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The interest in identifying volatiles responsible for specific sensory properties in food has lead scientists to correlating data obtained from gas chromatography (GC), GC-olfactometry (GCO), mass spectrometry (MS) and descriptive sensory analysis (DSA). GC, MS and DSA are well established methodologies; however, few scientists maintain a sustained commitment to the use and improvement of GCO techniques. In an attempt to create a suitable GCO technique, our sensory laboratory developed a new method entitled Osme. Osme is a time-intensity approach for evaluating the odor significance of compounds in the GC effluent. In our study, we explored the potential of Osme in establishing a compound's odor significance in a flavor system.

First, Osme capability and psychophysics was assessed by four subjects who directly recorded the intensity and duration, and reported the quality of sample odorants in the GC effluent. Samples consisted of five model solutions; each solution contained the same six aroma compounds but at

different concentrations. The subjects were capable of establishing psychophysical functions ($p \leq 0.05$) between *maximum odor intensity* and *odorant concentration* and between *area under the odor peak* and *odorant concentration*. Individual standard deviations in odor ratings ranged from 1.45 to 2.07 (16-point scale). Overall, Osme was comparatively quantitative with traditional olfactometry techniques.

Second, Osme was used jointly with GC, MS and DSA to determine the flavor properties and stability of a corn snack. Samples held for 0, 3, 6, 9 and 12 months were evaluated for aroma and flavor by a descriptive panel. Sample volatiles were extracted with methanol, isolated in dichloromethane, and separated by GC. Four panelists evaluated the GC effluents using the Osme technique to locate and describe significant aroma compounds. The compounds were identified by GC/MS. The fresh product was characterized by toasted-corn aroma and flavor. Among the volatiles associated with the snack aroma and flavor were methional, t,t-2,4-decadienal and a series of pyrazines and pyrrolines. Storage effected ($p \leq 0.05$) the aroma, flavor and volatile composition of the snack. Relating Osme, GC, MS, and DSA data was critical for understanding flavor properties and stability of the snack.

Flavor Properties and Stability of a Corn-Based Snack: Aroma Profiles By Gas Chromatography (GC), GC-Olfactometry, Mass Spectrometry, and Descriptive Sensory Analysis

by

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INTRODUCTION

In the snack food area, a highly competitive field in modern food marketing, the flavor of the product is a vital point in gaining and keeping customer preference. Though convenience, size, shape, color, texture and cost are all important, it is the flavor which makes the greatest impact, the most lasting impression and is the key to repeat sales (Heath et al., 1978; Van Osnabrugge, 1989).

Most of our food flavor impressions are stimulated by complex mixtures of volatile odorous chemicals, belonging to different chemical classes and occurring at variable concentrations (Dürr, 1983). In the snack area, identification of particular odorants responsible for specific sensory properties in the product can aid development of a novel synthetic snack flavor or flavor enhancer. In addition, it may help the postulation of mechanisms for formation of the objectionable aroma and flavor notes developed during the snack storage, with further development of practical methods to prevent or retard them.

The isolation, separation and identification of a large number of volatiles present in foods is possible for today's flavor chemists due to the improvement of chemical and physical methods of flavor extraction, associated with the sophistication of gas-liquid chromatography and mass

spectrometry. The current challenge has been to identify, among those volatiles, the particular ones responsible for specific sensory properties in the food product.

One approach to assess a compound's individual odor significance in a flavor system has been to have individuals actually smell the gas stream as it escapes from the GC-column and to record their sensory estimate of each eluted component. Such methodology, which combines sensory analysis with gas chromatography, is titled gas chromatography olfactometry (GCO).

Among the GCO techniques generated in the last decade, Charm (Acree et al., 1984; Cunningham et al., 1986; Marin et al., 1988) and AEDA (Schieberle and Grosch, 1987; Schiberle and Grosch, 1988) are most often cited in the flavor literature. Indeed, they have been useful in identifying areas of the chromatogram which are likely to be important for the products' flavor; however, both techniques has been subject to severe criticisms. The assessment of a compounds' individual odor significance by both Charm and AEDA is founded on the compounds' odor detection threshold rather than the estimation of its odor intensity (Maarse, 1991).

In an attempt to create a new GC-olfactometry methodology strongly founded on current psychophysical views, the Sensory Science Laboratory of Oregon State University developed a new GCO technique named Osme (McDaniel et al., 1990; Miranda-Lopez et al, 1992 and Sanchez et al., 1992). With Osme, trained subjects sniff the GC effluent mixed with humidified air, and directly record the odor intensity and duration time of each odor active compound while describing its odor quality. The plot of the retention time versus odor intensity, called an Osmegram, provides a graphical representation of the compound's odor significance in the flavor extract;

higher peaks suggest greater importance. According to Piggot (1990), Osme is a more satisfactory GCO approach than the afore mentioned methods.

Despite the advantages offered by the use of GCO techniques in general and Osme in particular, a significant number of flavor chemists hesitate in using them based on the following assertions: i) data generated by GCO techniques are only semi-quantitative (Burr, 1964); ii) rapidly eluted peaks allow little time for aroma characterization (Clark and Cronin, 1974); iii) the relationship between the compound concentration and the response by the human nose is not linear (Wick, 1965) and ; iv) fatigue decreases assessor's efficiency during a long run (Burr, 1964; Clark and Cronin, 1974). However, data obtained with traditional olfactometry support the feasibility of Osme and remaining GCO techniques: i) identification of odor compounds at their recognition threshold levels is achieved with a single sniff, whose average duration is just 0.45 seconds (Laing, 1986); ii) perception of odorants maximum intensity takes between 0.39 and 0.64 seconds (Laing, 1985) and iii) no observable decrease in sensitivity due to fatigue or adaptation was observable in subjects submitted to a 100-sample presentation in an 80 minute continuous sniffing session (Pangborn et al., 1964).

Since most of the criticism directed toward traditional GCO techniques question a subject's capability and reliability to establish a significant relationship between odorant concentration and odor intensity, questioning as a consequence the techniques capability in establishing a compound's odor significance in a flavor system, the objectives of the present study were:

(1) To determine subjects capability and reliability to establish a significant relationship between compound concentration and sensory response when using Osme;

(2) To determine the nature of the relationship between physical stimulus concentration and subject response with Osme;

(3) To determine Osme's capability in assessing the volatiles responsible for specific sensory properties in a corn-based snack food and;

(4) To determine Osme's capability in identifying volatiles responsible for the changes in the snack aroma and flavor during product storage under nitrogen.

LITERATURE REVIEW

GC-OLFACTOMETRY

The ultimate goal of basic food flavor research is to establish the identity of the stimuli responsible for the characteristic flavor of foodstuffs. Identification of chemical compounds responsible for specific food odor qualities can aid: i) postulation of the mechanisms for the formation of objectionable flavors in foods and thus development of practical methods to prevent or retard their formation; ii) specification and control of the flavor qualities; iii) development of a synthetic food flavor (Wick, 1965).

Among the recent analytical techniques used for the structural elucidation of complex food aroma systems, gas chromatography associated with mass spectrometry is the most common. However, while odor is the integrated and highly subjective response of an individual to a number of different stimuli, gas chromatography/mass spectrometry (GC/MS), at its best, merely differentiate the chemical compounds present in the aroma system, where only some of which serve as aroma/flavor stimuli. The challenge in flavor research at present is to establish which of the compounds isolated, separated and identified by GC/MS or infrared techniques are responsible for what sensory properties in the food product (Stewart, 1963).

A very practical approach to determine odor active compounds, and their respective odor quality in flavor extracts, is to have individuals actually smell the gas stream as it escapes from the GC column and to record their estimate of the odor quality of each eluted component. This approach has

been successfully employed by many researchers (Williams et al., 1977; Paule and Powers, 1989).

However, there is still the necessity to arrange the list of odor constituents into an order of decreasing odor significance. Historically, to circumvent this dilemma, the concept of 'odor units' (Guadagni et al., 1966) has been suggested whereby GC peak area is recalculated relative to the odor-detection threshold of each compound, thus giving a picture of the relative odor impact of each compound on overall sample flavor. However, odor units methodology is very laborious because threshold data must be determined for a great number of compounds (Maga, 1990).

To alleviate some of the above limitations, recent techniques based on gas chromatographic analysis and effluent sniffing of a dilution series of the original odor extract were created. Among these techniques, generally classified as GC-olfactometry techniques (GCO), Charm (Acree et al., 1984) and AEDA (Ullrich and Grosch, 1987; Schieberle and Grosch, 1988) are most often cited.

Data generated from Charm analysis are based on the length of time which an odor substance can be detected by a subject sniffing the effluent of a GC and the number of dilutions of the sample that produced an odor response at a particular retention index.

The equation that produces the Charm response chromatogram is:

$$C = D^{n-1}$$

where C, the instantaneous Charm, is equal to the dilution factor D, raised to the power "n-1", and "n" is the number of dilutions of the sample that produced an odor response at a particular index.

A Charm response aromagram is made by plotting C against retention index. Acree et al. (1984) suggest that the resulting peak areas are relative measures of the odor intensity of the substances eluting from the GC in a particular region. Usually from 4 to 5 dilutions are necessary to draw a Charm chromatogram.

Another alternative approach, titled Aroma Extract Dilution Analysis (AEDA) and introduced by Grosch and co-workers, involves diluting the sample successively with solvent prior to GC analysis and determining the flavor dilution factor, the D-value, which corresponds to the highest dilution at which a component is still detectable by sniffing at the end of the GC column (Schmid and Grosch, 1986; Ullrich and Grosch, 1987; Schieberle and Grosch, 1988 and Maga, 1990). The aromagram obtained in this way represents the plot of D-values against Kovats retention index.

Charm and AEDA are based on the determination of odor-detection thresholds of the compounds eluted from the GC-column rather than the psychophysical estimation of their individual odor intensity. In this aspect both methods are similar to the odor unit technique proposed by Guadagni et al. (1966) and furthermore, subject to the same criticisms (Maarse, 1991).

As first pointed out by Fritjers (1979) in regards to the Guadagni odor unit concept, and later by Piggot (1990) and Maarse (1991) with respect to Charm and AEDA, the fact that different odorants may show different intensity functions above their threshold, limit threshold-based techniques' firm conclusions about the relative contribution of individual odorants to a flavor system, even though those techniques still give some guidance in

identifying areas of the chromatogram which are likely to be important. Current psychophysical views represented by Steven's Law (Stevens, 1957; Stevens, 1961), establish that the relationship between the odor intensity (**I**) of a compound grows with the compound's concentration (**C**) raised to a power **n**:

$$I=k(C-T)^n \ ;$$

where T is the compound's effective threshold and **k** is the constant of proportionality. Furthermore, it is evident that under Steven's Law, two different compounds at the same concentration (**C**) and possessing very close threshold (**T**) but showing different exponents (**n**), may yet produce different individual odor intensities (I) and consequently different individual odor contributions to the intensity and quality of a flavor system.

One more direct approach to quantify odor relevance of flavor compounds present in isolates would be to directly measure each compound's odor intensity in the GC effluent. However, few researchers have reported the collection of odor intensity data from gas chromatography effluent (Dirinck et al., 1976; Person and von Sydow, 1973; and Drawert and Christoph, 1984 cited in Maga, 1990). In general, the intensity data was collected using a 3-6 point category scale, ranging from very weak to very strong. Panelists smelling the GC effluent reported orally the compounds' odor intensities.

A more reliable approach for the evaluation of odor intensity of compounds in GC-effluent was reported by Selke et al. (1972). In their technique, effluent from the GC-column was split three ways: Approximately one-fourth went to a Flame Ionization Detector (FID) and three-fourths to a

mass spectrometer for compound identification and into the atmosphere for "sniffing". Adjacent to the GC exit port was an auxiliary chart record and a voltage dividing resistor taped with a 10-position switch which controlled the recorder's pen deflection. A person sitting at the GC exit port, sniffing the effluent, would turn the switch whenever an odor was detected in the GC-effluent; how far the switch was turned depended upon the intensity of the odor detected. Also, a description of the odorant quality was given by the subject. Both the GC and auxiliary recorders had identical chart speeds; furthermore, odor descriptions could be easily assigned to particular GC peaks.

Combining the modern concepts of sensory descriptive analysis with novel techniques of computerized data collection and applying the Selke et al. (1972) time-intensity approach of evaluating the odor significance of compounds in the GC-effluent, McDaniel et al. (1990), Sanchez (1992), and Miranda (1992), created a new GC-olfactometry methodology, named Osme. With Osme, previously selected and trained subjects sniffing the GC-effluent mixed with humidified air, directly record the odor intensity and duration time of each odor active compound while describing its odor quality. Duration time and intensity values are collected using a data acquisition device consisting of a variable resistor with a pointer that the subject can move from left to right and back again across a 16 point structured scale (0=none; 15=extreme). Time is monitored and data collected by a software system installed in an IBM XT personal computer. Odor intensity values are collected every quarter second as indicated by a change in electrical resistance from a variable resistor inside the data acquisition device. Each panelist evaluates each sample in four replicates, each replicate being a different GC run. For each odorant, Osme provides: i) the odor peak, obtained by plotting

retention time x odor intensity values, ii) the odor duration time (d), which is the total time that the panelist could detect the compound in the GC-effluent, iii) the maximum odor intensity (I_{max}), iv) the area under the odor peak, v) the Kovats index based on panelist response and, vi) the odor quality. Osme methodology has many advantages: i) it is strongly founded on current psychophysical views because it directly collects each compound's odor intensity as it is present in the extract rather than estimating it by indirect approaches based on a diluting series as done in Charm and AEDA; ii) it is less time consuming since it does not require a dilution series; and iii) it provides one aromagram which, similar to the GC, represents the exact sensory phenomena occurring during the compound elution: the increasing odor intensity phase followed by one steady-phase with subsequent decreasing odor intensity.

Even though many flavor chemistry researchers have been using GC olfactometry (GCO) techniques for quantitative purposes, a considerable number of researchers still hesitate in using it. Current criticisms of the use of GCO are based on the following assertions: i) data are only semi-quantitative (Burr, 1964); ii) rapidly eluted peaks allow little time to characterize the aroma (Clark and Cronin, 1974); iii) the relationship between the compound concentration and the response provided by the GCO technique is not linear (Wick, 1963) and; iv) fatigue decreases assessor's efficiency during a long run (Burr, 1964; Clark and Cronin, 1974). In other words, researchers may be apprehensive about the reliability of the GCO methodology when used for quantifying the effluent odor intensities. However, a review of the literature does not show evidence which could restrict the use of olfactometry associated with gas chromatography as suggested by the above researchers.

In contrast, comprehensive investigations of human sniffing support the feasibility of GCO: i) identification of odor compounds at their recognition threshold levels is achieved with a single sniff, whose average duration is just 0.45 seconds (Laing, 1986), ii) perception of an odorant's maximum intensity takes between 0.39 and 0.64 seconds (Laing, 1985) and iii) in odor threshold and intensity tests, results achieved with a single normal relaxed breathing were not improved upon when subjects used an unlimited number of sniffs or their strongest single sniff (Laing, 1983). All these findings contradict Clarke and Cronin's (1974) conjecture that rapidly eluted peaks would not allow enough time for a subject to characterize their odor. In contrast, increasing the flow rate of an odorant from an olfactometer to a subject's nose such as in GCO, is known to decrease the odorant threshold level, supposedly due to enhancing odorant transport and concentration at the receptor level (Schneider et al., 1966; Tucker, 1963). Recent findings suggest in fact that a subject's odor sensitivity can even be enhanced as he/she performs using a GCO technique.

SUBJECTS VARIABILITY IN OLFACTOMETRY

Psychophysics is the branch of psychology that describes in quantitative terms the relationship between physical stimuli and psychological responses. It is primarily concerned with perceived intensity as a function of concentration (Engen, 1982).

The tradition of psychophysics from the early work of Weber until recent times, has emphasized laws relating group or mean responses to stimulus conditions, with little attention to individual differences (Tucker,

1960). However..."There never was in the world two opinions alike, no more than hairs or two grains, the most universal quality is diversity" Michel de Montaigne (1533-1592) (Pangborn, 1981).

Indeed, sources of individual differences in sensory studies have been identified. These include gender, menstrual status, genetic endowment, age and personality as defined by various psychological tests. Some of the dependent variables in which individual differences have been found in responses to odorants, tastants and oral irritants include absolute and differential sensitivity, perceived quality, hedonic ratings, identification, rate of salivation, and relative sensitivity of receptors' loci (Stevens, 1991).

The inherently wide variation in olfactory sensitivity is readily evident in threshold studies. Operationally, a threshold can be defined as the minimum concentration that can be perceived, or recognized, at some arbitrary level of probability, usually $p \leq 0.05$. At any brief moment in time, the threshold is a fixed value, but over time, a threshold is a fluctuating, statistically-determined end-point along a stimulus continuum. Threshold fluctuations reflect the particular measurement procedures employed, environmental factors, and individual judge sensitivity (Pangborn, 1981).

Pangborn et al. (1964) reported large variations in individual sensitivities between subjects regarding the detection threshold for 2-heptanone. The authors reported that the least sensitive subject sometimes never perceived the highest concentration of the most sensitive subject. Similarly, Erickson et al. (1976) reported a wide between-subjects variation in odor thresholds for 13 individuals in response to n-hept-trans-2-en-1-ol and of n-hept-trans-2-enal.

Stevens et al. (1988) believe that in fact the day-to-day variability for a given subject odor threshold is comparable to that reported across subjects

and suggest that the extreme fluctuations of threshold from one test to another may tend to obscure real and stable average differences among subject's olfactory keenness or dullness. Stevens and O'Connell (1991) reported a significant age effect ($p \leq 0.02$) in thresholds of human subjects to various odors. They found that generally, subjects over 35 years of age had lower thresholds for pemenone, the compound studied, than younger subjects.

More recently, Laska and Hudson (1991) investigating whether the perception of odor mixtures differed from the perception of their individual components with regard to reliability of detection threshold measurements, reported an interindividual variability of 1.2 orders of magnitude which decreased with the increase of stimulus complexity.

Even though a significant number of researchers have pointed out possible limitations in the use of the GC-olfactometry techniques for quantitative purposes, Dravnieks and O'Donnell (1971), conducting a cursory comparison of odor thresholds of several compounds smelled from a continuous stimulus olfactometer with those thresholds obtained through a GCO technique, reported that reasonable agreements in the threshold values of both techniques were attained when it was assumed that the odorant was equally distributed in 30 ml of effluent which corresponded to the peak width (Table 1). Unfortunately, in this research, thresholds obtained using GCO were generated basically on observation of one subject only.

Similarly, Marin et al. (1988), using the GCO technique associated with the Charm procedure, evaluated seven standard aroma compounds in order to study the variation in odor detection thresholds for four groups of individuals, cross-classified by sex and age. They reported that the threshold of *l*-carvone was affected by age. Significant threshold differences between

individuals of the same sex and age were found for menthone, *l*-menthol and *l*-carvone. Subjects duplicate error was small and homogeneous for all the compounds tested, suggesting that individuals show good reproducibility using the cited procedure.

Table 1.1 Comparison of odor thresholds obtained by olfactometry with those estimated by sniffing gas chromatograph effluent (GCO).

ODORANT	THRESHOLD (pg/ml)	
	Olfactometer ED Value[a]	Estimated using Gas Chromatograph[b]
m-Xylene	1,300	5,200
Toluene	45,000	42,000
Benzene	38,000	43,000
1,2-Dichloroethane	190,000	245,000

[a] These are values averaged from observations using a panel of five to seven judges, and they signify that 50% of the judges will begin to detect the odor above the quoted concentration. [b] Based on observation of mostly one observer only; a cross-check by occasional other observers essentially agreed with the principal observer (Dravnieks and O'Donnell, 1971).

The assessment of individual differences in the perception of taste and smell has been based predominantly on threshold measurements. However interindividual variability regarding estimates of the intensity of the odor of suprathreshold concentrations of many odorants have been reported. A single compound can also elicit multiple intensity and quality reports from different subjects. There are a large number of different constructs which can explain the quantitative and qualitative differences in odor perception observed across individual osmic and anosmic subjects for a

particular odorant. For example, the compound may interact with multiple types of perceptual channels which are unequally distributed within the human population. Thus, anosmics may differ from osmics in that they lack, or are deficient in some subset of these processes (O'Connell et al., 1989).

In fact, Berglund et al. (1971), obtained the psychophysical function for 28 different chemical compounds and reported for each odorant a large individual difference regarding psychophysical exponents. Individuals' exponents for acetone ranged between 0.12 to 1.02, for benzaldehyde between 0.07 and 0.38 and for pyridine between 0.06 to 0.70. Between-subject variability regarding the odor quality reported for several odorants cited by Schiffman and Lockhead (1982) showed differences between young and elderly groups regarding the descriptor used to characterize food odors diluted in odorless dipropylene glycol. The weight space of the Principal Component Analysis (PCA) for the individual subjects, revealed that young subjects were more distant from the origin while the elderly subjects fell closer to the origin. In addition, the elderly group presented a larger within-subjects variability.

In an attempt to verify individual differences that possibly occur in the perception of odor intensity, Enns and Hornung (1988) studied the absolute magnitude estimates of smell in young (18-21 years) and elderly (61-94 years) people and reported that on average, when compared with young adults, the elderly people gave significantly lower odor intensity ratings ($p \leq 0.05$) to the smell of almond extract solutions. However, there were no statistically significant differences ($p \leq 0.05$) in either the estimates of the intensity of the odor for the elderly and the young subjects.

