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CORRELATION OF ANALYSES OF ODOR PROFILES OF HDPE FILMS COATED WITH DIFFERENT ADHESIVES USING ELECTRONIC NOSE, SENSORY EVALUATION, AND GC-MS

By

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ABSTRACT

CORRELATION OF ANALYSES OF ODOR PROFILES OF HDPE FILMS COATED WITH DIFFERENT ADHESIVES USING ELECTRONIC NOSE, SENSORY EVALUATION, AND GC-MS

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The two dimensional PCA (Principle Component Analysis) module of the E-nose system differentiated successfully five groups of HDPE films (one uncoated film and four films coated with different adhesives) and a DI (Discrimination Index) of 82 was obtained. A DFA (Discriminative Function Analysis) model with a POR (Percentage of Recognition) of 94 was built and proved effective in identifying unknown samples as one of the training samples.

Both the pairwise ranking test and the quantitative affective consumer test indicated the control sample (HDPE base film) had the weakest odor while one type of coated film had the strongest odor among the five groups of samples. In addition, there was no significant difference in terms of the intensity of the perceived odor among the other three types of coated films. The "Acceptability" and "Intensity" sensory scores of the odor profiles were also obtained.

Two different sample preparation techniques, DH-TD (Dynamic Headspace – Thermal Desorption) and SPME (Solid Phase Microextraction), were used to concentrate volatile compounds released from the HDPE film

samples before the compounds were analyzed by GC-MS (Gas Chromatography – Mass Spectrometry). The DH-TD technique seemed to collect more volatile compounds from the sample matrix but its repeatability was affected by the connection between the transfer line of the thermal desorption unit and the gas chromatograph. In comparison, the SPME technique proved to be easier, faster, and cheaper to use. The same groups of potential volatile compounds were identified in the odor profiles of all five HDPE films, including ketones, aldehydes, aromatics, and hydrocarbons, among which were acetone, hexanal, benzaldehyde, nonanal, and 1,3-di-tert-butylbenzene. An SGE ODO II olfactory detector was used in the sniffing tests to describe the odor profiles of two coated HDPE films. Sniffing tests proved 1,3-di-tert-butylbenzene was not the crucial compound responsible for the stronger odor of one of the coated films.

PLS (Partial Least Square) models with correlation coefficients of 0.92 – 0.99 in the E-nose system were proved effective and robust in predicting the "Acceptability" sensory scores when the models were built for pairs of samples. On the other hand, the effectiveness and robustness of PLS models for predicting the amounts of acetone and nonanal in the odor profiles were affected by which pair of samples and what volatile compound were being investigated. Nevertheless, the E-nose system showed its potential as a quality control tool in packaging systems if the correlation between the E-nose system, sensory evaluation, and GC/MS was established. Moreover, the study showed the importance of complementing the three analytical techniques with one another.

谁言寸草心
报得三春晖

To Those Whom I love, Who Love and Sacrifice for Me

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Life is full of ups and downs. Looking back to the past six years, I am proud of myself. In the meantime, I feel grateful for everything, good and happy ones, as well as unfortunate ones. There are so many people who have helped me and whose name I cannot list here, but their kindness will be with me forever.

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CHAPTER 1 INTRODUCTION

BACKGROUND INFORMATION

The food packaging market has seen increasing applications that require adhesives to be in direct or indirect contact with food. Several examples include fruit labeling, microwave popcorn bags, paper food wraps and plastic food wraps. These applications require various levels of FDA regulation compliance. The level is dependent upon the type of food (aqueous, fatty, dry, and acidic) and the conditions in which the adhesive will come into contact with food.

In the past, much attention has been given to the safety of components used in food packaging systems. In most cases an extraction test is completed. However, of equal importance is the effect of components in a food packaging system on the odor and/or flavor quality of the food. Off-odor and off-flavor can become a big food quality issue and can be the result of processing, storage, and preparation of the food packaging system. In many cases, the problem originates from the adhesives used in the package, especially when the package is exposed to high temperatures, such as in microwave heating, in which more volatiles are generated.

Adhesives are being used in increasing amounts in food packaging situations where there may be direct contact of the adhesive with the product, or

other circumstances which permit migration of substances from the adhesive to the contained food. Compliance with FDA regulations does not in itself ensure that volatile components of the adhesive will not have an adverse impact on food odor. Off-odor is often associated with transfer of small quantities and complex mixtures of volatiles, which are difficult to detect and characterize using traditional analysis methods such as gas chromatography. Use of sensory panels can provide valuable information about odor and taste concerns, but responses of panels can be highly variable. Further, the panel response does not in itself provide any information about the source of the objectionable taste or odor, and therefore is not always of value in efforts to remediate the problem.

Since the introduction of electronic nose technology, such systems have been used with considerable success to differentiate between problematic and acceptable samples of products of a variety of types. Patterns of responses to the set of e-nose sensors provide qualitative differentiation between samples. Correlation of these responses to results from sensory panels can then allow the e-nose to be used as a quality control tool. Further, combining e-nose technology with gas chromatography/mass spectrometry or other suitable analysis tools can permit identification of particular compounds as those predominantly responsible for the odor and flavor problems.

Willing et al (1998) investigated the correlation between sensory panel evaluation and electronic nose analysis of odors associated with paperboard.

Several groups of e-nose responses were identified which had good correlation with problem odors in the paperboard. Some problem odors did not correlate to any pattern of e-nose responses, and some e-nose patterns did not match any smells identified by the test panel. No attempt was made to identify the compounds responsible for the odors. The e-nose was considered to be a useful tool for such characterization, although further optimization was recommended. The work also identified a statistical technique for correlation of e-nose responses with test panel responses.

Culter (1999) surveyed the use of electronic nose technology in quality control of products and packages. He found that most of the work with the technology had been for food products, flavor ingredients, and water. Little had been published about use in evaluation of packaging materials and problems associated with package/product interaction. He presented a procedure for development of methods for such use, since suitable standard methods were not yet available. The discussion includes sample preparation, purging and equilibration time, sampling, and statistical procedures, along with discussion of reproducibility. Examples cited include identification using e-nose of paperboard from different mills, and identification of laminations done by different processes.

Gruner and Piringer (1999) studied migration of adhesive components in paper and paperboard packaging into foods. Adhesives studied were an EVA (ethylene vinyl acetate) hot melt, dextrin, starch, a PVAc (polyvinyl acetate)

homopolymer dispersion, and a VAE (vinyl acetate-ethylene) copolymer dispersion. Components in the adhesives which could potentially migrate were determined by extraction using iso-octane and ethanol. Simulation of actual migration from the adhesives applied to paperboard was also carried out. Maximum global migration for several sample package/product systems was then calculated. In most cases, no attempt was made to identify particular migrants.

Galotto and Guarda (1999) examined overall migration from plastic packaging materials intended to be in contact with foods during thermal and microwave treatment. Microwave heating (compared to thermal treatment) was found to increase overall migration for PVC samples, but not for the other samples studied. These included polypropylene, and four different multilayer systems containing adhesives.

Heydanek (1978) examined the prediction of flavor effects of packaging materials, concluding that gas chromatography could be used to help forecast off-flavors and maintain product quality. Examples cited include a pine or spruce-like odor associated with waxed glassine cereal liners, and an insecticide or plastic off-flavor associated with cereal products stored in polystyrene foam containers.

Hollifield (1980) studied off-flavor in maple syrup associated with container-derived contamination. Headspace GC and GC-MS were used to determine the trace volatiles and verify that the methyl methacrylate/styrene/butadiene copolymer containers were the source of the taste and odor problem. The technique was successful in verifying the presence of methyl methacrylate, styrene, and toluene, and showed its usefulness in conducting difficult analyses of volatile contaminants in foods.

Ziegleder (1998) studied volatiles extracted from unprinted paperboard using steam distillation and analyzed by GC-MS. A list of 50 volatile compounds commonly found in paperboard was presented. Those found to be significant in contributing to odor intensity include 2,4-decadienals, 2-nonenal, 2-octen-3-ol, and a variety of aldehydes and short-chain fatty acids.

Ho et al (1994) used purge-and-trap GC-MS to identify 47 volatile compounds, belonging to alkane, alkene, aldehyde, ketone, phenolic, olefin, and paraffin groups, released from blow-molded HDPE bottles, as well as evaluate the effectiveness of anti-oxidants in reducing off-odor and off-taste associated with these compounds. Aldehydes and ketones were found to be the most important odor compounds. Taste and odor tests using an untrained panel were conducted.

Freire et al (1998) used Tenax trapping and GC-MS to examine volatiles released at high temperatures from PET-based packaging materials. Most volatiles were found to likely have originated from printing inks and adhesives. Materials tested included PET bottles, roasting bags, susceptor film, PET-coated paperboard, and several PET laminates.

SIGNIFICANCE OF WORK

Adhesives are widely used in food packaging, especially in primary packaging applications such as paperboard/seal material/flange, sealing pouches and bags, and bonding susceptors in microwave packaging (IOPP, 1995). Potentially, using adhesives poses problems for the odor quality of packaged food. Fast and accurate analysis of off-odor due to adhesives is very important for quality control purposes. Using an electronic nose makes it possible to detect the pattern of responses associated with off-odor as the result of adhesives used in food packaging systems.

Off-odor associated with components from food packaging is a significant concern. In many cases, the origin is adhesives used in the package. This concern is particularly great for packages which are exposed to high temperatures, such as in microwaving, which increases the generation of volatiles, but packages used at room temperature or lower are not immune. Identification and characterization of such volatiles is complicated by the fact that

many have effects at extremely low concentrations. Using electronic olfactory sensing technology enables detection of patterns of response that are associated with such problems, without requiring identification and quantification of individual components. Further, it permits very rapid detection of potential problems. Therefore, if a relationship between the response of e-nose systems and the presence of objectionable odor can be determined, e-nose systems can be used as an efficient and effective quality control measure in packaging systems. It is possible that the methodology could be used as an on-line monitoring system.

Further, through the correlation of electronic olfactory sensing, organoleptic testing, and GC-MS analysis, components of adhesives which contribute to objectionable odors may be identified. Successful completion of this research would permit consideration of their elimination or minimization in adhesive systems, and will also allow electronic nose technology to be used as a potential quality control measure in adhesive manufacturing and application.

OBJECTIVES

The adhesive/food systems selected for the study are pressure-sensitive formulations used in coating HDPE film for food packaging applications. The Alpha MOS Fox 3000 Electronic Nose system is used to determine the response patterns of the E-nose sensors to the headspaces of HDPE films coated with different adhesives. The resultant odor profiles will be evaluated with sensory

evaluation tests; with the suspect component volatile compounds being identified using GC-MS.

By correlating the analyses of the odor profiles with the E-nose system, sensory evaluation, and GC-MS, this study will investigate the potential of the E-nose system as a quality control tool.

CHAPTER 2 LITERATURE REVIEW

ADHESIVES

Adhesives are substances that are used to bond materials together. Adhesives take various forms including solids, liquids, or pressure sensitive formulations. They can be of natural origin, such as collagens, starches, dextrins, casein, rubber, etc. They can be synthesized, such as synthetic rubber, block copolymer, thermosetting resin, and thermoplastic resin adhesives, etc. Depending on the applications of adhesives, the formulations vary dramatically and are usually proprietary information. Some factors affecting an adhesive formulation include (IOPP, 2002):

Manufacturing process	Tackifiers
Material base	pH additives
Adhesive polymer base binders	Viscosity additives
Carriers	Extenders
Anti-foaming agents	Fillers
Anti-mold growth additives	Plasticizers
Humectants	Antioxidants

Adhesives are widely used in electronics, wood, pharmaceuticals, health care, automotive industries, and packaging applications (Gutcho, 1983; Pizzi & Mittal, 1994). In the late 1980s, about 35% of adhesives used around the world went to packaging-related applications such as paper, paperboard, glass, metal, and plastics (Brody & Marsh, 1997). One packaging-related application of adhesives is in food packaging. Examples are adhesives used in tube lidding,

microwave packaging, fruit labeling and food wraps. Adhesives that are widely used in packaging applications include starches, dextrins, resin emulsions, hot melts, and pressure-sensitives.

In early years, there were more nature-derived adhesives, but synthesized adhesives find a wider range of applications nowadays due to their variety. Moreover, natural adhesives are prone to attacks by microorganisms and their performance is usually sensitive to environmental factors such as temperature and humidity. In recent years, the adhesive industry is being pushed into two new trends (Brody & Marsh, 1997). One is the elimination of solvents or solvent-based adhesives, mostly because of the pressure from both governmental agencies and consumer concerns. The other trend is more and more need for recyclable adhesives, especially in recycled packaging applications.

Table 2.1 Chronological developments of adhesives in the U.S.

Year	Material
1814	Glue from animal bones (patent)
1872	Domestic manufacture of fish glues (isinglass)
1874	First U.S. fish glue patent
1875	Laminating of thin wood veneers attains commercial importance
1909	Vegetable adhesive from cassava flour (F.G. Perkins)
1912	Phenolic resin to plywood (Baekeland-Thurlow)
1915	Blood albumen in adhesives for wood (Haskelite Co.)
1917	Casein glues for aircraft construction
1920 – 1930	Developments in cellulose aster adhesives and alkyd resin adhesives
1927	Cyclized rubber in adhesives (Fisher-Goodrich Co.)
1928	Chlorized rubber in adhesives (McDonald-B. b. Chemical Co.)
1928 – 1930	Soybean adhesives (I. F. Laucks Co.)
1930	Urea-formaldehyde resin adhesives
1930 – 1935	Specialty pressure-sensitive tapes: rubber base (Drew-Minnesota Mining & Mfg. Co.)
1935	Phenolic resin adhesive films (Resinous Products & Chemical Co.)
1939	Poly (vinyl acetate) adhesives (Carbide & Carbon Chemicals Co.)
1940	Chlorinated rubber adhesives
1941	Melamine-formaldehyde resin adhesives (American Cyanamid Corp.) and Redux by de Bruyne (Aero Research Ltd.)
1942	Cycleweld metal adhesives (Saunders-Chrysler Co.)
1943	Resorcinol-formaldehyde adhesives (Penn. Coal Products Co.)
1944	Meltbond adhesives (havens, Consolidated Vultee-Aircraft Corp.)
1945	Furane resin adhesives (Delmonte, Plastics Inst.) and Pliobond (Goodyear Tire & Rubber Co.)
1946	Neoprene-phenolic adhesives
1948	Epoxy adhesives
1949 – 1952	Nitrile-phenolic and Nylon-phenolic adhesives
1960	Nylon-epoxy adhesives, modified epoxy-phenolic adhesives (service temperature ~ 550^0C)
1962	Polybenzimidazole (service temperature ~ 1000^0C)
1966	Polyimide
1969	Polymercaptan sealant commercialized
1974	Polybenzothiazole (service temperature ~ 1000^0C)

Modified, (Delmonte, 1947; Pizzi & Mittal, 1994)

Pressure-Sensitive Adhesives

According to a survey by Business Trend Analysts in 1990, pressure-sensitive adhesives accounted for 44.6% of adhesives sold in the United States with a sales value of $4.9 billion (Pizzi & Mittal, 1994). They remain tacky (i.e. sticky) at room temperature and form the bond with the substrate by applied pressure, which makes them easy to use. The three most traditional applications of pressure-sensitive adhesives are packaging tape, labeling, and identification and special marking labels (IOPP, 2002; Pizzi & Mittal, 1994). Recently, they are being used in plastic food wrap in the consumer products market.

Formulations of Pressure-Sensitive Adhesives

The US FDA regulates the ingredients used in formulating pressure-sensitive adhesives for food applications, in which adhesives can be in either direct or indirect contact with food. Approved ingredients can be found in various sections of the Code of Federal Regulations, 21 CFR 175 and 21 CFR 176 (Rosenberg, 1985). Typical ingredients used in pressure-sensitive adhesives are (IOPP, 2002):

Resin or rubber base

Tackifiers

Plasticizers

Fillers

Antioxidants

Carrier if pressure-sensitive is solvent or water borne

Two major base formulations for pressure-sensitive adhesives are acrylics and rubber/resin blends (IOPP, 2002; Soroka, 2002). Acrylic-based systems are usually composed of acrylic acid ester monomers and other co-monomers. The selection of monomer, ratios of co-monomer, and degree of polymerization directly affect the performance of adhesives including cohesion and adhesion. Rubber/resin blends are usually composed of block copolymers with tackifying resins, oils, and antioxidant. The selection of rubber and resin and the choice of tackifiers control the performance of adhesives (Soroka, 2002). In the book "Adhesives in Packaging" published by IOPP in 2002, the two major formulations of pressure-sensitive were compared (See Table 2.2) (IOPP, 2002):

Table 2.2 Comparison of acrylic- and rubber/resin-based pressure-sensitive adhesives

Property	Acrylics and Acrylic Co-polymer	Rubber/Resin
Tack	Good	Excellent
Peel	Good	Excellent
Hold, Room Temp.	Good	Excellent
Hold, Higher Temp.	Good	Fair
Aging	Excellent	Fair
Clarity, Non-Discoloration (Sunlight, UV)	Excellent	Poor
Resistance		
Oxidation	Excellent	Poor
Oils, Plasticizers	Excellent	Poor
Solvents Polar	Fair	Good
Non-polar	Good	Poor
Humidity (High)	Good	Good
Adhesion		
Polar Plastics, Metals	Excellent	Fair
Non-polar plastics	Fair	Good
Low Temperature	Good	Poor
Cost	Medium-High	Low-Medium

Reprint from (IOPP, 2002)

Coulding (1994) listed the most commonly used elastomers:

Table 2.3 Elastomers in common use

Elastomer	Used in
Rubbers	
Natural rubber	Solvent-based and water-based
Butyl rubber	Solvent-based
Styrene-butadiene rubber	Solvent-based and water-based
Block copolymers	
Styrene-butadiene-styrene	Solvent-based and hot-melt
Styrene-isoprene-styrene	Solvent-based and hot-melt
Other polymers	
Polybutene	Solvent-based and hot-melt
Poly(vinyl ether)	Solvent-based and water-based
Acrylic	Solvent-based and water-based
Ethylene-vinyl acetate	Hot-melt
Atactic polypropylene	Hot-melt
Silicon	Solvent-based

Reprint from (Goulding, 1994)

Forms of Pressure-Sensitive Adhesives

Three physical forms exist for pressure-sensitive adhesives: solvent-based, water-based, and hot-melt adhesives. In the book edited by Pizzi and Mittal, a variety of examples of formulations of solvent-based, water-based, and hot-melt pressure-sensitive adhesives are listed (Pizzi & Mittal, 1994).

Solvent-Based Adhesives

Solvent-based adhesives have three major components: an elastomer, the tackifier, and the carrier. Examples of elastomers are natural rubber and

synthetic rubbers such as butyl rubber, styrene-butadiene rubber, poly-isoprene, acrylic polymers, block-copolymers of styrene with butadiene or isoprene, silicone elastomer, vinyl ethers, and poly-isobutylene. Two common tackifying resins nowadays are wood rosin derivatives and hydrocarbon resins. The former is usually made through hydrogenation or esterification, and the latter is usually aliphatic, aromatic, or terpenes. The choice of the elastomer and the tackifier, as well as their ratio, determines the suitability for the end-use application, with the tackifier being mainly responsible for tack, peel, and shear properties (Goulding, 1994).

Solvent-based pressure-sensitive adhesives used to dominate the market. However, with more available synthetic elastomers, along with the health and environment concern toward the use of solvent, pressure-sensitive adhesives in the form of either water-based or hot-melt have cut into the market (Brody & Marsh, 1997; Goulding, 1994; IOPP, 2002; Soroka, 2002).

Water-Based Adhesives

Water-based adhesives are usually in the form of dispersions that are composed of at least two monomers. The combination of two monomers and their ratio give a wide range of properties (Goulding, 1994). Compared to solvent-based adhesives, water-based adhesives have advantages of resistance to heat, UV light, and oxidation. Thus antioxidant can be eliminated from the

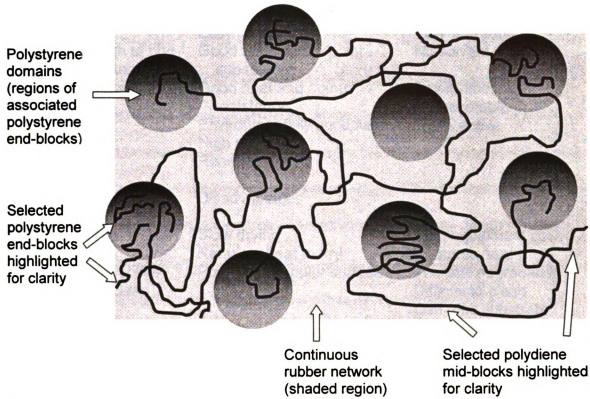
formulations. However, drying speed can sometimes be a concern in certain applications.

Hot-Melt Adhesives

A distinction between hot-melt and solvent-based adhesives is the mechanism to control the viscosity. With solvent-based adhesive, the viscosity is controlled with solvents, while the viscosity of hot-melts is controlled through temperature or the appropriate selection of tackifier resin. Unlike water-based adhesives, antioxidant is essential for hot-melt adhesives because they are normally applied at higher temperatures.

The primary advantage of hot-melt pressure-sensitive adhesives is their ability to develop bonds very fast, which make them suitable for high-speed processing (Wieczodek, 1990).

