figure 1 B revealed that other aroma attributes discriminated the aroma profiles of dried green and red fruits as compared to the fresh fruits. Hay, cacao, caramel, and nutty aroma appeared typical for the dried green bell peppers, whereas dried tomato, fruity/fresh, and rubbery were typical for the red fruits. Additionally, the rubbery aroma was also typical for the fresh red bell peppers. GC-sniffing port evaluation showed that several odour active compounds were formed or increased such as, 2-methylpropanal (cacao, spicy, sweaty), 2,3-pentanedione (fruity, sweet, caramel), 4-octen-3-one (mushroom/fruity), 2- and 3-methylbutanal (cacao, sweaty/rotten) (Chapter 6). Principal component analysis on the combined set of GC-sniffing and sensory data suggested that 2-methylpropanal correlated with the aroma attribute cacao in the dried green fruits (Chapter 7). However, no relationships were observed for the other aroma attributes. Possibly, the combination of volatile odour compounds were responsible for these attributes. Accordingly, Belitz and Grosch (1986) suggested that the aroma of processed food often consisted of a large number of volatile compounds, usually without a key compound. In conclusion, drying not only decreased the intensity of the typical fresh aromas of green and red bell peppers, but it also changed the aroma profile drastically by the production of thermally formed odour compounds (secondary volatiles).

The changes in the composition of volatile compounds during hot-air drying were assumed to be due to several processes like, evaporation, inactivation of volatile forming enzymes, Maillard reaction, Strecker degradation, autoxidation of unsaturated acids or a combination of these processes (Chapter 6). Numerous studies were focused on the evaporation and retention of volatile compounds during drying (Flink and Karel, 1970; Chirife et al., 1973; Mazza and LeMaguer, 1979; Bangs and Reineccius, 1981; King, 1988; Rosenberg et al., 1990). Although, evaporation appeared an important factor in decreasing the levels of volatile compounds, the other processes also had a considerable effect on the levels of several odour active compounds in bell peppers, and consequently had a large influence on the changes in aroma (Chapters 6 and 7). As mentioned in Chapter 1, the control of quality parameters such as, nutritional value, colour, and aroma required more knowledge about the influence of drying conditions on compounds determining them. Therefore, the observed changes of these compounds at different time and temperature conditions was modelled based on four kinetic mechanisms (Chapter 8). The model FORT (First Order Reaction at constant Temperature) explained over 90% of the time and temperature dependence of browning, loss of free α-amino groups, and formation of Strecker degradation volatiles. Based on the energy of activation and reaction rates of the different processes it appeared that at lower drying temperatures browning is more relevant than the formation of 2- and 3-methylbutanal, whereas at higher temperatures the Strecker degradation becomes increasingly important. The model DEED (Dislocated Exponential Enzyme Decay), which was based on the first-order kinetics of enzyme degradation and on first-order kinetics of enzymatic formation of volatiles upon tissue disruption, explained over 90% of the time and temperature dependence of the decrease of several enzymatically formed volatiles. The parameters estimated indicated that a same rate limiting factor during drying, e.g., inactivation of LOX, determined the production of the volatile compounds studied. The model COFR (Consecutive First-order Reaction) was based on first-order formation and subsequent first-order decrease. This model only explained a part of the time and temperature dependence of volatile compounds derived from autoxidation of lipids. The catalytic character of the autoxidation of lipids may be a source of variation, because a small difference in the level of catalysts in the fresh bell peppers could have a considerable effect on the amount of autoxidation volatiles formed during drying. Therefore, the development of these autoxidation volatiles can be cotrolled by more knowledge about the effects of catalysts on the behaviour of these compounds during drying. The combination model COFR-FORT, explained more than 80% of the time and temperature dependence of complex processes, which determined for example the levels of 1-penten-3-one and hexanal during drying. Appearently, the second degradation process was more susceptible to temperature than the first degradation and subsequent formation process. The final levels of the volatile odour compounds 1-penten-3-one and hexanal will be higher at lower drying temperatures. In conclusion, the four kinetic mechanisms yielded information about the time and temperature dependence of several volatiles and Maillard related compounds during drying.