O'Connell et al. (1989), obtained quantitative and qualitative odor reports from selected human subjects who were presented with various concentrations of pemenone, androstenone, ammonium hydroxide,

isovaleric acid, pentadecalactone, pepper pyrazine, phenyl ethyl alcohol and pyridine in order to determine if subjects were likely to rate them in a uniform fashion. Subjects presented the largest individual differences regarding the estimates of odor intensity for pemenone, androstenone and phenyl ethyl alcohol. The authors reported that subjects who appeared to be osmic for pemenone, because they provided relatively high ratings for its intensity were also likely to describe its odor quality as urinous, while the subjects who appeared to be anosmic for pemenone, because they provided relatively low ratings for its intensity, were unlikely to described its odor quality as urinous. In a subsequent study, Stevens and O'Connell (1991) reported that subjects relatively osmic for pemenone generally reported a putrid odor for pemenone, but anosmics reported mostly other qualities.

ODORS GENERATED IN DEEP-FRIED FOODS

Odors are generated in foods primarily by three processes (Parliment, 1989):

1- Enzymatic and microbial processes which liberate low molecular weight volatile chemicals. Both pathways are particularly important in the odors of foods such as fermented dairy products, and beverages.

2- Production of biologically-derived odor precursors with subsequent heating which generates the product final odor and flavor. Cocoa and bread odors are two examples of this type of odor generating process.

3- Non-enzymatic processes resulting from thermal treatment such as cooking and roasting. These reactions typically include thermal

decomposition of carbohydrates, lipids and proteins, and are responsible for the odor of foods such as meat, cereal, and deep-fat fried foods among others.

Deep-fat frying involves the continuous submersion of moisture-containing foods in oil which has been heated to approximately 190°C. Volatile compounds are generated from the food itself during the frying process. They are important to the food flavor and are formed primarily through the Maillard reaction and Strecker degradation, involving the food's primary constituents, such as carbohydrates and proteins (Ho et al., 1987).

Volatile products are also generated during the frying process due to thermal oxidation, isomerization, hydrolysis, pyrolysis and polymerization of the triglycerides. Those volatile products are essential for the development of the fried food flavor (Ho et al., 1987).

Finally, recent studies have indicated that interactions between constituents of the food and the frying fat, such as lipid-protein reactions, could result in the generation of volatile compounds important to the fried food flavor (Tang et al., 1983).

Thermal decomposition of carbohydrates

When reducing sugars are heated in the presence of ammonia, primary, or secondary amines or amino acids, a wide variety of odor and browning compounds are generated in a multi-step reaction named the Maillard reaction (Parliment, 1989). The Maillard reaction gives us some of the most pleasant flavors, e.g. chocolate, coffee, bread; and conversely, some very unpleasant ones, e.g. stale and gluey. Indeed, of the nearly 5,000 volatile flavor compounds identified to date, about half are known to be formed via the Maillard reaction (Reineccius, 1990).

The Maillard reaction is best divided into three stages. The initial stage involves the condensation between the carbonyl group of a reducing sugar, and the free amino group of an amino acid or peptide to produce a N-glycosylamine or fructosylamine. These glucosyl- or fructosyl- amines can rearrange to produce amino-deoxy-aldose or ketose via Amadori (from glucose) or Heyns (from fructose) rearrangements (Hodge, 1953; Parliment, 1989).

The intermediate stage of the Maillard reaction comprises: i) dehydration of the aldose or ketose either by loss of three molecules of water to furfural or by loss of two molecules of water to reductones; ii) fission of the aldose or ketoses by dealdolisation; and iii) Strecker degradation (Nursten, 1986).

The Strecker degradation is the interaction of amino acids with dicarbonyl compounds which may be either dehydroreductones or dehydration/fission products generated in earlier steps of the Maillard reaction. The Strecker degradation results in both odorous products as well as reactive intermediates. These intermediates can undergo a retroaldolization reaction to produce α-dicarbonyl compounds, such as pyruvaldehyde and diacetyl as well as reactive monocarbonyls, such as glycolaldehyde and glyceraldehyde (Parliment, 1989; Whistler and Daniel, 1985, Shallenberger and Birch, 1975).

The final stage of the Maillard reaction consists of the conversion of carbonyl compounds, be they furfurals, fission products, dehydroreductones or Strecker aldehydes, into high molecular weight products, the melanoidins, with further involvement of amines, where these are available (Nursten, 1986).

According to Nursten (1986), the odor compounds formed in the Maillard reaction can be classified into three categories:

1- Simple sugar dehydration/fragmentation products such as furans, pyrones, cyclopentenes, carbonyls and acids.

2- Simple amino acid degradation products such as aldehydes and sulphur compounds.

3- Volatiles produced by further interactions: pyrroles, pyridines, imidazoles, pyrazines, oxazoles, thiazoles and compounds from aldol condensations.

Furans, furanones, pyranones, and related compounds: Structurally, these substances are generally cyclic ethers, mainly furanoid compounds. While these compounds can be formed directly by sugar pyrolysis, they can be formed much more rapidly via the Maillard reaction (Reineccius, 1990). Indeed, furans are the most abundant products of the Maillard reaction and, as a rule, furan derivatives are considered important food odor constituents from a sensory point of view. Furan and furan derivatives not containing sulfur are mainly associated with sweet, fruity, nutty or caramel-like odor impressions (Hodge, 1967; Fors, 1983, Bailey, 1983). For instance, maltol, isomaltol, 4-hydroxy-5-methyl-3(2H)-furanone, 2,5-dimethyl-4-hydroxy-3(2H)-furanone, and 2-hydroxy-3-methyl-2-cyclopentene-1-one (cyclotene), have odors usually described predominantly as caramel or burnt sugar (Nursten, 1980). However furfural, which was reported in the nonbasic fraction of potato chip (Buttery and Ling, 1972) and in volatile concentrates of puffed dehydrated potato (Sapers et al., 1971), has a characteristic penetrating toasted odor (Flament, 1991); Similarly, 2,5-dimethyl-3(2H)-furanone has been reported to carry a strong odor of freshly baked bread (Flament, 1991). The

furan-3-one was reported as an odorant imparting important characteristics to the odor of baked potato (Coleman et al., 1981).

Distinctly, the S-containing furans and furan derivatives are extremely important contributors to "meaty" flavors (Bailey, 1983). Two interesting examples are the S-containing furan derivatives 2-methyl-3-furanthiol and the (2-methyl-3-Furyl)-bis-disulfide which provide meatlike odors (Evers et al., 1976; Wilson, 1975).

Aldehydes: Quantitatively, the major path for degradation of the Amadori or Heyns intermediate is the dehydration of the aldose or ketose yielding furfural or hydroxymethylfurfural. However, of greater flavor significance, are the minor pathways such as the Strecker degradation (Parliment, 1989; Shallenberger and Birch, 1975; Reineccius, 1990).

In the Strecker degradation, an α-amino acid suffers oxidative decarboxylation to an aldehyde (Nyhammar et al., 1983).

Hodge (1967) has tabulated various odorant aldehydes produced from the Strecker degradation reaction, some of which are shown in Table 2.

Guadagni et al. (1972) reported that phenylacetaldehyde, yielded in the Maillard reaction, was an important compound to overall potato chip flavor.

According to Buttery and Ling (1972), methional is one of the more important compounds in the nonbasic fraction of the steam volatile oil from potato chips. Methional was described as possessing a strong potato chip-like odor and was generated by deep-frying a cotton ball previously embebed with a solution of methionine and sugar (Lee et al., 1973). Similar results were reported by Tressl et al. (1989) when methionine was heated with monossacchrides for 1 to 1.5 h at 150 ^{o}C.

Accordingly, Guadagni et al. (1972) using the odor thresholds of the major components found in steam-volatile potato-chip oil, reported methional as probably one of the most important contributors to potato-chip odor.

Table 1.2 Flavor of heated 1:1 α-amino acid-glucose mixtures[1]

α-amino acid	Volatile Strecker Aldehyde	Odor Noted by the Sensory Panel
glycine	formaldehyde	burnt sugar
α-alanine	acetaldehyde	burnt sugar
valine	isobutyric	penetrating chocolate
leucine	isovaleric	burnt cheese
isoleucine	2-methyl-butanal	burnt cheese
threonine	lactic	burnt
methionine	methional	potato
phenylalanine	α-toluic	violets, lilac
proline		bakery odor
histidine		cornbread, buttery
lysine		bread-like
aspartic		caramel
glutamic		burnt sugar
glutamine		butterscotch

[1]Source: Hodge, 1967

Pyrazines: The sugar degradation compounds such as furans and furanones may undergo secondary reactions to yield Maillard reaction products such as pyrazines, pyrroles, thiophenes, and pyridines, among others (Shibamoto, 1989).

Reineccius et al. (1972) have shown that during the roasting of 100g of cocoa beans, about 1 g (total) of amino acids and sugars are consumed via the

Maillard reaction, but only 142-698 μg of total pyrazines are formed. This corresponds to only 0.2% of the reactants being channeled into pyrazine formation; however, the flavor produced is exceedingly important. This is due to the fact that many of the volatiles formed have very low sensory thresholds.

Alkyl pyrazines were among the first compounds to be recognized as important contributors to the flavors of all roasted, toasted, or similarly heated foods (Maga, 1982). The most direct route for their formation results from the interaction of α-dicarbonyl compounds (intermediate products in the Maillard reaction) with amino acids through the Strecker degradation reaction (Lindsay, 1985). Many pyrazines are somewhat similar in character having roasted, toasted or nutty odors (Reineccius, 1990).

Acetylpyrazine was proposed by Walradt et al. (1970) as an important flavor compound among the 58 volatiles identified in an extract of microwave-popped corn.

Sapers et al. (1971) partially attributed the toasted flavor of explosion puffed dehydrated potato, to the presence of alkylpyrazines, among them, 2-methylpyrazine and 2,5-dimethylpyrazine.

Buttery et al. (1971), identified 18 pyrazines in the basic fraction of the steam volatile oil from potato chips. Informal sensory evaluation indicated that 2-ethyl-3,6-dimethylpyrazine, a major component of the oil, was one of the most potent odorants isolated.

Alkyl-substituted pyrazines were the most important group of volatile compounds isolated by Deck and Chang (1965) and Deck et al. (1973) from potato chips; particularly 2,5-dimethylpyrazine and 2-ethylpyrazine, the latter being described by the panel as either strong potato or roasted peanut, depending upon the concentration. Similarly, the 2-ethyl-3,6-

dimethylpyrazine was one of the most important compounds in baked potato odor (Buttery et al., 1973). Guadagni et al. (1972), cited 2,5-dimethyl-3-ethylpyrazine as one of the most important volatiles in the odor of potato chips.

More recently, acetylpyrazine and 2-ethyl-3-methylpyrazine, both described as roasty, diethylmethylpyrazine and 2,5-dimethyl-3-ethylpyrazine, both described as fried potato-like, 2,6-dimethyl-3-ethylpyrazine described as cooked potato-like and 2,3,5-trimethylpyrazine described as musty potato-like were reported as volatiles of secondary importance to the odor of the crumb of rye bread (Grosch and Schiberle, 1991).

Studying the formation of volatile compounds from extruded corn-based model systems, Ho et al. (1989) identified 2-methyl-3(or 6)-pentyl-pyrazine, 2-methyl-3(or 6)-hexylpyrazine and 2,5-dimethyl-3-pentylpyrazine; they suggested that lipid-derived aldehydes might be involved in the formation of substituted pyrazines.

Pyrroles and Pyrrolidines (tetrahydropyrroles): Pyrroles have not received as much attention as flavor components as other heterocyclic Maillard reaction products such as pyrazines even though the number of derivatives determined is almost the same as that of pyrazines (Fors, 1983; Shibamoto, 1989). Pyrrolidines can be formed by heating proline with either glyceraldehyde or erythrose, arabinose, glucose and rhamnose at 150°C for 30 minutes (Tressl et al., 1985). The compound 2-acetylpyrrole was identified as a product formed in the L-cysteine/D-glucose model system upon microwave irradiation (Yeo and Shibamoto, 1991).

Some pyrrole derivatives have a pleasant flavor. For example, pyrrole-2-carboxyaldehyde gives a sweet, corn-like odor and 2-acetylpyrrole has a

caramel-like odor (Shibamoto, 1989). The compound 2-acetylpyrrole was reported in the nonbasic fraction of the steam volatile oil from potato chips (Buttery and Ling, 1972).

The compound 2-acetyl-1-pyrroline, has a popcorn-like odor and possesses a very high odor potency; it has been reported as an important flavor compound of several aromatic rice varieties (Buttery et al., 1983; Buttery et al., 1988). These results were confirmed by Tanchotikul and Hsieh (1991), who reported 2-acetyl-1-pyrroline ranging between 76 to 156 ppb in three varieties of aromatic rice.

Thermal decomposition of lipids

Flavor compounds generated from the decomposition of lipids during deep-fat frying are the most important contributors to food flavor (Ho et al., 1987). At elevated temperatures, oxidative decomposition of unsaturated fatty acids proceeds very rapidly. Unsaturated lipids (LH) form carbon-centered alkyl radicals (L·) and peroxyl radicals (LOO·), which propagate in the presence of oxygen by a free radical chain mechanism forming hydroperoxides (LOOH) as the primary products of autoxidation. Lipid hydroperoxides are readily decomposed into a wide range of carbonyl compounds, hydrocarbons, aldehydes, ketones and other compounds. For example, autoxidation of linoleate produces two conjugated hydroperoxides, the 9- and 13-hydroperoxides; the cleavage of both hydroperoxide produces hexanal and 2,4-decadienal. In contrast, octanal, nonanal, 2-decenal and 2-undecenal are the major aldehydes predicted from oleates (Nawar et al. 1978; Frankel 1980; Nawar, 1985; Frankel, 1991).

The pattern of the major polar compounds in heated fats is typical and consists of a series of alkanals, alkenals and dienals as well as smaller

amounts of some methyl ketones. In corn and soybean oil, hexanal, heptanal and *t,t*-2,4-decadienal are the three aldehydes produced in greatest quantity (Nawar et al., 1978). The odor of hexanal is described as green (Ullrich and Grosch, 1987), while the 2,4-decadienal formed by linoleic decomposition under conditions of deep-fat frying is described as deep fat fried (Patton et al., 1959, Ullrich and Grosch, 1987). The *t,t*-2,4-decadienal is an important odorant identified in the non-basic fraction of potato chip odor (Buttery and Ling, 1972) and was distinguished as a desirable fresh deep fat fried odorant among the 53 compounds identified by Deck and Chang (1965) in the volatiles from potato chips.

There is a significant reduction in the amount of decadienals when fats are heated at 250 °C as compared to heating at 185 °C (Nawar, 1978). One possible explanation is that saturated aldehydes can easily oxidize to form the corresponding acids and they can participate in dimerization and condensation reactions. For oxidation of the aldehydes with conjugated double bonds, Matthews et al. (1971) proposed that epoxides are formed by oxygen attack at the olefinic centers. In the case of 2,4-decadienal, either the 2,3-epoxy or the 4,5-epoxy derivative, can be produced as an intermediate. Similarly, hexanal, 2-butenal, hexane, and 2-butene-1,4-dial can be formed from decomposition of the 4,5-epoxide.

Various other reactions involving products of lipids oxidation may occur : i) unsaturated aldehydes can undergo classic autoxidation with oxygen attack at å-methylenic positions giving rise to short chain hydrocarbons, aldehydes, and dialdehydes such as malonaldehyde, ii) alkoxy radicals can also abstract a hydrogen atom from the å-methylene group of another molecule, producing a hydroxy acid, or can lose a hydrogen and give a keto acid, iii) reaction of alkoxy and peroxy radicals can produce epoxides, vi)

dimerization and polymerization reactions of lipids by thermal and oxidative mechanisms may occur. Linoleate, for example, can develop a conjugated double-bond system during thermal oxidation and then react with another molecule of linoleate (or oleate) to produce a cyclic dimer (Nawar, 1985).

Chang et al. (1978) identified 220 volatile degradation products from the deep-fat frying of corn oil, hydrogenated cottonseed oil, trilinolein and triolein. Among those compounds, the γ-lactones with unsaturation at the 2 or 3 position, 4-hydroxy-2-nonenoic acid lactone and 4-hydroxy-3-nonenoic acid lactone imparted a characteristic deep-fat fried flavor to cottonseed oil when added at 2.5 ppm. The responses of the panel to the description of the cottonseed oil, plus 4-hydroxy-2-nonenoic acid lactone, included nutty, fried fat notes, plus a butter-like note.

When ethyl linoleate was heated for 5 hr at 250 °C, the volatile decomposition pattern was dominated by the following major components: pentane, hexanal, ethyl hexanoate, 2-heptenal, ethyl octanoate, *t,c*-2,4-decadienal, *t,t*-2,4-decadienal, ethyl-8-oxooctanoate, ethyl-9-oxononanoate, 2-pentylfuran, monoethyloctanedioate. Qualitatively, the same pattern of major decomposition products of ethyl octanoate was obtained when the ester was heated at 250 °C for 1 hr and 180 °C for 1 hr (Henderson et al., 1980).

Linoleic acid heated between 4 to 154 minutes, gives rise to the following oxidation products: pentane, butanal, 4-methyl-2-pentene, pentanal, hexanal, heptanone, 2,4-decadienal, heptanal, 2-pentylfuran, 2-(1-pentenyl)-furan and 2-octenal (Dawson et al., 1991).

Studying the volatiles contributing to the flavors of deep-fried potatoes, Ha and Lindsay (1991) reported that *c*-4-heptenal (described as anchovy-like with green notes), cis and trans-2-octenoic acids (the later described as fresh nut, oatmeal, hand soap notes with a slight citrus overtone) and *t*-nonenoic

acid (green, fatty and soapy notes) were expected to provide general contributions to the overall fried-food flavor properties.

Eighty-seven volatile compounds were identified in the headspace of overheated beef fat including 7 alkanes, 31 alkenes, 18 aldehydes and 6 ketones. Aldehydes, which constituted 23.41% of the total GC peak area of the volatiles isolated, were the major components identified (Umano and Shibamoto, 1987). Similarly, Yasuhara and Shibamoto (1989), identified 9 aldehydes and 4 ketones in the headspace of heated pork fat. Hexanal was one of the major aldehydes produced and formaldehyde, which had never been reported prior to this study, was found in appreciable amounts.

Interaction between the food and the oil

It is generally recognized that lipid-protein interactions are involved in a variety of physical and chemical changes which are important to food quality, as for example in the aging of meat or the frozen storage of fish (Nawar, 1989). Aldehydes, the most important volatile products of rancid fats and oils react with free amino acids forming Schiff bases, which are later transformed by aldolization and condensation reactions into brown macromolecular substances. When mixtures of casein and propanal, hexanal, and nonanal were stored at 60 °C for 40 hr, the amount of available lysine decreased by 81%, corresponding with a binding of 0.53% of aldehydes with formation of Schiff bases and the odor of the aldehydes was effected (Pokorny, 1976). These specific interactions and their effects on the sensory characteristic of food may occur during product storage.

Reports on lipid-protein interactions at elevated temperature are scarce and the specific role of temperature in such interactions is imperfectly understood (Nawar, 1989). One of those reports states that when linoleic acid

or its esters were heated in the presence of valine, a number of interaction products were formed. The major product was 2-pentylpyridine which is thought to be produced by the reaction of 2,4-decadienal with ammonia. Additional interaction products found included secondary amides, isobutyloctanemide, isobutyllinoleylamide, alkylpyridines and alkylpyrroles (Henderson et al., 1980). The authors did not investigate the effects of those volatiles on the samples' odor and flavor, but it is known that pyridines have a unique odor at high concentration and an acceptable fatty or tallow-like odor in diluted solutions. The presence of alkylpyridines in cooked lamb may cause its rejection by some consumers (Shibamoto, 1989; Buttery et al., 1977). Pyrroles have not received as much attention as flavor components, but some pyrroles gives a sweet and corn-like odor while others have been found to contribute to off-flavor of food products (Shibamoto, 1989).

From their study concerning the mechanism of formation of volatile flavor constituents in potato chips, Chang et al. (1978) suggested that the following compounds could be produced from the interaction between decomposition products of amino acids and decomposition products of oils at frying temperature: 2-methyl mercaptosulfoxide-2-pentenal, 2-methyl mercapto-2,4,6-octatrienal and 2-methyl mercapto-5-methyl-2-hexenal. Similar results were obtained by Macku and Shibamoto (1991) while studying the influence of glycine on the volatile compounds generated by heating corn oil. They reported that the addition of glycine to corn oil decreased the amount of volatile unsaturated aldehydes by almost 110 times suggesting that secondary reactions occurred between glycine and the aldehydes. The amount of nitrogen-containing compounds, such as nitriles, pyridines and pyrroles in various corn oil/glycine mixtures increased with an increasing amount of

glycine in corn oil. The authors reported the presence of 1-methyl-2-propylpyrrole, 1-methyl-2-butylpyrrole and 1-methyl-2-pentylpyrrole.

Lipid oxidation at room temperature

The basic mechanism of autoxidation at room temperature is similar to that at elevated temperatures, i.e., a free radical chain reaction which involves the formation and decomposition of hydroperoxide intermediates. However, there are some unique aspects of room-temperature oxidation. Even though hexanal and 2,4-decadienal are typical scission products of linoleate hydroperoxides, there is a marked dominance of hexanal in the volatile products of low temperature oxidation, while at higher temperature, 2,4-decadienal is the major aldehyde formed (Nawar, 1989).