Different from solvent-based and water-based adhesives, pressure-sensitive hot-melt adhesives have a two-phase structure as shown below:



Reprint from (Goulding, 1994)

Figure 2.1 Structure of thermoplastic rubber

Goulding explained how the two-phase structure displayed in Figure 2.1 works and gives holt-melt adhesives vulcanized rubber-like properties at room temperature and the ability to flow at elevated temperatures or in solvent: "thermoplastic regions of styrene end blocks lock the elastomeric mid-sections of butadiene or isoprene at room temperature but allow the elastomer to move freely at elevated temperatures or in solvent" (Goulding, 1994).

Table 2.4 Advantages and disadvantages of pressure-sensitive adhesives

Solvent-based	Water-based	Hot-melt
	Advantages	
Quick drying	Easy cleaning	Very fast setting
Good adhesion to non-polar substances	Good adhesion to polar substances	No solvent waste
Good key on certain plastics	Good heat and aging resistance	Environmentally acceptable
Versatile	Environmentally acceptable	100% active
	High solids	
	Ready to use	
	Disadvantages	
Flammability	Slow drying	High equipment cost
Toxicity	Requires heat to dry	Requires heat
Relatively low solids	Poor on non-polar surfaces	Thermal degradation
Less easy to clean		Difficult to clean
		Can melt substrate
		Difficult to package

Reprint from (Goulding, 1994)

Off-odor from Packaging Systems Containing Adhesives

Flavor scientists attribute off-flavor/odor in a food system to the following sources (Baigrie, 2003):

Packaging Materials

Microorganisms

Oxidative rancidity

Millard reaction

Interactions between food components

Cleaning and disinfecting agents

In packaging-related applications, especially in food packaging, the packaging materials are sometimes exposed to high temperatures and/or extended storage periods, which may lead to off-odor. Printing inks, additives, and adhesives, as part of most packaging systems, all can contribute to off-odor because of their low-molecular weight components (Lord, 2003).

Kim et al (1988) used purge-and-trap and GC/MS techniques to characterize the off-odor released from two different PVC films. Various volatile compounds were identified, including alcohols, aldehydes, and ketones. It was concluded that those volatile compounds originated from the degradation of the bis-(diethylhexyl) phthalate, one major plasticizer used in PVC film.

Freire et al (1998; 1999) studied the formation of volatiles from various food packaging forms including laminates, bottles, and roasting bags made of PET (polyethylene terephthalate). It was found printing inks and adhesives were probably the packaging components that caused the formation of volatile compounds during the thermal processing, while PET itself was not a main factor in the formation of volatiles.

McNeal and his colleagues (1993) investigated and identified various volatile compounds released from several commercially-available microwaveable packaging systems with different susceptor designs, which were composed of

metalized polyester film, adhesives, and paper packaging materials. It was concluded every component of the susceptors, metallized PET film, adhesives, and paper materials, all contribute to the formation of the complicated volatile profile.

In a similar study, Booker and his co-workers (1989) studied and compared the volatiles released from microwave-interactive paperboard packaging materials that were heated in a microwave oven and in a conventional oven. Solvents from adhesives were quoted as one of the sources of volatile compounds such as 1,1,1-trichloroethane.

Czarnecki (1997) focused his work on the solvent retention and its contribution to odor in flexo packaging systems. It was concluded odor is an important attribute of any packaging system, and solvent used in printing inks should be selected carefully to alleviate the odor problem. In his work, Czarnecki also mentioned some water-based adhesives degraded and generated a strong vinegar-like odor.

In Anderson's work (1988), samples from a thermoformed and microwaveable container made of PP/Saran/PP were enclosed in a vial and heated in a microwave oven. The released volatiles were collected from the headspace, and four hydrocarbons and BHT (butylated hydroxytoluene) were identified and quantified.

In the article authored by Larson (1991), off-flavors and off-odor originated from food packaging systems were quoted as one of the major factors that contributed to less-than-satisfactory food quality. Printing inks, adhesives, paperboard, and plastics were all mentioned as major sources of volatiles.

ANALYSIS OF OFF-ODOR IN PACKAGING SYSTEMS

Challenges

Even though the importance of off-odor in food and consumer products has been widely accepted, the analysis of volatile compounds in an off-odor system has never been an easy task due to the following facts.

First, a lot of volatile compounds can be perceived by a human nose at a very low concentration, sometimes even being in the range of ppb (parts per billion) to ppt (parts per trillion), which makes it extremely difficult to detect those compounds with analytical instruments with moderate sensitivity (Baigrie, 2003; Marsili, 1997, 2002).

Table 2.5 Taste thresholds of halophenols and haloanisoles

Compound	Parts per billion (10^9), in water
2-Cholorophenol	0.1
2-Bromophenol	0.03
2,6-Dichlorophenol	0.3
2,6-Dichloroanisole	0.04 (odour)
2,6-Dibromophenol	5×10^{-4}
2,4,6-Trichlorophenol	2
2,4.6-Trichloroanisole	0.02
2,4,6-Tribromophenol	0.6
2,4,6-Tribromoanisole	8×10^{-6} (odour)

Reprinted from (Kilcast, 2003)

As shown in Table 2.5, the taste thresholds of 2,6-dibromophenol and 2,4,6-tribromoanisole are in the range of 10^{-4} and 10^{-6} ppb respectively, which are both out of the detection limits of most analytical instruments.

Second, the analysis of off-odor is complicated by the inconsistency of reported thresholds. Threshold, a term commonly used by scientists studying flavors and odors, is the concentration of a compound "in a specified medium that is detected by 50% of a specified population" (Kilcast, 2003). As hinted by the definition, the value of the threshold of a specific compound can vary dramatically from one reference to another (Devos *et al.*, 1990; Kilcast, 2003; Saxby, 1996a), because it is significantly affected by the nature of the medium such as temperature and pH, the test method and procedure, experience and number of test subjects, etc.

Table 2.6 lists the thresholds of several compounds above water and in water (Fazzalari, 1978; Lord, 2003; Saxby, 1992, 1996a).

Table 2.6 The effect of medium on the thresholds of several aromatic compounds

Compound	Odor threshold ppm in water*	Taste threshold ppm in water**
2,4,6-Trichloroanisole	3×10^{-8}	2×10^{-5}
2,3,4,6-Tetrachloroanisole	4×10^{-6}	2×10^{-4}
Chlorophenol	1.2	6×10^{-3}
2,4-Dichlorophenol	0.2	3×10^{-4}
2,4,6-Trichlorophenol	0.3	2×10^{-3}

* Odor thresholds above water; ** Taste thresholds in water.
Modified from (Lord, 2003)

As shown in Table 2.6, a threshold is affected by the means by which human senses interact with the compound. In addition, the threshold of the same compound in different mediums varies significantly.

The British Standards Institute expanded the meaning of "threshold" and defined two different thresholds; one is the "Detection threshold" and the other is the "Recognition threshold" (BSI, 1992). The former is the lowest concentration of a chemical entity that can be perceived and the latter is the lowest concentration that can be correctly identified.

Table 2.7 Different odor thresholds for hexanal in water

Threshold	Value/range (ppb)
Odor detection	0.19 - 30.0
Odor recognition	4.5 - 400
Taste detection	0.2 - 10

Modified from (Kilcast, 2003)

Third, the analysis of an off-odor system is especially difficult considering the fact that most such systems can be composed of hundreds of volatile compounds, interacting with and affecting each other (Acree & Teranishi, 1993; Jackson & Linskens, 2002). For example, more than 114 chemical components were identified in various citrus essential oils (Ruberto, 2002), 200 different odor regions were detected from a concentrated Chardonnay wine extract (Ferreira *et al.*, 2002), and 850 compounds were reported responsible for the unique taste of beer (Meilgaard, 1982). The aroma of coffee is composed of 791 unique compounds, which belong to 18 classes of chemical entities such as hydrocarbons, alcohols, aldehydes, ketones, acids, esters, phenols, amines, sulfur compounds, etc. (Parliment, 1997). The sensed odor characteristic of one volatile compound can vary because of the presence of another volatile compound. In a similar way, the threshold of one volatile compound might change because of the presence of another compound. For example, it was reported that taste detection thresholds of 2, 3, 6-trichloroanisole in tea, whisky, and blancmange were 0.016, 100, and 500 ppb, respectively. The reason is quite simple; that is, presence of other flavor compounds in the medium affects the sensory characteristics of 2, 3, 6-trichloroanisole (Kilcast, 1996).

Adding to this complexity is the fact that the perceived odor or taste characteristics of a compound can change, as its concentration in a medium changes.

Table 2.8 Effect of concentration on the taste description of trans-2-nonenal in water

Concentration (µg/l)	Taste
0.2	Plastic
0.4 – 2.0	Woody
8 – 40	Fatty
1000	Cucumber

Reprint from (Saxby, 1996b)

As shown in Table 2.8, the perceived taste of the same compound changes from an unpleasant plastic-like to a pleasant cucumber-like, as its concentration increases.

Fourth, a volatile compound in a food or consumer product system can come from a variety of sources, including ingredients, manufacturing process, packaging system, environment during storage and distribution, etc. This makes it extremely difficult to identify the origin of volatile compounds. For example, it was reported that both the characteristic aroma of rose flowers and the bitter taste of soybean protein vary at their different ripening stages, and thus chemical compounds responsible for their aroma and taste change during their life cycle too (Helsper *et al.*, 2002; Maehashi & Arai, 2002).

Last, the description of an odor system is always complicated and unreliable (Kilcast, 2003). Griffith asked a group of panelists to describe the sensed odor of 2, 3–dichloroanisoles, 30%, 20%, 15%, and 20% of panelists described it as "musty", "medicinal", "solvent and alcoholic", and "sweet and fruity", respectively (Griffith, 1974). This can be understood easily considering the experience and sensing capability of one panelist might be totally different from that of another. Moreover, as mentioned earlier, an odor system is usually composed of hundreds of volatile components, and thus the perceived odor is the integrated interactions among those components. This is why screening and training a panel is so important for an accurate and consensus description of the odor system in a descriptive analysis. Nevertheless, odor and/or flavor descriptions of various volatile compounds have been published (Acree & Teranishi, 1993; Baigrie, 2003; Saxby, 1996a).

Table 2.9 lists the odor/flavor description of various chemical compounds. Terry Acree and Heinrich Arn at Cornell University set up an online database called Flavornet (www.flavornet.org) with 738 odorants. A similar database with more than 1500 entries was built by Don Mottram at University of Reading in UK (http://www.odour.org.uk/).

Table 2.9 Odor/flavor description of various compounds

Compound	Description
1,1-Diethoxyethane	Jasmine odor
2-Ethyl-5,5-dimethyl-1,3-dioxane	Sweet, nutty, woody
3-Isopropyl-2-methoxypyrazine	Musty odor
4,4,6-Trimethyl-1,3-dioxane	Musty odor
2,2,6-Trimethyl-1,5-dioxane	Sweet, camphor odor
2,2,4,5-Tetramethyl-1,3-dioxane	Camphor, liniment odor
2-Ethenyl-2,5-dimethyl-1,3-dioxane	Musty, liniment odor
4-Methyl-4-mercaptopentan-2-one	Catty urine odor
4-Phenylcyclohexene	Synthetic latex odor
Acetaldehyde	Pear-like odor taste
Benzophenone	Geranium odor
Aliphatic acids	Short chain lengths particularly odorous, e.g. butyric acid has a rancid off odor
Alkyl acetates (ethyl-, propyl-, butyl-acetate)	Fruity odor
Alkyl substituted benzenes	Hydrocarbon
α-Methyl styrene	Hydrocarbon plastic
Butyl acetate	Pear drop odor, fruity taste
Chlorocresol	Medicinal odor and taste
Cumene (Isopropyl benzene)	Hydrocarbon
Cyclohexanone	Sweet pungent odor
Di/tribromophenol	Medicinal taint
Di/trichlorophenol	Medicinal taint
Dichlorobenzene	Medicinal taste
Diphenyl sulfide	Cabbage-like odor
Glycol ethers, e.g. 2-butoxyethanol	Soapy taste
Guaiacol	Smoky phenolic
Hexanal	Board/mown grass odor
Isophorone	Pungent brown sugar odor
Methyl benzaldehyde	Almond odor
Methyl benzoate	Pungent herbal odor
n-propyl benzene	Hydrocarbon
Naphthalene	Petroleum odor/taste
p-Cresol	Phenolic
Pentan-1,2-dione	Medicinal, chemical taint
Styrene	DIY fiber glass car repair odor
Thioglycollic acid alkyl esters	Pungent strong stale beer
Toluene	Petroleum odor/taste
Tribromoanisoles	Musty odor

Modified from (Lord, 2003)

Sample Preparation

As discussed earlier, concentrations of volatile compounds in an odor system are usually too low to be detected directly with most analytical instruments. Thus a sample preparation step is necessary to separate, purify, and concentrate analytes of interest from a sample matrix.

Many sample preparation techniques are available, including solvent extraction, steam distillation, direct (or static) headspace, dynamic headspace (DH), purge and trap, stripping, direct thermal desorption (DTD), solid-phase extraction (SPE), solid-phase micro-extraction (SPME), and supercritical fluid extraction (SFE) (Marsili, 1997, 2002; Zhang *et al.*, 1994). Some of these techniques involve the use of toxic organic solvents, multiple steps, costly equipment, or comprehensive training of the operator. In his report, Zhang listed the criteria for an ideal sample preparation technique: "solvent-free, simple, inexpensive, efficient, selective, and compatible with a wide range of separation methods and applications" (Zhang *et al.*, 1994).

Dynamic Headspace and Thermal Desorption

The prototype of dynamic headspace and thermal desorption (DH-TD) technique was invented in the 1960s by a group of scientists in California but the technique did not draw much attention until Tenex (poly-2,6-diphenyl-p-phenylene oxide) was introduced as a universal adsorbent material by Zlatkis

and his colleagues at the University of Houston in 1970s (Ettre, 2001). Since then the technique has been widely accepted as an effective way to concentrate analytes from various sample matrices, such as air, water, soil, pharmaceutical, food, and packaging materials. Compared to traditional sample preparation techniques, DH-TD is advantageous because it eliminates the use of solvent.

Though several versions of thermal desorption techniques exist, including direct thermal desorption, short-path desorption, automatic thermal desorption, and thermal desorption cold trap-injection, there are always two stages involved. The first stage is to trap analytes of interests by using a thermal desorption tube filled with single- or multi-bed adsorbent material, and the second step is to desorb the trapped analytes to the separation instruments such as GC (Gas Chromatography) by heating the desorption tube.

Theory of DH-TD

The term "dynamic headspace" is used to differentiate itself from static headspace, in that an inert carrier gas is continuously flushed into the sample vial and thus the headspace above the sample is always changing.

In static headspace sampling, no sample will be taken until equilibrium has been established between the sample matrix and the headspace, and thus concentrations of volatile compounds have been stabilized.

In contrast, volatile compounds are instantly swept out of the headspace and trapped in the adsorbent tube at the exit when an inert gas is continuously flushed into the sample vial in dynamic headspace sampling. As a result, the thermodynamics favors the transfer of volatiles from the sample matrix to the headspace, and an exhaustive extraction becomes possible.

A mathematical model was quoted in a paper authored by Nunez to calculate the theoretical time required to strip 95% of an analyte out of the sample matrix, $T_{0.05}$ (Nunez & Gonzalez, 1984):

$$T_{0.05} = \frac{3}{F}(V_G + K_i V_L)$$

where F is the volumetric flow rate of the purge gas in the sampling process, V_L and V_G are the volumes of the sample and the gaseous phase, and K_i is the capacity factor, which is dependent on the analyte and the trapping tube.

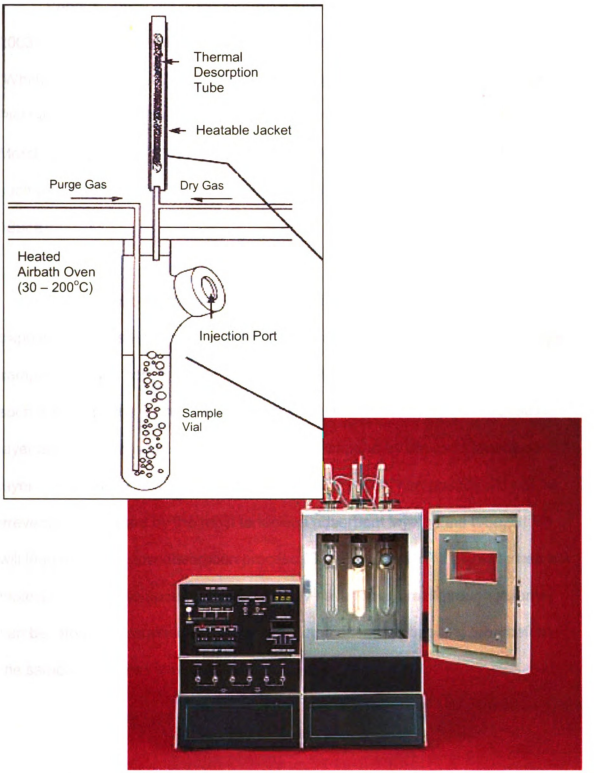
In the same report, a second theoretical model was quoted to predict the value of the breakthrough volume for a particular compound (Nunez & Gonzalez, 1984):

$$V = V_R^i\left(1 - 2/\sqrt{N}\right)$$

where N is the number of theoretical plates of the trapping tube and V_R^i is the retention volume of a compound i, which is dependent on the properties of the compound itself, the trapping tube and the purge gas.

"Breakthrough volume" is used as a limiting factor in the trapping process (Nunez & Gonzalez, 1984; Reid, 2003). As the purge gas continuously flows through the trapping tube, trapped volatile compounds will slowly move upward and eventually reach the end of the tube and begin to be eluted. Several factors were mentioned as important in dictating the value of the breakthrough volume (Nunez & Gonzalez, 1984), including 1) properties of the trapping tube, including the size of the tube, the adsorbent material used in the tube such as porosity, surface area, polarity, amount, and interaction with the analytes; 2) properties of the purge gas, including flow rate, temperature, and purity; 3) properties of the analytes, including concentrations, chemical structures, and sample matrix. If the sample matrix contains water, moisture carried over and retained in the adsorbent bed can lead to difficulty in the following thermal desorption process. However, because the breakthrough volume for water is usually much lower than those for volatile compounds, the trapping tube can be purged with dry clean gas after the sampling to eliminate the retained moisture from the sample matrix (Reid, 2003).

Figure 2.2 is a picture of a Dynamic Thermal Stripper Model 1000 used in dynamic headspace sampling to trap volatile and semi-volatile compounds, in which the oven temperature can be maintained between ambient and 150^0C.



Reprint from (Supelco, 1998a)
Figure 2.2 Dynamic thermal stripper model 1000

Various adsorbent materials are available (Nunez & Gonzalez, 1984; Reid, 2003), including Poropak series (Horwood *et al.*, 1981), Chromosorb series (Whitfield *et al.*, 1983), Tenax-GC (Durst & Laperle, 1990; Kwo, 1991; Mazza & Pietzak, 1990; Wellnitz-Ruen *et al.*, 1982), Tenax-TA (Kanavouras, 2003; Morales *et al.*, 1997; Werkhoff & Bretschneider, 1987a), and multi-bed tubes such as Carbotrap 300 (Kanavouras, 2003) and other Carbotrap series (Supelco, 1998a).

Compared to single-bed tubes, multi-bed tubes are more efficient in trapping a wider spectrum of volatile compounds with varying polarity in a single sampling. In addition, the order of different adsorbent materials in a tube is in such a way that the least volatile compounds will be trapped by the least active layer and the most volatile compounds will be trapped by the most tenacious layer. This arrangement ensures high molecular weight compounds will not be irreversibly adsorbed by the most tenacious adsorbent layer in the tube, which will lead to a very slow desorption process (Supelco, 1998a). It also ensures low molecular weight compounds, which usually have lower breakthrough volume, can be effectively retained without being eluted out of the trapping tube before the sampling process is completed.

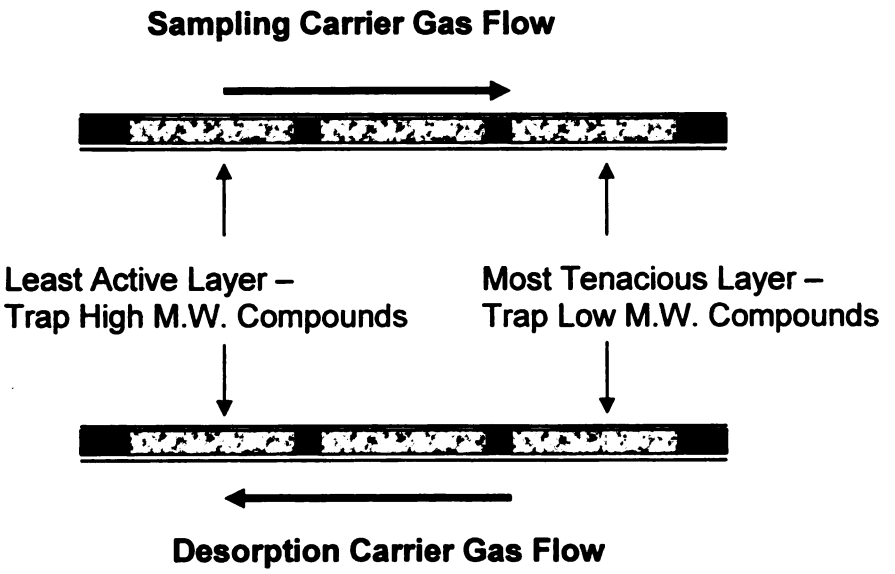


Figure 2.3 Carrier gas flow directions in sampling and desorption

Following the sampling is the thermal desorption step, in which the desorption tube with retained analytes is loaded onto the thermal desorption unit and heated to a high temperature instantly so that the analytes are released very quickly from the tube to the head of a GC column for separation.