This thesis provided basic knowledge about the flavour of fresh and hot-air dried bell peppers. This knowledge can be used to study effects of, for example, harvest time, growth conditions, sample variation, cultivars, and seasons on the flavour of fresh and/or dried bell peppers. Furthermore, in this thesis a biochemical pathway was proposed for the enzymatic development of bell pepper volatiles, which can be used as basis for detailed studies on the enzymatic formation of bell pepper volatiles. Finally, the complex behaviour of volatile compounds during hot-air drying of bell peppers was described by four different kinetic mechanisms. This kinetic approach may be used to investigate the behaviour of volatiles in other dynamic processes.

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Summary

Fruits of *Capsicum annuum* and *C. frutescens* are commonly used in the diet because of their typical colour, pungency, taste, and distinct aroma. The fruits are eaten fresh or processed, as unripe (green) or ripe (e.g., red, yellow, orange, white) peppers. In the last decade, attention is shifting towards flavour as an important quality parameter for fruits and vegetables. The flavour of fruits and vegetables, as perceived during consumption, is the overall sensation provided by the interaction of taste, odour, mouth feel, sight, and sound. The composition of non-volatile compounds influences mainly the sensory perceived taste, while the aroma is affected by volatile compounds.

In this thesis, the flavour, including taste, aroma, non-volatile and volatile compounds, of fresh and dried bell peppers were evaluated. The major Dutch bell pepper cultivar for export, i.e., *C. annuum* cv. Mazurka, was used as basic cultivar throughout the thesis, others were included for comparison of results.

Instrumental and sensory flavour evaluation of fresh bell peppers

Development of non-volatile bell pepper compounds. In chapter 2, some growth characteristics and changes in compositions of non-volatile compounds (sugars, organic acids) during ripening of cv. Mazurka and cv. Evident were investigated. The development of the fruits showed a growth (between 4-7 weeks after fruit set) and ripening phase (7-10 weeks). During growth, fresh weight increased rapidly, while during ripening the colour turned from green to red, which was reflected in a decreased level of chlorophyll *a* (i.e., green pigments) and an increased level of carotenoids (i.e., orange/red pigments). Two high performance liquid chromatography (HPLC) methods were developed for the analysis of sugars and organic acids in bell peppers. The sugars detected were, sucrose, glucose, and fructose, and the major organic acids detected were ascorbic, citric and malic acid, while oxalic, fumaric, shikimic, and pyroglutamic acid were present at lower

concentrations. The levels of sugars and organic acids showed distinct ripening related patterns, i.e., glucose, fructose, citric and ascorbic acid increased while sucrose and malic acid decreased.

Relationships between non-volatile compounds and taste. Based on these time series, three distinct ripening stages (i.e., green, turning, and red) of cv. Mazurka and cv. Evident were selected for instrumental and sensory evaluation, in Chapter 3. The composition of sugars and organic acids were analysed and the fruits were evaluated by a sensory descriptive panel on flavour attributes, perceived while eating. The flavour of green bell peppers was characterised by bitter taste and grassy, green bell pepper, and cucumber aroma, while red bell pepper aroma, sweet and sour taste were typical for the turning and red fruits. The relationships between sugars and sweetness, and organic acids and sourness, and dissociated organic acids and sourness were examined using principal component analysis (PCA). It appeared that sweetness, which was typical for the ripe stages, closely related to glucose, fructose, total sugar, and dry matter content, while sourness, also characteristic for the ripe stages, closely related to total citric and total ascorbic acid. PCA on calculated undissociated and dissociated acids revealed that, mainly undissociated ascorbic acid and citric acid in the "-1" and "-2" dissociation forms related closely with sourness. Based on these results and the available literature, it was suggested that undissociated ascorbic acid might be directly important for the sour perception, while the dissociated forms of citric acid influenced the sourness by increasing the proton (H^+) concentration.