The most intense odor compounds formed during the autoxidation of methyl linolenate stored for 48 hr at room temperature were *t,c*-2,6-nonadienal (green odor), followed by 1,*c*-5-octadien-3-one (metallic), *t,c*-3-5-octadien-2-one (fatty/fruity) and *c*-3-hexenal (green apple-like). After 102 hr of storage, 1,*c*-5-octadien-3-one was by far the most important odor compound, followed by *c*-3-hexenal and *t,c*-2,6-nonadienal (Ullrich and Grosch, 1988a).

Among the volatile compounds formed during the autoxidation of linoleic acid and methyl linoleate at 22-24 °C, hexanal (described as a green odor), 2(Z)-octenal (fatty/fruity/slight-green) and 2(E)-nonenal (cucumber-tallowy) were the most potent odor compounds produced from both lipids. The 1-octen-3-ol (mushroom) followed in the case of linoleic acid and the 1-octen-3-one (spicy/fatty/nutty) in the case of methyl linoleate (Ullrich and Grosch, 1987). The 2-pentylfuran has been also suggested to be an autoxidation product of linoleic acid; its distinct beany and grassy flavor characteristics

have been associated with flavor reversion in soybean oil (Whitfield et al., 1988).

The unsaturated aldehydes undergo further oxidation to shorter chain aldehydes. In model systems, the 2,4-decadienal oxidizes producing hexanal and 2-octenal (Josephson and Lindsay, 1987).

In a study of oxidation at room temperature of three soybean oil samples, Guth and Grosch (1990) reported that (Z)-3-hexenal, 1-octen-3-one (mushroom-like odor), (Z)-1,5-octadien-3-one (geranium-like/metallic), 1-octen-3-hydroperoxide, (Z)-1,5-octadien-3-hydroperoxide (geranium-like/metallic), (E)- and (Z)-2-nonenal, 3-methyl-2,4-nonandione and *t*-4,5-epoxide-(E)-2-decenal (metallic/green), were the primary odorants formed. The authors reported that the major differences in the intensity of the reversion odor of the three soybean oil samples were due to an increase in the concentration of 3-methyl-2,4-nonandione during storage.

In cottonseed oil, 1-decyne generated from the photooxidation of cyclopropenoid fatty acids naturally present in the oil, produces a distinct off-flavor which is described as "light struck" (Fan et al., 1983).

The condition of fat in dehydrated potato granules, however, is quite different from that used by the workers mentioned above, who usually oxidized the fatty acid ester in bulk form by flushing with oxygen or air for a few days. In the later case, there is a relatively high concentration of fatty acid ester and a low concentration of air. With potato, or any fried snack, however, the fat is finely dispersed throughout an inert medium and is exposed to an abundance of oxygen, it might therefore be expected that the autoxidation products of the potato granules would be more degraded than those of liquid fatty acid esters. This was actually the case in the identification of the volatile compounds present in the autoxidized dehydrated potato reported by Buttery

(1961). The products identified were suggested to have arisen from the autoxidation of linoleic and linolenic fatty acids and included methane, ethane, propane, butane, pentane, acetaldehyde, propanal, 2-methylpropanal, butanal, pentanal, 2- and 3-methylbutanal, and hexanal.

Chemical indicators of lipid oxidation

There is a special interest among flavor researchers to link individual compounds isolated from oil volatiles with the degree of lipid oxidation in food and correspondent flavor panel scores. Linoleic acid, a predominant unsaturated fatty acid in many food products can be oxidized to hexanal, and to 2,4-decadienal which further decomposes into hexanal, which is in turn used as an indicator of lipid oxidation in food and in model systems.

Rho et al. (1986) reported that hexanal appears to be a good indicator of the development of oxidative rancidity in deep-fried instant noodles. In uncured meat, hexanal showed a significant linear correlation (R^2=0.96) with TBA values and sensory scores; the larger the hexanal content, the lower the acceptability of the meat (Shahidi et al., 1987). A correlation of 0.97 was reported between the hexanal content and the mean flavor scores in cookies prepared with a marginal animal fat shortening and held at ambient temperature in a cellophane wrapper (Jacobson et al., 1989). Özilgen and Özilgen (1990), developed a general simple kinetic model simulating lipid oxidation in which the oxidation time was correlated with hexanal production in order to describe quality loss in lipid food storage. Similarly, Koelch et al. (1991) developed a cubic model representing a mechanistic basis by which lipid oxidation could be followed and predicted over time by means of hexanal production.

Dupuy et al. (1977) reported that pentane, total volatiles and *t*-2-*t*-4-decadienal presented a significant linear correlation ($p \leq 0.05$) with flavor scores of soybean oils subjected to light exposure. From the three peak indicators, the *t*-2-*t*-4-decadienal was the best predictor, showing an R^2 of -0.97 ($p \leq 0.05$) being suggested as a reliable indicator of the quality of vegetable oils.

Several researchers concluded that the use of a single compound, such as hexanal, does not adequately assess the degree of lipid autoxidation and perceived flavor intensity. Min (1981) and Min (1983) correlated the levels of aldehydes formed in the oxidation of soybean oil with the oil flavor scores rated by a sensory panel and reported that the oil flavor scores (FS) could be predicted by means of the following equation:

$$FS = 17.85 - (1.12 \log A_{hc} + 1.04 \log A_{oc} + 0.95 A_{dc})$$

where A_{hc}, A_{oc} and A_{dc} are values of the peak areas observed for t-2-heptenal, *t*-2-octenal and *t,t*-2,4-decadienal respectively. In the range of 50 to 2000 ppb, the regression values (R^2) exceed 0.99 (N=5) with relative standard deviations of under 2% for heptenal and octenal and 5.8% for decadienal.

Drumm and Spanier (1991), found that during the storage of ground beef patties at 4 °C, lipid autoxidation products showed a significant ($p \leq 0.05$) increase; the rate of formation of these compounds generally followed zero-order kinetics and was specific to each individual compound showing R^2 values ranging from 0.27 to 0.83. Among those compounds were: pentanal, hexanal, heptanal, 2-heptenal, octanal, 2-octenal, nonanal, decanal, 2,4-nonadienal, 2,4-decadienal, 2-ethylfuran, furfural, 2-pentylfuran, pentanol, 2-hexen-1-ol, hexanol, heptanol, 1-octen-3-ol, 2-octenol, 1-octanol, 2-heptanone,

2,3-octanedione, 3-octen-2-one, 2-nonanone, 3-nonen-2-one, benzaldehyde and phenylacetaldehyde.

DESCRIPTIVE SENSORY ANALYSIS

Descriptive sensory analysis is a unique and highly specialized form of sensory analysis by which the individual attributes of a food material or product are identified, described, and quantified using human subjects who have been specially trained for this purpose (Einstein, 1991). It is founded on the concept that a person can be trained to perceive and recognize individual sensory characteristics of a product, rate their intensities and with the use of appropriate training aids, reach an agreement with his or her fellow panel members. Descriptive sensory methods replace the "individual expert" with a group of trained subjects that provide a collective response which in most descriptive methods can be statistically analyzed (Stone et al., 1974).

All descriptive methods require that the subjects verbalize their perceptions, developing a set of terms or words that form the basis for their scorecards. Usually, open sessions are held with small groups of judges to develop descriptive terms, to define attributes and descriptors, and to recommend physical references such as food and non-food substances, chemical compounds, etc. Language is the key element in a descriptive test; the subjects must agree as to the meaning of the words. In a quantitative descriptive test, subjects must also indicate how much of each sensory quality is perceived (Stone and Sidel, 1985). Among the many applications of descriptive analysis in research laboratories are: i) comparison of sensory with physical or chemical analyses, e.g. aroma vs. chromatographic analysis of

volatiles; ii) characterization of new varieties of fruits and vegetables, or new breeds or feeding practices for meat animals; iii) quantification of sensory changes due to time, temperature of storage, and/or to processing method, preservatives, packaging, etc, and; iv) in psychophysical studies to characterize the subtle differences among similar items, e.g. caffeine vs. quinine; sucrose vs. synthetic sweeteners, etc. (Pangborn, 1988).

Over the last 40 years many descriptive analysis methods have been developed, and some have gained and maintained popularity as standard methods: the Flavor Profile® (Cairncross and Sjöström, 1950), the Texture Profile® (Brandt et al., 1963; Szczesniak et al., 1963), Quantitative Descriptive Analysis (QDA Method)® (Stone et al., 1974) and the Spectrum® method (Meilgaard et al., 1988).

Flavor Profile® method

The Flavor Profile® method was developed by Arthur D. Little, Inc. in the late 1940s. It presents a descriptive analysis of flavor expressing in common language terms the characteristic notes of both aroma and flavor, their order of appearance and intensities, and the amplitudes of total aroma and total flavor. Groups of six to ten people are selected and trained with a broad selection of reference samples representing the product range, as well as examples of ingredient and processing variables for the product type. Intensity of individual character notes is judged initially by an arbitrary scale based upon the recognition threshold, using the following designations: not detectable, just detectable, slightly strong, moderately strong, and strong. The panelists individually evaluate one sample at a time for both aroma and flavor and record the attributes (called character notes), their intensities, order of appearance, and aftertaste. The results are reported to the panel leader, who

then leads a general discussion with the panel to arrive at a "consensus" profile for each sample. The data may be expressed in either diagrammatic or a tabular form, with no further statistical analysis (Cairncross and Sjöström, 1950; Meilgaard et al., 1988). The need for statistical analysis was noted by the developers of the method who emphasized that there would be confidence based on collective professional judgement and reliance on results, rather than on statistics (Stone and Sidel, 1985). As traditionally used, the method has several disadvantages: i) selection, training, and conducting of panels is extremely time-consuming and therefore a very expensive method of analysis; ii) results are not analyzed statistically; and iii) exclusive use is made of an open-discussion technique wherein judges continually influence each other's opinions (Pangborn, 1988).

Texture Profile® method

The Texture Profile® method was developed at General Foods Corporation to define the textural parameters of foods. Brandt et al. (1963) defined the method as the sensory analysis of the texture complex of a food in terms of its mechanical, geometrical, fat and moisture characteristics, the degree at which each attribute is present and the order in which they appear from the first bite through complete mastication. In developing the method, the objective was to eliminate problems of subject variability, allow direct comparison of results with know materials, and provide a relationship with instrumental measures (Szczesniak et al., 1963). These objectives are accomplished with the use of standard rating scales for each texture term and specific reference materials to represent each scale category for each of the terms. At the onset, texture terms and definitions are compiled, sorted and categorized (Stone and Sidel, 1985). Panelists selected for training are exposed

to a large range of products from the category under investigation in order to provide a wide frame of reference. In addition, panelists are introduced to the underlying textural principles involved in the structure of the products under study. Category, line and magnitude estimation scales have been used for judgement of attributes' intensities (Meilgaard et al., 1988).

QDA method®

This method was developed by Stone and Sidel (Stone et al., 1974). It uses from 10 to 12 subjects, each one qualified prior to participation. The language development process is free from leader influence and is considered a type of focus group, with the panelists evaluating products and verbalizing their perceptions. In addition, the subjects must define each term and where possible, designate an appropriate reference material which represents the particular sensation and the term used to describe that sensation. The QDA Method® uses a 15 cm line scale with word anchors, always moving from left to right with increasing intensity; e.g. weak to strong. The method suggests the analysis of variance (AOV) as the most appropriate statistical procedure for analyzing responses and uses a spire graph to display the results (Stone and Sidel, 1985).

Spectrum® method

This method was developed by Gail Civille (Meilgaard et al., 1988) and requires that all terminology is developed and derived by a panel which has been exposed to the underlying technical principles of each modality to be described. For example, a panel describing color must understand color value, hue and chroma. The intensity of each attribute can be rated in different types of scale; line, category or magnitude estimation; however, one must use at

least two reference intensity standards. Meilgaard et al. (1988) believe that a set of well-chosen reference intensity standards greatly reduces panel variability, allowing for comparison of data across time and products. Data can be statistically analyzed.

Free Choice Profile method

There are a number of sources of variation which might not be eliminated completely by panelist training (Arnold and Williams, 1986): i) assessors vary in their overall level of scoring; ii) assessors use descriptors in different ways; iii) assessors vary in their range of scoring; iv) assessors vary in their use of terms and scales between sessions and v) assessors might perceive different stimuli in the same products .

To overcome some of these problems the Free Choice Profile (FCP) procedure was developed (Williams and Langron, 1984; Williams and Arnold, 1985). This new method allows the assessors to choose their own vocabularies, thus eliminating the need for extensive training in descriptor use. The method uses Generalized Procrustes Analysis (GPA), eliminating the first three sources of variation listed above. GPA consists of three logically distinct steps: firstly, the centroids of each assessor's data are matched so as to eliminate the effect of use of different parts of the scales; secondly, isotropic scale changes remove the differences in the scoring range used by different assessors; thirdly, the configurations are matched as closely as possible by rotation and reflection of the axes (Arnold and Williams 1986). This produces a perceptual space for each assessor, which is matched as closely as possible with the other assessors. A consensus configuration is then calculated as the average configuration from all the assessors. This is usually simplified as a reduced dimensional plot by principal components analysis (PCA). The

residual errors (distances between the assessors' individual configurations and the consensus) can then be used to calculate coordinates for plotting the assessors, in order to identify outliers or groups (Piggot and Watson, 1992). FPC permits the use of untrained assessors, because there is no need for training in the use of descriptors. However, this may lead to misuse of descriptors and difficulties in interpretation of results (Guy et al., 1989). In addition, some assessors have difficulty in generating sufficient descriptors (Piggot et al., 1991).

RELATING INSTRUMENTAL WITH SENSORY DATA

Instrumental analyses supplement, but can never substitute for human measurement. By definition, colors, textures, flavors, and tastes are human sensations. Colorimeters measure absorbed or transmitted light, texturometers measure resistance to pressure or stress, and GC is used to separate and quantify volatile compounds (Pangborn, 1984). One of the most valuable applications of analytical sensory measurement is the relationship to corresponding physical and chemical measures (ASTM, 1968; Noble, 1975).

Perceived odor and flavor originate from stimulation of the sensory cells with compounds present in the food. With few exceptions it is a matter of several or many compounds taking part in the stimulation. The compounds responsible can be characterized in terms of identity and quantity, using GC/MS techniques. Perceived flavor, can be dealt with using sensory profiling techniques and in principle one can arrive at a set of qualitative sensory data corresponding to the set of chemical data. As mentioned earlier

in this chapter, there are theoretical and practical reasons for investigating the relationships between these sets of data (von Sydow and Akesson, 1977).

When correlating sensory with instrumental data, important requirements for making either statistical or non-statistical correlations include: i) the test samples must be identical for both measurements; ii) the test samples must cover a sufficiently wide range of the variability under observation; iii) there must be sufficient replication of both measurements; iv) it is necessary to calibrate sensitivity and reproducibility of both measurements; v) the same judges must participate in all sensory replications within a test; and vi) the same person should administer both tests (Guadagni, 1968; Dravnieks, 1976).

The contribution of volatiles to the odor of clingstone peaches was investigated by exit-port sniffing, descriptive flavor analysis and GC analysis of fresh samples of Halford peaches and of canned samples of Halford and nine other peach varieties (Spencer et al., 1978). Equations from stepwise multiple regression indicated the sensory characteristics were dependent on the relative concentrations of the volatile compounds present. Correlation studies indicated that differences among peach varieties were due more to the relative concentration of esters and monoterpenes than to the gamma-lactones. The latter contribute the necessary peachy background while the lower-boiling compounds contributed to the fruity and floral notes.

Paule and Powers (1989) correlated data from a trained sensory panel with GC/MS and sniffing of the GC effluents to examine odorous and non odorous rice varieties. The peaks possessing characteristic odor were identified by GC/MS and correlated with the desirable aroma terms from the sensory panel. The compound 2-acetyl-1-pyrroline showed highly significant

positive correlations with the descriptive terms; hexanol showed negative correlations.

Very recently, Spanier et al. (1992) correlated descriptive sensory data with chemical parameters developed during storage of meat. The authors reported that thiobarbituric acid (TBA) reactive substances, hexanal and pentanal showed a strong negative correlation ($p \leq 0.05$) with a cooked beef/brothy flavor attribute, but showed a strong positive correlation ($p \leq 0.05$) with both painty and cardboard flavor attributes. Even though the chemical parameters can be an easier and quicker way for monitoring meat flavor quality, the authors suggested that descriptive sensory panels are a requirement when studying meat flavor quality.

Correlating the intensity of bitterness of virgin olive oil samples with a new analytical method based on the extraction of the bitter constituents of the oil with methanol/water and measurement of the absorbance at 225 nm, Rosales et al. (1992), suggested the cited method as a real alternative to the use of the panel test for the evaluation of this taste attribute.

With the objective of developing an enzymatic method for quality evaluation of frozen stored fresh water and brackish water fish, Nambudiri and Gopakumar (1992), correlated results from a trained sensory panel with several chemical and enzymatic parameters from the fish muscle. The authors reported significant correlations ($p \leq 0.05$) between sensory scores and free fatty acid levels, peroxide values, ATPase and LDH activities in the fish muscle. The authors suggested the chemical and enzymatic parameters could be used for quality evaluation for the freshness of fish.

THE CAPABILITY AND PSYCHOPHYSICS OF OSME: A NEW GC-OLFACTOMETRY TECHNIQUE

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ABSTRACT

Osme is a time-intensity approach for evaluating the odor significance of compounds in the GC-effluent: it provides an easily interpretable FID-style aromagram called an Osmegram. Osme capability and reliability was assessed by four trained subjects who directly recorded the intensity, duration, and quality of each sample odorant in the GC-effluent. Samples consisted of five model solutions; each solution contained the same six aroma compounds but at different concentrations. The subjects were capable of establishing significant psychophysical functions ($p \leq 0.05$) between *maximum odor intensity* and *odorant concentration* and between *area under the odor peak* and *odorant concentration*. The R^2 values ranged from 0.82 to 0.94. Individual standard deviations in odor ratings ranged from 1.45 to 2.07 (16-point intensity scale). Subjects were in good agreement in rating each compound's odor potency and quality; overall, Osme was comparatively quantitative with traditional olfactometry techniques.

INTRODUCTION

Gas chromatography olfactometry (GCO) deals with sensory measurements of odors present in GC effluents. Its use in flavor research has three objectives: i) to establish odor active compounds in flavor extracts, ii) to determine a compound's single odor quality, and ii) to quantify a compound's individual odor significance in flavor systems.

The interest in determining the individual contribution of volatile compounds present in flavor systems has lead to a generation of new GCO techniques, among which Charm (Acree et al., 1984; Cunningham et al., 1986; Marin et al., 1988) and AEDA (Schieberle and Grosch, 1987; Schieberle and Grosch, 1988) are most often cited in the flavor literature.

Charm and AEDA are based on the determination of odor-detection thresholds of the compounds eluted from the GC-column rather than the psychophysical estimation of their individual odor intensity. In this aspect both methods are similar to the odor unit technique proposed by Guadagni et al. (1966), and furthermore are subject to the same criticisms (Maarse, 1991).

As first pointed out by Fritjers (1979) in regards to the Guadagni odor unit concept and later by Piggot (1990) and Maarse (1991) with respect to Charm and AEDA, the fact that different odorants may show different intensity functions above their threshold, limit threshold-based techniques' valid conclusions about the relative contribution of individual odorants to a flavor system, even though these techniques still give some guidance in identifying areas of the chromatogram which are likely to be important. Current psychophysical views represented by Stevens' Law (Stevens, 1957; Stevens, 1961), establish that the relationship between the odor intensity (I) of

a compound grows with the compound's concentration (**C**) raised to a power **n**:

$$I=k(C-T)^n \quad ;$$

where **T** is the compound's effective threshold and **k** is the constant of proportionality. Furthermore, it is evident that under Stevens' Law, two different compounds at the same concentration (**C**), and possessing very close thresholds (**T**) but showing different exponents (**n**), may yet produce different individual odor intensities (**I**) and consequently provide different individual odor contributions to the intensity and quality of a flavor system.

In an attempt to create a new GC-olfactometry methodology which would be strongly founded on current psychophysical views, our sensory laboratory has developed a new method called Osme (McDaniel et al., 1990; Miranda-Lopez et al., 1992; Sanchez et al., 1992 and Da Silva et al., 1992a). Osme combines the modern concepts of sensory descriptive analysis with novel techniques of computerized data collection and applies the Selke et al. (1972) time-intensity approach of evaluating the odor significance of compounds in the GC effluent. With Osme, trained subjects sniffing the GC effluent mixed with humidified air, directly record the odor intensity and duration time of each odor-active compound while describing its odor quality (Figure 2.1A). The plot of the *retention time* versus *odor intensity*, called an Osmegram, provides a graphical representation of the compound's odor significance in the flavor extract; higher peaks suggest greater importance (Figure 2.1B). According to Piggot (1990), Osme is a more satisfactory GCO approach than the afore mentioned methods.

Some past criticisms to the use of GCO are based on the following assertions: i) data generated by GCO techniques are only semi-quantitative (Burr, 1964); ii) rapidly eluting peaks allow little time for aroma characterization (Clark and Cronin, 1974); iii) the relationship between the compound concentration and the response provided by the human nose is not linear (Wick, 1965) and; iv) fatigue decreases assessor's efficiency during a long run (Burr, 1964; Clark and Cronin, 1974). However, a review of the literature does not provide evidence which could restrict the use of olfactometry associated with gas chromatography as suggested by the early mentioned researchers.