Figure 2.4 is the Dynatherm Thermal Desorption Unit Model 890, in which the tube chamber can be heated up to 399°C very quickly to help desorb trapped analytes from the desorption tube to the head of the GC column in a few seconds. In addition, all transfer lines can be heated to 250°C to avoid condensation of compounds with higher boiling points.

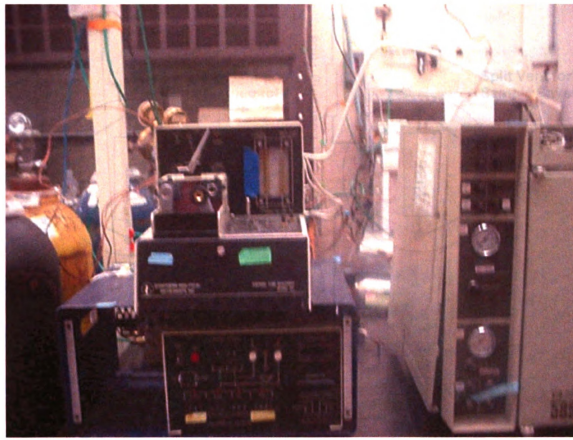


Figure 2.4 Dynatherm thermal description unit model 890

The unit features a multi-port valve design, as shown in Figure 2.5, which makes two distinct flow paths possible; one is for sample preparation (or focusing) and the other is for sample desorption.

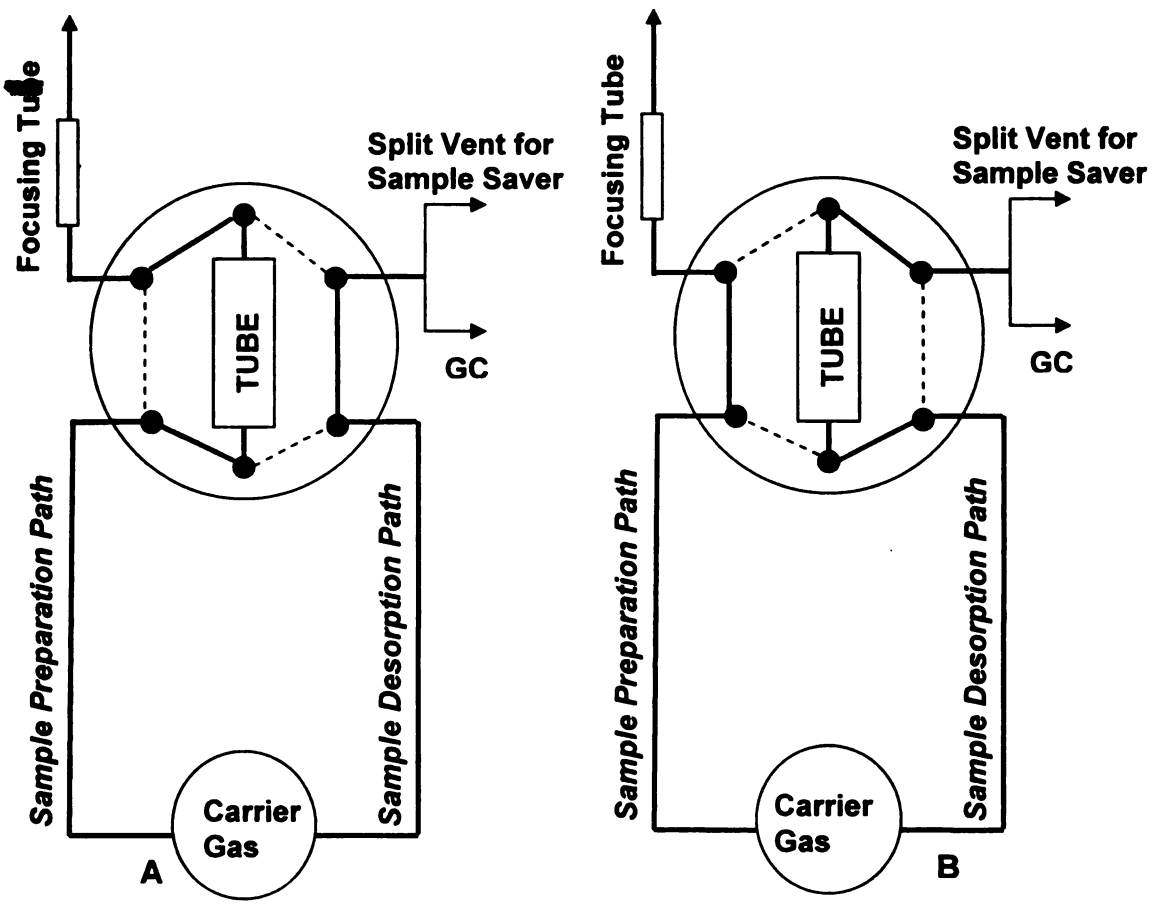


Figure 2.5 Six-port valve dictating sample preparation (A) and sample desorption (B) paths

Path A is used to clean the desorption tube after analytes have been eluted to the GC column and before the tube is used for the next sampling. Alternatively, path A can be used to focus analytes by desorbing them from the relatively bigger thermal desorption tube, which is 4 ½" x 6 mm O.D., to a small-bore adsorbent tube. The smaller internal volume of the focusing tube helps improve desorption efficiency, especially when a capillary column is used in the GC (Dynatherm, 1989).

Path B is used to introduce thermally desorbed analytes from the desorption tube to the head of the GC column for separation. An optional split path is connected to a sample saving vent, where a portion of desorbed analytes from the original desorption tube can be re-collected.by a second tube.

Applications of DH-TD in Packaging Analysis

In packaging, the dynamic headspace and thermal desorption technique has been used to study the odor systems originating from various food and consumer products packaged in different packaging materials, as well as the permeability properties of polymer materials.

Werkhoff and his co-workers (1987a; 1987b) optimized operating parameters in dynamic headspace and thermal desorption, including sampling temperature, desorption temperature, and desorption gas flow rate. Several flavor and fragrance applications using the thermal desorption technique were demonstrated.

Kwo (1991) used a Carbotrap-300 thermal desorption tube to trap volatile compounds released from a heated susceptor and then thermally desorbed the compounds to a GC column for analysis. Six volatile compounds were confirmed in her study.

A modified purge and trap/thermal desorption system was used to measure the organic vapor permeability of various high barrier polymer membranes (Chang, 1996). The approach proved to be more sensitive than a standard isostatic procedure using a MAS 2000^{TM} permeation instrument.

In his study investigating the effect of co-permeant on the permeability of various binary organic vapor mixtures through OPP and PVdC coated OPP films, Laoharavee (1998) used a purge and trap/thermal desorption system to measure the concentrations of permeated organic vapors. The author concluded that the compositions of the studied binary vapor mixtures did not affect the mass transfer properties of the co-permeant through the two films used in the study.

Kanavouras (2003) evaluated flavor compounds generated from packaged olive oil with the dynamic headspace and thermal desorption approach using Carbotrap-300 and Tenax-TA desorption tubes. It was concluded the method was capable of isolating and concentrating flavor compounds in olive oil, which were then identified with a coupled GC/MS instrument.

Direct thermal desorption and short-path thermal desorption, two other revised versions of the thermal desorption technique, were used by some researchers to study various flavor and fragrance problem, from food packaging films (Hartman *et al.*, 1991a), to forest products (Coello-Perez *et al.*, 1997), to air and soil samples (Manura & Hartman, 1992), to food and food products (Hartman

et al., 1991b; 1991c; Manura & Hartman, 1992), and to pharmaceutical applications (Manura & Hartman, 1992).

Solid-Phase Microextraction (SPME)

SPME (Solid-Phase Microextraction) is a solvent-free sample preparation technique that was first introduced in 1990 at the University of Waterloo in Canada (Arthur & Pawliszyn, 1990). Compared to DH-TD, SPME involves easier sampling procedures and requires simpler instrument setup.

SPME has almost all the qualities of an ideal sample preparation technique (Zhang *et al.*, 1994): "solvent-free, simple, inexpensive, efficient, selective, and compatible with a wide range of separation methods and applications". Moreover, SPME has more advantages such as linear results over a wide range of concentrations of analytes (Arthur *et al.*, 1992/1993; 1992a; 1992b; 1992c; Potter & Pawliszyn, 1992, 1994; Supelco, 1998b), automatic sample introduction to GC or HPLC, fast sampling process, reusability, and its applicability to field sampling (Supelco, 2005). Table 2.10 compares SPME and other sample preparation techniques (Supelco, 1998b).

Table 2.10 SPME compared with other sample preparation techniques

Detection Limit	Precision (% RSD)*	Expense	Time	Solvent Use	Simplicity
Purge & Trap					
ppb	1 – 30	High	30 min	No	No
Stripping					
ppt	3 – 20	High	2 hr	No	No
Headspace					
ppm	N/A	Low	30 min	No	Yes
Liquid-Liquid Extraction					
ppt	5 – 50	High	1 hr	Yes	Yes
Solid Phase Extraction					
ppt	7 – 15	Medium	30 min	Yes	Yes
SPME					
ppt	< 1 – 12	Low	5 min	None	Yes

Reprinted from (Supelco, 1998b)
* RSD%, percentage of relative standard deviation, also called CV (Coefficient of Variation), equals the ratio of the standard deviation to the mean.

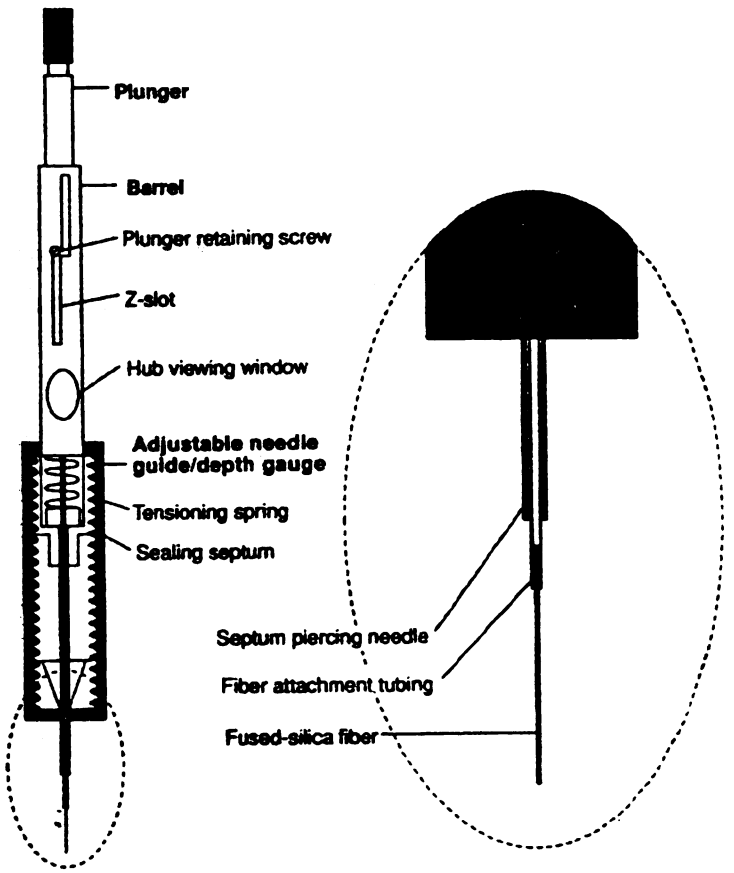
In the past sixteen years, SPME has been widely accepted as a powerful and easy-to-use technique in extracting odor and flavor compounds from solid, gaseous, and liquid sample matrices, environmental analysis, forensic analysis, and toxicology applications (Supelco, 2001a; 2005).

Theory of SPME

The core of the SPME technique is a chemically-inert and stable fused-silica fiber coated with liquid-phase polymer material, and in some cases mixed

with a solid adsorbent. Various coating materials are available, with varying polarities for extracting different volatile compounds (see Table 2.11).

The coated silica fiber is very fragile and thus needs some mechanical protection. The SPME device is designed in such a way that the fiber is connected to a stainless steel plunger via a spring. The fiber assembly is then contained in a syringe-like holder whose end is a hollow needle (see Figure 2.6).



Reprinted from (Zhang *et al.*, 1994)

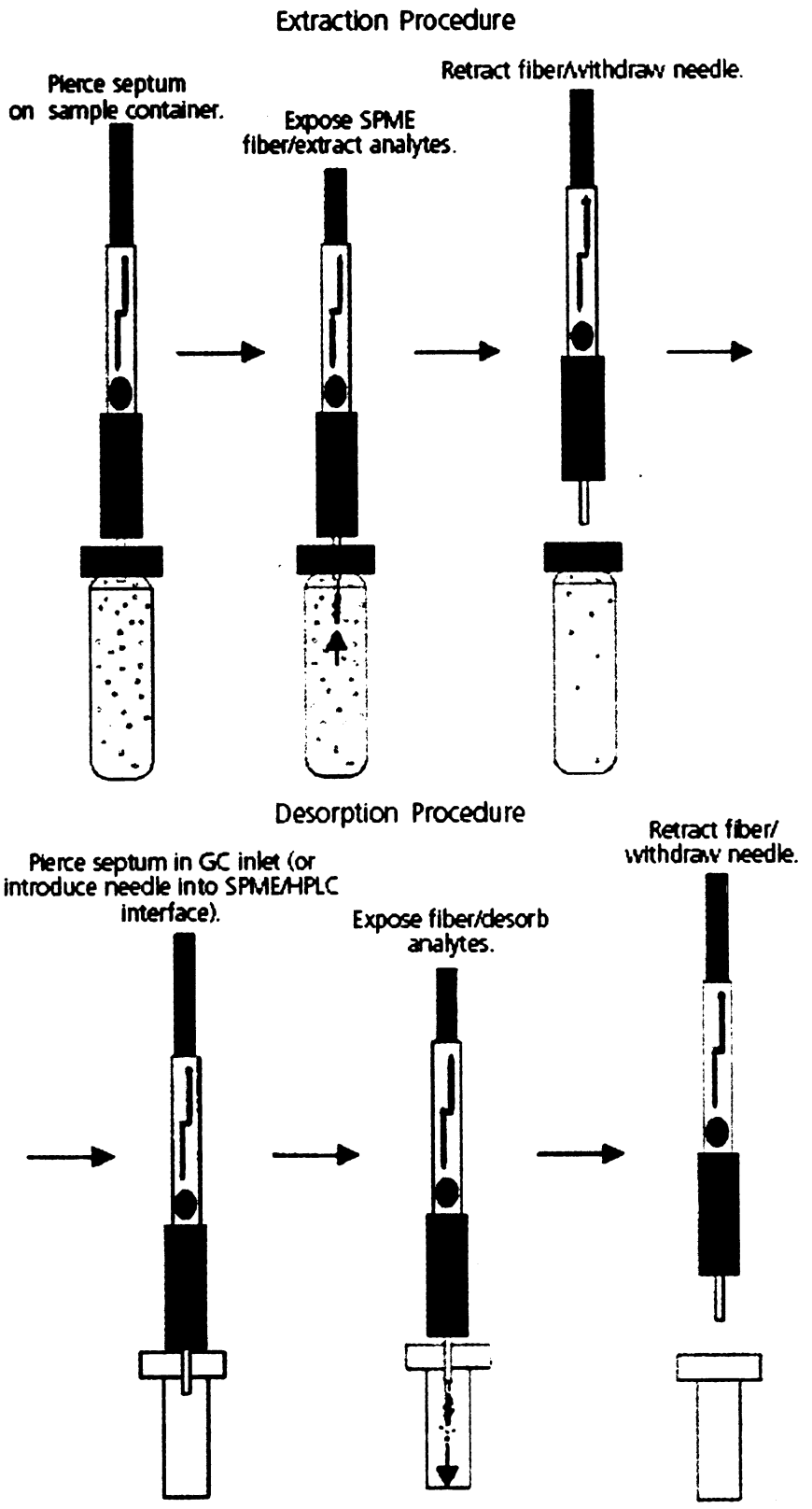
Figure 2.6 SPME fiber assembly

Table 2.11 Various SPME fiber coatings and coating thickness and their recommended applications

Fiber	Recommended use
PDMS (Polydimethylsiloxane)	
7 µm	Moderately polar to non-polar high molecular weight compounds (MW 125 – 600)
30 µm	Non-polar semi-volatiles (MW 80 – 500)
100 µm	Volatiles (MW 60 – 275)
PDMS/DVB (Polydimethylsiloxane/Divinylbenzene)	
60 µm	Amines and polar compounds, for HPLC only
65 µm	Polar volatiles, amines and nitro-aromatic compounds (MW 50 – 300)
CAR/PDMS (Carboxen/Polydimethylsiloxane)	
75 µm, 85 µm	Trace-level volatiles, and gases and low molecular weight compounds (MW 30 – 225)
PA (Polyacrylate)	
85 µm	Polar semi-volatiles (MW 80 – 300)
CW/DVB (Carbowax/Divinylbenzene)	
65 µm, 70 µm	Alcohol and polar analytes (MW 40 – 275)
DVB/CAR/PDMS (Divinylbenzene/Carboxen on Polydimethylsiloxane)	
50/30 µm	Flavor compounds: volatiles and semi-volatiles, C3 – C20 (MW 40 – 275)
CW/TPR (Carbowax/Templated Resin)	
50 µm	Surfactants (for HPLC only)

Modified from (Supelco, 1998b; 2005)

Similar to the HD-TD technique, two steps are involved in SPME. The first is sampling and the second is desorption. In the first step, the coated silica fiber is either exposed to the headspace of a solid or liquid sample matrix or directly submerged into the gaseous or aqueous sample to extract analytes of interest onto the fiber. The former is called 'Headspace SPME' and the latter is termed "Direct SPME'. In the second step, the fiber assembly is inserted into a GC injection port or an HPLC interface port and the fiber is exposed again to release analytes by heat (GC) or by the mobile phase (HPLC).



Reprinted from (Supelco, 1998b)

Figure 2.7 SPME extraction and desorption procedure

Thermodynamics of Direct and Headspace SPME

The extraction process in SPME sampling is essentially a partitioning process of analytes between the coating on the fiber and the extraction medium (headspace or the sample matrix itself). Equilibrium will not be reached until the chemical potentials of the analyte in all phases are equal (Zhang *et al.*, 1994). Theoretical models were proposed to describe the thermodynamics of the partition process.

In direct SPME, the equilibrium involves two phases: the sample matrix (gaseous or aqueous) and the liquid coating on the fused silica fiber. The extracted amount of a particular compound can be calculated using this theoretical equation (Yang & Peppard, 1994; Zhang & Pawliszyn, 1993; Zhang *et al.*, 1994):

$$n = \frac{K_{fs} V_f V_s}{V_s + K_{fs} V_f} C_0$$

in which n is the mass of a compound adsorbed by the coating after equilibrium has been reached; V_f and V_s are the volumes of the coating and the sample; K_{fs} is the partition coefficient of the compound between the coating and the sample matrix; and C_0 is the initial concentration of the compound in the sample matrix.

This equation indicates the linear relationship between n and C_0. Moreover, if the affinity between the compound of interest and the coating is strong, the value of K_{fs} is high, which leads to good sensitivity and concentrating effect. In the extreme case where $K_{fs}V_f >> V_s$, the above equation can be simplified as:

$$n = V_s C_0$$

and an exhaustive extraction is reached, which further underlines the importance of choosing the right coating material for a particular analysis. However, K_{fs} cannot be realistically large enough for every volatile compound in an odor or flavor system.

Another conclusion can be deduced from this equation, which is the suitability of SPME to field sampling. If the sample volume is extremely large, e.g. open air, a lake, or a river, $V_s >> K_{fs}V_f$ and the equation is simplified as:

$$n = K_{fs} V_f C_0$$

As indicated by this new equation, n is not dependent on the sample volume anymore, making the SPME technique a suitable tool for field sampling purposes.

On the other hand, three phases are involved in the thermodynamics of headspace SPME and a different model was proposed (Yang & Peppard, 1994; Zhang & Pawliszyn, 1993):

$$n = \frac{K_{fs}V_fV_s}{V_s + K_{fs}V_f + K_{gs}V_g}C_0$$

in which V_s is the volume of the headspace and K_{gs} is the partition coefficient of the analyte between the headspace and the sample matrix.

Comparing the two equations for direct and headspace SPME, the only difference is the term $K_{gs}V_g$, which means n based on headspace SPME is always smaller than n based on direct SPME. In the other words, direct SPME is always more sensitive than headspace SPME. However, K_{gs} is relatively small for most analytes (Zhang & Pawliszyn, 1993), which makes the amount of extracted compound, designated as 'n' in these equations, in headspace SPME similar to that in direct SPME, if V_g (headspace volume) is much smaller than V_s (sample volume) as well (Supelco, 1998c).

Kinetics of Direct and Headspace SPME

The kinetics of the extraction process in SPME is controlled by the mass transfer of the analyte among the sample matrix, the headspace, and the coating on the fiber.