Development of volatile bell pepper compounds. In chapter 4, the same ripening stages of cv. Mazurka were selected for evaluation of the volatile compounds, for which samples were cut into pieces or completely homogenised. Sixty four compounds were analysed and identified in the headspaces of the different samples by gas chromatography (GC) and GC-mass spectrometry. A trained sniffing port panel detected and characterised 30 odour compounds, of which 21 could be identified. The GC-sniffing profiles of all ripening stages and sample preparations had several odour compounds in common, i.e., 2,3-butanedione (caramel odour), 1-penten-3-one (chemical/pungent, spicy), hexanal (grassy), 3-carene (red bell pepper, rubbery), (*Z*)-ß-ocimene (rancid, sweaty), octanal (fruity), and 2-isobutyl-3-methoxypyrazine (green bell pepper). Furthermore, major ripening and preparation related differences were observed. During ripening from green to red, many volatile compounds having green-related odour notes e.g., (*Z*)-3-hexenol (grassy, lettuce, cucumber), nonanal (mushroom, herbal), 2-*sec*-butyl-3-methoxypyrazine (carrot, lettuce, grassy), linalool (floral, green bell pepper), (*Z*)-3-hexenal (grassy, green bell pepper, fruity), and 1-hexanol (fruity, green bell pepper, herbal), decreased or even disappeared. Only the levels of (*E*)-2-hexenal and (*E*)-2-hexenol, which have almond, fruity, sweet odours, were higher in the turning and red fruits. Disruption of the cell structure by homogenization of the fruits favoured oxidation of unsaturated fatty acids by lipoxygenase and other enzymes, and stimulated the formation of related alcohols, aldehydes, and ketones; several of these compounds had distinct odour notes.

Biochemical aspects of the formation of specific volatile bell pepper compounds. The development and origin of these enzymatic formed volatiles were studied in more detail in Chapter 5. Extracts of green bell peppers of cv. Mazurka were analysed for the presence of lipoxygenase (LOX); a pH-optimum of 5.5-6.0 was obtained and enzyme activity was 100% and 51% inhibited by two specific LOX inhibitors (10 mM of *n*-propyl gallate and 340 µM of 5,8,11,14-eicosatetraynoic acid). During bell pepper ripening, LOX activity decreased 70% between 6 and 8 weeks after fruit set. The composition of enzymatic formed volatiles showed four typical ripening related patterns. The origin of these compounds was investigated by adding specific LOX substrates to homogenates of green and red bell peppers. Addition of linoleic acid to green homogenates increased significantly the levels of hexanal, hexanol, 2-pentylfuran, (*E*)-2-heptenal, (*E*)-2-octenal, (*E*)-2-nonenal, but also (*Z*)-3-hexenal, whereas addition of linolenic acid to the same homogenates increased the levels of (*Z*)-3-hexenal, (*E*)-2-hexenal, (*Z*)-2-hexenal, (*Z*)-2-pentenal, (*E*)-2-pentenal, (*Z*)-2-pentenol, (*E*)-2-pentenol, and 1-penten-3-one; similar qualitative effects were observed in the red pepper homogenates. A biochemical pathway was proposed for the formation of volatile bell pepper compounds upon tissue disruption based on experimental data and comprehensive literature.

Influence of hot-air drying on the instrumental and sensory flavour evaluation of fresh bell peppers

Effects of hot-air drying on the composition of non-volatile and volatile flavour compounds. In chapter 6, the composition of non-volatile and volatile odour compounds were analysed before and after drying of four bell peppers with distinct flavours, i.e., green and red cv. Mazurka, white cv. Blondy, and yellow cv. Kelvin. Hot-air drying affected both the composition of non-volatile and volatile compounds. The levels of glucose, fructose, ascorbic, citric, and oxalic acid decreased, while the levels of sucrose, malic, fumaric, and *cis*-aconitic acid increased. Glucose, fructose, and ascorbic acid possibly participated in the Maillard reaction during heating. The effects of hot-air drying on the composition of volatile bell pepper compounds could be distinguished in three groups i.e., 1) compounds which decreased or disappeared, 2) compounds which increased or were formed, and 3) compounds which showed no clear changes.

The first group represented the majority of volatiles, which apparently evaporated during drying. Most of these compounds were partly retained in the dried bell peppers, but some disappeared completely such as, (*Z*)-2-pentenal, (*E*)-2-pentenal, (*Z*)-3-hexenal, (*Z*)-2-pentenol, (*E*)-2-pentenol, (*E*,*E*)-2,4-hexadienal, and (*E*,*Z*)-2,4-hexadienal. The majority of these compounds were ascribed to the enzymatic breakdown of unsaturated fatty acids upon tissue disruption (Chapter 5). Therefore, it was suggested that the disappearance of these compounds was due to heat inactivation of enzymes more than evaporation during drying. Sniffing port evaluation showed that the compounds, which decreased or disappeared after drying, had mainly "fresh" odour notes like lettuce/grassy/green bell pepper ((*Z*)-3-hexenal), fruity/almond ((*E*)-2-hexenal), fruity (octanal), lettuce/green bell pepper ((*Z*)-3-hexenol), and grassy/green bell pepper ((*Z*)-2-hexenal).