In contrast, comprehensive investigations of human sniffing support the feasibility of GCO: i) identification of odor compounds at their recognition threshold levels is achieved with a single sniff, whose average duration is just 0.45 seconds (Laing, 1986), ii) perception of odorants maximum intensity takes between 0.39 and 0.64 seconds (Laing, 1985) and iii) in odor threshold and intensity tests, results achieved with a single normal relaxed breath were not improved upon when subjects used an unlimited number of sniffs or their strongest single sniff (Laing, 1983). However, increasing the flow rate of an odorant from an olfactometer to a subject's nose such as in GCO, decrease the odorant threshold level, supposely due to enhancing odorant transport and concentration at the receptor's level (Schneider et al. 1966; Tucker, 1963).

Since most of the criticisms directed at traditional GCO techniques question a subject's capability and reliability to establish a significant relationship between compound concentration and sensory response, this study was conducted not only to answer those questions, but also to determine the nature of the relationship between physical stimulus

concentration and subject response when using the recently developed Osme.

MATERIALS AND METHODS

Samples

A set of six odorants representing various chemical classes and degrees of trigeminal irritability was used. Samples consisted of five pentane model solutions, each one containing the same six odorants, but at randomly assigned different concentration levels as indicated in Table 2.1. The five concentration levels of each odorant were chosen in such a way that their odor intensity as perceived by the subject sniffing the GC effluent, should nearly cover the entire range of a sixteen point intensity scale (0= none, 15= extreme). All compounds were purchased from Aldrich Chemical Co. (Milwaukee, WI) and had a nominal purity of at least 98% except nonyl aldehyde whose purity was 95%.

GC-olfactometer

The gas chromatograph (Hewlett Packard model 5890, Avondale, PA) was equipped with an FID detector and a 30m Supelcowax10 capillary column (Supelco, Inc., Bellefonte, PA) whose internal diameter and film thickness were 0.53mm and 0.50μm, respectively. For the sensory evaluation of the GC effluents, the chromatograph was modified so that the column was moved from the FID to another detector base, but without the flame. A sniffer, consisting of a 60 cm x 1 cm glass tube, coated with silicone (Sylon CT, Supelco, Inc., Bellefonte, PA) was set on the top of the second detector base in order to allow the eluted compounds to be directed to the subject (Figure 2.1A). Effluents were mixed with humidified and charcoal filtered air. The air

flow rate and relative humidity at the end of the sniffer were 10.2L/min and 60%, respectively. The GC was run with an injector temperature of 200^{0}C, a detector temperature of 250^{0}C, and He flow rate of 3.1 ml/min. For each run, the temperature was programmed as follows: initial temperature (60^{0}C), rate (2.0^{0}C/min), final temperature (155^{0}C), rate A (5.0^{0}C/min.), final temperature (240^{0}C), isothermal conditions for 30 min. at 240^{0}C. For each sample, 2 μL was injected. Retention data for a series of hydrocarbons were obtained under the same operating conditions as the GC-olfactometer but using the FID. The compounds's retention times for both GC-olfactometer and GC/FID were converted into Kovats' indexes (Kovats et al., 1958).

Data acquisiton

Using a data acquisition device which contained a category scale (Figure 2.1B), subjects, sniffing the GC effluent mixed with humidified air, recorded the intensity and duration of each odor active compound while describing its odor quality to the researcher. The device consisted of a variable resistor with a pointer that the subject could move from left to right and back again across a 16 point structured scale (0= none; 1= just detectable; 2; 3= slight; 4; 5=slight to moderate; 6; 7= moderate; 8; 9= moderate to large; 10; 11= large; 12; 13= large to extreme; 14; 15= extreme). Time was monitored and data collected by a software system installed in an IBM XT personal computer. Odor intensity values were collected every quarter second as indicated by a change in electrical resistance from a variable resistor inside the data acquisition device. Each panelist evaluated each sample in four replicates, each replicate being a different GC run. The sniffing time in each run was 20 min. For each odorant, Osme provides (Figure 2.1B): i) the odor peak, obtained by plotting *retention*

Table 2.1 Composition of model system pentane solutions.

Solution number	Compound Concentration (µl/ml)					
	Butyl Sulfide	Hexyl Alcohol	Nonyl Aldehyde	Ethyl Caprylate	2-Decanone	Linalool
1	1.20	22.50	0.75	1.84	6.30	7.72
2	4.90	92.00	2.30	7.36	58.35	124.81
3	3.05	57.50	4.00	0.92	27.80	1.68
4	0.75	147.50	0.43	14.72	13.25	30.69
5	1.90	14.06	1.32	0.46	3.00	0.43

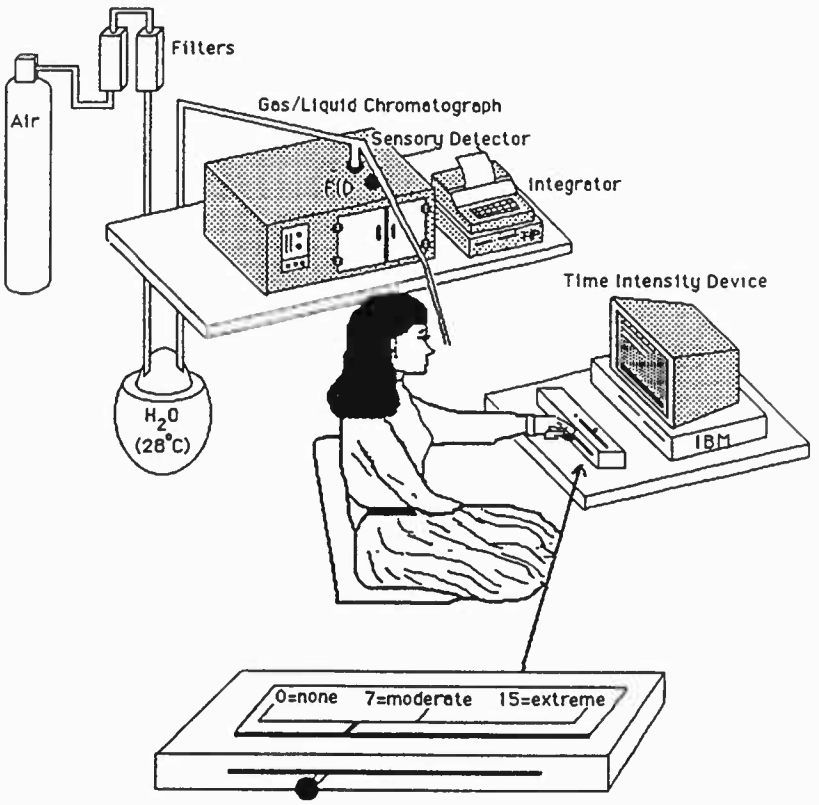

Figure 2.1 - Osme equipment and Osmegram.

time x *odor intensity* values, ii) the odor duration time (d), which is the total time that the panelist could detect the compound in the GC-effluent; iii) the maximum odor intensity (I_{max}); iv) the area under the odor peak; v) the Kovats index based on panelist response and; vi) the odor quality.

Training procedure

Subjects consisted of four student volunteers from the Food Science and Technology Department at OSU, selected on the basis of availability and interest. Subjects completed twelve training sessions prior to data collection in order to become familiar with the time intensity device and correspondent category scale ranges, to become familiar with the sensory properties of the compounds evaluated, and to reduce variability between and within the subjects. Two solutions were used for training purposes: one solution containing all of the six compounds at their highest tested concentration levels, and the other at their lowest tested concentration levels. In addition, intensity standards were provided as reference points for the scale in order to reduce panelist variability (Meilgaard et al., 1988). The intensity standards were anchored at point 3 (40 ml of safflower oil, Saffola Quality Foods Inc., Los Angeles, CA), point 7 (30 ml of orange drink; Hi-C, Coca Cola Foods, Houston, TX), point 11 (30 ml of grape juice, Welch's, Concord, MA), and point 13 (one stick of cinnamon bubble gum, Plen T-Pak Big Red, Wrigley Jr. Co., Chicago, Il.) of the intensity scale. The standards were presented at ambient temperature in 210 ml tulip shaped wine glasses covered with aluminum lids. After evaluation of each eluted aroma compound panelists were asked to evaluate the standard whose odor intensity was closest to the odor intensity of the eluted compound in order to confirm their intensity rating. During the first training sessions, subjects evaluated the GC effluent of

each model solution and generated their individual descriptor terms for each odorant. Based on those descriptors, reference standards were provided to the Osme panel prior to each session as an aid to the development of consensus descriptors. However, when a consensus could not be obtained, the subject was allowed to remain with his/her own descriptor.

Statistical analysis

Assessment of subjects' capability in establishing a psychophysical function between stimulus concentration and sensory response was conducted by fitting the *compounds' concentrations* vs. both *odor intensities* and *area under the odor peak* generated by each panelist with a simple linear function, a logarithm function, or a power function. Assessment of the best-fit function between *maximum odor intensity* (I_{max}) vs. *compound concentration*, and between *area under the odor peak* vs. *compound concentration* was conducted by plot analysis, residual analysis and the lack-of-fit test as detailed by Neter et al. (1989). Panelist reliability was determined by the traditional coefficient of determination of the fitted function R^2, as suggested by Moskowitz (1985). Estimation of subjects reproducibility in rating odor intensity and area under the odor peak was conducted by calculating individuals' pooled standard deviations over the odorants tested. Finally, the minimum significant difference (MSD) ($p \leq 0.05$) between mean ratings was obtained for each panelist using the individuals pooled standard deviations to perform a pooled *t*-test (Devore and Peck, 1986).

RESULTS AND DISCUSSION

Subject capability

All subjects were capable of establishing a significant ($p \leq 0.05$) relationship between *odor intensity* and *physical stimuli* and between *area under the odor peak* and *physical stimuli* for all odorants except ethyl caprylate independent of the mathematical model tested: linear, logarithmic and power (Table 2.2 and Table 2.3). These results suggest that the short period over which subjects are exposed to the odorant in the GC-effluent is indeed sufficient to allow them to perform qualitative and quantitative sensory measurements. Our findings are supported by Laing (1985) who reported that under the inhalation rate of 38.6 L/min., the average human takes between 0.39 and 0.64 seconds to perceive the maximum odor intensity of a specific concentration of odorants independent of its degree of irritability. In the chromatogram, peaks presenting 0.04 min width at their half-height, a typical value for GC high resolution capillary column (Anonymous, 1985), provide subjects with at least 2.4 seconds to perform their evaluation. Therefore it should not be surprising that these subjects were able to produce quantitative odor measurements from the GC effluent. The odor intensity level for ethyl caprylate reached only 6 (16 point intensity scale) at the highest odorant concentration. Consequently, we were not able to cover the entire range of the odor intensity scale and data related to ethyl acetate were dropped from further analyses.

Nature of the relationship between physical stimulus and subject response

Evaluation of the best model relating two or more variables is often conducted by examining the magnitude of correlation coefficients, however it is not an adequate technique since correlations sometimes can be higher for

Table 2.2 Significant correlations and lack-of-fit for the linear, logarithmic and power functions relating maximum odor intensity in the GC-effluent and odorant concentration.

Odorant	Linear				Logaritmic				Power			
	S_1	S_2	S_3	S_4	S_1	S_2	S_3	S_4	S_1	S_2	S_3	S_4
Butyl Sulfide	***	***	***	**	***	***	***	**	***	***	^{**Lf}	**
Hexyl Alcohol	***	**	n.s.	*	***	***	n.s.	*	***	***	n.s.	*
Nonyl Aldehyde	*	**	**	**	**	**	**	**	**	***	*	*
Ethyl Capryolate	*	n.s.	n.s	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
2-Decanone	***	^{***Lf}	**	**	***	***	**	**	***	^{***Lf}	**	^{**Lf}
Linalool	^{**Lf}	^{***Lf}	***	*	***	***	***	*	^{***Lf}	***	***	n.s.

^{S}Subject. *, **, *** Significant correlation at: * $p \leq 0.05$, ** $p \leq 0.01$ and $^{***}p \leq 0.001$. ^{Lf}Significant lack-of-fit at $p \leq 0.05$
$^{n.s.}$Not significant at $p \leq 0.05$

Table 2.3 Significant correlations and lack-of-fit for the linear, logarithmic and power functions relating area under the odor peak and odorant concentration.

Odorant	Linear				Logaritmic				Power			
	S_1	S_2	S_3	S_4	S_1	S_2	S_3	S_4	S_1	S_2	S_3	S_4
Butyl Sulfide	***	***	***	***	***	***^{Lf}	***	***	***	***	***^{Lf}	**
Hexyl Alcohol	***	***	**	***	***	***	***	***	***	***	***	**
Nonyl Aldehyde	***	***	*	**	***	***	*	**	**	***	**	*
Ethyl Capryolate	***	n.s.	n.s	n.s.	**	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.
2-Decanone	***	***	***	***	***^{Lf}	***^{Lf}	**	***^{Lf}	***	***^{Lf}	**	***^{Lf}
Linalool	***^{Lf}	***^{Lf}	***	****	***^{Lf}	***^{Lf}	***	***^{Lf}	***	***^{Lf}	***	***^{Lf}

^{S}Subject.*, **, *** Significant correlation at: * $p \leq 0.05$, ** $p \leq 0.01$ and ***$p \leq 0.001$. ^{Lf}Significant lack-of-fit at $p \leq 0.05$. $^{n.s.}$Not significant at $p \leq 0.05$

incorrect than for correct models (Birnbaum, 1973). In this study, appropriateness of the three functions, linear, logarithmic or power, in relating *compound concentration* x *maximum odor intensity* as well as in relating *compound concentration* x *area under the odor peak* perceived by the subjects sniffing the GC effluent was conducted by the lack-of-fit test. Results indicated that in general, the three mentioned functions fit the data very well and it is not clear which one would be the best fitting function (Table 2.2 and Table 2.3).

The logarithmic function showed slightly better fit for relating *compound concentration* x *maximum odor intensity*: a significant correlation ($p \leq 0.05$) between the maximum odor intensity and the log of compound concentration was found in 20 of the 24 sets of data and the logarithmic function was rejected as being the best fit function in only two of these 20 sets; and the power function provided a significant correlation ($p \leq 0.05$) in 18 of the 24 sets of data being rejected as the best fit function in four of the 18 sets (Table 2.2). Reports in the literature are also conflicting regarding which is the appropriate function relating categorical sensory data with stimulus concentration. While Galanter and Messick (1961), Pangborn (1981) and Moskowitz (1983) suggest that category scales produce data which are often linear with the log of the stimulus intensity, Stevens (1961), Marks (1968) and Foley et al. (1983) point to the power function as best describing the relationship between categorical responses and physical stimuli. Since current psychophysical views assume that the relationship between sensory magnitude and stimulus-concentration is a power function and our study did find a reasonable fit for the single power function, we decided to fit the later function for estimating subjects' reliability in rating *odor intensity* of odorants present in the GC effluent.

The linear function showed a better fit for the relationship between *compound concentration* and *area under the odor peak*: a significant correlation ($p \leq 0.05$) between the area under the odor peak and the compound concentration was found in 21 of the 24 sets of data (Table 2.3); and the linear function was rejected in only two of those sets; while the logarithmic and the power functions were rejected in 7 and 5 of the 24 sets of data, respectively. Furthermore, the linear function was used for estimating subjects' reliability in assessing *area under the odor peak* of odors present in the GC effluent.

Subject reliability

Testing subjects' reliability corresponds to estimating how much of the variability reported by the individual in odor rating was indeed due to the stimuli changing instead of to the subject's physiological and/or psychological effects. Moskowitz (1985) recognizes this test as "construct validity" and uses the Pearson's correlation coefficient ($\sqrt{R^2}$) to determine how well panelists' sensory responses correlate with actual stimuli intensities. In the present study, testing of subjects' reliability was conducted by using the R^2 of the individual fitted odor intensity functions (*odor intensities* vs. *odorant concentrations*). Values of R^2 ($p \leq 0.05$) close to 1.0 indicate a perfectly reliable subject, while an R^2 close to zero suggests a totally unreliable subject. In addition, since in psychophysics, results are commonly expressed as mean responses, in this study arithmetic means were computed over replications and subjects' averaged odor intensity functions across compounds were obtained ($p \leq 0.05$) with the correspondent R^2 values (Table 2.4). The R^2 values of the subjects who where capable of generating odor intensity functions ($p \leq 0.05$), ranged between 0.82 and 0.94 (Table 2.4). This implies that the model accounted for more than 82% of the variation reported by subjects (over 4

Table 2.4 [a]**Determination coefficients (R^2, $p \leq 0.05$) for individuals' averaged odor intensity functions (*odor intensity* vs. *odorant concentration*).**

Odorants	Subjects			
	S1	S2	S3	S4
Butyl sulfide	0.94	0.87	n.s.	0.82
Hexyl Alcohol	0.91	0.89	n.s.	0.83
Nonyl Aldehyde	0.92	0.88	n.s.	0.93
2-Decanone	0.92	0.86	n.s.	n.s.
Linalool	0.82	0.89	0.85	n.s.

[a] Computed on basis of mean over four replications.

replications) in maximum odor ratings. Furthermore, less than 18% of the variation reported by the subjects where due to psychological and psychophysical errors. These results are comparable to those reported by Hall and Anderson (1983) which by using a dynamic olfactometer determined the odor intensity functions of 14 odorants and reported R^2 values ranging from 0.81 to 0.98. The authors also averaged the data over the panel responses.

The averaging procedure substantially decreases the degrees of freedom, futhermore not all subjects were able to generate significant ($p \leq 0.05$) odor intensity functions over the averaged results (Table 2.4). Subjects' inability to perceive odor intensity changes with odorant concentration changes was also reported by Berglund et al. (1971); this phenomenon was associated with 13 of the 28 odorants they investigated.

Estimation of subjects' reproducibility is often obtained by assessing individuals' standard deviations. In the present study, the individual pooled standard deviations of maximum odor intensity ratings (16-point scale) values ranged from 1.45 to 2.07 (Table 2.5). It is difficult to validate these results with literature data because the traditions of psychophysics from the early work of Weber until recent times, have emphasized generalized laws relating group or mean responses to stimulus conditions with little attention to individual differences (Tucker, 1960). Within the variability reported in Table 2.5, the more reliable subject would be capable of detecting significant differences ($p \leq 0.05$) between mean ratings differing from 2.5 and the less reliable subject would be capable of detecting significant differences ($p \leq 0.05$) between mean ratings differing from 3.6 points on the 16-point intensity scale (3=slight, 7=moderate), as indicated by the pooled *t*-test. Hence, by using Osme, subjects can provide quantitative data when rating odor intensities of

Table 2.5 Subjects' pooled standard deviation (sd) by odorant (n= 5) and averaged minimum significant difference (MSD) at $p \leq 0.05$ for odor intensity ratings.

	Subjects			
Odorants	S_1	S_2	S_3	S_4
Butyl Sulfide	1.32	1.49	1.34	2.56
Hexyl Alcohol	1.59	1.77	1.94	2.46
Nonyl Aldehyde	1.41	1.70	1.76	2.16
2-Decanone	1.22	1.65	1.90	1.61
Linalool	1.70	1.35	1.09	1.57
sd_{avg}[a]	1.45 (0.20)	1.59 (0.17)	1.61 (0.37)	2.07 (0.46)
$\overline{MSD}$[b]	2.51	2.75	2.79	3.59

[a]Subject's pooled standard deviations averaged over odorants. [b]Averaged minimum significant ($p \leq 0.05$) difference between two means ratings in order to establish a significant difference ($p \leq 0.05$).

GC effluent. Higher discriminating power can be obtained by increasing the number of replications for each subject.

All subjects, by evaluating the GC effluents, were able to generate data which yielded linear functions ($p \leq 0.05$) between compound concentration and the averaged area under the odor peak (average over four replications). Subjects' coefficients of determination most often were above 0.90 ranging from 0.82 to 0.97 (Table 2.6). Furthermore, assuming that a linear function is appropriate to fit the variation of peak area across compound concentration, we can estimate that subjects' physiological and psychological effects contribute less then 18% of the total variability.

Overall, individual pooled standard deviations in rating area under the odor peak ranged from 0.53 to 0.92 (Table 2.7). Such variability would allow the more reliable subject of detecting significant sample differences ($p \leq 0.05$) where mean area under the odor peak responses differed by at least 0.82 units and the less reliable subject would be capable of detecting significant differences ($p \leq 0.05$) where mean ratings differed at least by 1.59 units.

There were no observable fatigue effects during the GC-sniffing run since the individual R^2 and the pooled standard deviations of butyl sulfide and linalool, the first and the last eluted compounds respectively, did not show any noticeable difference (Table 2.4, 2.5, 2.6, 2.7). Similar findings were reported by Pangborn et al. (1964) who studied the influence of methodology on olfactory response; they did not find any observable decrease in sensitivity due to fatigue or adaptation. These subjects were subjected to a 100-sample presentation in a 80 minute continuous sniffing session. Analogously, Berglund et al. (1971) reported no adaptation and cross-adaptation effects for subjects performing from 70 to 77 comparison stimuli (odor) per session; each session lasted 20 minutes with intervals of 15 seconds between stimuli.

Table 2.6 Determination coefficients[a] (R^2, $p \leq 0.05$) for individuals' averaged linear functions between area under the odor peak and compound concentration.

Odorants	Subjects			
	S_1	S_2	S_3	S_4
Butyl sulfide	0.95	0.96	0.84	0.99
Hexyl Alcohol	0.95	0.94	n.s.	0.93
Nonyl Aldehyde	0.92	0.86	0.91	0.82
2-Decanone	0.97	0.98	0.91	0.91
Linalool	0.94	0.86	0.98	0.97

[a] Computed on basis of mean over four replications.