In direct SPME, the coated fiber is immersed in the gaseous or the aqueous sample. Equilibrium can be reached very quickly if the mass transfer is controlled by the diffusion of analytes in the thin coating (3 – 100 μm) (Louch *et al.*, 1992) and if the sample is gaseous, which leads to a large diffusion coefficient. Vigorous agitation, e.g. magnetic stirring or sonication, is necessary to help reach equilibrium quicker in an aqueous sample. However, the equilibrium time is usually longer for an aqueous sample in reality even with agitation because of a thin static aqueous layer adjacent to the coating on the fiber (Arthur *et al.*, 1992/1993; Zhang *et al.*, 1994).

Headspace SPME needs to be adapted for solid samples or other sample matrices where direct SPME is not suitable, such as oil, wastewater, and sludge. The mass transfer in headspace SPME is more complicated because two inter-phases among three phases (matrix, headspace, and coating) are involved. It is easier to extract low molecular weight compounds, which have lower boiling points and high volatility, than to extract semi-volatiles. The speed of extraction in these cases is usually dependent on how fast analytes can escape from the sample matrix into the headspace (Pawliszyn, 1993; Zhang & Pawliszyn, 1993). One way to speed up this process is to heat the sample as well as stirring the sample to continuously create a fresh surface between the sample matrix and the headspace (Zhang & Pawliszyn, 1993; Zhang *et al.*, 1994).

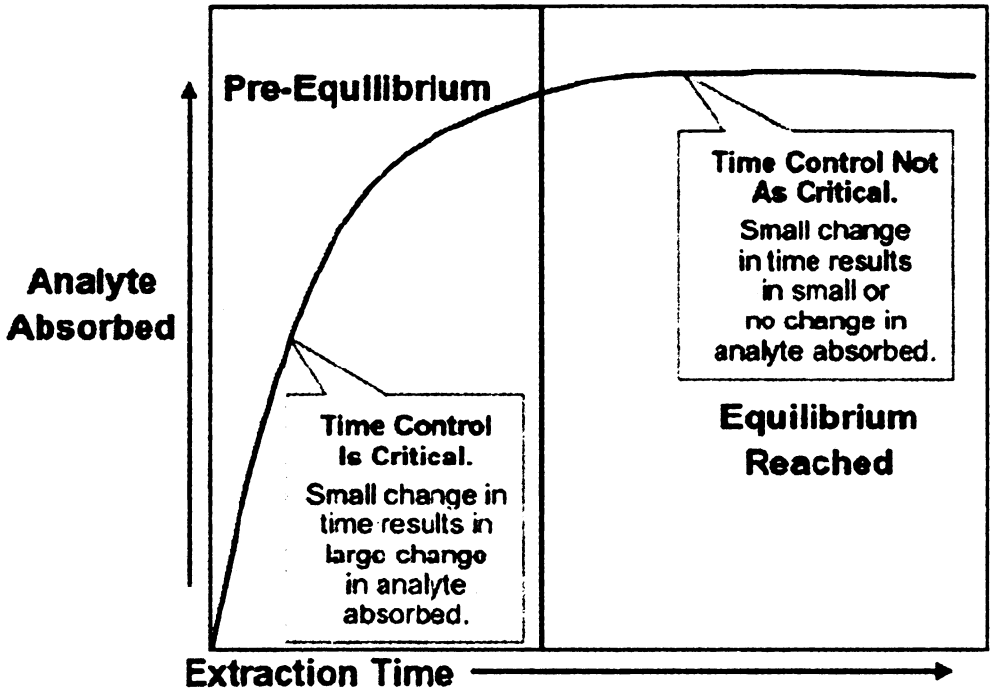
Table 2.12 Comparison of kinetics and applications of direct and headspace SPME

SPME	Kinetics	Applications
Direct SPME	Controlled by the diffusion of the analyte in the coating	Gaseous or clean/low-viscosity aqueous sample (if the solution is well stirred)
Headspace SPME	Controlled by the mass transfer from the sample to the headspace	Any sample matrix

More detailed theoretical analysis of the mass transfer process in both direct SPME and headspace SPME can be found in other papers (Koziel *et al.*, 2000; Louch *et al.*, 1992; Martos & Pawliszyn, 1997; Zhang & Pawliszyn, 1993).

Parameters in SPME

Because the extraction in SPME is a partitioning process, the amounts of extracted analytes will not be stabilized until equilibrium is reached. In some applications, equilibrium can be reached in less than 1 min (Arthur *et al.*, 1992/1993; Louch *et al.*, 1992). However, a much longer time (2 – 30 min) is usually required in most applications (Supelco, 1998b). Figure 7 explains how the extraction time affects the amount of analyte extracted by the fiber in SPME.



Reprinted from (Supelco, 2001a)

Figure 2.8 Extraction time profile curve in SPME

Time

As shown in Figure 2.8, the amount of extracted analytes will become independent of the extraction time once equilibrium has been reached. On the other hand, that amount is dependent on the extraction time during the pre-equilibrium stage. It becomes very critical to keep the extraction time consistent if working in the pre-equilibirum period.

Temperature

It is easily understood that temperature is another critical parameter because it affects the kinetics of the extraction process. Partition coefficients of analytes between the headspace and the sample matrix increase with temperature at first, and then start to decrease after reaching an optimum temperature. In the other words, more analytes are released from the sample matrix as the temperature rises, which leads to higher concentration of analytes in the headspace and favors the SPME sampling process. This trend will start to reverse after passing an optimum temperature, at which partition coefficients of analytes between the coating on the fiber and the headspace start to decrease.

Coating

The type and the thickness of the coating polymeric materials on the fiber, along with the addition of adsorbent materials such as Carbowax and Carboxen, affect the effectiveness of the extraction process, as well as the amount of analytes that can be extracted after equilibrium is reached (see Table 2.11 and thermodynamics equations). Generally speaking, a thick coating is used to extract volatile compounds, and a thin coating is preferred for extracting semi-volatile compounds because a thick coating in this case will prolong the desorption time and semi-volatile compounds can be carried over to the next sampling (Supelco, 1998b).

Other factors

Other important factors in the SPME sampling process include: sample volume, headspace volume, sample vial size, pH and salt content of the sample solution, sample agitation, and fiber immersion depth (Supelco, 1998b; 2001a; 2001b). Important factors in the desorption process include: time between extraction and desorption, fiber positioning during injection, injector temperature, desorption time, etc (Supelco, 1998b; 1998c; 2001a; 2001b).

In SPME sampling, it is more important to keep these sampling parameters consistent than to reach a full equilibrium before taking samples. Various studies have proved that a reliable, precise and reproducible quantitative analysis using the SPME technique is possible when the sampling practice is well controlled and kept consistent (Martos & Pawliszyn, 1997; Supelco, 1998b, 2001a; Zhang *et al.*, 1994).

In one study, ten duplications of SPME/GC analysis were conducted with a solution of chlorinated pesticides, which contained 20 compounds. The results showed the % RSD (percentage of relative standard deviations) of the relative response of the 20 compounds in the gas chromatographs ranged from 4.8% to 28.6% (Supelco, 1998b). When Potter and his colleagues used a 15 μm PDMS-coated SPME fiber to extract volatile and semi-volatile compounds from water

samples, it was concluded the detection limits in the SPME analysis exceeded the regulatory requirements of the standard analysis method US EPA 525 (Potter & Pawliszyn, 1994).

Applications of SPME in Packaging Analysis

Supelco compiled a SPME applications guide with over 500 references (Supelco, 2001c), which covers a large variety of applications using the SPME sampling technique, from food to packaging materials, to pharmaceuticals, to toxicology, to forensics, to environmental analysis (air, wastewater, pesticides, surfactants, and soil), and to field sampling.

A 75 μm CAR/PDMS fiber was used to extract US EPA method 624 volatile organic compounds from water in field sampling (Supelco, 2004). Concentrated compounds adsorbed on the fiber were then stored at -40^{0}C for 3 days before being analyzed with a GC. The results proved the fiber was capable of retaining compounds effectively. Excellent linearity was obtained (R^2 of 0.953 to 0.990) for a standard mixture composed of 9 volatile compounds including acrylonitrile, 1,3-butadiene, and vinyl chloride, whose concentrations were in the range of 1 and 400 ppb.

Various flavor analyses using the SPME sampling technique were quoted in the report by Supelco, including fruit juice beverage, whole fruit, flavor oils,

punch flavor in the presence of glycerin, rancid corn oil, odor in wines (trichloroanisole), and off-flavor compounds from light-oxidized milk (Supelco, 2000). One study conducted by Song and Beaudry at Michigan State University attributed the characteristic flavor of fresh, ripe tomato to Z-3-hexenal, E-2-hexenal, 1-pentene-3-one, 2-isobutylthiazole, and 6-methyl-5-hepttene-2-one, and the flavor of strawberries to methyl butanoate, ethyl butanoate, methyl hexanoate, hexyl acetate, ethyl hexanoate, 2,5-dimethyl-4-hydroxy-3(2H)furanone and its methyl ether, 2,5-dimethy-4-methoxy-3(2H)furanone. In total 30 and 34 volatile compounds were identified for tomato and strawberry, respectively, using SPME sampling coupled with time-compressed gas chromatography (TCGC) and time-of-flight mass spectrometry (TOFMS). The study proved that the SPME sampling can significantly cut short the total analysis time compared to purge-and-trap and/or simultaneous steam distillation techniques (Supelco, 2000).

Chai et al (1993) compared the sensitivity of direct SPME and headspace SPME and investigated the linearity of concentrations of volatile chlorinated hydrocarbons in air and water samples. It was concluded the two techniques had comparable sensitivity. Results showed direct SPME held linearity over a wider range of concentrations but required longer sampling time relative to headspace SPME.

Potter and Pawliszyn (1992) used a 100 μm PDMS fiber to extract BTEX compounds (benzene, toluene, ethyl benzene, and xylene) in water. The results showed the detection limit of benzene in water was 15 pg/ml, which was well below the requirement in the US EPA method 524.2 (30 – 80 pg/ml). The study also proved the SPME sampling technique coupled with ion-trap mass spectrometry provided good linearity, which extended over five orders of magnitude, and good reproducibility with a RSD% in the range of 2.7 to 7.5% for the concentrations of the BTEX compounds.

Four different SPME fiber assemblies, 100 μm PDMS, 65 μm PA, 65 μm PDMS/DVB, and 65 μm CW/DVB, were used to investigate the flavor additives used in tobacco products (Clark & Bunch, 1997). The headspace sampling technique was proved effective for the application and a total of 31 characteristic tobacco flavor compounds were detected.

Tombesi and Freije (2002) used SPME-GC-MS to monitor the concentration of BHT (Butylated hydroxytoluene) in fifteen commercial branded water samples packaged in plastic containers. The study focused on the fiber exposure time, detection limits, linearity and precision of the analysis.

Hill and Smith (2000) investigated volatile and semi-volatile sulphur compounds from beer at trace levels with various SPME fibers using headspace SPME sampling combined with GC and found the CAR/PDMS fiber was the most

effective among the fibers studied. The technique proved simple, effective and provided good sensitivity for the flavor analysis of beer.

SENSORY EVALUATION

Analysis of off-odor is always complicated because an odor system might be composed of hundreds of volatile compounds. Nevertheless, both quantitative and qualitative tools are available for investigating and describing off-odor and/or off-flavor problems. Quantitative analyses such as GC-MS focus on locating and identifying volatile compounds in the odor system, while qualitative analyses such as sensory evaluation focus more on the description of the odor system.

The Sensory Evaluation Division of the Institute of Food Technologists defined sensory evaluation as "the scientific discipline used to evoke, measure, analyze and interpret those reactions to characteristics of foods and materials as perceived through the senses of sight, smell, taste, touch and hearing" (IFT, 1975).

Sensory evaluation is considered "a child of industry" (Lawless & Heymann, 1998) because its systematic formation as a scientific discipline in the middle of the 20th century was the result of rapid growth of consumer product and

food industries. However, sensory evaluation is widely recognized as a useful and effective tool to help improve quality of consumer and food products.

Human Senses

As explained in the definition (IFT, 1975), human senses are used in sensory evaluation to evaluate the quality of products in terms of color, shape, sound, taste, mouth-feel, texture, flavor, etc.

In his book published in 1996, Schiffman described the process of perceiving a sensory attribute as: stimulus triggers sensation by the sense organ. The signal is transferred to the brain via the nerve system and processed there. By comparing the processed signal (the sensation) with the database, which is generated via past experiences, saved in the brain, a perception results (Schiffman, 2001).

Compared to most commonly used analytical instruments, a well-trained panelist can be more sensitive (Grimm *et al.*, 2002; MacRae & Falahee, 1995; Peled & Mannheim, 1977). For example, it was reported (Baigrie, 2003) that a human nose is capable of detecting some volatile compounds with a concentration as low as 0.01 ppb (parts per billion), making a human nose a powerful tool in studying odor/flavor problems.

Table 2.13 lists the major human senses and their corresponding perceived sensory attributes.

Table 2.13 The human senses and their perceptions

Sense	Perception
Vision	Appearance (color, shape)
Hearing	Sound
Gustation	Flavor/taste/mouth feel
Olfaction	Odor/aroma/fragrance
Chemical/trigeminal	Irritant
Touch	Texture and consistency

Modified from (Kilcast, 2003)

Some people use the term "odor" and "flavor" interchangeably. However, most sensory experts agree there is a distinction between them (Baigrie, 2003; Lawless & Heymann, 1998; Meilgaard *et al.*, 1999).

Odor is sensed when a volatile compound enters the nasal passage, either via the nose or via the retro-nasal path in the mouth, and stimulates the olfactory epithelium found in the roof of the nasal cavity (Baigrie, 2003; Kilcast, 2003; Meilgaard *et al.*, 1999). In comparison, flavor involves more complicated processes and is the combination of aromatics perceived by the olfactory system, tastes perceived by the gustatory system, and the chemical feelings perceived by the chemical/trigeminal system (Meilgaard *et al.*, 1999).

Types of Sensory Evaluation

There are three major categories of test methods used in sensory evaluation, and they serve different purposes (Lawless & Heymann, 1998; Poste *et al.*, 1991).

Table 2.14 Various test methods used in sensory evaluation

Category	Question of Interest	Type of Test	Test Methods
Discriminative	Are products different in any way? (i.e. determine whether a difference exists)	"Analytic"	Triangle test Duo-trio test 2-out-of-5 test Paired comparison test Ranking test (Friedman)
Descriptive	How do products different in specific sensory characteristics? (i.e. determine the nature and intensity of the difference)	"Analytic"	Scaling methods Descriptive analysis
Affective	How well are products liked or which products are preferred? (i.e. determine relative preference)	"Hedonic"	Paired comparison preference test Hedonic scaling test Ranking test

Modified from (Lawless & Heymann, 1998)

Discriminative Tests

In the discriminative category, the main purpose is to decide whether or not one test sample is different from another. Either trained or untrained panel

can be used in this type of test. The advantage of discriminative tests is they can be used as a quick way to screen panelists to improve the accuracy and precision of later sensory evaluation results. The disadvantage is they do not answer questions like how big the difference is. Among the test methods in the discriminative category, each has certain pros and cons (Poste *et al.*, 1991).

Table 2.15 Comparison of different methods in the discriminative sensory test

Test Method	Advantages	Disadvantages
Triangle test	Used in quality control Used to screen panelists	Samples must be homogeneous (i.e. samples can only be different in one aspect) No nature or intensity of difference is determined Do not specify any particular characteristic
Duo-trio test	Easier than triangle test	Same as triangle test Statistically less effective than triangle test Always one-tailed test
2-out-of-5 test	Statistically more effective than triangle test Suitable for visual, auditory, and tactile analysis	Strongly affected by sensory fatigue Not suitable for flavor and odor analysis
Paired comparison test	Same as triangle test Determine if a difference exists in a characteristic Can be either one-tailed or two-tailed test	No indication of the intensity of the difference Statistically less effective than triangle test
Ranking test	Can study several samples at once Used to screen samples Significance study possible	No indication of the intensity of the difference Results are sample group dependent Fixed ranking unit

Modified from (Poste *et al.*, 1991)

Descriptive Tests

Different from discriminative tests, descriptive tests answer questions such as how big the difference of the perceived sensory attribute is, or what sensory characteristics lead to the detected difference. More than one sensory characteristic can be evaluated at once, which is considered more advantageous than discriminative tests. In addition, they can be used to describe the profile of the perceived sensory attribute such as flavor and texture.

In scaling methods, both structured scaling and unstructured scaling are used. In structured scaling, the determined intensity of difference does not necessarily disclose the true intensity of difference because the numerical distance between any two consecutive anchor points is always fixed as one. Moreover, because each panelist might have different psychological perceptions of descriptive words and the structured scale, the panel is usually trained so everyone on the panel can agree upon the meaning of those terms and thus an accurate and meaningful result can be obtained. However, a trained panel does not necessarily represent a typical consumer group. As a result, the findings from this test can not always be generalized as the opinion of consumers.

In comparison, panelists have the freedom to mark the perceived intensity of the investigated sensory attribute anywhere on the unstructured scale.

Nevertheless, the significance of the difference can be determined with the appropriate statistical analysis in both structured and unstructured scaling tests.

Affective Tests

In order to obtain a more complete profile of the sensory attributes of a product, a descriptive analysis method should be used. A lot of such methods have been developed, including flavor profile, texture profile, quantitative descriptive analysis (QDA), spectrum descriptive analysis, time-intensity descriptive analysis, and free-choice profiling (Meilgaard *et al.*, 1999; Poste *et al.*, 1991). Usually screening and extensive training of panelists are involved in descriptive analysis.

Affective tests are also referred to by many as hedonic tests. As disclosed by the term itself, this type of tests focuses mainly on the subjective opinion, such as preference, liking, and acceptance, of panelists toward a product based on its perceived sensory attributes. Usually a large number of untrained panelists are used so the result can be generalized as the opinion of consumers. However, the panelists can be selected from a specific region, age group, gender, income level, and so on, so that a target market can be studied for the product. Three most commonly used methods in this category are paired comparison preference test, hedonic scaling test, and ranking test (Poste *et al.*, 1991).

Table 2.16 Characteristics of various affective sensory tests

Test	Characteristics
Paired comparison preference test	Used to investigate the preference by the panel; Either one-tailed or two-tailed test; No data on the size of the difference; No indication of what characteristic the preference is based on.
Hedonic scaling test	Used to measure the degree of liking; Either unstructured or structured scale; Order of sample presentation is randomized for each panelist.
Ranking test	Either overall preference or a specific sensory attribute can be studied; Uniform distance between two consecutive rankings; Only study the relative nature of the rank; No indication of degree of difference; Results are sample group dependent.

Modified from (Poste *et al.*, 1991)

Sensory Evaluation in Packaging Applications

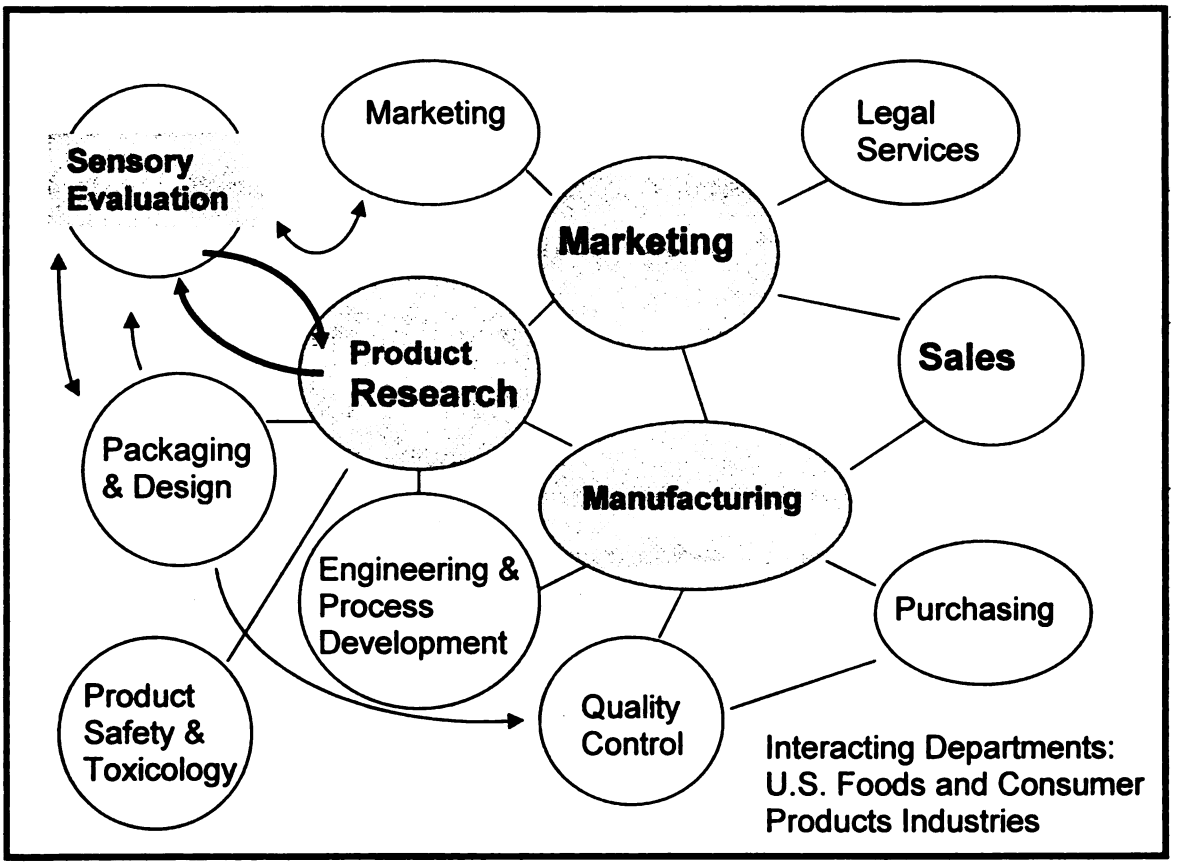
Sensory attributes are inherently crucial parts of the overall quality of consumer products and processed food products, which are almost definitely contained and protected in some forms of packages. Naturally, sensory evaluation is used in packaging applications as a complimentary and helpful tool to study the off-odor and off-flavor of various products due to packaging materials. Different from other instrumental analysis, subjective results are obtained by the panelists in a sensory evaluation to disclose the liking, preference, and acceptance by consumers.