The second group included compounds which increased or were formed during drying. The increased levels of 4-octen-3-one, (*E*)-2-heptenal, (*E*)-2-octenal, (*E*,*Z*) and (*E*,*E*)-2,4-heptadienal, decanal, and (*E*)-2-nonenal were possibly due to autoxidation of unsaturated fatty acids. Whereas, the increased levels of 2-

methylpropionic and 2- and 3-methylbutyric acid, 2-methylpropanal, and 2- and 3-methylbutanal seemed to be due to Strecker degradation. Sniffing port evaluation showed that the latter three compounds had distinct cacao, sweaty, and spicy odour characteristics.

Changes of the aroma profile of fresh bell peppers due to hot-air drying. In chapter 7, the effects of hot-air drying on the fresh aromas of the four different bell peppers were investigated in more detail. The percentage composition of odour active compounds in the fresh and dried samples were analysed, and the fruits were evaluated by a sensory descriptive panel on aroma attributes, as perceived while smelling. On one hand, hot-air drying decreased the intensity scores of several characteristic fresh aroma attributes. For example, in the green and white fruits, mainly “green” attributes like herbal, grassy, green bell pepper, and cucumber decreased after drying. Moreover, the intensity of the aroma attributes fruity/fresh and floral decreased in the green, white, and red but not in the yellow samples. GC-analysis of the odour active compounds showed that the percentage peak areas of (*Z*)-3-hexenal, 2-heptanone, (*Z*)-2-hexenal, (*E*)-2-hexenal, hexanol, (*Z*)-3-hexanol, (*E*)-2-hexenol, and linalool, which possessed green, vegetable-like, fruity, and floral characteristics, decreased during drying, which may be responsible for the decreased intensity scores of corresponding aroma attributes in the dried samples. On the other hand, hot-air changed the aroma profile. Five extra attributes, i.e., cacao, caramel, nutty, savoury, and dried tomato, were necessary to describe the aroma of the bell pepper samples after drying. The major odour active compounds detected in the dried samples were 2-methylpropanal, 2 and 3-methylbutanal, hexanal, and nonanal. Also, the levels of 2,3-butanedione, 2,3-pentanedione, 4-octene-3-one, (*E*)-2-octenal, and (*E*)-2-nonenal were significantly higher in the dried bell peppers. Several of these compounds having cacao, spicy, sweaty/rancid, and sweet odour characteristics may contribute to aroma attributes like savoury, sweet/sickly, rancid/sweaty, cacao, and caramel, which were characteristic for all dried samples.

Modelling the lipid oxidation, Strecker degradation, and Maillard reaction products during hot-air drying of bell peppers. In Chapter 8, the complex

behaviour of volatile bell pepper compounds during drying at different time and temperature conditions was described by four distinct kinetic mechanisms.

This thesis provided basic knowledge about the flavour of fresh and hot-air dried bell peppers. This knowledge can be used to study effects of, for example, harvest time, growth conditions, sample variation, cultivars, and seasons on the flavour of fresh and/or dried bell peppers. Furthermore, in this thesis a biochemical pathway was proposed for the enzymatic development of bell pepper volatiles, which can be used as basis for detailed studies on the enzymatic formation of bell pepper volatiles. Finally, the complex behaviour of volatile compounds during hot-air drying of bell peppers was described by four different kinetic mechanisms. This kinetic approach may be used to investigate the behaviour of volatiles in other dynamic processes.

Samenvatting

Paprika's (*Capsicum annuum* en *C. frutescens*) worden veel gebruikt in de dagelijkse voeding vanwege hun karakteristieke kleur, aroma, en scherpe smaak. Ze worden zowel vers als verwerkt, zowel onrijp (groen) als rijp (bijv. rood, geel, oranje, wit) gegeten. In het afgelopen decennium is het aroma een steeds belangrijker aspect van de produktkwaliteit geworden. Het aroma van fruit en groente, zoals we dat waarnemen tijdens consumptie, is de totale gewaarwording tengevolge van de interactie van uiterlijk, smaak, geur, mondgevoel en geluid. De samenstelling van niet-vluchtige componenten bepaalt hoofdzakelijk de waargenomen smaak; de geur wordt bepaald door vluchtige componenten.