Table 2.7 Subjects' pooled standard deviation (sd) by odorant (n= 5) and averaged minimum significant difference (MSD) at $p \leq 0.05$ for area under the odor peak ratings.

	Subjects			
Odorants	S_1	S_2	S_3	S_4
Butyl Sulfide	0.25	0.18	0.36	0.46
Hexyl Alcohol	0.53	0.63	0.86	1.07
Nonyl Aldehyde	0.29	0.33	1.08	0.45
2-Decanone	0.92	0.62	1.34	0.61
Linalool	0.67	0.60	0.96	0.60
sd_{avg}[a]	0.53 (0.28)	0.47 (0.20)	0.92 (0.36)	0.64 (0.23)
$\overline{MSD}$[b]	0.92	0.82	1.59	1.11

[a]Subject's pooled standard deviations averaged over odorants. [b]Averaged minimum significant ($p \leq 0.05$) difference between two mean ratings in order to establish a significant difference ($p \leq 0.05$).

Assessment of a compound's odor significance

In odor research, the primary use of a psychophysical function is the assessment of odorant potency. Results are commonly expressed as mean responses such as those presented in Figure 2.2. Subjects #1, #2 and #4 agreed that both nonyl aldehyde and butyl sulfide were odorants of similar potency and had considerably higher odor strength than hexyl alcohol. For these odorants, intensity values ranged from nearly 3 (slight) to 12 (11= large) on the 16-point scale over concentrations of 0.2 to 7.0 μL/ml. Nearly 10 times more hexyl alcohol was required to produce the same sensory response. Similarly, subject #1 and #2 agreed that both linalool and 2-decanone were more potent odorants than hexyl alcohol . Further, subjects #1 and #2 agreed that nonyl aldehyde was a more powerful odorant then 2-decanone. These results also are an indication that it is possible, to some extent, to obtain a measure of the odor potency and consequently estimate the relative significance of odorants present in the aroma extract by directly assessing their odor intensities in the GC-effluent.

There was a better agreement among subjects regarding the functions relating area under the odor peak and odorant concentration (Figure 2.3).

The relationship between area under the odor peak and odorant potency is not straight forward; however, one could postulate that at the same concentration and elution time, compounds showing larger values for area under the odor peak are likely to be more potent odorants then compounds presenting small area values. Under this assumption, as was observed from the odor intensity data, the area under the odor peak functions in Figure 2.3 suggest that both nonyl aldehyde and butyl sulfide had considerably higher odor strength than hexyl alcohol; in order to generate similar area under the odor peak values, much larger concentrations of hexyl alcohol are required, as

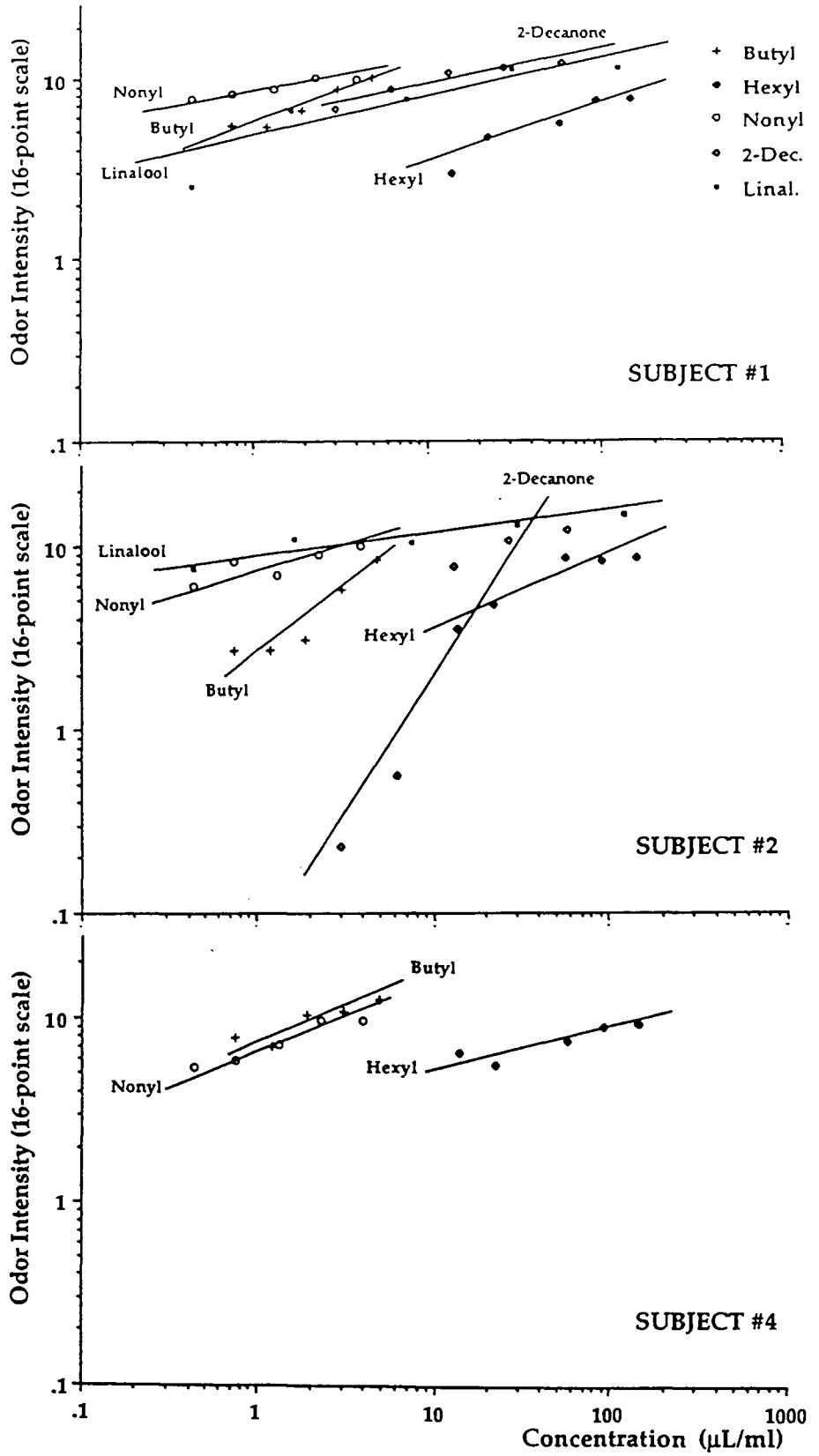

Figure 2.2- Subjects' intensity functions of odorants present in the GC-effluent as determined by Osme.

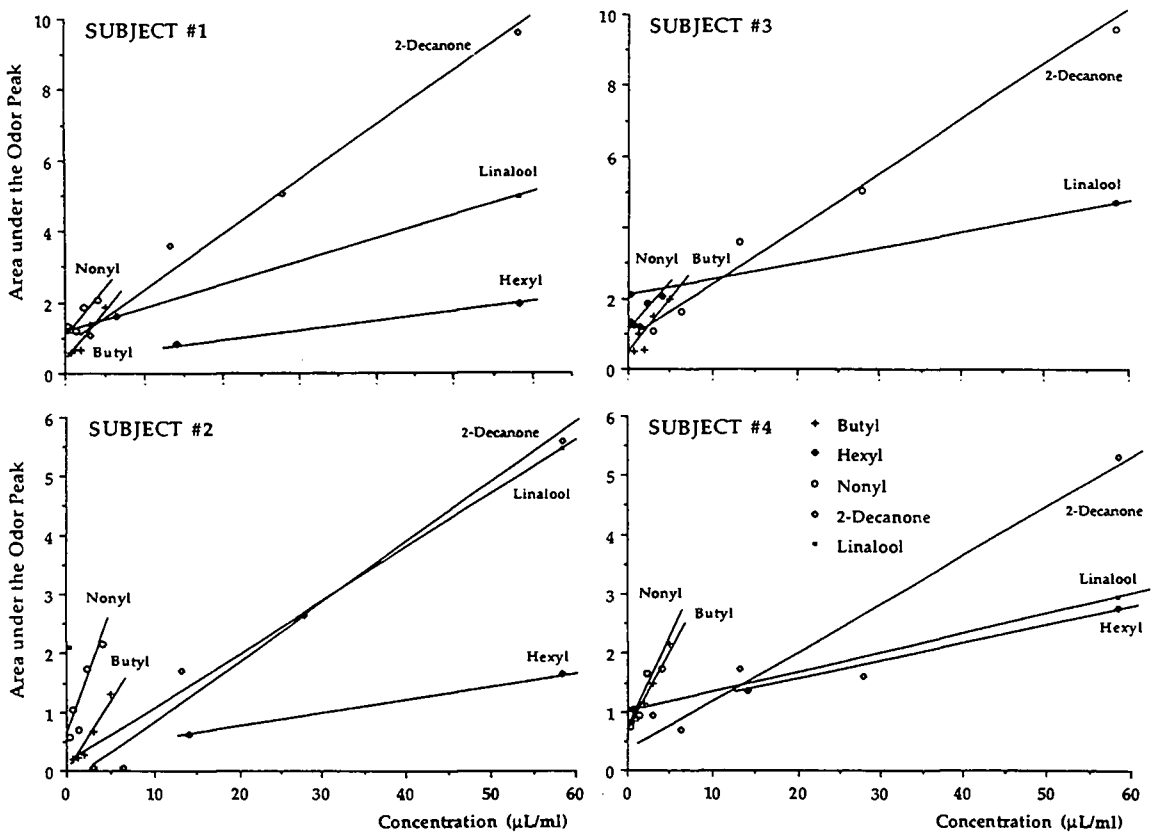

Figure 2.3- Subjects' individual functions relating area under the odor peak and odorant concentration of compounds present in the GC-effluent as determined by Osme.

compared with nonyl aldehyde and butyl sulfide. Similarly, subjects indicated that 2-decanone and linalool are more potent odorants than hexyl alcohol. Overall, conclusions obtained by the analysis of the area under the odor peak functions correlated very well with those conclusions obtained with the odorants intensity functions.

Another measure of the relative strengths of odorants are the slopes of the odor intensity functions, also refered to as exponents of power functions (Table 2.8 and Table 2.9). For butyl sulfide, individual exponents of the intensity functions ranged from 0.30 to 0.64; for hexyl alcohol values ranged from 0.21 to 0.40; for nonyl aldehyde and linalool individual exponents varied between 0.13 and 0.30 and between 0.11 and 0.26 respectively. Substantial individual variation occurred regarding the 2-decanone exponents, which ranged between 0.20 and 1.47. Individual differences are customarily reported in psychophysics measurements and should not be perceived as unique to GCO. Differential sensitivity to odorants, tastants and oral irritants may be attributed to gender, menstrual status, genetic endowment, age and personality as defined by various psychological tests (Stevens, 1991). Piggot and Harper (1975) found that magnitude estimation of the odor intensity of 1-butanol resulted in power function exponents ranging from 0.25 to 0.49 for eleven subjects. Between individual differences in the power exponents of odorants was consistently found by Berglund et al. (1971) who reported power function exponents for acetone ranging from 0.12 to 1.02; for pyridine from 0.06 to 0.70; for eugenol from 0.08 to 0.69, among others. Berglund et al. (1971) attributed those variations to perceptual differences among subjects rather them to response bias. Tuorila (1981) reported individual exponents of odor intensity function

Table 2.8 Slopes[a] and correspondent standard deviations for individual odor intensity functions (log I= log C + n log k ; p≤0.05).

Odorants	Subjects			
	S_1	S_2	S_3	S_4
Butyl Sulfide	0.38 (0.05)	0.64 (0.15)	n.s.	0.30 (0.08)
Hexyl Alcohol	0.38 (0.07)	0.40 (0.08)	n.s.	0.21 (0.06)
Nonyl aldehyde	0.13 (0.02)	0.21 (0.05)	n.s.	0.30 (0.05)
2-Decanone	0.20 (0.04)	1.47 (0.34)	n.s.	n.s.
Linalool	0.26 (0.07)	0.11 (0.02)	0.11 (0.03)	n.s.

[a] Computed on basis of mean over four replications. I, odor intensity; C, odorant concentration. n.s., not significant at p≤0.05.

Table 2.9 Slopes[a] and correspondent standard deviations for individual functions of area under the odor peak x odorants concentration.

Odorants	Subjects			
	S_1	S_2	S_3	S_4
Butyl Sulfide	0.33	0.28	0.35	0.32
	(0.04)	(0.03)	(0.09)	(0.01)
Hexyl Alcohol	0.03	0.02	n.s.	0.03
	(0.01)	(0.01)		(0.01)
Nonyl aldehyde	0.44	0.44	0.78	1.29
	(0.08)	(0.10)	(0.14)	(0.35)
2-Decanone	0.15	0.10	0.10	0.08
	(0.02)	(0.01)	(0.02)	(0.01)
Linalool	0.07	0.06	0.05	0.03
	(0.01)	(0.01)	(0.01)	(0.00)

[a] Computed on basis of mean over four replications. n.s., not significant at $p \leq 0.05$.

ranging from 0.34 to 0.77 for vanillin, from 0.31 to 1.10 for n-butyl acetate, from 0.15 to 0.81 for trimethylamine, from 0.30 to 1.02 for diacetyl, and from 0.47 to 0.97 for limonene. Algom et al. (1986) in a study of the power function of electrocutaneous stimulation, reported individual subjects' exponents ranging from 0.70 to 2.48. More recently, Yau and McDaniel (1990) reported the power law exponents of carbonation perception in water ranging from 2.17 to 4.16. Hence, once more, it seems that the sensory measurements obtained with GCO using the Osme technique, are very comparable with a wide range of other psychophysical studies.

In contrast, with the odor intensity functions the slope of the linear regression between *odorant concentration* and the *area under the odor peak* resulted in very close values across panelists, but differed among compounds (Table 2.9). This finding may be significant in terms of odor power discrimination among compounds and should be further explored.

Variation occurred among subjects in the use of the structured intensity scale, even though they were trained with intensity standards (Figure 2.2). For butyl sulfide at 0.75 μL/ml, subject # 1 reported an average odor intensity of nearly 5.5 while subjects #2 and #3 scored 2.8 and 7.9 respectively. Similar variation on categorical scaling was reported by Drake et al. (1969) and by Tuorila (1981) who verified significant correlation ($p \leq 0.001$) between individual detection thresholds and intensity estimates of five odorants. Tuorila suggested that greater sensitivity to an odorant leads a subject to scale it on a higher level, while lower sensitivity causes the panelist to use low categories when assessing the odorant.

Assessment of a compound's odor quality

There was good agreement among panelists as to the descriptor characteristics of GC-effluents, but occasionally subjects used different descriptors to characterize the odorants tested. For subjects #1, 3 and 4 the odor descriptors of butyl sulfide included terms such as "green leaves" and "pungent herb", for subject #2 "rubber" was the best descriptor even though the subject agreed that butyl sulfide indeed resembled the odor of green leaves.

Hexyl alcohol was described as floral/fruity with pungent and musty aroma notes.

Slight disagreement among subjects was also noted regarding the odor characterization of nonyl aldehyde. While subjects #1, #3 and #4 used the descriptors "floral" and "fruity with watermelon rind and citrus notes" in order to best characterize that odorant, subject #2 described nonyl aldehyde as gasoline/kerosene/chemical-like at its lower concentrations, and as floral/musty/gasoline-like at its two highest concentration levels.

Overall 2-decanone was described as floral/fruity with a soapy note for subject #1 while for subject #3 and #4 the floral/fruity aroma presented a hint of citrus and tropical fruit notes.

Linalool was described as floral/citrus/orange blossom-like.

Individual differences in judging odor qualities is usual and may be especially great because of subjects' unique experiences and special associations with odors (Engen, 1974). In addition, individual differences in odor quality reported for specific odorants, has been suggested to arise from individual differences in perceptual pathways. Any individual alteration in the relative specificity or number of individual binding sites or, for that matter, in any of the other normal receptor neuron processes in any one neural channel should alter both the overall pattern of interaction for any

one odor and thus should give rise to alterations in both intensity and quality of the odor perceived (O' Connell, 1991).

CONCLUSIONS

Subjects sniffing the GC effluents were reliable for reporting odor itensity changes ($p \leq 0.05$) with changes in odorant concentration in the GC effluent. Similarly, by recording the total time that the subject could detect the compound in the GC effluent and integrating it with the correspondent odor intensity values, subjects were reliable for generating aroma peaks whose areas systematically reflected the changes in odorant concentration in the GC effluent ($p \leq 0.05$). All three functions; linear, logarithm and power, provided good fit to the category scale data in relating odorant concentration in the GC effluent with odor intensity as assessed by Osme; the linear function provided the best fit in relating area under the odor peak and odorant concentration. Usually, more then 82% of the variation reported by subjects in both intensity ratings and area under the odor peak ratings (means over four replication) were due to true physical stimulus variation; the remaining 18% can be attributed to subjects' physiological and psychological effects. Overall, all psychophysical parameters obtained in this study using the Osme technique were comparable with correspondent parameters reported in the literature where compounds' odor intensities were assessed by different psychophysical methodologies. Furthermore, despite the short time for aroma characterization and possible fatigue effects, panelists assessing the GC effluents by Osme, seem to be comparatively quantitative with panelists performing more traditional olfactometry techniques. In addition, there was

good agreement among panelists as to the descriptor characteristics of GC effluents. Hence, it seems to be possible, to some extent, to obtain a measure of the odor potency and consequently estimate the relative significance of odorants present in the aroma extract by directly assessing their odor intensities in the GC effluent using Osme.

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FLAVOR PROPERTIES AND STABILITY OF A CORN-BASED SNACK: RELATING SENSORY, GAS CHROMATOGRAPHY, AND MASS SPECTROMETRY DATA

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ABSTRACT

Corn-based snacks were packed in nitrogen-flushed cans and stored at 70°F. Samples held for 0, 3, 6, 9, and 12 months were evaluated for aroma and flavor by a trained descriptive panel. For each sample, volatile compounds were extracted with methanol, isolated in dichloromethane, and separated by gas chromatography (GC). Four panelists evaluated the GC effluents using the Osme technique to locate significant aroma compounds. The compounds were identified by GC/MS. The fresh product was primarily characterized by toasted-corn aroma and flavor. Some volatiles associated with the snack aroma and flavor were methional, 2,5-dimethyl-4-hydroxy-3(2H)-furanone (furaneol), vinyl guaiacol, *t,t*-2,4-decadienal, *o*-amino acetophenone, vanillin, 4,5-dimethyl-3-hydroxy-2(5H)-furanone, 2-acetyl-1-pyrroline, trimethylpyrazine, 2-propionyl-1-pyrroline, 2-vinyl-pyrazine, 3-ethyl-2,5-dimethyl-pyrazine and 2,3-diethyl-5-methylpyrazine. Univariate and multivariate analyses indicated that storage time significantly ($p \leq 0.05$) affected the aroma, flavor, and volatile composition of the samples. Correlation between sensory and chemical data was critical for understanding flavor properties and stability of the snack.

INTRODUCTION

Most food flavor and odor impressions are stimulated by complex mixtures of volatile odorous chemicals (Dürr, 1983). Identification of those volatiles can aid not only the development of a synthetic flavor, but also the elucidation of the mechanisms of formation of objectionable flavors, contributing to the development of practical methods to prevent or retard flavor change.

The isolation, separation and identification of a large number of volatiles present in foods is possible for today's flavor chemists due to the improvement of chemical and physical methods of flavor extraction associated with the sophistication of gas-liquid chromatography and mass spectrometry. The current challenge has been to establish from the extracted volatiles which are responsible for specific sensory properties in the food product.

A very practical approach to determine odorous volatiles, their odor quality and importance in flavor extracts has been to have individuals smell the gas stream as it escapes from the GC column and to record their sensory estimates; such methodology is called GC-olfactometry (GCO).

Among the GCO techniques generated in the last decade, Charm (Acree et al., 1984; Cunningham et al., 1986; Marin et al., 1988) and AEDA (Schieberle and Grosch, 1987; Schieberle and Grosch, 1988) are most often cited in the flavor literature. Indeed, they have been useful in identifying areas of the chromatogram which are likely to be important for the products' flavor; however, both techniques have been subject to criticism. Charm and AEDA are based on the determination of odor-detection thresholds of the compounds eluted from the GC-column rather than the psychophysical

estimation of their individual odor intensity. As pointed out by Piggot (1990) and Maarse (1991), the fact that different odorants may show different intensity functions above their threshold, limits threshold-based techniques' valid conclusions about the relative contribution of individual odorants to a flavor system, even though those techniques may still give some guidance in identifying areas of the chromatogram which are likely to be important.

In an attempt to create a new GCO methodology which would be strongly founded on current psychophysical views, our sensory laboratory has developed a new method called Osme (McDaniel et al., 1990; Miranda-Lopez et al., 1992 and Sanchez et al., 1992, Da Silva et al., 1992b). Osme combines the modern concepts of sensory descriptive analysis with novel techniques of computerized data collection and applies the Selke et al. (1972) time-intensity approach of evaluating the odor significance of compounds in the GC effluent. With Osme, trained subjects sniffing the GC effluent directly record the odor intensity and duration time of each odor active compound while describing its odor quality. By combining the information provided by Osme with the information provided by the gas chromatogram, it is possible to identify which volatile is important for each flavor attribute, assessing each volatiles' significance in the product's flavor.

In the present study, information obtained with GC/MS techniques, was combined with information provided by GCO and descriptive sensory analysis in order to: i) characterize the aroma and flavor of a corn-based snack, ii) identify volatile compounds important to the snack's aroma and flavor, iii) determine the product's aroma and flavor stability under nitrogen packing conditions and, iv) identify changes of aroma-active compounds over storage.