Maneesin (2001) stored water samples in HDPE containers and glass bottles for 6 months and evaluated the off-flavor of the water by a pre-screened and trained panel, using the Difference-from-Control Test (6-point ranking test with a reference sample). The results helped the researcher investigate the effect of different packaging materials and additives such as antioxidants on the flavor quality of water samples.

Das (2003) used an untrained sensory panel in his study to rank water samples based on their perceived off-flavor after the water samples were stored in direct contact nine different food grade HDPE resins at 40 ± 2^{0}C for one week. Sensory evaluation results were used to provide subjective analysis of HDPE resins, in addition to the objective analysis of those resins using an electronic nose system.

Chung (2004) focused her work on the off-flavors of reduced fat milk and cheddar cheese that was exposed to light oxidation. The effect of packaging materials, including glass, HDPE, HDPE-TiO_2, PET, and PE-coated paper cartons, was also investigated. Sensory evaluation was used to study the perceived off-flavor using both consumer and trained panelists. Chung's work proved that sensory evaluation is a powerful and complimentary tool to other instrumental analysis such as GC-MS.

Besides being used as an analytical tool, sensory evaluation is also used in packaging design to improve the quality of packaged products (Anon, 2006). Figure 2.9 (Lawless & Heymann, 1998) explains how a sensory evaluation department interacts with other functional groups in a food or consumer product company in the process of research and development, quality control, packaging design, etc.



Reprinted from (Lawless & Heymann, 1998)

Figure 2.9 Role of sensory evaluation department in a food or consumer product company

ELECTRONIC NOSE

Gardner and Bartlett defined the electronic nose (E-nose) as "an instrument, which comprises an array of electronic chemical sensors with partial specificity and an appropriate pattern recognition system, capable of recognizing simple or complex odors" (Mielle, 1996).

Since the first discovery by Taguchi in 1971, electronic sensors have gained more and more attention due to their superior advantages, which include consistent, objective, nondestructive, prompt responses and lower running cost compared to a trained sensory panel (Mielle, 1996). Later, some researchers found that arrays of these sensors are capable of recognizing complex food aroma systems (Bartlett *et al.*, 1997). By now, the electronic nose has been widely used in the food, personal care, cosmetics, tobacco, and automobile industries, and, of course, in packaging applications (Chung, 2004; Culter, 1997, 1998; Gardner & Persaud, 2000; Harper, 2001; Haugen, 2001; Hurst, 1998; Maneesin, 2001; Mielle, 1996; Siripatrawan, 2002; Siripatrawan *et al.*, 2004a, 2004b, 2006a, 2006b).

Table 2.17 lists some commercially available electronic nose systems and their sensor technologies.

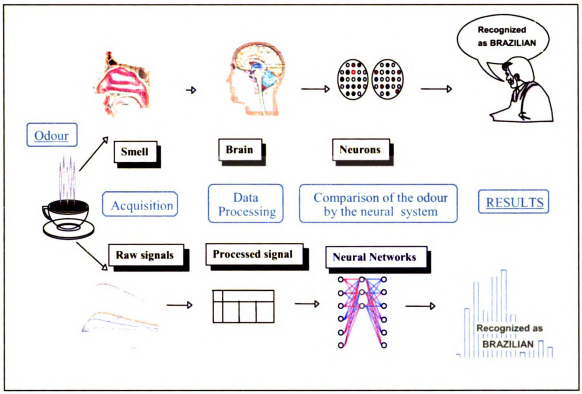
Table 2.17 Various electronic nose systems and their sensor technologies

Electronic Nose System	Sensors
Alpha MOS	Metal oxide sensors (6 – 18 sensors)
Aroma Scan	Organic polymer (32 sensors)
Bloodhound	Organic polymer (6 – 12 sensors)
Moses ii	Combination array (multiple sensors)
Hewlett Packard	Headspace with mass selective detector
Neotronics	Organic polymer (12 sensors)
Perkin-Elmer	Quartz crystal sensors

Modified from (Harper, 2001)

Electronic Nose and Human Nose

In the human nose, a vast number of chemical sensors are activated and react with the compounds when the odorous compounds enter the nasal cavity. This process is called data acquisition. The chemical reactions are transformed into a photoelectrical signal, which is transferred to the brain via the chemosensory nerves in the nasal cavity and the central neural system (Boudreau, 1983). The signal is analyzed and sorted by the brain, which is called data processing. By comparing the odor with the stored information in neurons, we can recognize an unknown simple or complex odor system.



Reprinted from (Alpha-M.O.S., 1999)

Figure 2.10 Recognition of Brazilian coffee by the E-nose and the human nose

The term electronic nose originated from the fact that it works in a similar way to the human nose. The electronic nose recognizes an unknown odor system via the process of data acquisition, data analysis, comparison and recognition (see Figure 2.10). Electronic chemical sensors resemble the chemical sensors in the human nasal cavity. Pattern recognition is similar to that occurring in the brain of a human being.

Moreover, the electronic nose does not separate the individual components in the odor system. Instead, similar to the human nose, it

recognizes the odor system via a global analysis by comparing the unknown sample with the database, which is input into the electronic nose system during the training period.

According to a report by Harper (1998), sensitivity of the sensors used in the Alpha MOS Fox 3000 electronic nose system is comparable to that of the human nose (see Table 2.18).

Table 2.18 Comparison of detection threshold values of several chemical compounds in water determined by an electronic nose system (Alpha MOS Fox 3000) and human nose

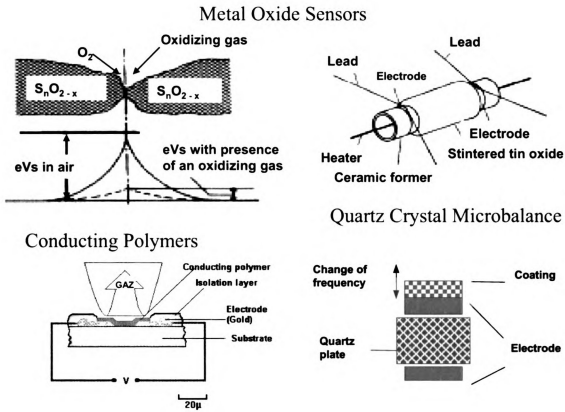
Compound	Fox 3000	Human Nose
Ethanol	< 25 ppm	24.9 – 100 ppm
1-octen-3-ol	10 ppb	1 ppb – 1ppm
Octanal	1 ppm	0.7 – 0.8 ppb
Butyric Acid	1 ppm	50 ppb – 2.15 ppm
Diacetyl	100 ppb	4 – 15 ppm
p-cresol	50 ppb	55 – 680 ppb
Nookatone	1 ppb	1 ppb – 1ppm

Modified from (Harper & Kleinhenz, 1998)

Electronic Sensors

Because most electronic sensors only offer partial specificity, an array of sensors is commonly used (Bartlett *et al.*, 1997; Mielle, 1996). The electronic sensor array is the heart of the electronic nose system. The various sensors in the array are used to create an olfactory picture of the system by reacting with the odorous compounds. There are three main types of sensors that are

commercially available and widely used. These are M.O.S. (Metal Oxide Sensors), conducting polymer sensors, and Q.C.M. (Quartz Crystal Microbalance) sensors.



Reprinted from (Alpha-M.O.S., 1997)

Figure 2.11 Different types E-nose sensors

Deventer and Mallikarjuman (2002a) published a comparison of performance of three electronic nose systems based on three different sensors: Alpha M.O.S. Fox 3000 system based on metal oxide sensors, Cyranose 320 system based on conducting polymer sensors, and QMB6 system based on quartz crystal microbalance sensors. They concluded all three systems were

capable of differentiating film samples with different levels of retained solvents. However, metal oxide sensors and conducting polymer sensors demonstrated better discriminatory ability.

Metal Oxide Semiconductor Sensors (M.O.S. Sensors)

Metal oxide sensors were firstly discovered by Taguchi in 1971 (Mielle, 1996). They are the most commonly used ones nowadays (Aishima, 1991a, 1991b; Tan *et al.*, 1995). The detection principle is based on the measure of the variation of the resistance of the sensors (Culter, 1999). These sensors are made of a ceramic coated with a semi-conducting film, usually in a thickness of 50 μm (Alpha-M.O.S., 1997). Selectivity of the sensors toward different chemical compounds can be obtained by either doping the film with noble catalytic metals or by modifying the working temperature of the sensing element in the range of 50-400°C. In addition, the selectivity can be affected by the particle size of the polycrystalline semiconductor (Alpha-M.O.S., 1997; Mielle, 1996).

The resistance of the sensor is changed with the reaction of an odorant with the sensor, which generates a measurable electronic signal. The magnitude of the response depends on the nature of the volatile molecules and the type of metal oxide.

These sensors offer good sensitivity in the ppm or even ppb range to a very wide range of chemical compounds (Table 1). They are relatively resistant to humidity and to aging. However, they can be easily poisoned by sulphurous compounds and weak acids and they are extremely sensitive to ethanol, which could "blind" the sensors to any other analyte of interest. Caution is needed when high molecular weight compounds are analyzed because these compounds result in slow baseline recovery (Alpha-M.O.S., 1997; Culter, 1999; Mielle, 1996).

Conducting Polymer Sensors

Conducting polymer sensors are made of a thin layer of polymer across the gap between electrodes via electro-polymerization (Mielle, 1996). Such conductive polymers include polypyrroles, polyanilines, and poly(3-methylthiophenes). It was found that doping the polymer with various ions could enhance its conductivity (Culter, 1999; Vetenskapsakademien, 2000). Similar to M.O.S., the adsorption of the volatile compound into the polymer will change the polymer's resistance (Culter, 1999; Mielle, 1996).

Table 2.19 Comparison of different sensors used in electronic nose systems

Metal oxide sensor	Low-medium selectivity High sensitivity (ppb-ppm) Low to medium sensitivity to humidity Low temperature dependence Medium desorption time Fast recovery time Long lifetime (18 – 36 months)
Conducting polymer sensors	Medium to high selectivity Good sensitivity (ppm) High sensitivity to humidity High temperature dependence Long desorption time Slow recovery time Shorter lifetime (6 – 9 months)
Q.C.M. sensors	Medium to high selectivity High sensitivity (ppb-ppm) Dependent on humidity (coating) Moderate temperature dependence Quick desorption process Slow recovery time Reasonable lifetime (9 – 12 months)

Modified from (Alpha-M.O.S., 1997; Haugen, 2001)

Their main advantages are that they operate at room temperature and in some cases they are more selective for certain compounds. However, they are not good for high temperature applications because the inherent shift with temperature is very high. Moreover, they are very sensitive to humidity, which means external variables such as temperature and humidity must be controlled in using these sensors (Bartlett *et al.*, 1997). The analysis time could be extended due to long response time (Culter, 1999; Mielle, 1996).

Compared to metal oxide sensors, conducting polymer sensors are expensive and have poor reproducibility.

The success of the analysis using conducting polymer sensors is critically dependent on the choice of polymer sensors. In addition, the choice of appropriate signal preprocessing and subsequent choice of pattern recognition technique is of paramount importance, which could mean either success or failure for a particular application (Bartlett *et al.*, 1997).

Motion et al (2004) used an electronic nose system based on conducting polymer sensors to identify wood chip samples of various species. It was found the system was capable of differentiating different samples but this capability was affected by the ratio of mixtures if two different wood chip samples were mixed and analyzed with the electronic nose system. Garneau et al (2004) used the same type of electronic nose system to successfully differentiate wood samples of different species.

Quartz Crystal Microbalance Sensors (Q.C.M. Sensors)

The sensing element for this type of sensor is a coated quartz resonator. The sensor is made by depositing a gas-sensitive coating such as silicone or polyglycol onto a quartz support. The volatile compounds can absorb or adsorb onto the coating upon exposure, which leads to a change of mass of the crystal and correspondingly a change of the frequency of the oscillation.

The sensitivity and selectivity of the Q.C.M. sensors can be controlled by the coating material and the quantity deposited. Compared to the other two electronic sensors, this type of sensor is less used (Culter, 1999).

Deventer and Mallikarjunan (2002b) analyzed volatiles released from printing inks used in 9 different food packaging films using an electronic nose system based on an array of 6 resonating quartz sensors.

Pattern Recognition Systems

In most cases, an odor system is composed of hundreds of volatile compounds and each of them will react with each sensor in the sensor array to a different extent. Thus, the response of each sensor is the integrated result of reactions with every volatile compound contained in the odor system. An array of sensors will thus give a global profile of the odor system.

The database created for analysis is generally presented as a matrix of n rows and p columns with each row standing for one analyzed odor sample (objects) and each column equivalent to one sensor response (variables) (see Figure 2.12). This database is created by measuring the interaction of the electronic nose with the odor samples. It is used for comparison and identification purposes with the help of a pattern recognition system.

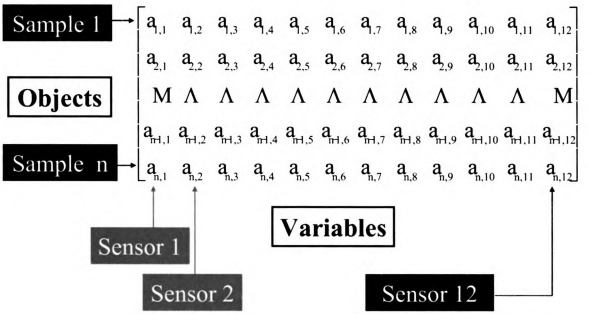


Figure 2.12 Data matrix created by analyzing n samples with an electronic nose system with an array of 12 sensors

The most popular pattern recognition systems include principle component analysis, discriminative function analysis, partial least squares, and good-bad analysis.

Principle Component Analysis (PCA)

One easy way to discriminate two samples is to visually compare the sensor response profiles for these two samples. As mentioned above, a sensor array will give a matrix that is difficult to represent in the three-dimensional world. Principle component analysis is used to reduce the number of variables by linearly combining the original variables with maximal variance. In other words,

the technique seeks a dimension along which the observations are maximally separated or spread out (Alpha-M.O.S., 1999; Krzanowski, 1988; Rencher, 1995).

Then it is possible to compare two samples by projecting the original data (matrix of n x p) into a two or three dimensional space, with the chosen principle components (the linear combination of original variables) being the vectors (see Figure 4). By doing so, we can assess the similarities between samples or the relation between sensors.

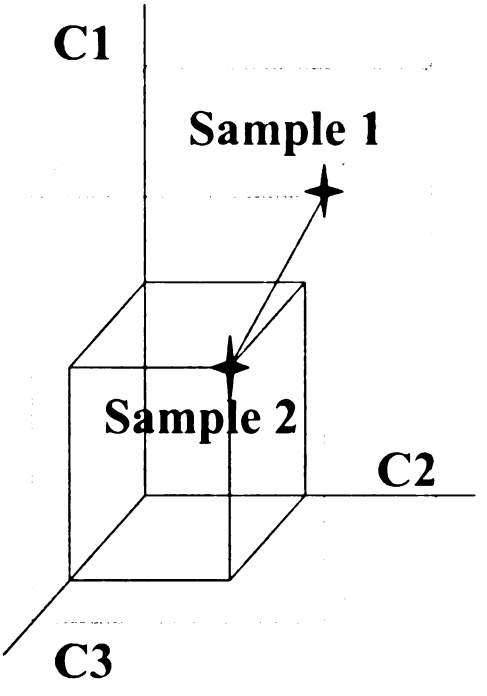


Figure 2.13 Differentiate two samples with a PCA with three principle components in an electronic nose analysis

Discriminative Function Analysis (DFA)

Discriminative function analysis is a dimension reduction technique similar to principle component analysis. However, a mathematical model is developed and used to classify unknown samples into one of the training sample groups.

In order to do so, the first step involves defining new variables, the linear combination of the descriptive variables, which has the characteristic of separating as much as possible the different groups of samples.

Then the technique can be used to place a new unknown sample into the known training groups by using computation of the distance between the centers of gravity between different groups (Alpha-M.O.S., 1999).

Culter (1999) reported work using discriminative function analysis to classify different paperboard samples and study the effect of humidity on the odor profile of paperboard.

Partial Least Squares (PLS)

Partial Least Squares (PLS) is a quantitative technique. Two types of quantitative information are related to volatile compound analysis. One is the concentration of specific compounds in the analyzed sample. The other is the sensory evaluation score. The objective of PLS is to build a model with which

such quantitative results can be obtained through the sensor response profile in the E-nose system (Alpha-M.O.S., 1999).

PLS is based on linear regression analysis. If Y is the matrix of the database of the quantitative measurements (e.g. concentration of a specific compound or sensor score), Y' is the matrix of the corresponding prediction values, and X is the matrix of database from the sensors' response, then partial least squares is the technique to find a matrix B that is used to predict the quantitative values by minimizing the distance between Y and Y' with Y' = XB.

The scientists at Alpha M.O.S. used the electronic nose Fox 4000 system to build a mathematical model to predict the concentration of peppermint flavor in drinking water in the range of 0.01% to 0.50% (Alpha-M.O.S., 2001). A high correlation coefficient of 0.97 was obtained and the model was validated with two different samples. A similar study was conducted at the School of Packaging at Michigan State University to predict the concentration of nonanal, which was claimed by the investigator to be the main odorous compound in drinking water stored in HDPE containers. A correlation coefficient of 0.99 was obtained (Maneesin, 2001).

Good-Bad Analysis

This technique is used to optimize a model and then use this model to classify the unknown sample as either good or bad product. This technique needs an extensive training process in which all the possible differences between samples due to formulations, production conditions, and batch-to-batch variations must be taken into consideration and are presented to the electronic nose system during the training process (Alpha-M.O.S., 1999).

Alpha M.O.S. used this technique to study the quality of the raw material used in producing cherry fragrance during a food treatment process (Alpha-M.O.S., 2001). Six batches of samples were classified as acceptable and the technique was found successful in recognizing unknown good samples and unknown bad samples.

APPLICATIONS OF THE ELECTRONIC NOSE SYSTEM

There have been various applications of the electronic nose system in the fields of food, cosmetics, automobiles, environment, pharmaceuticals, packaging, etc. (Bartlett *et al.*, 1997; Chen & Ou, 2001; Chung, 2004; Culter, 1999; Maneesin, 2001; Mielle, 1996).

Maneesin (2001) analyzed the off-flavor compounds in water stored in two different plastic containers made of HDPE with two different antioxidant system, using an electronic nose system, sensory evaluation, and GC-MS.

Siripatrawan (2002) investigated the possibility of using an electronic nose system to detect and identify pathogens including E. Coli and Salmonella growing on various food systems.

Chung (2004) used an electronic nose as a tool to investigate the quality of light-oxidized reduced fat milk and cheddar cheese. Off-flavors were analyzed with the electronic nose system as well as other traditional analytical methods such as GC-MS. The off-flavors were also evaluated with human sensory evaluation and the results were correlated with the sensor responses of the electronic nose system.

Haugen (2001) listed a variety of food applications using the electronic nose system, including odor analysis, lipid oxidation, freshness and spoilage, taints and of-flavor, food safety, process control, and packaging. At Ohio State University, Harper and his team (2001) worked with three electronic nose systems, Aroma Scan, Neotronics, and Alpha MOS Fox 2000, to study flavors and quality of various dairy products such as milk, butter, cheese, and whey protein.

ELECTRONIC NOSE AND OTHER ANALYSIS TECHNIQUES

There are often misconceptions about the electronic nose technology. One is that the electronic nose is a replacement for sensory evaluation. Another is that the electronic nose is superior to the traditional separation techniques such as gas chromatography (GC), and thus can replace the latter. As we will discuss here, neither is valid.

Electronic Nose and Sensory Evaluation

The electronic nose is pictured by some people as similar to the human nose. From some standpoints, they are right because the electronic nose system can do some of the jobs a human nose does, such as recognizing an unknown odor system.

A human nose can make subjective judgments based on personal life experience. An individual can recognize Brazilian coffee because he has encountered it in the past and stored the "image" of the coffee odors in his brain. In a similar way, an individual can judge whether an unknown sample is good or bad quality because he has built up a database from his past experience, which helps to make such subjective judgments later on.

Human olfactory systems can be made very sensitive and reliable by a pre-screening and training process. A trained panel can give precise and

consistent results. For the last half century, evaluation of odor and flavor of food and food packaging systems has been done by organoleptic evaluation technology, which gives complex sensations via the interactions of human senses and the tested items (Lawless & Heymann, 1998; Meilgaard *et al.*, 1999; Poste *et al.*, 1991). However, panelists are prone to off-days, colds and hay-fever. Their judgments are always subjective and susceptible to their body health and mood. Besides, human beings are not suitable in the case of harmful chemicals. Most importantly, it takes time and it is costly to train an effective panel and run human sensory evaluation tests.