In dit proefschrift is het aroma van zowel verse als gedroogde paprika gekarakteriseerd; met name zijn de vluchtige en niet-vluchtige componenten in relatie tot de geur en smaak onderzocht. Het belangrijkste Nederlandse ras voor export is *Capsicum annuum* Mazurka, dit ras is gebruikt als modelmateriaal voor het proefschrift.

De instrumentele en sensorische evaluatie van het aroma van verse paprika

Ontwikkeling van niet-vluchtige paprika componenten. In hoofdstuk 2, zijn enkele groeikarakteristieken en de veranderingen in de samenstelling van niet-vluchtige componenten, zoals suikers en organische zuren, tijdens de rijping van de rassen Mazurka en Evident, onderzocht. De ontwikkeling van de paprikavruchten bestond uit een groeifase (tussen 4-7 weken na vruchtzetting) en een rijpingsfase (7-10 weken). Gedurende de groei nam het gewicht snel toe, terwijl tijdens rijping de kleur veranderde van groen naar rood. Deze verandering in kleur ging gepaard met een snelle afname van het chlorophyl gehalte (groene pigmenten) en een toename van het gehalte aan carotenoiden (oranje en rode pigmenten). Twee HPLC (High Performance Liquid Chromatography) methoden zijn ontwikkeld voor de bepaling van suikers en organische zuren in paprika's. Drie suikers m.n., sucrose, glucose, en fructose en zeven zuren m.n., ascorbine- (vitamine C), citroen-, appel-, oxaal-, fumaar-, shikimine- en pyroglutaminezuur zijn gedetecteerd in de onderzochte paprika's. Zowel de suikers als zuren hadden een specifiek rijpingspatroon. Glucose, fructose, citroen- en ascorbinezuur namen toe, terwijl sucrose en appelzuur afnamen tijdens rijping.

Relaties tussen niet-vluchtige componenten en smaak. Voor de instrumentele en sensorische analyses werden drie rijpheidsstadia, gebaseerd op de tijdreeks, geselecteerd met name groen, intermediair en rood van de rassen Mazurka en Evident (Hoofdstuk 3). De samenstelling van suikers en organische zuren werd bepaald met de HPLC. Met behulp van een getraind sensorisch beschrijvend panel werd de smaak geëvalueerd op aroma-attributen zoals waargenomen tijdens eten. De groene paprika werd in het bijzonder gekarakteriseerd door de bittere smaak, en "grassig", "groene paprika" en "komkommer" aroma. De rode paprika werd getypeerd door de zoete en zure smaak en "rode paprika" aroma. De relaties tussen de instrumentele en sensorische data werden onderzocht met behulp van Principale Componenten Analyse (PCA is een multivariate statistische analyse techniek). Uit de PCA bleek dat de zoetheid gerelateerd was aan de gehaltes aan glucose, fructose, totaal suiker en drogestof. De zuurheid was gerelateerd aan de totaal gehaltes van respectievelijk citroen- en ascorbinezuur. PCA van de berekende gedissocieerde zuren toonde aan dat, hoofdzakelijk ongedissocieerd ascorbinezuur en gedissocieerd citroenzuur in de "-1" en "-2" vorm gerelateerd waren aan de zuurheid. Gebaseerd op deze resultaten en de beschikbare literatuur, is het aanemelijk dat ongedissocieerd ascorbinezuur direct de waargenomen zuurheid beïnvloedt, terwijl de gedissocieerde vormen van citroenzuur de proton concentratie (H^+ ionen) verhogen en op deze wijze indirect de zuurheid beïnvloeden.