MATERIALS AND METHODS

Samples

Samples consisted of flavored corn snacks whose ingredients included: corn, vegetable oil, Romano, Cheddar and Parmesan cheeses, buttermilk solids, whey, salt, wheat flour, tomato solids, monosodium glutamate, onion and garlic powder, dextrose, citric acid and, sugar among others. Two batches of the flavored snack were tested. Samples from each batch were packed in nitrogen flushed canisters and stored at 21.1°C for 0, 3, 6, 9 and 12-months.

Immediately after batch processing, the 0 month samples were placed in frozen storage at -35 °C to prevent further aging effects; and the remaining samples were stored at 21.1°C in a controlled-temperature room. At 3, 6, 9 and 12 months, samples were taken from the controlled temperature room and placed in frozen storage (-35°C).

At the end of 12 months, samples collected to date were tested as described below.

Sensory descriptive analysis

The characterization of the product aroma and flavor and their changes during storage were monitored by a trained descriptive panel.

Twelve volunteers from the Food Science and Technology Department of Oregon State University were trained in 20 sessions over a period of 10 weeks to describe and rate the aroma and flavor intensity of the corn snack .

In the initial training sessions, panelists evaluating the samples generated their own aroma and flavor terms. In subsequent sessions, reference materials were provided to help standardize the panelists in the use of each descriptive term. Further training sessions and group discussions

under the panel leader's guidance, resulted in panel agreement on the use of terms and a final ballot. A written, consensus definition of each descriptive term was developed and reviewed by the panelist before each testing session. Terms, definitions, and reference standards used are listed in Table 3.1.

Samples held for 0, 3, 6, 9 and 12-months were evaluated for aroma, while the product flavor was evaluated in samples stored for 0, 9, and 12 months. The selection of the samples to be evaluated for aroma and flavor was based on results from a previous difference-from-control test (control=sample 0-months storage) using an untrained panel of 27 subjects. Because results indicated a significant difference ($p \leq 0.05$) between the aroma of the 0-months stored sample and all the others, all samples were tested by the descriptive panel for aroma. On the other hand, no significant flavor difference ($p \leq 0.05$) was detected among the 0, 3 and 6 months stored samples, hence just the samples stored for 0, 9 and 12 months were tested for flavor by the trained descriptive panel.

For the sensory evaluation, chips were crushed through a stainless steel sieve with hole diameters of 1.6 cm and 10g samples were served in opaque, tulip shaped wine glasses (0.25L) coded with 3-digit random numbers and capped with an aluminum lid. Sample evaluation took place in individual booths under red lighting.

The intensity of each descriptor was rated on a 16-point structured scale, (0=none, 1=just detectable, 2, 3=slight, 4, 5=slight to moderate, 6, 7=moderate, 8, 9=moderate to large, 10, 11=large, 12, 13=large to extreme, 14, 15=extreme). Intensity standards were provided as scale reference points to reduce variability among panelists. The standards were anchored at point 3

Table 3.1 Attribute definitions and reference standards[1] used by the descriptive sensory panel during the evaluation of the snack aroma and flavor.

Descriptors	Definition and reference preparation
Overall aroma	The overall impact (intensity) of all odorants as perceived by the nose.
Overall flavor-by-mouth	Total impact (intensity) as perceived in the mouth which includes all aromatic, taste and feeling factors contributing to the product flavor.
Toasted corn aroma and flavor-by-mouth	Primary aroma and flavor-by-mouth of a a reference prepared for baking one tortilla (Diane's 12 thin corn tortillas for tacos, Temcsa-Mex, McMinnvile, OR 97128) for 15 minutes at 177°C.
Rancid aroma and flavor-by mouth	The snack off-aroma and off-flavor notes associated with the primary odor and flavor-by mouth of 60 ml of corn oil (Mazola, Best Foods, CPC International Inc., Englewood Cliffs, NJ 07632) previously stored for 5 months at 37.8°C and described as paint thinner, shoe polish, turpentine, used fish oil, beany and pungent.
Stale aroma and flavor-by mouth	The primary aroma and flavor-by-mouth of references prepared as follows: 1-One 10 cm x 4 cm piece of cardboard soaked in 10 ml of water, 2-30 gr of yellow corn flour previously stored for 11 months at room temperature, with no rancidity.
Cheese aroma and flavor-by-mouth	The dairy, sour and pungent aroma and flavor-by-mouth notes associated with flavor-by mouth Romano and Parmesan cheeses, added with both the moldy, raw mushroom, sweet and pungent notes of Blue cheese and the sweet, dairy and fruity aroma and flavor-by-mouth notes of mild Cheddar cheese. References were individually prepared as follows: 1- 17.5 gr of 1.5 cm^2 cubes of mild cheddar cheese (Tilamook, OR 97141) 2- 5 pieces of 0.5 x 0.5 x 2.6 cm of Romano cheese (Frigo, Green Bay, WI 54307-9024),

Table 3.1 (cont.)

Descriptors	Definition and reference preparation
	3- 5 pieces of 0.5 x 0.5 x 2.6 cm of Parmesan cheese (Frigo, Green Bay, WI 54307-9024), 4- 5 pieces of 0.5 x 0.5 x 2.6 cm of Treasure Cave Blue cheese (Beatrice Cheese, Inc., Waukesha, WI 53186).
Sweet aroma	The primary aroma of 10 g of a reference standard prepared by baking (1.5 h at 220°C) 60 g of fresh corn granular flour naturally processed (Cub Foods, bulks, Corvallis, OR 97330) added with 60 ml of bottled water (Aqua-Cool, Portland, OR 97232).
Onion aroma and flavor-by-mouth	The primary aroma and flavor-by-mouth notes associated with 1 teaspoon of coarse flakes of dried onion (Cub Foods, bulks, Corvallis, OR 97330).
Garlic aroma and flavor-by-mouth	The primary aroma note associated with 1/2 teaspoon of coarse flakes dried garlic (Cub Foods, bulks, Corvallis, OR 97330).
Pepper flavor-by-mouth	The burnt flavor-by-mouth note associated with ground Cayenne red pepper (Schilling, McCormick&Co, Inc., San Francisco, CA).
Bitter taste	The primary taste associated with a standard solution of 0.04% of caffeine (Fisher Scientific, Fairlawn, NJ 07410) in bottled water (Aqua-Cool, Portland, OR 97232).
Salty taste	The primary taste associated with standard solutions of 0.3% NaCl (Morton International, Inc., Chicago, Il).

[1]All the reference standards were placed in opaque, tulip shaped wine glasses (0.25L) capped with an aluminum lid.

(40 ml of safflower oil, Saffola Quality Foods Inc., Los Angeles, CA 90023), point 7 (30 ml of orange drink, Hi-C, Coca Cola Foods, Houston, TX 77252), point 11 (30 ml of grape juice, Welch's, Concord, MA 01742), and point 13 (cinnamon bubble gum, Plen T-Pak Big Red).

A randomized, complete-block design was used to test the samples' flavor and a randomized incomplete block design was used to test the samples' aroma; both designs provided three replications over the treatments. Assessments by panelists were analyzed per attribute through a four-way ANOVA (factors= batch (B), panelist (P), treatment (T), and replication (R)). Panelist and replication were treated as random effects (Lundahl and McDaniel, 1988), while batch and treatment were considered fixed effects. The appropriate F values and degrees of freedom were calculated according to Steele and Torrie (1980), providing the following formulas:

$$F_B = \frac{MS(B) + MS(PxRxB)}{MS(PxB) + MS(RxB)} \qquad F_T = \frac{MS(T) + MS(PxTxR)}{MS(TxR) + MS(PxT)}$$

$$F_P = \frac{MS(P)}{MS(PxR)} \qquad F_R = \frac{MS(R)}{MS(PxR)}$$

The PxT, PxB, BxT and TxB interactions for each attribute were tested for significance by using the MS(PxBxT) in the denominator of the F test. In the event of a significant PxT interaction, the data were interpreted cautiously.

Pairwise comparisons of treatment means were conducted by using Fisher's least significant difference (LSD) test ($p \leq 0.05$). When using a compound F-test, as detailed above, it is possible to encounter a significant F value for treatment effect, but not be able to show differences among treatment means because the LSD value is too great. For these situations, a *t*-

test was used to test for significant differences among treatments as detailed by Lederer et al. (1991).

Flavor extraction

Samples held for 0 (control sample), 3 (first significant difference from the control for aroma as detected by the trained descriptive panel) and 12 months (end of the storage study) were selected for extraction.

A 500 g, finely ground sample was mixed with 50 ml distilled water and allowed to stand at room temperature for 1 hr. The sample was extracted twice with 500 ml methanol. After filtering the extract through Whatman 934-AH filter paper, the combined extracts were mixed with 800 ml of distilled water and placed in a -20°C freezer overnight for separation of the oil phase.

The methanol-water phase was filtered and extracted three times with a total of 500 ml of dichloromethane. The combined dichloromethane extract was dried with anhydrous sodium sulfate and concentrated to 2 ml using a Kuderna-Danish apparatus. The concentrated extract was transferred to a minivial with a Minivert valve, and an internal standard (n-undecane) was added. The extract was stored in a -20°C freezer.

The aroma of the extracts was evaluated by the trained descriptive panel and described as toasted corn, cheese and onion/garlic, being reported as very similar to the aroma of the original samples.

Recovery studies

To evaluate the efficiency of the extraction procedure, stock solutions of 13 aroma volatiles expected to be present in the samples were individually spiked into 500 g of snack sample which was subsequently extracted as previously described. Spiked levels ranged between 5 to 10 ppm. Recovery

level was 14% for diacetyl, 50% for pentanal, 39% for dimethyl disulfide, 52% for hexanal 45 % for methylpyrazine, 47% for allyl sulfide, 62% for 2-heptanone, 74% for methional, 48% for allyl disulfide, 70% for 2,4 decadienal, 60% for vanillin, 83% for ∂-decalactone, and 77% for ∂-dodecalactone.

Extraction or other procedures necessary for the isolation of adequate quantities of odor concentrates, by their nature cause preferential accumulation of certain components and loss of others; the degree of success obtained by any particular isolation technique is measured by comparison of the aroma of the extract with the aroma of the original product (Teranishi et al., 1971). Overall, the extraction procedure was considered successful because the extracts' aroma resembled closely the original products' aroma. The low recovery of diacetyl was accounted for by losses during the extract concentration due its high volatility.

Volatile separation by gas chromatography (GC)

Flavor extracts from samples stored for 0, 3 and 12 months were analyzed using a Hewlett Packard 5890 GC equipped with an FID. A Supelcowax10 fused silica capillary column [30m x 0.32mm (i.d.), 0.50 μm film thickness, Supelco, Inc.; Bellefonte, PA] was used to separate the volatile compounds extracted from the snack samples. The GC was run with a splitless injector held at 150°C, a detector temperature of 240°C, and a helium carrier flow rate of 3.1 ml/min. The oven temperature was programmed as follows: initial temperature (50°C for 6.0 min), rate (5.0°C/min.), final temperature (250°C), isothermal conditions for 30 min at 240°C. Injection volume was 0.2 μl and was measured in a 2 μl syringe (Hamilton Co., Reno, NV 89520) designed to hold the sample in the needle. The 7 cm needle was fitted with a 3 cm needle spacer for more reproducible injections. Kovats'

indices (Kovats et al., 1958) for the volatile compounds were calculated by using retention data for a series of n-paraffin standards (C_{11}-C_{30}).

Osme analysis

Osme data collection: Flavor extracts from samples stored for 0, 3, and 12 months were assessed by Osme. For the sensory evaluation of the GC-effluents, the chromatograph was modified as described by Sanchez et al. (1992). The column was moved from the FID to a second detector base and a sniffer which consisted of a 60 cm by 1 cm (i.d.) glass tube coated with silicone (Sylon CT, Supelco, Inc., Bellefonte, PE) was set on top of the detector base. GC-effluents emerging from the column were continuously mixed with humidified and charcoal filtered air (60% RH, flow rate 10.2 L/min) and directed to the subject for evaluation (Figure 3.1A).

Subjects verbally described the quality of each odorant while recording its intensity and duration time by using an electronic device containing a 16-point structured intensity scale. This device consisted of a variable resistor with a pointer that could be moved from left to right and back again across the 16-point intensity scale.

Time and intensity values were collected and stored by a software system named DASSIE (Data Acquisition System for Sensory Input and Evaluation) developed in the Sensory Science Laboratory in the Department of Food Science and Technology at Oregon State University. The program was installed in an IBM XT personal computer.

For each odorant perceived in the GC-effluent, the following data were accessed (Figure 3.1B): i) the odor peak, obtained by plotting retention time values x intensity values, ii) the odor duration time (d), which is the total

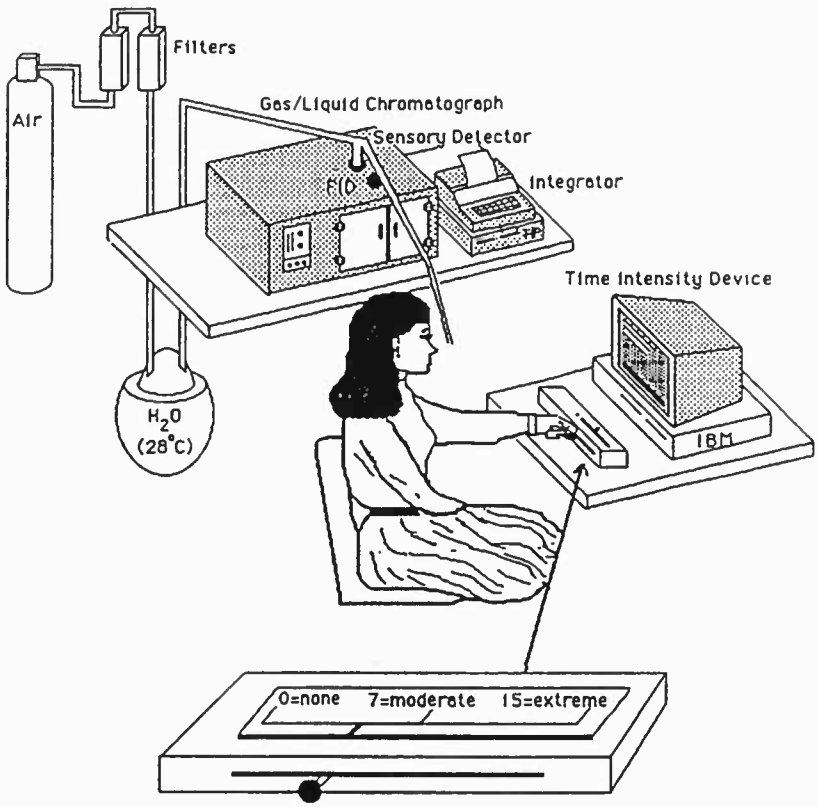

Figure 3.1- Osme equipment and Osmegram.

time that the subject could detect the compound in the GC-effluent; iii) the odor maximum intensity (I_{max}), iv) the area under the odor peak; v) the Kovats index based on subject response, and vi) the odor quality. The combination of all this information across peaks provides the sample Osmegram (Figure 3.1B), which is the aromagram of each extract as assessed by using the Osme technique.

The GC conditions were the same as previously described for volatile separation. The sniffing time in each run was 35 min.

Osme subjects: The subjects consisted of four volunteers from the Food Science and Technology Department at OSU, all of whom were members of the trained descriptive panel. Subjects were given six training sessions prior to data collection. Training samples consisted of the same samples subsequently tested.

During the first sessions, subjects evaluated the GC effluent of each sample extract and generated their individual descriptors for each compound. Reference standards, based on each descriptor, were provided to the Osme panel. Prior to each session, subjects evaluated the reference standards.

Osme data analysis: For this study, Osme data analysis as described by Sanchez et al. (1992) was modified. To summarize the Osme panel results in a "consensus aromagram", those researchers initially averaged, for each subject, times and intensities of those peaks that were detected at least 50% of the time (in four replication by each subject). Missing values were not computed in the averaging process. Then, in the next step, the times and intensities of those peaks that were detected for at least three of the four subjects were averaged

again resulting in a "consensus Osmegram" which was used to summarize the panel response for the tested sample.

This data analysis is very conservative in reporting the existence of an aroma peak, even possibly leading the researcher to miss information. To exemplify this problem, consider that aroma peaks detected in all four replications by two of the four subjects, would not be present in the consensus Osmegram, and would be considered absent from the sample, despite the fact that it is quite unlikely that two of the four subjects would report a peak with similar odor quality in the same retention-time range just by chance. In fact, it is more reasonable to accept that the panelists which could not detect that peak are less sensitive to that odorant.

Similarly, aroma peaks reported once in four replications by all four panelists, will also be deleted from the consensus Osmegram and would be considered absent from the analyzed sample, when in fact it is possible that the odorant is present at a concentration near the detection threshold.

As a consequence of being conservative in reporting aroma peaks, the Sanchez et al. (1992) data interpretation will tend to be liberal in accepting the absence of aroma peaks; furthermore, when this procedure is used to compare Osmegrams from different samples, it will be liberal in reporting differences among samples.

In the present study, the previous method was modified to generate a data analysis which would be less conservative in reporting the existence of an odorant in the aroma extract and, consequently less liberal in reporting between-samples difference. In the present study, odor peaks detected at least once over all of the samples by at least two subjects, were computed as actual peaks; missing observations over the 4 subjects were computed as 0 ratings in the averaging process. As a consequence, peaks reported at least once by only

half of the panelists, will be present in the aromagram as small peaks, and peaks frequently reported by all subjects will be represented by a bigger peak in the aromagram. For example, consider the extreme case where, for sample 1, panelist 1 reported in one replication the existence of an aroma peak at intensity level 5 (16-point scale), and a second panelist reported the same aroma peak (same descriptor/same retention time) in one replication of sample 2 at intensities level 4, and this peak was not reported in any other samples or replications. Thus, this peak will be reported in sample 1 and 2; aromagrams at intensities of 0.31 and 0.25 respectively (16-point scale) because all observations missed (4 panelists evaluated each sample in four replications) will be computed as 0 scores. However, if a second aroma peak is perceived by all four subjects, in all four replications, at intensity 4 for sample 1 and at intensity 5 for sample 2; then, this second aroma peak will be reported in sample 1 and 2 aromagrams as peaks showing intensities of 4 and 5 respectively.

Hence, in extreme cases, where we really cannot be sure if an aroma peak is a true signal or a false alarm, the modified data analysis will report that peak at a level similar to the noise signal in the GC.

The modified Osme data analysis is expected to provide the following advantages: i) it takes into account the concept that panelists show different sensitivities across compounds, ii) it is less conservative in admitting the presence of an odorant in the flavor extract, iii) it is less liberal in reporting differences among samples, and iv) it treats data similarly to other sensory techniques, allowing statistical data analysis.

The odor significance of each compound in the flavor extract was assessed by using both the compound's maximum odor intensity (I_{max}) and the area under the odor peak: high odor intensity (I_{max}) and large area

indicated a major odor-active compound in the flavor extract, while low odor intensity and small peak area indicated a minor aroma/flavor contribution to the extract.

For each sample, comparison of odor intensities (I_{max}) and areas across compounds was performed by using a three-way ANOVA (factors=panelist (P), peak (PE), and replicates (R)). In this case, panelist and treatment were considered as fixed effects, and replicate was viewed as a random effect. Each factor was tested for significance by using the MS(PxPE) in the denominator of the F test. Comparison of means was performed by the Tukey test for means ($p \leq 0.05$).

Storage time effects were evaluated by comparing, for each peak, the odor intensities and areas across samples using a three-way ANOVA (factors=samples (S), P, and R). Panelist and sample were considered fixed effects, replication was a random effect, and MS(SxP) was used as the error term. Mean comparisons was performed by the Tukey Student Range test (HSD) for means ($p \leq 0.05$).

Identification of aroma compounds by gas chromatography-mass spectrometry

Electron impact mass spectra were obtained on a Hewlett-Packard 5985 gas chromatography mass spectrometer (GC/MS). The GC analysis conditions were the same as for Osme analysis. Peaks were tentatively identifyed by matching a computerized library or published mass spectra. Pure chemicals were purchased from commercial sources, and chemical identities were confirmed by a match of retention indices and mass spectra.

After chemical identification, a standard solution was prepared with pure chemicals at approximately the same concentration as in the sample

extracts. Identification of the aroma compounds was confirmed by Osme analysis of the standard solution.

Headspace analysis

Hexanal content in samples held by 0, 3, 6, 9, and 12 months was measured by static headspace-GC analysis according to Robards et al. (1988).

Oxygen in the canisters headspace was measured in samples held by 0, 3, 6, 9 and 12 months by a Systeck instrument model ZR891HS.

Changes in hexanal and oxygen content over time ($p \leq 0.05$) were assessed by correlating hexanal and oxygen content in the canisters headspace with storage time. Assessment of the best-fit-function between hexanal content and storage time was conducted by plot analysis, residual analysis and lack-of-fit test as detailed by Neter et al. (1989). Correlations between hexanal content in the canisters' headspace and rancid aroma and flavor were also conducted.

RESULTS

Aroma and flavor characterization

The 0-months snack had a moderate to large overall aroma and flavor, both primarily characterized by a toasted corn note (Figure 3.2).