On the other hand, running the electronic nose system is lower cost, and it offers objective judgments, prompt response, consistent results, and comparable sensitivity to the human nose in some applications. However, it must be noted that the electronic nose itself only can make objective judgments. Sometimes, subjective judgment is preferred or required.

The untrained electronic nose system cannot judge which sample is good or which is bad. As discussed earlier, PCA can decide whether two samples are similar or not, without any training. But in order to identify an unknown sample (DFA), or decide the sample is good or bad (Good-Bad Analysis), or determine the concentration of a specific compound in the unknown sample (PLS), the electronic nose system must be trained in advance. In other words, the system

must be told which training samples are good or bad, what they are, or the concentration of the compound of interest.

Human sensory evaluation can help the training process of the electronic nose system by offering information such as good or bad judgments, or sensory scores based on the perceived sensory characteristics of the samples. Such information can be used for the purpose of DFA or PLS functions in the electronic nose system.

Willing et al (1998) studied the odor from paperboard samples with an electronic nose. The correlation between the results from the electronic nose system to the results from human sensory evaluation was built with a PLS model. A similar approach was used in Maneesin's and Chung's work (Chung, 2004; Maneesin, 2001).

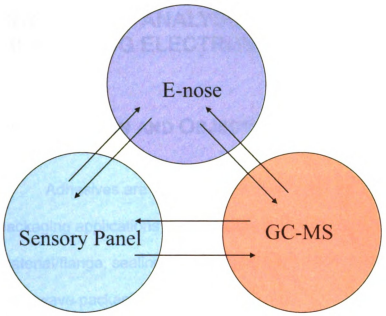
Electronic Nose and GC-MS

Some people think the electronic nose system is superior to GC analysis. The fact is the electronic nose system does not and cannot separate a compound mixture into individual components. As mentioned earlier, the electronic nose system acquires the global odor profile, based on which the pattern recognition system is applied. GC, on the other hand, is used to separate the mixture into individual components.

With the help of gas chromatography-mass spectrometry (GC-MS), two types of information can be obtained and used in the electronic nose system. First, volatile compound(s) contributing to the objectionable odor may be identified. Moreover, the concentration of volatile compound(s) can be determined. Such information can be used in DFA or PLS in the electronic nose system.

Actually, the electronic nose technique, human sensory evaluation, and GC-MS can be correlated with each other to get a more comprehensive analysis of an odor system. Sensory evaluation offers subjective judgment and quantitative results (sensory scores) and GC-MS offers qualitative as well as quantitative results. Such information can be used for training purposes for the electronic nose system. After the training process, the electronic nose is ready for quick, easy and objective analysis.

Moreover, the results obtained with GC-MS can be used in the sensory evaluation test. Identification of major compounds and their concentration can help in the training process by selecting the appropriate training standards. Figure 2.14 demonstrates the idea of correlation between these three analysis techniques.



Modified from (Hodgins, 1997)

Figure 2.14 Correlation of the E-nose nose, sensory evaluation, and GC-MS

Forsgren et al (1997; 1999) used GC, an electronic nose system, and human sensory analysis to study the volatile compounds released from two food packaging board products. Data obtained from GC was processed with multivariate analysis and was correlated satisfactorily with the results from the electronic nose analysis and human sensory evaluation. It was concluded that the electronic nose system could be potentially used as an on-line instrument for monitoring the quality of board products.

Heinio et al (2002) studied volatile compounds from printed paperboard samples using an electronic nose system, GC-MS, and sensory evaluation. PLS (Partial Least Square) models were built to correlate the sensory evaluation data with the sensor responses obtained from the electronic nose.

CHAPTER 3 ANALYSIS OF HEADSPACES OF HDPE FILMS USING ELECTRONIC NOSE

INTRODUCTION AND OBJECTIVES

Adhesives are widely used in food packaging, especially in primary packaging applications such as sealing lidding stock, paperboard/seal material/flange, sealing pouches and bags, and bonding susceptors in microwave packaging (IOPP, 1995).

In the past, focus has been on the adhesive's compliance with FDA regulations, which emphasize the safety of adhesives in indirect contact with food. Little attention was paid to the contribution of adhesives to off-odor and/or off-flavor of food packaging systems. Predictably, identification and characterization of such volatile compounds could help us to resolve the problem, improve the formulation of the adhesive, and optimize the conditions in which the adhesive will come into contact with food.

In the last half century, evaluation of odor and/or flavor of food and food packaging systems has often been done by organoleptic evaluation techniques, which give complex sensations via the interaction of human senses and the tested item (Poste *et al.*, 1991). Human olfactory systems can be made very sensitive by pre-screening and training procedures. Trained panels can provide precise and consistent results, but they are prone to off-days, colds and hay-

fever. Their judgments are always subjective and human beings are not suitable in the case of harmful chemicals. More importantly, it takes time to train an effective panel and running human sensory evaluation tests is costly (Poste *et al.*, 1991).

Since the first discovery by Taguchi in 1971, electronic chemical sensors have gained more and more attention due to their superior advantages, which include consistent, objective, non-destructive, prompt responses and lower running costs compared to a trained sensory panel (Mielle, 1996). Researchers have found that arrays of these sensors are capable of recognizing complex food aroma systems (Mermelstein, 1997). So far, the electronic nose has been widely used in the food industry, personal care, cosmetic industry, tobacco industry, automobile industry, and packaging applications (Mielle, 1996).

Gardner and Barlett defined the electronic nose (E-nose) as "an instrument, which comprises an array of electronic chemical sensors with partial specificity and an appropriate pattern recognition system, capable of recognizing simple or complex odors" (Mielle, 1996).

Using an electronic nose makes it possible to detect the pattern of responses associated with off-odor and off-flavor as the result of adhesives used in food packaging systems, without requiring identification of the individual components. Furthermore, if a relationship between the response of the E-nose

and the objectionable odor and/or flavor (presence and/or concentration) could be established, the E-nose system could then be used as an efficient and effective on-line QC measure.

The objectives of this part of the research were to: 1) develop and optimize the data acquisition parameters so a meaningful and processable sensor response pattern can be obtained for the analyzed sample headspace; 2) determine the response patterns of odor associated with adhesives used to coat HDPE films using an Alpha MOS Fox 3000 electronic nose system; 3) differentiate the four adhesive formulas and the base film with PCA (Principle Component Analysis); 4) develop a DFA model and validate the model by identifying "unknown" HDPE film samples.

MATERIALS AND METHODOLOGY

Four different pressure-sensitive adhesive formulations were used to coat the control sample HDPE film, which were all provided by National Starch, Inc.

Table 3.1 Codes and formulas of adhesives and base film

Code from NSC*	Polymer	Tackifier	Code used in the study
11753 – 36A	A	A	PATA
11753 – 36B	B	A	PBTA
11753 – 36C	B	B	PBTB
11753 – 45A	C	A	PCTA
7613 – 48 – 1	/	/	CONT (HDPE base film)

* National Starch & Chemical

More details of the formulations are:

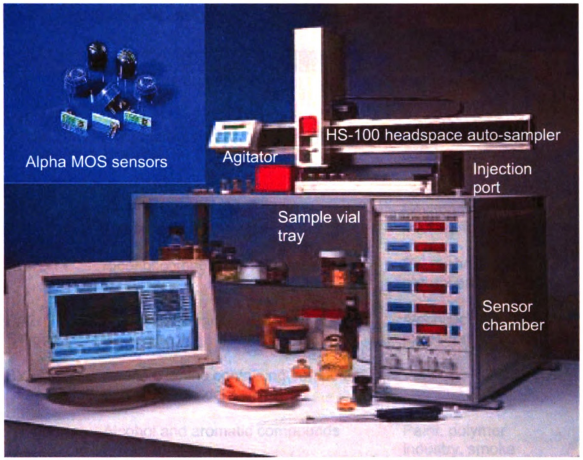
11753-36A: rubber block copolymer, food grade rosin ester, mineral oil, and antioxidant;

11753-36B: different rubber block copolymer, same food grade rosin ester as 11753-36A, mineral oil, and antioxidant;

11753-36C: same polymer as 11753-36B, food grade terpene, mineral oil, and antioxidant;

11753-45A: rubber polymer (random architecture), same food grade rosin ester as 11753-36A, mineral oil, and antioxidant.

Figure 3.1 is a picture of an Alpha MOS Fox 3000 electronic nose system with HS-100 auto-sampler, which has an array of 12 sensors (see Table 3.2), and MOS sensors.



(Reprinted from Alpha MOS picture database, with permission)

Figure 3.1 Alpha MOS Fox 3000 E-nose system with HS-100 auto-sampler and MOS sensors

Table 3.2 Sensor array used in Alpha MOS Fox 3000 E-nose system

Set	Sensor 1	Sensor 2	Sensor 3	Sensor 4	Sensor 5	Sensor 6
1	SY-LG	SY-G	SY-AA	SY-gH	SY-gctL	SY-gcT
2	T30/1	P10/1	P10/2	P40/1	T70/2	PA2

Even though MOS sensors generally have low to medium selectivity, each sensor does have specificity to certain compounds (see Table 3.3).

Table 3.3 MOS sensors and their specificity to organic compounds

Sensors	Specificity to organic compounds	Applications
T30/1	Polar compounds ethanol	Liquors, beers
P10/1 P10/2 SY-AA SY-gcT	Hydrocarbons such as methane and propane	Cooking, roasting of coffee, petro-chemistry
P40/1 SY-LG	Fluorinated and chlorinated compounds, aldehydes	Environment, packaging
T70/2	Alcohol compounds, food aroma and volatiles	Petro-chemistry, natural aromas, coffee
PA2 SY-gctL	Alcohol, solvents	Alcoholic perfumes, fermentation
SY-G	Amines, amine containing compounds, and ammonia derivatives	Various
SY-gH	Alcohol and aromatic compounds (toluene)	Paint, polymer industry, smoke detection

Modified from (Alpha-M.O.S., 1996)

As shown in Figure 3.1, the Alpha MOS Fox 3000 E-nose system consists of three major parts: an agitator, a HS-100 auto-sampler, and the sensor chamber.

HDPE samples were cut and sealed into 10 ml glass vials, which were loaded onto the sample tray. Glass vials were transported individually and consecutively by the HS-100 headspace auto-sampler to the agitator, where the sample vial was heated at a pre-specified temperature for a pre-specified period of time before a sample syringe took a sample of the headspace from the vial

and introduced it into the injection port automatically. The sample vial was then taken back to the sample tray and a new sample vial was moved to the incubator for the next analysis. Volatile compounds from the headspace were transferred into the sensor chamber by the carrier gas (zero-grade air composed of 80% N_2 and 20% O_2), where they reacted with the MOS sensors, whose resistances changed due to the reactions. Variations of resistances were then used to generate measurable electric signals, which were recorded and processed by the software of the E-nose system.

The generated response pattern for each headspace analysis by the Alpha MOS Fox 3000 E-nose system was a plot that was composed of 12 curves. Each curve represents the variation of the sensor resistance with time, calculated as $\Delta R/R_0$, where ΔR equals $(R - R_o)$, and R_0 and R are the sensor resistance at t = 0 and time t, respectively.

Because the compositions of the headspaces (the odor systems) of different samples vary both qualitatively and quantitatively, the resultant E-nose sensor response patterns will be different as well, based on which the headspaces and thus the original samples can be differentiated from each other and/or recognized, if the E-nose system is pre-trained.

RESULTS AND DISCUSSION

Parameter Optimization for the E-nose System

Justification and Criteria

The first step of using the electronic nose system is always developing the data acquisition method (Culter, 1999). The most important parameters are incubation temperature, incubation time, acquisition time, delay, and sample size.

Incubation temperature and time are the temperature and time the sample vial is held in the agitator. Acquisition time is the time the E-nose system records generated signals. Delay is the time after the last analysis and before the next headspace sample is injected into the E-nose system, which is necessary for the sensor responses to come back to the baseline. These parameters should be studied and selected based on the following considerations (Alpha-M.O.S., 1996):

1) Return of sensor responses to the baseline is rapid and stable;
2) Able to acquire the peak (maximum) of the response curve;
3) Sensors are not saturated (front panel values of the sensor readings do not reach 0 or 4095);
4) Sensors react sufficiently (minimum of 5% and maximum 90% $\Delta R/R_0$).

Initial Settings of Acquisition Parameters

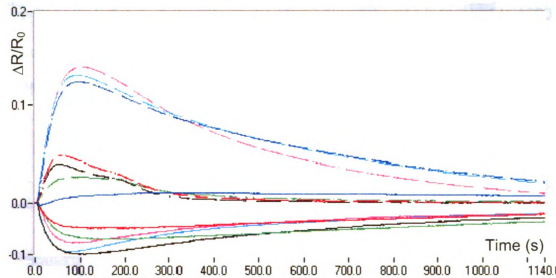
Table 3.4 lists the initial settings of the data acquisition parameters for the E-nose system, including all the crucial parameters as well as minor parameters, which were based on recommendations by Alpha MOS (Alpha-M.O.S., 1999). The incubation temperature was set at 100^{0}C based on the potential applications of the adhesive-coated HDPE films proposed by National Starch, Inc.

Table 3.4 Initial settings of the data acquisition parameters for the E-nose system

Crucial Parameters	
Incubation time (s)	600
Incubation temperature (^{0}C)	100
Acquisition time (s)	1140
Delay (s)	0
Sample size	1 strip of 10" x 1" HDPE film
Minor Parameters	
Agitation speed (rpm)	500
Syringe type (ml)	5
Fill speed (µl/s)[1]	500
Syringe temperature (^{0}C)	5 degrees higher than incubation temperature
Flushing time (s)[2]	120
Vial type (ml)	10
Injection volume (µl)[3]	5000
Injection speed (ml/min)[3]	2500
Acquisition period (s)[4]	1
Flow (ml/min)[5]	300

1. The speed at which the syringe takes the headspace sample from the vial;
2. The time the syringe flushes the remaining headspace sample out of itself after the injection;
3. The volume and the speed that the sampled headspace is injected into the E-nose system;
4. The frequency at which signals of sensors are recorded;
5. The flow rate of the carrier gas.

"Delay" was set at zero so the total run time equals the acquisition time, which made it possible to visually observe profiles of the sensor responses all through the analysis.



(original data was generated on 02/22/01)

Figure 3.2 Sensor response patterns of the 12 E-nose sensors in analyzing sample PBTA under the acquisition parameters listed in Table 3.4

Figure 3.2 is the sensor response pattern for the headspace sample from film PBTA. It was observed that an acquisition time of 600 seconds was long enough so that all the peaks of the sensor responses would be recorded. However, a total run time (acquisition time plus delay) of 1140 seconds was not long enough for the sensors to go back to the baseline.

Moreover, intensities of some sensor responses for some film samples were lower than 5% (data not shown), indicating insufficient reaction of those sensors with volatile compounds to give a reliable detection.

Based on these observations, the acquisition time was increased to 1500 seconds and the sample size was doubled in each glass vial to 2 strips of 10" x 1" HDPE films.

Effect of Sample Size

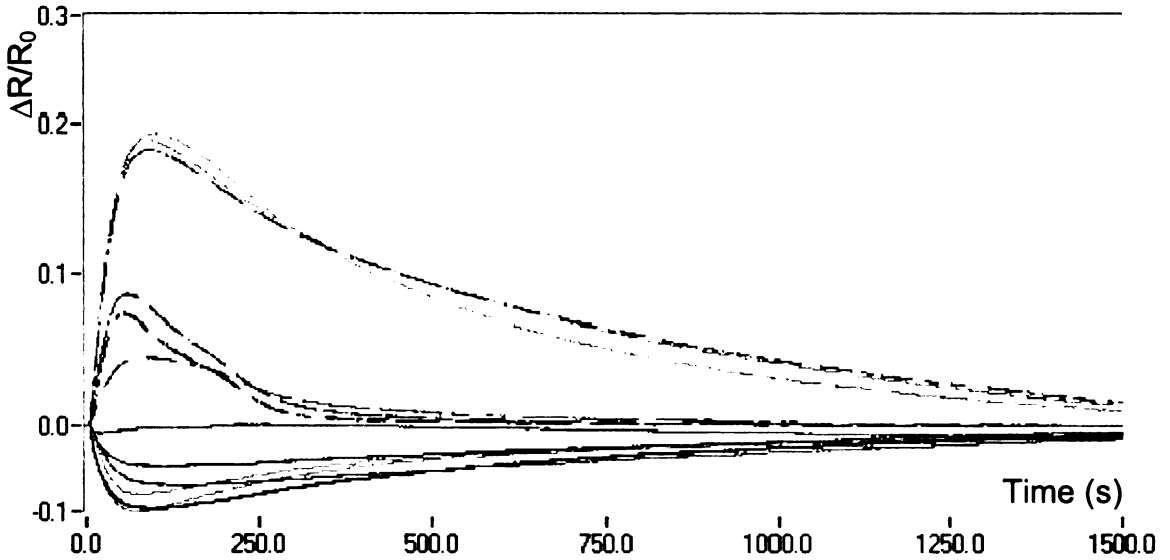
Table 3.5 lists the modified data acquisition parameters based on the initial test results discussed in the previous section.

Table 3.5 Modification to the data acquisition parameters for the E-nose

Crucial Parameters*	
Incubation time (s)	600
Incubation temperature (^{0}C)	100
Acquisition time (s)	1500
Delay (s)	0
Sample size	2 strips of 10" x 1" HDPE films

* All the minor parameters were same as those listed in Table 3.4

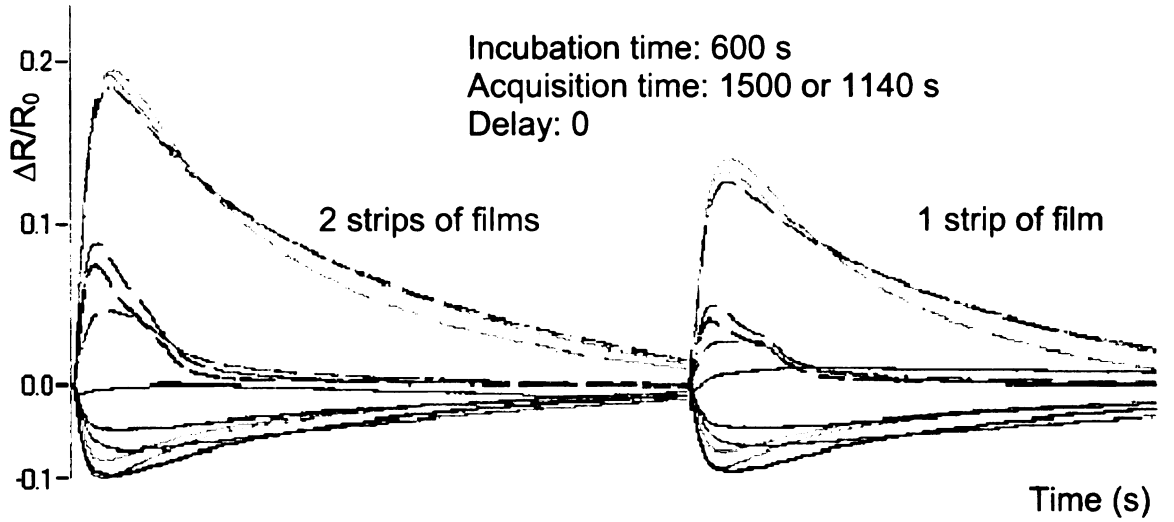
"Delay" was set as zero again but the total run time was extended to 1500 seconds to ensure all the sensors would have enough time to go back to the baseline before the next headspace sample was injected for analysis. Moreover, sample size was doubled to get higher sensor response intensities.



(Original data was generated on 03/13/01)

Figure 3.3 Sensor response patterns of the 12 E-nose sensors in analyzing sample PBTA under acquisition parameters listed in Table 3.5

As shown in Figure 3.3, a run time of 1500 seconds was long enough for the sensors to go back to the baseline. Figure 3.4 is the side-by-side comparison of the sensor response patterns of PBTA with different sample sizes. By doubling the sample size, intensities of sensor responses were increased to satisfactory levels, though not doubled.



(Original data were generated on 03/13/01 and 02/22/01 respectively)

Figure 3.4 Effect of sample size on the sensor response patterns of the 12 E-nose sensors in analyzing sample PBTA

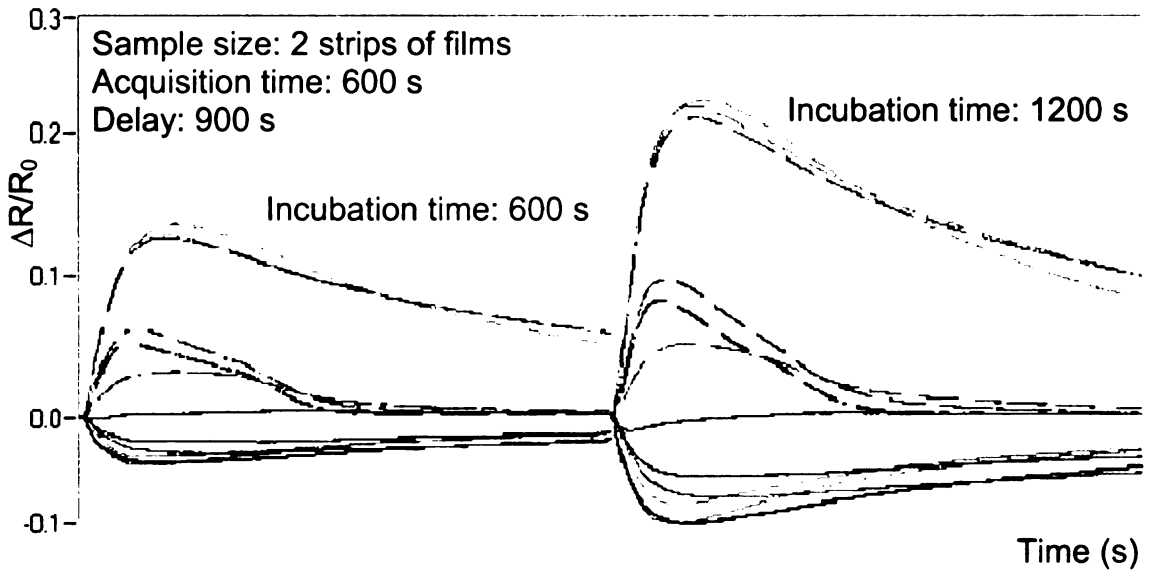
Effect of Incubation Time

Another concern was whether the incubation time of 600 seconds was long enough for equilibrium to be established between the HDPE film sample and the headspace inside the glass vial. There is no easy way to predict the time required to reach equilibrium. However, the effect of the incubation time on the sensor response pattern can be investigated using PCA (Principle Component Analysis).