Ontwikkeling van vluchtige paprika componenten. In hoofdstuk 4, zijn de zelfde rijpheidsstadia van het ras Mazurka onderzocht op de samenstelling van vluchtige componenten; zowel gesneden als fijngemalen paprika's werden geanalyseerd. In het totaal zijn 64 vluchtige componenten gedetecteerd en geïdentificeerd met behulp van gaschromatografie(GC) en GC-massa spectrometrie (MS). Een getraind snuffelpanel detecteerde en omschreef 30 geurcomponenten, waarvan 21 met de GC-MS zijn geïdentificeerd. Naar bleek hadden de GC-snuffelprofielen van alle onderzochte monsters een aantal geurcomponenten gemeenschappelijk met name 2,3-butaandion (caramel geur), 1-penteen-3-on (chemisch/scherp, kruidig), hexanal (grassig), 3-careen (rode paprika, rubber), (*Z*)-ß-ocimeen (ranzig, zweterig), octanal (fruitig), and 2-isobutyl-3-methoxypyrazine (groene paprika). Daarnaast waren er duidelijke verschillen tussen de rijpheidsstadia en de verschillende bereidingsmethoden (snijden versus malen). Tijdens rijping van groen naar rood deed zich een afname voor van de gehaltes van een groot aantal componenten met typische "groene" geuren, zoals (*Z*)-3-hexenol (grassig, sla, komkommer geur), nonanal (paddestoel, tuinkruiden), 2-*sec*-butyl-3-methoxypyrazine (wortel, sla, grassig), linalool (bloemen, groene paprika), (*Z*)-3-hexenal (grassig, groene paprika, fruitig) en 1-hexanol (fruitig, groene paprika, tuinkruiden). Slechts twee geurcomponenten met een zoetig, fruitig karakter, namelijk (*E*)-2-hexenal en (*E*)-2-hexenol, namen toe tijdens rijping van groen naar rood. Het kapotmaken van de celstructuur door malen van de paprika's resulteerde in een toename van het aantal enzymatisch gevormde vluchtige componenten, waarvan een aantal typische geurkarakteristieken hadden.

Biochemische aspecten van de enzymatische vorming van vluchtige paprika componenten. De ontwikkeling en de herkomst van de enzymatisch gevormde vluchtige componenten zijn nader onderzocht in hoofdstuk 5. De aanwezigheid van het enzym lipoxygenase (LOX) werd aangetoond in de extracten van groene paprika. Het enzym had een pH optimum tussen 5.5-6.0 en de activiteit werd 100% en 51% geremd door respectievelijk de specifieke remmers n-propylgallaat (10 mM) en 5,8,11,14-eicosatetraynoic acid (340 mM). Tijdens rijping nam de LOX activiteit met 70% af tussen 6 en 8 weken na vruchtzetting, wanneer de vruchten van groen naar rood kleurden. De enzymatisch gevormde vluchtige componenten vertoonden vier verschillende rijpingspatronen. De herkomst van deze vluchtige componenten werd onderzocht door toevoeging van specifieke LOX substraten, linol- en linoleenzuur, aan de gemalen groene of rode paprika homogenaten. Door toevoeging van linolzuur aan de groene homogenaten namen de gehaltes van hexanal, hexanol, 2-pentylfuran, (*E*)-2-heptenal, (*E*)-2-octenal, (*E*)-2-nonenal, en (*Z*)-3-hexenal significant toe. Toevoeging van linoleenzuur aan de groene paprika homogenaten resulteerde in een significante toename van de gehaltes van (*Z*)-3-hexenal, (*E*)-2-hexenal, (*Z*)-2-hexenal, (*Z*)-2-pentenal, (*E*)-2-pentenal, (*Z*)-2-pentenol, (*E*)-2-pentenol, en 1-penteen-3-on. Vergelijkbare effecten werden waargenomen voor de rode paprika's, alleen de hoeveelheden waren verschillend. Op basis van de experimentele data en de beschikbare literatuur is een biochemische route voorgesteld voor de enzymatische vorming van een aantal componenten uit paprika.

Invloed van hete-lucht drogen op de instrumentele en sensorische evaluatie van het aroma van verse paprika.

Effecten van hete-lucht drogen op de samenstelling van niet-vluchtige en vluchtige componenten. In hoofdstuk 6, is de samenstelling van niet-vluchtige en vluchtige componenten geanalyseerd van vier verschillende soorten paprika's (groene en rode cultivar Mazurka, witte cv. Blondy en gele cv. Kelvin), voor en na hete-lucht drogen. Hete-lucht drogen verlaagde het gehalte van glucose, fructose, ascorbinezuur, citroenzuur en oxaalzuur, terwijl het gehalte van sucrose, appel-, fumaar- en *cis*-aconinezuur toenam. De afname van glucose, fructose en ascorbinezuur werd mogelijk veroorzaakt door de deelname van deze stoffen aan de Maillard reactie die plaatsvindt tijdens het verhitten. Het effect van hete-lucht drogen op de samenstelling van vluchtige componenten kon ingedeeld worden in drie groepen: 1) componenten die verdwnenen of afnamen, 2) componenten die toenamen of nieuw gevormd waren, en 3) componenten die niet beïnvloed werden.