Overall, the aroma of the sample stored for 0 months was described as moderately toasted (toasted corn), slight to moderately cheesy and sweet, slightly stale, garlic and onion with a just detectable rancid note. The fresh snack flavor was characterized as moderately toasted and salty, slightly to moderately cheesy and onion, slightly stale, garlic and peppery with just

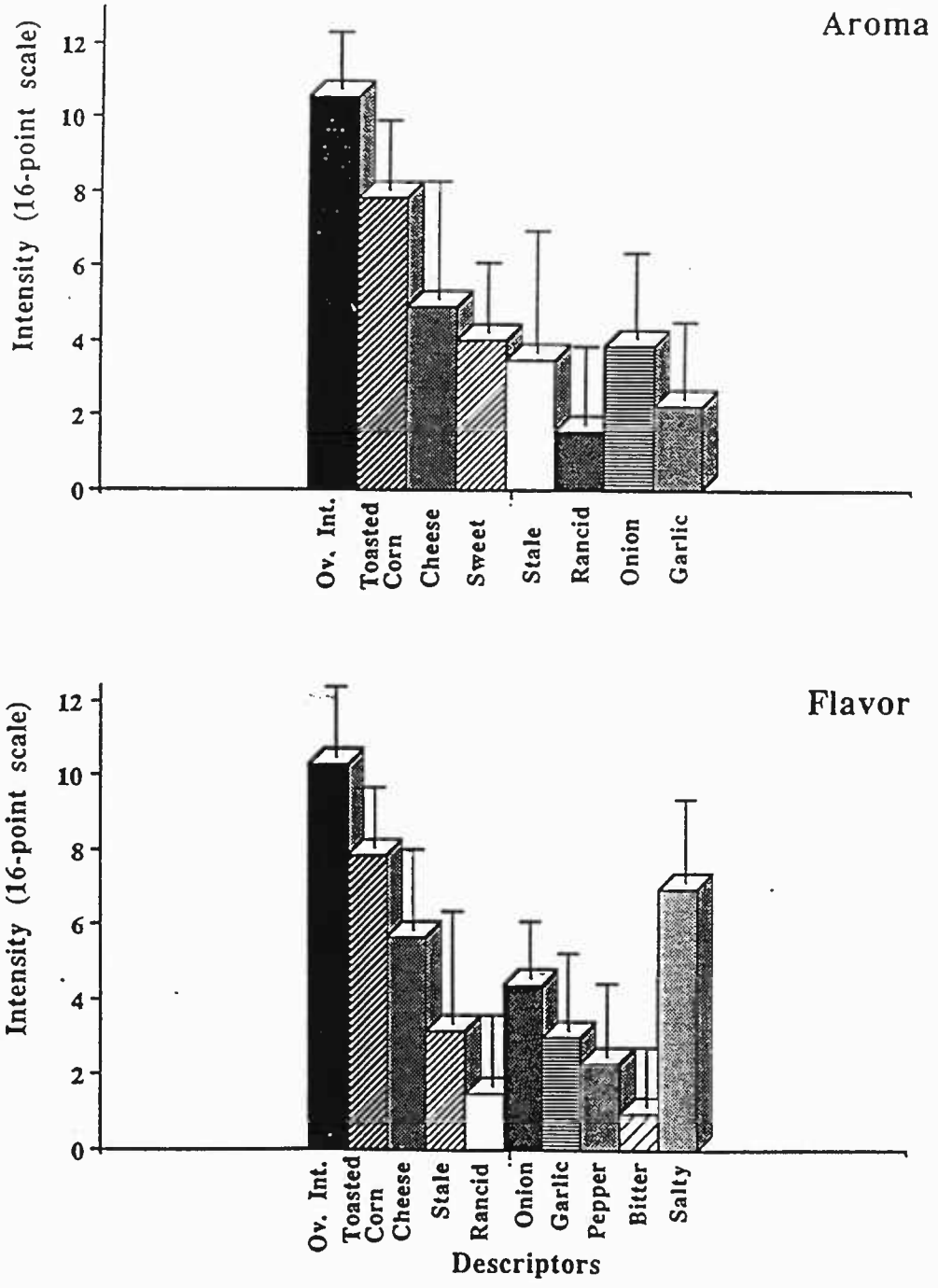

Figure 3.2- Aroma and flavor profile of the 0 month corn-based snack as perceived by the trained descriptive panel.

detectable bitter and rancid notes.

Stability of the Snack Aroma and Flavor

Storage significantly ($p \leq 0.05$) changed the snack aroma and flavor profile despite the product having been packed in canisters under nitrogen conditions. Aroma ratings indicated that the most significant changes in the snack during storage were the decreasing of its toasted note, simultaneous with the increasing of the rancid and stale notes ($p \leq 0.05$) (Table 3.2). The loss of the toasted aroma is of concern because panelists perceived this attribute as contributing the most to the overall aroma intensity of the 0-month snack and with storage, its significance in the product aroma decreased drastically (Table 3.2).

Similarly, the increase in the stale and rancid notes calls for attention since both were noticed as minor aroma notes in the 0-month snack, but with storage they became the most important contributors to the snack overall aroma intensity. Changes of such magnitude in the snack aroma could effect the product acceptability.

Conversely, the degree of changes which occurred in the snack overall aroma intensity, cheese, sweet and onion aroma notes were very small as compared with the toasted, stale and rancid notes. The impact of these changes on the aroma profile of the stored product is likely to be of less significance.

Additional changes in the snack flavor were noticed in the later stages of storage ($p \leq 0.05$). While a significant ($p \leq 0.05$) decrease in toasted flavor was perceived at 9 months of storage, the onion note presented a significant ($p \leq 0.05$) decrease only by the twelfth month of storage. The stale flavor note showed a significant ($p \leq 0.05$) increase by the ninth month of storage, the

Table 3.2 Mean ratings, standard deviations and least significant difference (LSD) for descriptors showing a significant change ($p \leq 0.05$) during the snack storage[1].

Descriptors	Storage time (months)					
	0	3	6	9	12	LSD($p \leq 0.05$)
AROMA						
Overall Intensity	10.53^{a} (1.53)	9.70^{b} (1.89)	9.63^{b} (1.76)	9.78^{b} (1.81)	9.92^{b} (1.80)	p≤005[2]
Toasted Corn	7.83^{a} (1.89)	5.09^{b} (2.02)	4.26^{bc} (2.07)	4.32^{bc} (2.15)	4.07^{bc} (2.03)	0.96
Cheese	4.88^{a} (3.17)	2.98^{b} (2.25)	2.69^{b} (2.48)	2.48^{b} (2.32)	2.50^{b} (2.29)	1.86
Stale	3.48^{b} (3.23)	6.44^{a} (2.72)	6.26^{a} (2.74)	6.76^{a} (2.73)	6.48^{a} (3.04)	1.84
Sweet	4.00^{a} (1.85)	2.83^{b} (1.88)	2.92^{b} (1.83)	2.88^{b} (2.23)	2.82^{b} (2.00)	1.19
Rancid	1.56^{b} (2.06)	4.92^{a} (3.08)	5.25^{a} (3.23)	5.48^{a} (2.98)	5.88^{a} (2.95)	2.88
Onion	3.81^{a} (2.34)	2.81^{b} (2.70)	2.69^{b} (2.87)	2.67^{b} (3.12)	2.65^{b} (2.98)	p≤0.05[2]
FLAVOR						
Toasted Corn	7.83^{a} (1.85)			6.77^{b} (1.61)	6.03^{c} (1.91)	p≤0.05[2]
Stale	3.17^{c} (1.60)			5.03^{b} (1.93)	6.08^{a} (1.89)	p≤0.05[2]
Rancid	1.48^{b} (1.89)			4.52^{ab} (2.79)	5.81^{a} (2.95)	3.33
Bitter	0.945^{b} (1.52)			1.79^{ab} (1.72)	2.68^{a} (2.17)	1.63
Onion	4.39^{a} (1.45)			4.17^{a} (1.68)	3.67^{b} (1.82)	p≤0.05[2]

Table 3.2 (cont.)

Means with the same superscript within the same row are not significantly different from each other, [1]Sixteen-point structured intensity scale (0=none, 15=extreme), [2]Significant F value for treatment effect but not being able to show differences among treatment means, a *t*-test was used to test for significant differences among treatments.

rancid and bitter notes significantly ($p \leq 0.05$) increased only after 12 months of storage (Table 3.2).

Changes in the snack flavor followed a slightly different pattern than changes which occurred in the snack aroma. The drastic loss of the toasted note perceived in the snack aroma was not noticed in its flavor; toasted corn was still one of the most significant attributes contributing to the snack overall flavor intensity after 12 months of storage. However, the stale and rancid notes, considered of less impact to the 0-month samples' flavor, became major notes over storage, similar to what occurred with the snack aroma.

Overall, changes in the snack aroma and flavor occurred with storage, however, they were limited. After 12 months of storage, the rancid and stale notes increased just from 3 up to 6 in the 16-point intensity scale (5= slight to moderate).

A correlation between hexanal content and storage time ($p \leq 0.05$) was found, evidencing that hexanal content increased over time and supporting the descriptive panel findings that oxidation did occur during storage even though the conditions inside the canisters were optimized to avoid such an effect.

The linear (Figure 3.3) and the logarithm functions (R^2= 0.94 and R^2= 0.92 respectively, $p \leq 0.05$) provided a good fit for the relationship between hexanal content in the canisters and storage time, according to the models coefficient of determination and residual analysis. However, neither criteria is an adequate technique for evaluation of the best model relating two or more variables, since correlations sometimes can be higher for incorrect than for correct models (Birnbaum, 1973). Furthermore, in this study appopriatness

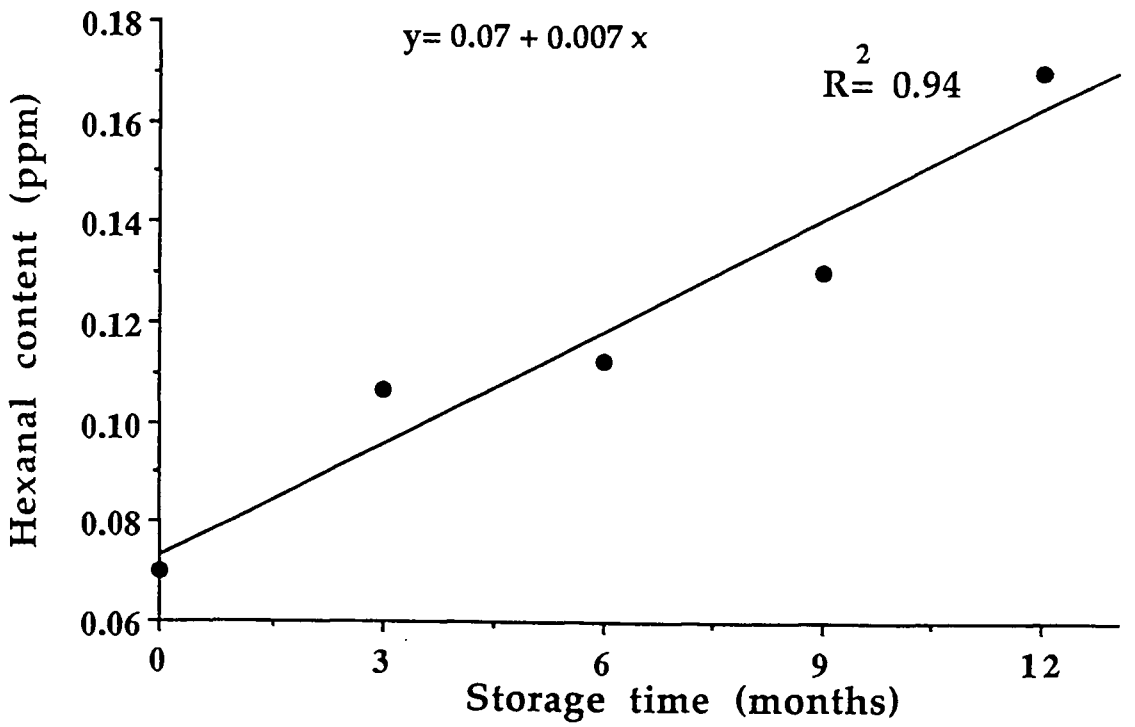

Figure 3.3- Correlation between storage time (months) and hexanal content (ppm) in the canister headspace.

of the best-fit-function relating hexanal content x storage time was conducted by the lack-of-fit test.

Results from the lack-of-fit-test indicated the linear function was not the best function to fit hexanal content vs. storage time. A third-order polynomial was the best function (R^2= 0.997, $p \leq 0.05$) relating hexanal content in the canisters headspace to storage time. Hexanal has already been reported as a potential indicator of oxidative reactions in lipid systems. Rho et al. (1986) found that hexanal concentration in deep-fried instant noodles was a good indicator of the development of oxidative rancidity; they graphically represented the relationship between hexanal content in the product and storage time as an exponential function, but no statistical test was reported. Indeed, our study indicated that an exponential function would fit the data (R^2= 0.93, $p \leq 0.05$), however the residual analysis and the lack-of-fit test suggested that, for our data, the exponential model was not appropriate.

Hexanal has also proved to be a reliable off-flavor predictor. Significant correlation was found between the logarithm of hexanal concentration (ppm) in the canister headspace and the sample's rancid aroma (R^2= 0.847, $p \leq 0.05$) and rancid flavor (R^2= 1.00, $p \leq 0.05$). Similarly, Shahidi et al. (1987) found that hexanal content and sensory scores were linearly correlated ($p \leq 0.05$). Jacobson et al. (1989), reported a linear determination coefficient (R^2) of 0.94 ($p \leq 0.05$) between hexanal content and flavor scores (9-point hedonic scale) of cookies prepared with animal shortening.

Oxygen in the canisters' headspace did not significantly change ($p \leq 0.05$) over storage time, remaining around 2% v/v.

Important volatile compounds related with the snack aroma and flavor

More than 150 volatiles were detected in the flavor extract of the 0-month sample, as shown by the gas chromatogram; 49 of those volatiles were indeed odor active compounds as indicated by the match with the samples' Osmegrams (Figure 3.4). However, the Osmegram of the flavor extracts reveal a different profile as compared with the gas chromatogram determined by gas chromatograph. Regions of the chromatogram showing very low responses for the Flame Inonization Detector, in fact contained several odorants which were perceived at moderate and high odor intensities by the GCO panel. That was the case for peaks 10, 19, 37, 48, and 49 among others. This occurrence stresses the need to complement GC/MS studies with GCO data.

For the product stored for 0 months, aroma descriptors, odor intensity, area under the odor peak and correspondent odorant (GC/MS) for aroma peaks and rating at least 4 (3=slight) on the 16-point intensity scale are listed in Table 3.3.

With Osme, the primary reason to rate each compound's odor intensity in the gas chromatograph effluent is to assess the odorant potency; high odor intensity (I_{max}) is indicative of a major odor-active compound in the aroma extract, while low odor intensity is indicative of a minor aroma/flavor contributor to the extract. Hence, since aroma peaks 10 (described as baked potato and identified as methional), 21 (described as Romano cheese and identified butyric acid) and 40 (clove, eugenol/2-methoxy-4-vinylphenol) showed the highest Imax values, rating between 8 and 9 in the 16-point intensity scale (9= moderate to large), they probably represent major odorants in the flavor extracts and are probable major contributors to the snack overall aroma and flavor. Those peaks showed

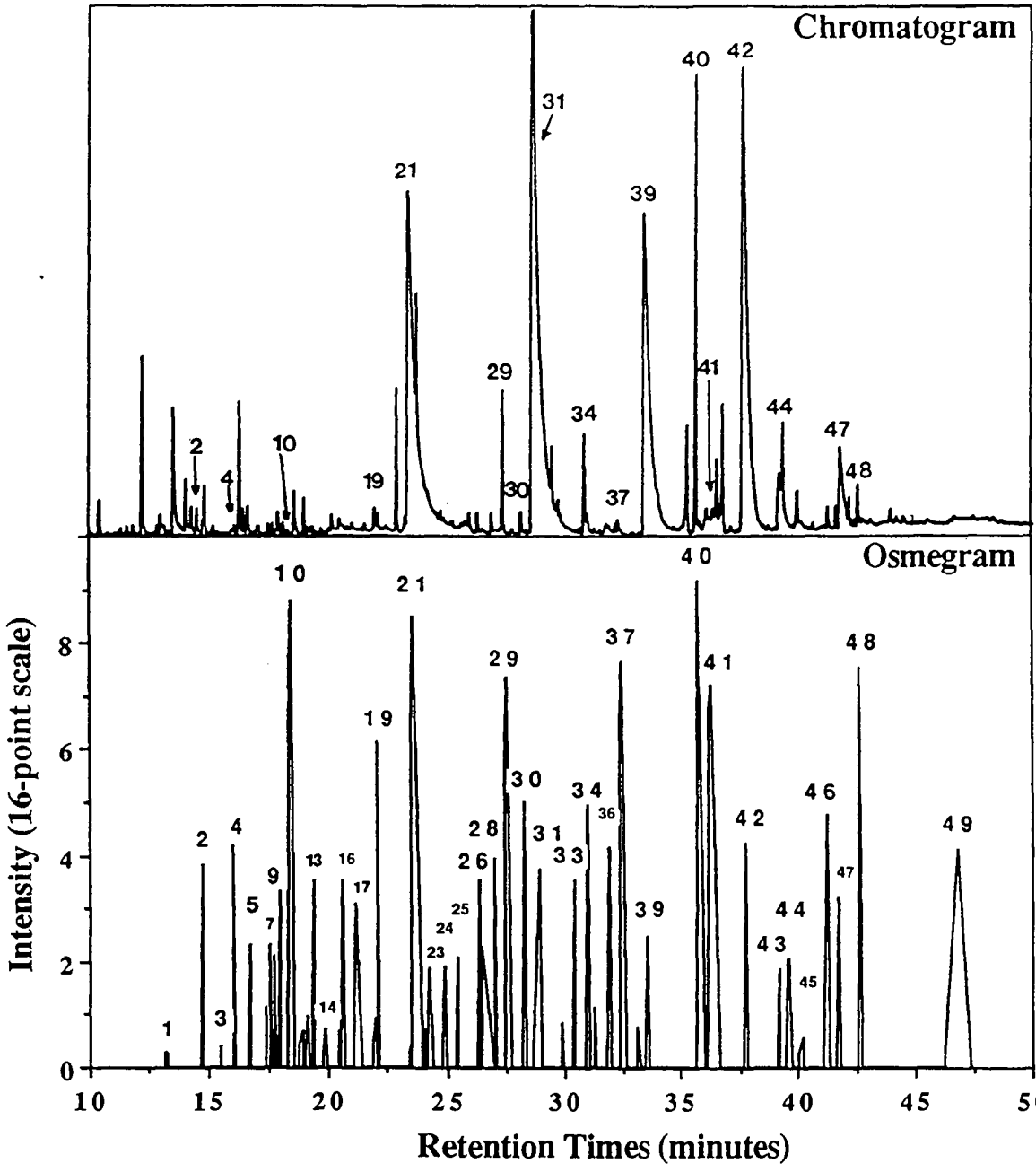

Figure 3.4- GC-chromatogram and Osmegram of the extract for the 0 month storage snack.

Table 3.3 Peak number, Kovats' index (KI_{20M}), odor descriptors, associated odorant, maximum odor intensity (Imax) and area under the odor peak for odorants showing high and intermediate odor intensity (I_{max}) in the 0 months stored snack as perceived by the GCO panel (means across panelists and replications).

Peak #[1]	KI_{20M}[2]	Descriptors	Identified (GC/MS)[3]	Osme[4]	
			Compound	I_{max}	Area
2	1342	toasted corn	2-acetyl-1-pyrroline[pi]	3.8^{f}	0.3^{f}
4	1383	garlic/onion	dimethyltrisulfide[pi]	4.2^{f}	0.4^{f}
9	1442	floral/plastic/oxidized	8-nonen-one[pi]	3.7^{f}	0.3^{f}
10	1463	cooked or baked potato	methional[pi]	8.8^{a}	1.7^{bc}
19	1596	garlic/onion	methyl allyl trisulfide[pi]	6.1^{bcdef}	0.8^{def}
21	1640	Romano cheese/butyric acid	butyric acid[pi]	8.5^{ab}	2.0^{ab}
28	1795	garlic/onion	diallyl trisulfide[pi]	4.0^{f}	0.7^{ef}
29	1818	body odor/rotted onion and garlic	*t,t*-2,4-decadienal[nc]	7.4^{abcd}	2.0^{ab}
30	1849	spicy/cooked onion/celery	2-vinyl-4H-1,3-dithiin[pi]	5.0^{def}	0.8^{def}
34	1967	caramel + strawberry/cotton candy	3-hydroxy-2-methylpyran-4-one (maltol)[pi]	5.1^{cdef}	0.7^{ef}
36	2012	musty/oxidized/vinyl/metallic	2-methyl-phenol[pi]	4.2^{f}	0.7^{ef}
37	2039	caramel+strawberry/cotton candy	2,5-dimethyl-4-hydroxy-3(2H)-furanone (furaneol)[nc]	7.6^{abc}	1.6^{bcd}
40	2204	clove/eugenol/medicinal	2-methoxy-4-vinylphenol[pi]	9.2^{a}	1.8^{b}
41	2225	stale corn chip/sweet floral	*o*-amino-acetophenone[pi]	7.2^{abcde}	1.5^{bcde}
42	2291	soapy/rubber/plastic/vinyl	decanoic acid[pi]	4.2^{f}	0.9^{cdef}
46	2491	soapy/bad breath	skatole[pi]	4.8^{ef}	0.8^{def}
48	2574	vanilla	vanillin[pi]	7.5^{abcd}	1.5^{bcde}
49	n.d.	burnt caramel/musky floral	4,5-dimethyl-3-hydroxy-2(5H)-furanone[pi]	4.8^{ef}	2.8^{a}

Table 3.3 (cont.)