Two groups of samples were analyzed with the E-nose system. One was incubated for 600 seconds and the other for 1200 seconds. Each group consisted of five different HDPE film samples (see Table 3.1) and each film sample had three duplicates.

All the samples (2 groups x (5 samples/group x 3 duplicates/sample) = 30) were analyzed on the same day and presented to the E-nose randomly to eliminate the effect of sensor drifts. The thirty samples generated thirty profiles of the sensor responses, based on which a library was built and analyzed with PCA.

Figure 3.5 shows the side-by-side comparison of the sensor response profiles of film PBTA under incubation times of 600 seconds and 1200 seconds.



(Original data were generated on 04/05/01)

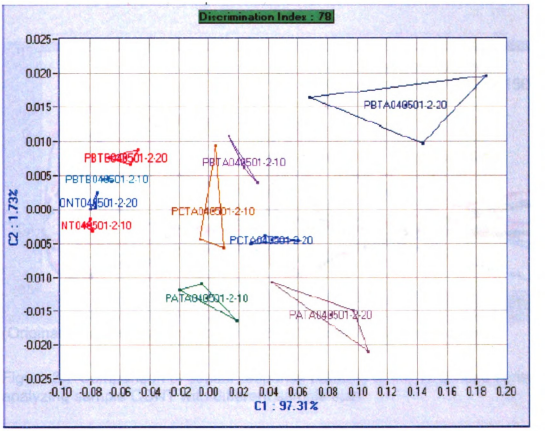
Figure 3.5 Effect of incubation time on the sensor response patterns of the 12 E-nose sensors in analyzing sample PBTA

As shown in Figure 3.5, sensor responses were almost doubled with the doubled incubation time, indicating the latter played an important role in the formation and thus the composition of the headspace of the HDPE film sample.

The effect of the incubation time was further investigated with the PCA module of the E-nose system (see Figure 3.6). The library consisted of the 30 profiles of the sensor responses corresponding to the 30 samples analyzed by the E-nose system. Each triangle in the PCA plot represented one group of HDPE film samples with three duplicates, which were pre-defined by the investigator before applying the PCA module to the library. For example, the triangle tagged with "PBTA040501-2-20" was headspaces of 2 strips of film samples of PBTA that were generated by being incubated at 100^0C for 20 minutes (1200 seconds) in the agitator of the E-nose on April 5, 2001.

The database in the library was a 30 x 12 (objects x variables) matrix, with the row and the column representing 30 samples and 12 sensors, respectively. The x-axis and y-axis were the primary and secondary components of the principle components, which accounted for 97.31% and 1.73% of the total variance, respectively. Ten (10) groups of samples were separated from each other in the PCA plot. That along with a Discrimination Index (DI) of 78 indicated the PCA module differentiated the headspaces of the same HDPE film generated at different incubation times, e.g. the control base film at 600 seconds and at 1200 seconds were detected as two separate groups. The PCA plot confirmed

the significance of the incubation time in optimizing the data acquisition parameters. By balancing the real-life situation and the requirement for reliable E-nose analysis, an incubation time of 1200 seconds was determined to be more appropriate.

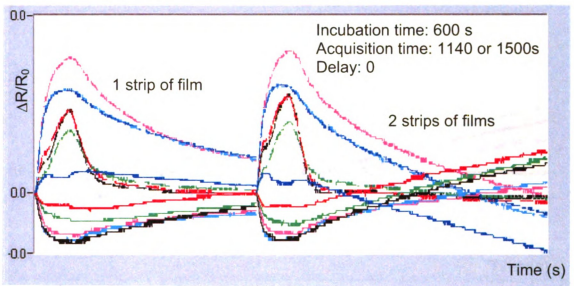


(Original data were generated on 04/05/01)

Figure 3.6 PCA of HDPE film samples after different incubation time

Sensor Response Pattern of Control Sample

During the investigation, it was found the intensities of the sensor responses for the control sample, base HDPE film, were always low and sometimes even lower than 5%.



(Original data were generated on 02/22/01 and 03/13/01 respectively)

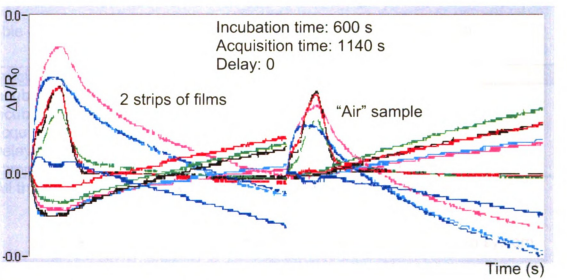
Figure 3.7 Comparison of sensor response patterns of the 12 E-nose sensors in analyzing sample CONT with different sample size

As shown in Figure 3.7, doubling the sample size did not significantly change the sensor response pattern of the control sample HDPE base film.

An "air" sample was prepared by sealing a vial without any HDPE film. This blank sample was prepared under the same data acquisition parameters as

those used for HDPE film samples. The headspace was extracted and injected into the E-nose system to obtain a sensor response pattern.

Figure 3.8 is the side-by-side sensor response patterns of a control film and air.



(Original data generated on 03/13/01)

Figure 3.8 Comparison of sensor response patterns of the 12 E-nose sensors of sample CONT and an "air" sample

Figure 3.8 showed that the sensor responses ($\Delta R/R_0$) were very low for both the control sample (HDPE base film) and the air sample, indicating the headspace of the control sample probably did not contain many volatile compounds or their concentrations were very low.

PCA Based on the E-nose Method

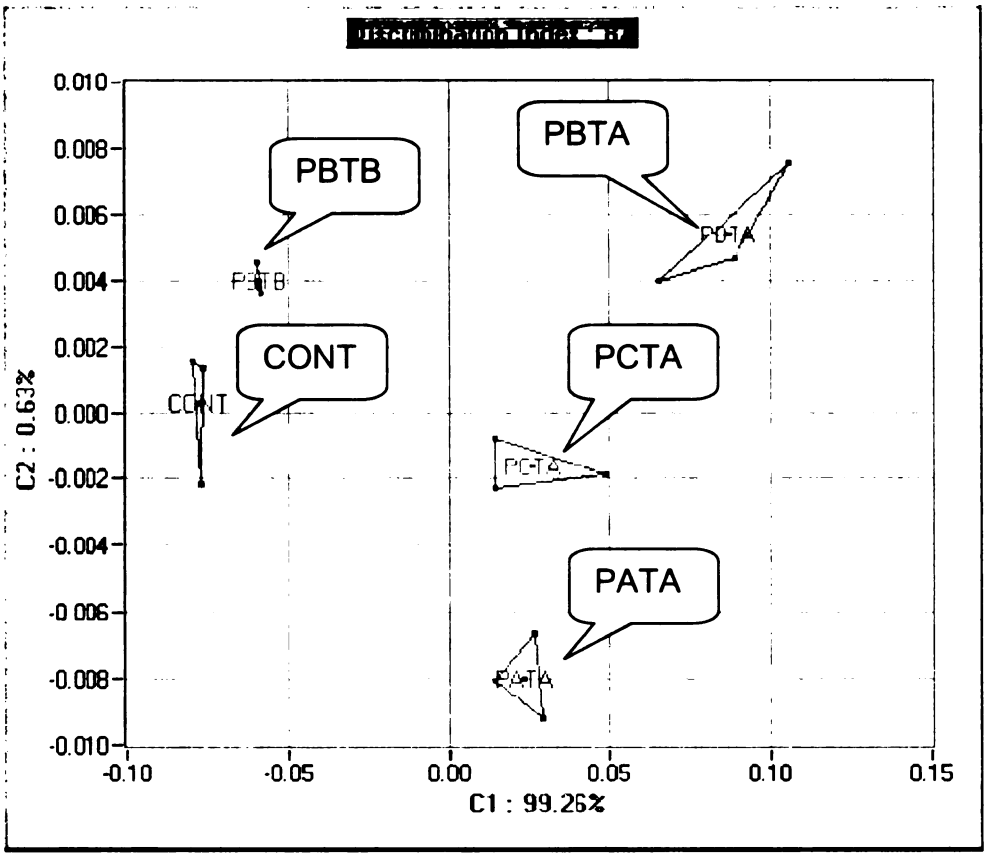
Figure 3.9 is the PCA plot based on the finalized E-nose method listed in Table 3.6. It shows that the PCA module of the E-nose system was able to differentiate the five different HDPE film samples.

Table 3.6 Finalized settings of data acquisition for the E-nose system

Crucial Parameters*	
Incubation time (s)	1200
Incubation temperature (^{0}C)	100
Acquisition time (s)	600
Delay (s)	900
Sample size	2 strips of 10" x 1" HDPE films

* All the minor parameters were same as those listed in Table 3.4

Alpha SOFT, the software for the Alpha MOS Fox 3000 E-nose system, has a function called "sensors optimization", which allows the investigator to select the most discriminating variables (sensors) for a given application. Sensors are automatically ranked based on their ability to differentiate the groups in the study (Alpha-M.O.S., 2002). Only the selected sensors and their responses are used in the multivariate statistical analyses.



(Original data were generated on 09/26/01)

Figure 3.9 Using PCA module of the E-nose to differentiate five different HDPE films

The reproducibility (%RSD = (σ/ $\bar{X}$) x 100%) of sensors and sample groups based on the PCA plot in Figure 3.9 is given in Table 3.7.

Table 3.7 Reproducibility of sensors and sample groups

Sensors	CONT	PATA	PBTA	PBTB	PCTA
SY/LG	260.75	-11.03	-7.36	11.29	-21.23
SY/Gh	-0.42	-10.42	-11.18	-7.98	-15.91
P10/2	3.13	5.79	9.69	2.93	11.94
PA2	5.84	4.86	8.63	1.49	12.40

Most %RSD values were below or close to 10%, indicating good reproducibility of the analysis (Alpha-M.O.S., 2002). Four sensors were selected by the E-nose system as the discriminating sensors, among which sensor SY/Gh, P10/2, and PA2 showed better reproducibility than sensor SY/LG. It was noticed responses of sensor SY/LG to the control sample (HDPE) were not consistent at all, indicating this particular sensor was not effective in analyzing the control sample. As discussed before, however, the odor system of the control sample was "seen" by the E-nose system as almost a blank system anyway (see Figure 3.8).

Group distances (Euclidean distance) were calculated to help evaluate the similarity between any two sample groups based on the PCA plot in Figure 3.9 (Rencher, 1995). The smaller the distance is, the more similar the two groups are to each other.

Table 3.8 Euclidean distances between each two groups of HDPE films

	CONT	PATA	PBTA	PBTB	PCTA
CONT		0.10	0.16	0.02	0.10
PATA			0.06	0.08	0.01
PBTA				0.15	0.06
PBTB					0.08
PCTA					

As shown in Table 3.8, the E-nose system determined the headspace of the control sample was very different from that of sample PBTA (distance = 0.16), when compared with other pairs of samples. The same conclusion was reached

for PBTA and PBTB (distance = 0.15). On the other hand, the headspace of the control sample was determined similar to that of sample PBTB, when compared with other pairs of samples. The same conclusion applied to PATA and PCTA.

Application of Principle Component Analysis (PCA)

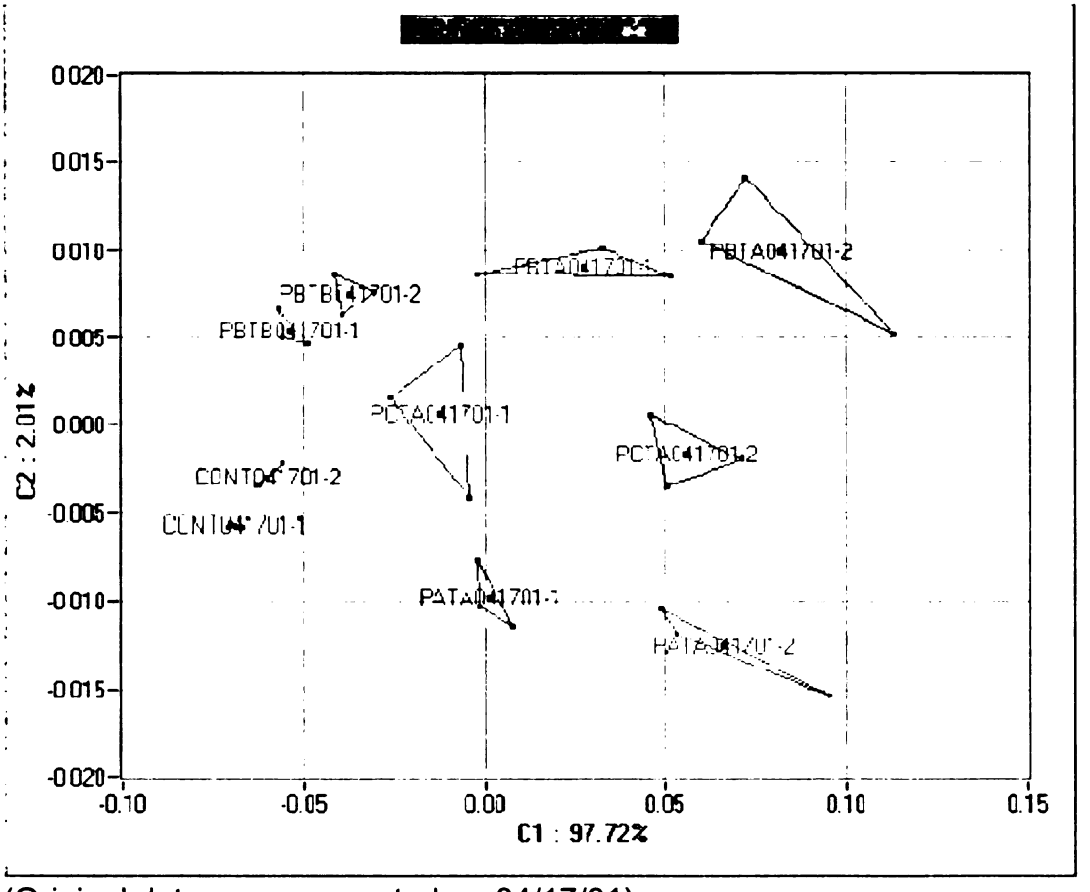
It was still worthwhile to further investigate the effect of sample size on the PCA result. No matter whether one strip or two strips of films was used, the proportion of all the volatile components in the headspace would be the same, if the incubation temperature and time were kept the same. The only difference between the resultant headspaces with different sample sizes of the same HDPE film would be the absolute quantities of the components. By using PCA, it became possible to find out whether the proportion or the absolute amount of the volatile compounds in a headspace would determine the PCA result. If the PCA module could not differentiate the headspaces of the same HDPE film, for example the base film, generated from different sample sizes, it would indicate the PCA analysis was dependent on the proportion of the volatile compounds in the headspace. The other way would indicate that the absolute amount of the volatile compounds in the headspace was more important.

Two groups of samples were analyzed with the E-nose system. One had one strip of film and the other had two strips of films. Each group consisted of

five different HDPE film samples (see Table 3.1) and each film sample had three duplicates.

All sample vials were incubated in the agitator at 100^0C for 1200 seconds before the headspace was sampled and injected into the E-nose system. The total run time for each analysis was 1500 seconds, with 600 seconds of acquisition time and 900 seconds of delay.

All the samples were analyzed on the same day and presented to the E-nose in a random order to eliminate the effect of sensor drifts. Thirty samples generated thirty profiles of the sensor responses, which were used to build a library for PCA analysis.



(Original data were generated on 04/17/01)

Figure 3.10 Using PCA module of the E-nose to differentiate the headspaces of HDPE films with different sample sizes

Each triangle represented one group of samples prepared under the same conditions. For example, PBTA041701-1 and PBTA041701-2 meant there were 1 strip and 2 strips of films in the group, respectively.

A DI (Discriminative Index) of 82 and the well-separated groups in the PCA plot showed the E-nose system can differentiate the headspaces of four different coated film samples and the base film. More importantly, it was shown

that the absolute amount of the volatile compounds in each headspace is important in determining the results of PCA.

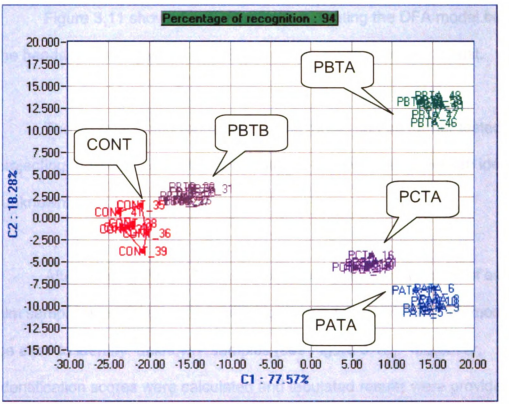
Discriminative Function Analysis (DFA)

PCA is considered an unsupervised learning technique because no prior knowledge of the analyzed samples is necessary (Delpha *et al.*, 2001; Krzanowski, 1988; Rencher, 1995). It reduced the 12-dimensional data generated by the Fox 3000 E-nose system to 2-dimensional data, making it easier to visually evaluate the similarity between sample groups.

On the other hand, Discriminative Function Analysis (DFA) is a supervised technique in which some prior information of the analyzed samples is known. Two steps are involved in DFA. The first is to develop a model by finding descriptive variables, which are the linear combinations of the sensors and are capable of separating as much as possible and thus classifying the samples. This step is similar to that used in PCA. The built model is then used as the prior knowledge of the sample groups, which is utilized in the second step of DFA to identify unknown samples. Some researchers call these steps the "training" stage and "validation" stage (Yuzay, 2004).

Ten duplicates of each five HDPE film samples were analyzed by the E-nose system, among which seven duplicates were used to build a training model

and the remaining three duplicates were used to validate the model. Figure 3.11 showed the DFA model built from the first seven duplicates of each film sample.



(Original data were generated on 10/03/06)

Figure 3.11 DFA training model of five different HDPE films

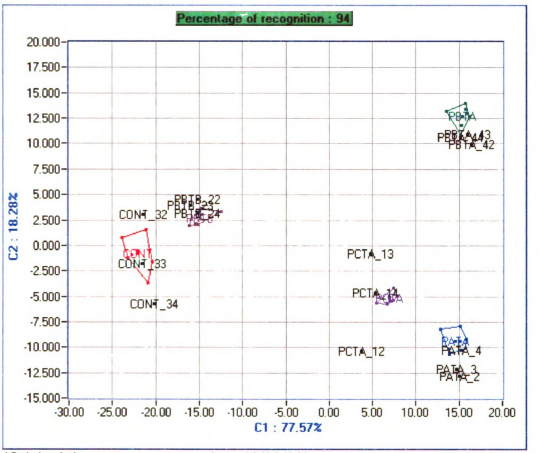
The "leave one out method" was used to calculate the percentage of recognition (POR) in the DFA model (Alpha-M.O.S., 2002). In this method, each data point of the 7 (duplicates) x 5 (samples) = 35 data points was removed from the data set at a time and treated as an "unknown". The "unknown" data point was then cross validated against all the remained data points so it could be recognized as one of them. The POR is the overall score of the validation of the

35 data points. A DFA model with a score close to or higher than 95% is considered a robust model (Alpha-M.O.S., 2002).

Figure 3.11 showed a POR of 94%, indicating the DFA model built from the headspace analyses of HDPE film samples was valid and robust.

The validation and robustness of the model can be further tested by projecting "unknown" samples to investigate whether the model can identify the "unknown" samples.

After building the DFA model successfully, three duplicates of each HDPE film samples were used as the validation group to test whether the model could be used to identify "unknown" samples (see Figure 3.12). Moreover, identification scores were calculated and tabulated results were provided to show what identity the "unknown" samples had been recognized as by the DFA model (see Table 3.9).



(Original data were generated on 10/03/06)

Figure 3.12 Validation of DFA model by projecting "unknown" samples

In Figure 3.12, each “unknown” sample was represented by a solid black dot and marked with its sample name followed by a number. The closer the unknown sample is to a training group (represented by a circle), the more likely this unknown sample belongs to that group.

Table 3.9 lists the validation data set “unknown” samples and their projected identity recognized by the DFA model.