Het grootste aantal componenten behoorde tot de eerste groep. Deze componenten namen waarschijnlijk af door verdamping. Echter de meeste van deze componenten waren voor een deel nog aanwezig na drogen, terwijl sommigen al volledig waren verdwenen zoals, (*Z*)-2-pentenal, (*E*)-2-pentenal, (*Z*)-3-hexenal, (*Z*)-2-pentenol, (*E*)-2-pentenol, (*E,E*)- en (*E,Z*)-2,4-hexadienal. Deze componenten worden waarschijnlijk enzymatisch gevormd (Hoofdstuk 5). Het verdwijnen van hiervan wordt mogelijk veroorzaakt door inactivatie van de enzymen tijdens drogen. Uit de GC-snuffelpoort analyses bleek dat hoofdzakelijk de vluchtige componenten met "verse" geuren zoals grassig/fruitig/sla ((*Z*)-3-hexenal), fruitig/amandel ((*E*)-2-hexenal), fruitig (octanal), sla/groene paprika ((*Z*)-3-hexenol) en grassig/groene paprika ((*Z*)-2-hexenal) afgenomen of verdwenen waren.

De tweede groep bestond uit vluchtige componenten die toenamen of gevormd waren tijdens drogen. De toename van de gehaltes aan 4-octeen-3-on, (*E*)-2-heptenal, (*E*)-2-octenal, decanal, (*E*)-2-nonenal, (*E,Z*) en (*E,E*)-2,4-heptadienal werd waarschijnlijk veroorzaakt door autoxidatie van onverzadigde vetzuren. De Streckerdegradatie is mogelijk verantwoordelijk voor de toename van 2-methylpropaanzuur en 2- and 3-methylbutaanzuur, 2-methylpropanal, en 2- en 3-methylbutanal. Uit de GC-snuffelpoort analyses bleek dat de laatst genoemde drie componenten typische cacao, zweterig, en kruidige geurkarakteristieken hadden.

Veranderingen in het aromaprofiel van verse paprika tengevolge van hete-lucht drogen. In hoofdstuk 7, zijn de effecten van hete-lucht drogen op het verse aroma van de vier verschillende kleuren paprika nader onderzocht. Van de verse en gedroogde monsters werd de procentuele samenstelling van de geuractive componenten geanalyseerd. Ook werden de monsters geëvalueerd op aroma-attributen tijdens ruiken door een beschrijvend panel. Uit het onderzoek bleek dat, hete-lucht drogen de intensiteitscores van diverse typische "verse" aroma-attributen verlaagde. In de groene en witte paprika's nam de intensiteit van groene aroma-attributen af zoals, tuinkruiden, grassig, groene paprika, en komkommer. De intensiteit van de attributen fruitig/fris en bloemen nam af in de groene, witte, en rode paprika's. Uit de GC-analyses van de geuractive componenten bleek dat door drogen de procentuele piekoppervlaktes afnamen van, (*Z*)-3-hexenal, 2-heptanon, (*Z*)-2-hexenal, (*E*)-2-hexenal, hexanol, (*Z*)-3-hexanol, (*E*)-2-hexenol en linalool. De genoemde vluchtige componenten hadden groene, groente-achtige, fruitige, of bloemenachtige geuren. De afname van deze componenten veroorzaakt mogelijk de afname in de intensiteitscores van de overeenkomstige sensorische aroma-attributen. Naast de afname in intensiteit van een aantal componenten veranderde hete-lucht drogen het ook aroma-profiel van verse paprika. Voor de beschrijving van het gedroogde produkt waren vijf extra aroma-attributen nodig m.n., cacao, caramel, noot-achtig, pittig en gedroogde tomaat. Hexanal, 2-methylpropanal, nonanal, 2 en 3-methylbutanal vormden de grootste procentuele bijdrage aan de samenstelling van geuractieve componenten in de onderzochte gedroogde paprika's. Ook de gehaltes van 2,3-butaandion, 2,3-pentaandion, 4-octeen-3-on, (*E*)-2-octenal en (*E*)-2-nonenal waren significant hoger na drogen. Verschillende van deze componenten hadden geurkarakteristieken zoals, cacao, caramel, kruidig, zweterig, ranzig, en zoetig. Deze dragen mogelijk bij aan de sensorische aroma-attributen, zoals pittig, zoet/weeïg, ranzig/zweterig, cacao, en caramel, die typerend waren voor de onderzochte gedroogde paprika's.