	Osme[4]	
	I_{max}	Area
MSD[5]	2.56	0.84

Means with the same superscript within the same column, are not significantly different from each other ($p \leq 0.05$), [1]Peak number as shown in Figure 3.4, [2]Kovats indices as detected by the GC/MS, [3]Odorant as identified by the GC/MS, [pi]Positive identification, [nc]Identification not 100% agreed to by the GCO panel when pure standard was evaluated, [4]Sixteen-point intensity scale (0=none, 15=extreme), [5]Minimum Standardized Difference at $p \leq 0.05$.

significantly higher I_{max} ($p \leq 0.05$) as compared with peaks 2, 4, 9, 28, 30, 34, 36, 42, 46, 48 and the remaining aroma peaks not listed on Table 3.3.

Peaks 29 (rotten onion, garlic/*t,t*-2,4-decadienal), 37 (caramel, cotton candy/furaneol), 41 (stale corn chip/o-amino-acetophenone) and 48 (vanilla/vanillin) were perceived as imparting a moderate odor intensity (7= moderate); furthermore, they are expected to be important to the product aroma and flavor. These peaks were not significantly different ($p \leq 0.05$) in peak height (I_{max}) from the major peaks 10, 21 and 40 but were significantly higher ($p \leq 0.05$) than peaks 2, 4, 9, 28, 36, 42 and the remaining compounds not listed in Table 3.3.

Aroma peaks, 2, 4, 9, 28, 36 and 42 and the remaining aroma peaks not listed on Table 3.3 were perceived as imparting very low odor impact to the GC effluent, rating around 4 on the 16-point scale (3= slight). They were significantly lower in intensity than from aroma peaks 10, 21, 29, 37, 40, 41 and 48 at $p \leq 0.05$. It is unlikely that these low aroma intensity peaks individually are major contributors to the snack aroma and flavor; nevertheless, the possibility of flavor enhancing or suppressing effects occasioned by interactions between compounds in the food-matrix should be considered. Those minor odorants should not be totally disregarded as contributors to the snack aroma and flavor.

The relationship between area under the odor peak and odorant potency is not straight forward; besides the odor intensity, area under the odor peak takes into account the odorant threshold and its elution time. However, one could postulate that at similar elution times, compounds showing larger values for area under the odor peak are likely to be more significant in the aroma extract then compounds presenting smaller area values. Under this assumption, peaks 10, 21, 29, 40 and 49 can be viewed as important odorants

in the snack flavor extract, since they showed the highest area under the odor peak. Peaks 10, 21, 29 and 40 were significantly higher in intensity ($p \leq 0.05$) than peaks 2, 4, 9, 19, 30, 34, 36, 46 and remaining odorants not listed in Table 3.3, which presented the lowest area under the odor peak values. Peak 49 particularly showed the largest area under the odor peak, significantly larger ($p \leq 0.05$) than aroma peaks 2, 4, 9, 10, 19, 28, 30, 34, 36, 37, 40, 41, 42, 46, 48 and remaining odorants not listed in Table 3.3.

Peaks 37, 41 and 48 showed intermediate area under the odor peak values, being significantly higher ($p \leq 0.05$) than peaks 2, 4, 9, and all the remaining peaks not listed in Table 3.3.

Except for peak 49, assessing compounds' odor significance in the aroma extract using area under the odor peak lead to similar conclusions as when assessing odor significance using I_{max}. The apparent inconsistency regarding peak 49 can be explained by the significantly poorer resolution of that odorant in the chromatographic column, as evidenced by the total ion chromatogram shown in Figure 3.4. Under better chromatographic resolution, it is probable that this odorant would present a high aroma intensity in the GC effluent. Hence, peak 49 should also be included in the list of the important odorants present in the snack flavor extract.

Methional is an extremely potent flavor compound (Guadagni et al. 1972), and is a product of the decomposition of methionine by Strecker degradation (Ballance, 1961). It has been previously cited as an important odorant in deep fried food (Buttery and Ling, 1972; Ho et al. ,1987), wheat bread crumb and rye bread crust (Schieberle and Grosch, 1989) among other food products. In the present study it seems likely that methional was a major contributor to the toasted note described by the trained descriptive panel.

Butyric acid, peak 21, is probably the major contributor to the cheese note described in the product's aroma and flavor.

The compound 2,5-dimethyl-4-hydroxy-3(2H)-furanone (furaneol) is a product of sugar degradation/fragmentation in the Maillard reaction (Shibamoto, 1989). Its odor has been described as caramel-like, becoming fruity and strawberry at low concentrations. The class of compounds it belongs to (furans) is particularly important in the quantity and quality of coffee flavor (Latrasse, 1991).

Vinyl guaiacol (2-methoxy-4-vinylphenol), possibly a product of the thermal decomposition of nonvolatile phenolics, has been reported to strongly contribute to the flavor of the crust of rye bread (Grosch and Schieberle, 1991a).

Under deep-fat frying conditions, linoleic acid undergoes decomposition forming 2,4-decadienal, which is described as possessing a deep-fried aroma and flavor (Patton et al., 1959). Decadienals were reported in several foods, among them potato chips (Deck and Chang, 1965). Buttery and Ling (1972) reported *t,t*-2,4-decadienal as one of the most important volatile compounds in the nonbasic fraction of potato chip aroma.

Acetophenone has been previously identified by Buttery (1973) in potato chip flavor extract.

Peaks 4, 19, 28 and 30 (Table 3.3) were described overall as spicy, with garlic/onion notes but imparted very low odor impact on the GC effluent; furthermore, they were regarded as minor odorants in the snack flavor extract. However, together with peak 19, additive interactions of those odorants in the food matrix would explain the spice/onion/garlic aroma and flavor notes perceived in the snack by the trained descriptive panel.

Similarly, a series of low intensity aroma peaks described as toasted corn and dusty/potato peel are listed in Table 3.4. They may be partially responsible for the toasted corn note attributed to the snack aroma and flavor. Most of these odorants are pyrazines and pyrrolines which have been previously associated in the literature with roasted, nut, popcorn and bread aroma and flavor. Both pyrazines and pyrrolines are produced in Maillard reactions, and are found in foods processed at high temperatures such as in the deep-frying process (Shibamoto, 1989). The compounds 2-acetyl-1-pyrroline and 2-propionyl-1-pyrroline were previously suggested as potent flavor compounds showing roasty odors in popcorn (Schieberle, 1991a). In addition, 2-acetyl-1-pyrroline was also identified as an important odorant in selected aromatic rice varieties (Paule and Powers, 1989; Tanchotikul and Hsieh, 1991). Trimethylpyrazine was tentatively identified and associated with the toasted flavor in explosion puffed dehydrated potatoes (Sapers et al., 1971). The compound 2,3-diethyl-5-methylpyrazine was reported in the basic fraction of the steam volatile oil from potato chips (Buttery et al., 1971) and toasted cocoa (Van Praag et al. 1968). Some pyrrolines and pyrazines such as 2-acetyl-1-pyrroline and 2-propionyl-1-pyrroline posses a very low odor threshold and are frequently cited as directly contributing to roasted or smoky flavors (Shibamoto, 1989; Schieberle, 1991a). In our study, their secondary importance to the product's aroma and flavor might be due to their lower extraction (45%) from the food matrix as compared with the 74% extraction for methional.

Overall, aromagrams showed good agreement with the product's aroma/flavor profile as described by the descriptive panel. If one did not know the specific product being tested, by the examination of Osme results, one would predict that the product would exhibit toasted, cheese, onion and

Table 3.4 Peak number, Kovats' index (KI_{20M}) as determined by GC/MS and correspondent odorant for odor peaks described as toasted corn/dusty/potato in the snack flavor extract.

Peak #[1]	KI_{20M}[2]	Descriptor	Odorants[3](GC/MS)
2	1342	toasted corn	2-acetyl-1-pyrroline[pi]
5	1407	dusty/potato peel	trimethylpyrazine[pi]
6	1429	toasted corn	2-propionyl-1-pyrroline[ti]
7	1441	dusty/cardboard	2-vinyl-pyrazine[pi]
8	1449	potato/dusty	3-ethyl-2,5-dimethyl-pyrazine[ti]
13	1494	dusty/potato peel	2,3-diethyl-5-methylpyrazine[pi]
24	1709	toasted corn	*t,t*-2,4-nonadienal[pi]
26	1773	toasted corn	c,t-2,4-decadienal[ti]

[1]Peak number as shown in Figure 3.4, [2]Kovats' indices as detected by GC/MS, [3]Odorant as identified by GC/MS, [pi]Positive identification, [ti]Tentative identification.

garlic aroma and flavor notes with a sweet aroma note, the exact description of the snack by the trained panel.

Changes of aroma-active compounds over storage

Several aroma compounds from oil oxidation showed a concentration increase from 50% to 400% over 12 months of storage (Table 3.5) as detected by GC/MS.

Among those lipid oxidation compounds listed in Table 3.5, 1-octen-3-ol, 1-octen-3-one and 3-octen-2-one presented the largest increase during storage. Both 1-octen-3-ol and 1-octen-3-one have been reported by Ullrich and Grosch (1987) as important flavor compounds formed during autoxidation of linoleic acid, however, less significant then hexanal, 2-(Z)-octenal and 2-(E)-nonenal. In addition, 1-octen-3-one was reported to provide a metallic flavor to milk, which was modified to an oxidized flavor by addition of small amounts of octanal, 2-heptanal, and 2,4-heptadienal (Hammond and Hill, 1964). However, despite the fact that the GC/MS analysis detected the increase over storage of compounds related to oil oxidation such as 1-octen-3-ol and 1-octen-3-one, Osme subjects, in most cases, did not detect any aroma peak correspondent to those compounds. This indicated that those compounds may have been below their detection threshold (in air) at the level they were presented to the subjects. It is reasonable to assume that lipid oxidation was reduced by the low level of oxygen in the canisters, and oil oxidation products, such as 1-octen-3-ol and 1-octen-3-one, had little impact over the stored product's flavor and aroma.
Thus, the increase in the rancid aroma and flavor notes perceived in the snack by the trained descriptive panel would be attributed to the additive effect of hexanal (which was not evaluated by the GCO panel due to its close

proximity to the solvent peak), the remaining compounds listed in Table 3.5, and all peroxides. The peroxides, even if present in the samples extract, would not be detected by GC/MS and Osme analysis because they are unstable on polar gas-chromatographic liquid phases such as the one used in this study (Guth and Grosch, 1990).

The concentration of compounds which were formed in the thermally activated Maillard reaction and Strecker degradation, increased over the 12 months of storage (Table 3.6). These effects were noticed by the GC/MS and confirmed in some cases by the Osme panel. In juices and similar systems, Maillard reactions are found to be significant during storage at ambient temperature, but the significance of these reactions is expected to decrease in systems with low water activity such as deep fried foods. Yet, our study suggests that interactions between reducing sugars, amino acids and dicarbonyls continue to occur, at low rates, in the snack during storage. The descriptors ratings for Osme subjects did not show statistically significant changes as often as the GC/MS possibly because those changes occurred at levels below the subjects' difference thresholds.

Two compounds were found to decrease in concentration over the snack storage: *c,t*-2,4-decadienal, described as toasted corn and *t,t*-2,4-decadienal described in our study as body odor/rotten onion and garlic odorant, but frequently cited as imparting deep-fried odor (Table 3.7). At 12 months of storage, both decadienals underwent a two fold decrease as compared with the 0-months sample; however, the Osme subjects detected a significant decrease ($p \leq 0.05$) just for the *c,t*-2,4 decadienal. More extreme decreases in decadienals were reported by Mookerjee et al. (1965) who found these compounds decreasing from 55% of the total monocarbonyl compounds in fresh potato chips to 7% in the stale potato chips. Based on reports of

Table 3.5 Oil oxidation products showing a significant increase over storage time as detected by GC/MS and Osme subjects.

Compound[1]	KI_{20M}[2]	GC/MS	Osme				
		% of Increase[3]	Aroma[4] Peak	Descriptor	(I_{max})[5] Storage Time (months)		
					0	3	12
hexanal	<1100	420	n.t.				
1-octen-3-one	1310	262	n.d.o.				
3-octen-2-one1	n.d.	399	n.d.o.				
1-octen-3-ol	1461	426	n.d.o.				
c-2-nonenal	1508	147	14	plastic	0.73[a]	0.44[a]	0.60[a]
c,t-3,5-octadien-2-one	1528	149	15	garlic/onion	0.71[a]	0.60[a]	0.88[a]
t,t-3,5-octadien-2-one	1577	174	n.d.o.				
gamma-octanoic lactone	1921	209	32	coconut	0.87[b]	1.96[ab]	2.70[a]

Means with the same superscript within the same row, are not significantly different from each other ($p \leq 0.05$), [1]Compound identity as determined by GC/MS, [2]Kovats' indices as detected by GC/MS, [3]Ratio of compound concentration at 12 months of storage as compared with the 0 months stored sample, [4]Aroma peak number as shown in Figure 3.4, [5]Maximum odor intensity of the aroma peak, n.t.Not tested, n.d.o.Not detected by the Osme panel, n.d. Not detected by GC/MS

Table 3.6 Maillard reaction products showing a significant increase over storage time as detected by GC/MS and Osme.

Compound[1]	KI_{20M}[2]	GC/MS	Osme				
		% of Increase[3]	Aroma[4] Peak #	Descriptor	I_{max}[5] Storage Time (months) 0	3	12
2-acetyl-1-pyrroline	1342	252	2	toasted corn	3.81[a]	4.30[a]	4.94[a]
trimethylpyrazine	1407	184	5	dusty/potato	2.32[a]	3.07[a]	2.46[a]
2-propionyl-1-pyrroline	1429	221	6	toasted corn	1.12[b]	1.49[ab]	2.64[a]
furfural	1474	153	11	plastic	0.70[a]	0.79[a]	1.77[b]
2,3-diethyl-5-methyl-pyrazine	1494	415	13	dusty/potato peel	3.52[a]	3.48[a]	3.16[a]
o-aminoacetophenone	2225	170	41	stale potato chip	7.20[a]	6.89[a]	7.02[a]

Means with the same superscript within the same row, are not significantly different from each other ($p \leq 0.05$), [1]Compound identity as determined by GC/MS, [2]Kovats' indices as detected by the GC/MS, [3]Ratio of compound concentration at 12 months of storage as compared with the 0 months stored sample, [4]Aroma peak number as shown in Figure 3.4, [5]Maximum odor intensity of the aroma peak.

Table 3.7 Products showing a significant decrease over storage time as detected by GC/MS and Osme.

Compound[1]	KI_{20M}[2]	GC/MS	Osme				
		% of Decrease[3]	Aroma[4] Peak	Descriptor	(I_{max})[5] Storage Time (months) 0	3	12
c,t-2,4-decadienal	1773	271	27	toasted corn	2.29[a]	1.56[b]	0.78[c]
t,t-2,4-decadienal	1818	206	29	body odor	7.37[a]	6.77[a]	7.67[a]

Means with the same superscript within the same row are not significantly different from each other ($p \leq 0.05$), [1]Compound identity as determined by GC/MS, [2]Kovats' indices as detected by the GC/MS, [3]Ratio of compound concentration at 12 months of storage as compared with the 0 months stored sample, [4]Aroma peak number as shown in Figure 3.4, [5]Maximum odor intensity of the aroma peak.

Table 3.8 Peak number, chemical name, Kovats' index (KI_{20M}) of odorants present in the 0 months stored snack, KI_{20M} of the reference compound and the source of the mass spectra used for the identification of the odorant.

Peak #[1]	Identified (GC/MS)[2] Compound	KI_{20M} Unknown[3]	KI_{20M} Reference[4]	Mass Spectra Source
2	2-acetyl-1-pyrroline[pi]	1342	1341	Tanchotikul and Hsieh (1991)
4	dimethyltrisulfide[pi]	1383	1383	McLafferty (1989)
5	trimethylpyrazine[pi]	1407	1406	McLafferty (1989)
6	2-propionyl-1-pyrroline[pi]	1429	n.d.	Schieberle (1991a)
7	2-vinyl-pyrazine[pi]	1441	1437	McLafferty (1989)
8	3-ethyl-2,5-dimethyl-pyrazine[ti]	1449	1449	McLafferty (1989)
9	8-nonen-one[pi]	1442	1449	McLafferty (1989)
10	methional[pi]	1463	1463	McLafferty (1989)
13	2,3-diethyl-5-methylpyrazine[pi]	1494	1497	McLafferty (1989)
19	methyl allyl trisulfide[pi]	1596	1591	Yu et al. (1989)
21	butyric acid[pi]	1640	1640	McLafferty (1989)
24	*t,t*-2,4-nonadienal[pi]	1709	1708	McLafferty (1989)
26	*c,t*-2,4-decadienal[ti]	1773	1772	McLafferty (1989)
28	diallyl trisulfide[pi]	1795	1795	Yu et al. (1989)
29	*t,t*-2,4-decadienal[nc]	1818	1817	McLafferty (1989)
30	2-vinyl-4H-1,3-dithriin[pi]	1849	1942	Yu et al. (1989)
34	3-hydroxy-2-methylpyran-4-one	1967	1967	McLafferty (1989)
36	2-methyl-phenol[pi]	2012	1012	McLafferty (1989)
37	2,5-dimethyl-4-hydroxy-3(2H) furanone (furaneol)[nc]	2039	2039	Sen et al. (1991)

Table 3.8 (cont.)

40	2-methoxy-4-vinylphenol[pi]	2204	2207	Ralph and Hatfield (1991)
41	*o*-amino-acetophenone[pi]	2225	2225	McLafferty (1989)
42	decanoic acid[pi]	2291	2291	McLafferty (1989)
46	skatole[pi]	2491	1491	McLafferty (1989)
48	vanillin[pi]	2574	2574	McLafferty (1989)
49	4,5-dimethyl-3-hydroxy-2(5H)-furanone[pi]	n.d.	n.d.	Schieberle (1991b)

[1]Peak number as shown in Figure 3.4, [2] The compound was identified by comparing it with the reference substance on the basis of the following criteria: Kovats' indices as detected by the GC/MS using a Supelcowax10 fused silica capillary column, mass spectra data and odor quality perceived at the sniffing port. [3]Kovats' indices of the unknown compounds (odorants) as detected by GC/MS, [4]Kovats' indices of the reference standards, [pi]Positive identification, [nc]Identification not 100% agreed to by the GCO panel when pure standard was evaluated, n.d. not detected by GC/MS.

Table 3.9 Chemical name, Kovats' index (KI_{20M})of oil oxidation products showing a significant increase over storage time of the snack , KI_{20M} of the reference compound and the reference of the mass spectra used for the identification of the oil oxidation product.

Identified (GC/MS)[1]	KI_{20M}		Mass Spectra
Compound	Unknown[2]	Reference[3]	Reference
Hexanal	<1100	<1100	McLafferty (1989)
1-octen-2-one	1310	1310	Swoboda and Peers (1977)
3-octen-2-one	n.d.	n.d.	McLafferty (1989)
1-octen-3-ol	1461	1461	McLafferty (1989)
c-2-nonenal	1508	1513	Ullrich and Grosch (1988b)
c,t-3,5-octadien-2-one	1528	1529	McLafferty (1989)
t,t-3,5-octadien-2-one	1577	1577	McLafferty (1989)
gamma-octanoic lactone	1921	1921	McLafferty (1989)

[1] The compound was identified by comparing it with the reference compound on the basis of the following criteria: Kovats' indices as detected by the GC/MS using a Supelcowax10 fused silica capillary column and, mass spectra data. [2]Kovats' indices of the unknown compounds (odorants) as detected by GC/MS, [3]Kovats' indices of the reference standards, n.d. not detected by GC/MS.

Pippen and Nonaka (1963), which state that 2,4-decadienal, on exposure to air at room temperature, develops first stale and then rancid odors, Mookerjee et al. (1965) proposed that these compounds may contribute to desirable aroma, explains why the loss of in the toasted note reported by those researchers and verified in this study was followed by an increase in stale aroma and flavor notes.

The Kovats' index of reference compounds used for the identification of the compounds cited in this study, as well as the correspondent references of the mass spectras, are sumarized in Tables 3.8 and 3.9.

CONCLUSIONS

Sensory descriptive analysis established that the snack aroma and flavor was primarily characterized by toasted corn and cheesy notes.

GC/MS and GCO techniques suggested that methional, *t,t*-2,4-decadienal, 2-methoxy-4-vinylphenol, furaneol, o-aminoacetophenone and vanillin are the most important volatiles related to the snack toasted corn aroma and flavor, while butyric acid probably is the major contributor to the cheese note described in the product's aroma and flavor.

Changes in the snack aroma and flavor profile occurred despite product packing in nitrogen-flushed canisters. The most important changes observed in the snack during storage were increases in the stale and rancid notes which occurred with a simultaneous dramatic drop in toasted corn aroma.

The additive effect of oil oxidation products such as hexanal, 1-octen-3-ol, 3-octen-2-one and 1-octen-3-one in the food matrix, may be accounted for

in the increase of rancid notes detected in the product by the descriptive panel.

The decline of toasted corn notes detected by the descriptive panel over storage can be attributed to the coincident decrease in decadienal concentration in the product over storage. Suppression effects caused by the rise of rancid and stale notes, and formation of complexes between carbohydrates, such as maltodextrins and aroma compounds, are two additional effects which could be contributing to the weakening of the toasted corn notes.

Finally, relating instrumental data (GC/MS) with sensory data (descriptive sensory panel and GCO), was an essential step to acquiring a better understanding of the product's flavor properties and stability.

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