Modelleren van vetoxidatie, Streckerdegradatie en Maillard reactie produkten tijdens hete-lucht drogen. In hoofdstuk 8, is het complexe gedrag van een aantal produkten uit de vetoxidatie, de Streckerdegradatie, en de Maillard reactie, onder verschillende tijd- en temperatuurcondities beschreven met vier verschillende kinetische mechanismen.

Dit proefschrift heeft een bijdrage geleverd aan de fundamentele kennis over het aroma van verse en gedroogde paprika's. Deze kennis kan als basis gebruikt worden voor onderzoek naar de effecten van bijvoorbeeld, oogsttijdstip, groeicondities, binnen-monster-variatie, rassen, en seizoenen op het aroma van verse en/of gedroogde paprika's. Daarnaast is in dit proefschrift een biochemische route voorgesteld voor de enzymatische vorming van vluchtige paprika-componenten. Deze kennis kan als basis gebruikt worden voor meer gedetailleerde studies naar de enzymatische vorming van vluchtige paprika-componenten. Tevens, is het complexe gedrag van een aantal produkten uit de vetoxidatie, Streckerdegradatie en Maillard reactie gemodelleerd met behulp van vier kinetische mechanismen. Deze kinetische benaderingswijze kan gebruikt worden in onderzoek naar het gedrag van vluchtige componenten in andere dynamische processen.

Nawoord

Na vijf jaar hard werken op het ATO-DLO maak ik de balans op en kom ik tot de conclusie die velen voor mij hebben getrokken....."Een promotie doe je niet alleen, maar de weg er naartoe is vaak eenzaam." De vele mensen die mij, ieder op zijn of haar manier, hebben gesteund en/of geïnspireerd wil ik graag bedanken.

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Kees, het citaat van Rudolf Steiner in jouw proefschrift "De mens is over de drempel gegaan" heeft zijn volle betekenis gekregen.

Curriculum Vitae

Pieternelleke Arianne Luning werd op 7 augustus 1963 geboren te Leiden. In mei 1982 behaalde zij het Atheneum B diploma aan het Maasland College te Oss. In datzelfde jaar begon zij aan de studie Levensmiddelentechnologie aan de toenmalige Landbouwhogeschool te Wageningen. In de doctoraal fase werden de hoofdvakken levensmiddelenchemie (dr ir J.P. Roozen) en levensmiddelenmicrobiologie (ir J. de Wit en prof.dr ir F. M. Rombouts) afgerond. In Barie (Zuid Italie) heeft zij 3 maanden stage gelopen bij het Centro Recerche Bonomo (prof. dr. W. Pilnik). In september 1988 is zij afgestudeerd aan de Landbouwuniversiteit. Aansluitend, heeft zij in het kader van een tweejarige ontwerpers opleiding gewerkt als onderzoeker aan de Landbouwuniversiteit van oktober 1988 tot april 1990. In deze periode heeft zij gewerkt aan "de invloed van broodverbetermiddelen op het aroma van wit brood" in samenwerking met de firma Sonneveld B.V.. Aansluitend is zij werkzaam geweest als wetenschappelijk onderzoeker bij het Instituut voor Agrotechnologisch Onderzoek ATO-DLO met een uitloop tot april 1995. Het onderzoek aan "het aroma van verse en gedroogde paprika" was mede gefinancieerd door de VIGEF en de commissie voor groenten en fruit. Het onderzoek, zoals beschreven in dit proefschrift, is uitgevoerd op het ATO-DLO (dr. ir. C. van Dijk en dr. H. J. Wichers) onder begeleiding van dr. ir. J. P. Roozen en prof dr. ir A. G.J. Voragen bij de sectie Levensmiddelenchemie en -microbiologie. Per 1 april 1995 is zij tijdelijk werkzaam bij de sectie Levensmiddelenchemie en -microbiologie ten behoeve van een samenwerkingsproject met Unilever Vlaardingen.

Naast haar werk organiseert zij zomerkampen voor kinderen, is zij actief in het vrouwennetwerk van Wageningse Ingenieurs (VWI), en is zij een zeer enthousiaste sportster.

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