Table 3.9 Validation of "unknown" samples and their projected identities

Sample code	Recognized	Projected identity	% of Recognition
CONT_32	Yes	CONT	23.9
CONT_33	Yes	CONT	100.0
CONT_34	Yes	CONT	100.0
PATA_2	Yes	PATA	68.6
PATA_3	Yes	PATA	100.0
PATA_4	Yes	PATA	100.0
PBTA_42	Yes	PBTA	73.0
PBTA_43	Yes	PBTA	100.0
PBTA_44	Yes	PBTA	100.0
PBTB_22	Yes	PBTB	100.0
PBTB_23	Yes	PBTB	100.0
PBTB_24	Yes	PBTB	100.0
PCTA_12	Yes	PCTA	17.2
PCTA_13	Yes	PCTA	45.9
PCTA_14	Yes	PCTA	100.0

The third column of Table 3.9 shows what the "unknown" samples were recognized as by the DFA validation process. The fourth column indicates how far each "unknown" sample (validation data) was from its recognized group (training data). Even though the percentage of recognition of a few validation samples was low, all fifteen "unknown" samples were recognized correctly, which further proved the robustness of the DFA model.

CHAPTER 4 EVALUATIONS OF ODOR PROFILES OF HDPE FILMS WITH SENSORY EVALUATION TESTS

INTRODUCTION AND OBJECTIVES

The sensory evaluation technique has long been used to evaluate various organoleptic properties of food and consumer products. The formalized, coded, and maybe structured methodology sometimes works better than certain analytical instruments in studying odor systems, depending on the applications and the characteristics of the sample (Peled & Mannheim, 1977).

In Chapter 3, the Alpha MOS Fox 3000 E-nose system was used to differentiate the headspaces generated from five HDPE film samples. The results indicated the E-nose system detected the difference among these samples.

However, the judgment made by the E-nose system was objective. No information in terms of preference or acceptance was obtained. Moreover, the question of whether the difference disclosed by the E-nose system was perceived in a similar way by the human nose had not been answered.

All these unanswered questions made it necessary to use sensory evaluation to further investigate the odor systems generated by the HDPE film samples.

Because human subjects would be used in the sensory evaluation, prior approval was received from the Institutional Review Board (IRB) of the Office of Regulatory Affairs at Michigan State University. The reference number was IRB# 02-033.

The objectives of this portion of the research were to: 1) evaluate the five different HDPE film samples to see if there was any difference among the generated odor systems; 2) investigate the preference and/or acceptance of the odor systems; 3) determine the intensity of the odor systems perceived by the human nose.

MATERIALS AND METHODOLOGY

The same HDPE film samples used in Chapter 3 were used in the sensory evaluation test (see Table 3.1): the control sample HDPE film (CONT) and four film samples coated with different adhesives (PATA, PBTA, PBTB, and PCTA).

Sample Preparation

A standard plastic cutter was used to cut the HDPE film samples into 10" inch long and 1 inch wide strips. Two strips of films were enclosed into 8 ml glass vials with poly-lined caps (Research Products International Corp.). The

glass vials were then heated in an oven at 100^{0}C for 20 minutes. Settings of the temperature and time were selected based on the finalized data acquisition parameters for the E-nose system in Chapter 3 (see Table 3.6). The vials were cooled down to room temperature before being presented to the sensory panel.

All the sensory evaluation tests were conducted at the Sensory Evaluation Facility located in the Department of Food Science and Human Nutrition at Michigan State University. The facility featured 7 individual booths in a quiet, clean, and air conditioned room with controlled air flow and lighting.

Pairwise Ranking Test

The pairwise ranking test with Friedman Analysis was used to evaluate the preference of the HDPE film samples. Fifty (50) people participated in this test: thirty-four (34) were ages 15 to 24, thirteen (13) were ages 25 to 34, three (3) were ages above 35. Participants were MSU faculty members, staff and students. All panelists were healthy on the day of the test and had no known allergic reaction to the HDPE film samples being studied.

The pairwise ranking test is recommended for evaluating a single sensory attribute of the testing samples by comparing them in all possible pairs (Meilgaard *et al.*, 1999). There were ten (10) possible pairs for the five (5) HDPE film samples (without considering the order of the two samples in each pair).

A randomized and balanced presentation was used to avoid positional bias (Poste *et al.*, 1991). To reduce the effect of fatigue, each panelist only received four (4) randomly selected pairs of the ten (10) possible pairs. Two hundred (200) pairs of samples were evaluated by fifty (50) untrained panelists.

Table 4.1 shows the sample codes and pair codes used in the pairwise ranking test. Each vial was coded with a random three-digit number. The shaded columns represent the second samples that the panelists were asked to evaluate in the pairs. Numbers 1 to 10 were given to the ten (10) possible pairs of five HDPE film samples, and numbers 11 to 20 were the same pairs in reverse order.

Table 4.1 Sample codes and pair codes used in the pairwise ranking test

Pair code	*	#	*	#	Pair code	*	#	*	#
1	A	119	B	322	11	B	498	A	949
2	A	293	C	952	12	C	675	A	491
3	A	926	D	383	13	D	634	A	352
4	A	455	E	536	14	E	781	A	378
5	B	834	C	128	15	C	563	B	131
6	B	662	D	637	16	D	857	B	495
7	B	341	E	873	17	E	245	B	967
8	C	787	D	764	18	D	196	C	153
9	C	578	E	285	19	E	918	C	228
10	D	814	E	516	20	E	479	D	815

* A – CONT, B – PATA, C – PBTA, D – PBTB, E – PCTA.

Refer to Appendix 1 for the worksheet used in the pairwise ranking test, which shows how the fifty (50) panelists received their four (4) pairs of samples.

Also as shown in Appendix 1, each pair (total of 20 pairs) of samples were presented to and evaluated by ten (10) panelists.

Quantitative Affective Consumer Test

The quantitative affective consumer test was used to evaluate the acceptability and intensity of the perceived odor of the HDPE film samples.

A randomized and balanced presentation was used to avoid positional bias. To reduce the effect of fatigue, each panelist only received five (5) samples. Five hundred vials containing the HDPE film samples were evaluated by one hundred (100) panelists. The SAS statistical software program was used to analyze the data.

Table 4.2 shows the three-digit random numbers that were used to encode the five different HDPE samples.

Table 4.2 Sample codes used in the quantitative affective consumer test

Sample	CONT	PATA	PBTA	PBTB	PCTA
Code	786	637	391	938	892

Table 4.3 shows that each panelist received five (5) different samples; the number of occurrences for each sample to be presented at one specific position in each group was 20. In the other words, the control sample was presented first

20 times, as were samples PATA, PBTA, PBTB, and PCTA. In a similar way, each sample was presented as the second sample, the third sample, the fourth sample, and the last sample 20 times.

Table 4.3 Worksheet used in the quantitative affective consumer test

	Position 1	Position 2	Position 3	Position 4	Position 5	Total
CONT	20	20	20	20	20	100
PATA	20	20	20	20	20	100
PBTA	20	20	20	20	20	100
PBTB	20	20	20	20	20	100
PCTA	20	20	20	20	20	100

Each panelist has different sensitivity and accuracy in evaluating the sensed odor of the HDPE film sample. Based on the following three assumptions, it made sense to use RCBD (Random Complete Block Design) to design the experiment, with each panelist as one block and within each block all five samples were evaluated once (Ott & Longnecker, 2001). These three assumptions were: 1) there were no time effects; 2) there were no carryover effects; 3) the observations within a subject were equally correlated with each other as in a compound symmetry assumption.

The block factor (panelists) was considered a random variable, representing a random draw from the on-campus population of the MSU community, while the treatment factor (five different HDPE film samples) was considered as a fixed factor. Refer to Appendix 2 for the detailed sample presentation.

RESULTS AND DISCUSSION

Pairwise Ranking Test

The pairwise ranking test has been determined to be "particularly useful for sets of three to six samples which are to be evaluated by a relatively inexperienced panel" (Meilgaard *et al.*, 1999).

In the pairwise ranking test, the panelists were asked to evaluated the perceived odor upon opening the glass vials, and then choose which sample in each pair they thought had stronger odor (see Appendix 3). The chosen sample in each pair was then given one (1) point. This was repeated until all two hundred (200) tested pairs of samples were evaluated, which gave the result shown in Table 4.4.

Table 4.4 Data analysis of the pairwise ranking test to rank the intensity of perceived odor of five HDPE films

		Column sample (weaker odor)				
		CONT	PATA	PBTA	PBTB	PCTA
Row Samples (stronger odor)	CONT	/	7	7	6	7
	PATA	13	/	11	11	7
	PBTA	13	9	/	9	8
	PBTB	14	9	11	/	/
	PCTA	13	13	12	18	2

Each pair of samples, for example CONT and PATA, was evaluated by twenty (20) panelists. The table shows the number of times each "row" sample was perceived as having stronger odor than each "column" sample.

As shown in Table 4.4, when sample PATA was presented with the control sample CONT as a pair to the panelists, it was perceived as having a stronger odor thirteen (13) times. Conversely, 7 panelists decided sample CONT had a stronger odor than sample PATA.

According to the method of Friedman Analysis (Meilgaard *et al.*, 1999), the rank sum for each sample was calculated. A rank of one (1) was assigned to the sample with "stronger odor" and a rank of two (2) was given to the sample with "weaker odor". The rank sums were then obtained by adding the sum of twice the column frequencies to the sum of the row frequencies.

For example, the rank sum for the control sample CONT was calculated as:

$$(7+7+6+7)+2\times(13+13+14+13)=133$$

Table 4.5 Rank sum of the tested samples in the pairwise ranking test

Sample	CONT (A)	PATA (B)	PBTA (C)	PBTB (D)	PCTA (E)
Rank sum	133^{a}	120^{ab}	121^{ab}	124^{a}	104^{b}

Note: Different letters indicate significant statistical differences.

The test statistic, Friedman's T value, was computed as follows:

$$T = \left(\frac{4}{pt}\right)\sum_{i=1}^{t}\left\{R_i^2 - \left[9p \times (t-1)^2\right]\right\} = 36.88$$

where p = the number of times the basic design was repeated, i.e. how many times each pair of samples were evaluated, which was twenty (20) in the test; t = number of treatments (tested samples), which was five (5); R_i = the rank sum for the i'th treatment (sample).

The critical χ^2 with a degree of freedom of 4 and $\alpha = 0.05$ was 9.49 (Meilgaard *et al.*, 1999).

The calculated T value of 36.88 is higher than the critical chi-square χ^2 of 9.49, indicating there was a significant difference among the perceived odor systems generated from the five different HDPE film samples.

On the same scale, the HSD (Honestly Significant Difference) for comparing two rank sums ($\alpha = 0.05$) was computed as:

$$HSD = q_{\alpha,t,\infty}\left(\sqrt{pt/4}\right) = 3.86 \times \sqrt{\frac{20 \times 5}{4}} = 19.3$$

where $q_{\alpha, t, \infty}$ was determined to be 3.86 (Meilgaard *et al.*, 1999).

HSD was used to determine whether there was a significant difference between any two rank sums. Any difference higher than the HSD was considered significant. Otherwise, the difference was not significant.

Figure 4.1 is a plot of the arrangement of all the rank sums of all five groups HDPE film samples on a scale of intensity of their perceived odor based on the results in Table 4.5 on the previous page.

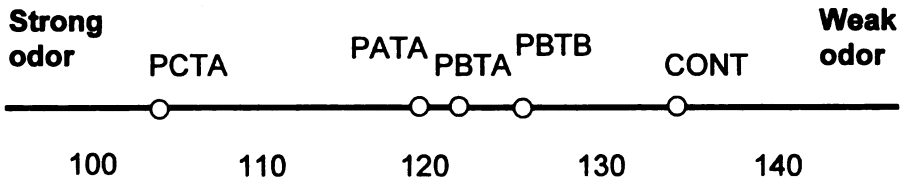


Figure 4.1 Rank sums of five HDPE film samples in the pairwise ranking test

Sample CONT had the weakest perceived odor while sample PCTA had the strongest odor. The intensity of the perceived odor of PCTA was significantly stronger than the odors of PBTB and CONT, but there was no significant difference in terms of the odor intensity among CONT, PATA, PBTA, and PBTB.

Quantitative Affective Consumer Test

Justification

Even though the pairwise ranking test proved effective in discriminating samples when more than twenty (20) panelists were used (Meilgaard *et al.*, 1999), the panel size was still considered small for the test to be qualified as a consumer test. Out of the five (5) HDPE film samples, there were 10 possible pairs. If the sequence of sample presentation in each pair was also considered, there were 20 pairs (see Table 4.1), which meant each pair of samples in that particular order of presentation was tested only ten (10) times. This brought up a question about the panel size and thus the accuracy of the obtained results.

Moreover, the panelists in the pairwise ranking test were neither pre-screened nor pre-trained with the test methodology, which might have affected the accuracy of the obtained results as well (Meilgaard *et al.*, 1999).

Another concern was the questionnaire (see Appendix 3) used in the pairwise ranking test. The panelists were asked to "pick one which has the stronger odor in each pair of samples". Basically the pairwise ranking test was designed to compare the perceived intensity of the odor systems. However, the acceptability or preference of the odor systems was more important for the purpose of this research. In the other words, it was possible that one sensed

odor would not be accepted by consumers even though its intensity was found weak.

Also, the rank sums in the pairwise ranking test were calculated based on a structured scale, which might not represent the real difference among the odor systems (Lawless & Heymann, 1998; Poste *et al.*, 1991).

A quantitative affective consumer test was thus designed to answer the questions mentioned earlier, using a panel with one hundred (100) panelists (see Appendix 4).

As shown in Appendix 4, each panelist was asked two questions. They were first asked to evaluate the overall acceptability of the perceived odor, and then asked to determine the intensity of the odor.

Unstructured scales were used in addressing both "acceptability" and "intensity" of the odor systems. The full length of each scale was 15 cm. The scaling was obtained for each question by measuring the length from the left end vertical line to the vertical line marked by the panelist.

Mixed Model and Validation of Residuals Assumption

The PROC MIXED function in the SAS statistical software program was used to analyze the data (see Appendix 5 for the SAS program and output). The issues to be addressed included: 1) was the assumption that the distribution of residuals was normal proved valid? 2) How did the different treatments (different HDPE film samples) affect the acceptability and intensity of the sensed odor; 3) did factor "panelists" play any role in the data analysis? In the other words, was blocking an effective way to control the variability?

Because RCBD (Random Complete Block Design) was used, the model used to describe the random blocks (panelists) and fixed-effect treatment factor (HDPE film sample) was:

$$Y_{ij} = \mu + \rho_i + \alpha_j + e_{ij}$$

in which Y_{ij} (sensory score) = acceptability or intensity of sensed odor of sample j determined by panelist i; i (block) = 1, 2, 3, ..., 100, representing one hundred panelists; j (treatment) = 1, 2, 3, 4, and 5, representing five HDPE film samples. The assumption regarding the model was:

$$\rho_i \sim NIID\left(0, \sigma_\rho^2\right), e_{ij} \sim NIID\left(0, \sigma_e^2\right)$$

in which NIID stands for Normal, Independent, and Identical Distribution.

The assumptions regarding the block and residuals were the basis for the subsequent pair-wise comparison of the acceptability and intensity of the sensed odor, and thus should be proved first.

Figures 4.2 and 4.3 show the distributions of residuals in the form of residuals versus treatment (five HDPE film samples).

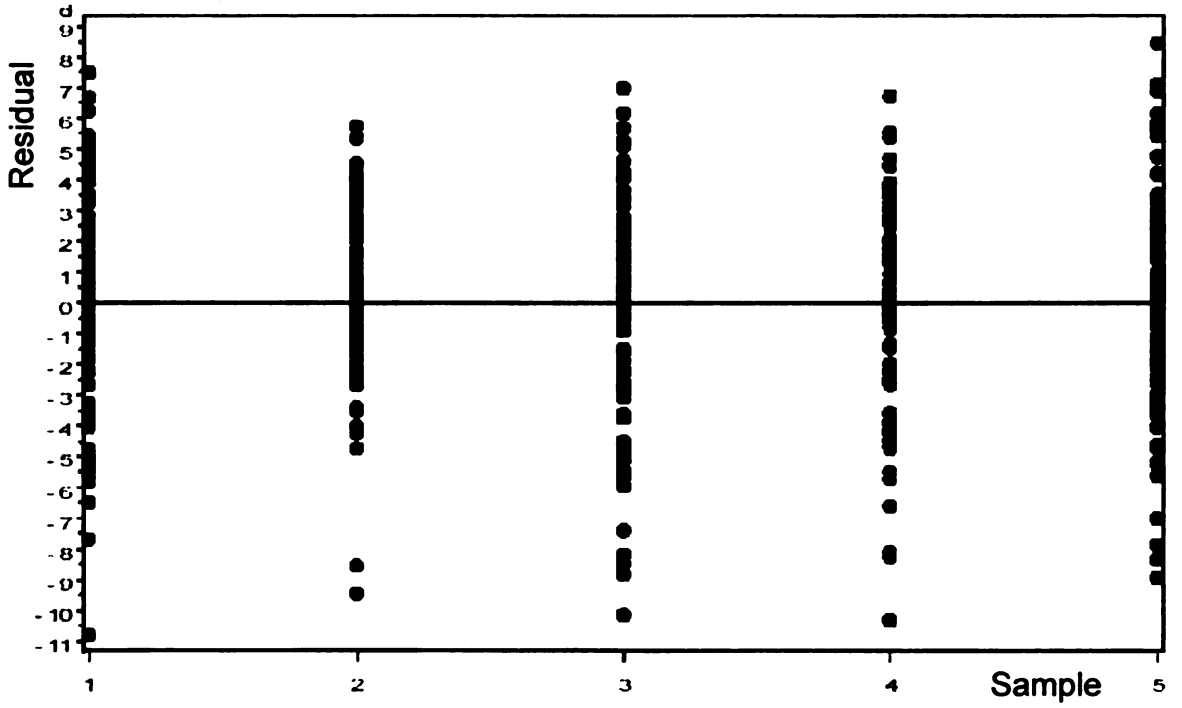


Figure 4.2 Residuals versus sample ID in analyzing acceptability scores

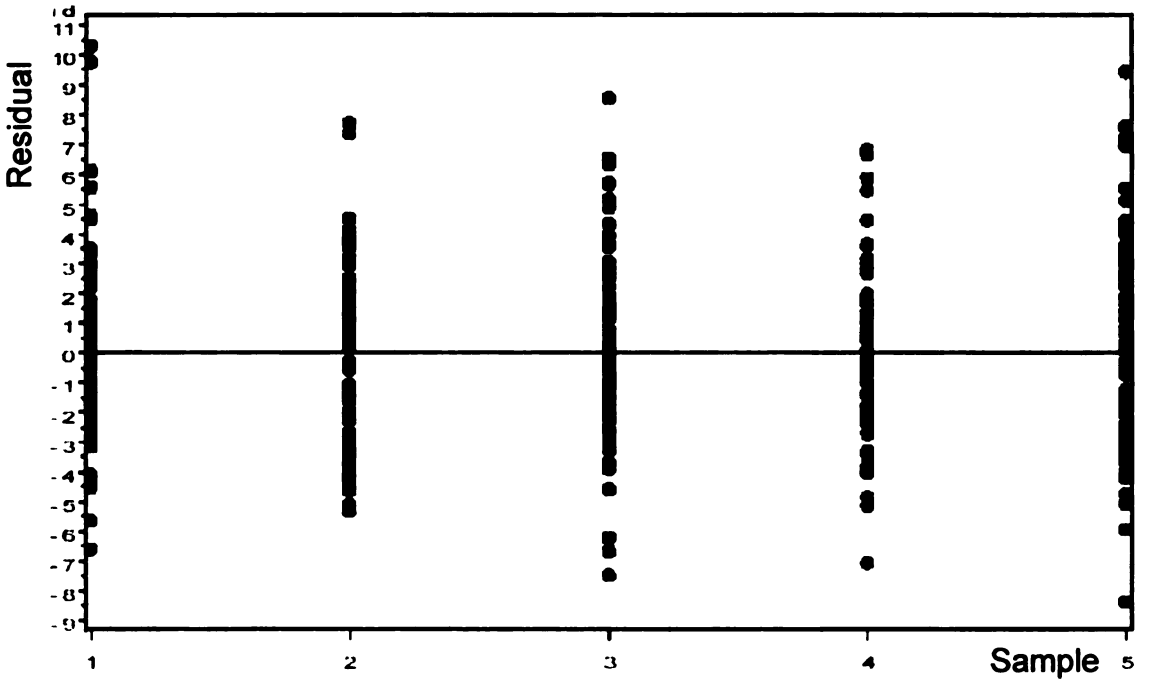


Figure 4.3 Residuals versus sample ID in analyzing intensity scores

Obviously, the distributions of residuals in analyzing both acceptability and intensity scores satisfied the NIID assumption as shown by the even and symmetrical distribution along the line across zero (0). Moreover, the PROC UNIVARIATE analysis also proved the normal distributions of the residuals by the box plots and normal probability plots (see Appendix 5).

Paired comparison of treatment means (acceptability and intensity scores)

With the assumptions regarding residuals and blocking being proved valid, pair-wise comparison was used to investigate the effect of different treatment (five different HDPE film samples) on the acceptability and the intensity of the sensed odor determined by the panelists.

The PROC MIXED function was used to analyze the sensory score (acceptability and intensity). Table 4.6 shows the ANOVA results for both the acceptability and intensity scores.

Table 4.6 ANOVA analyses of the acceptability and intensity scores

Acceptability					
Source	DF	Sum of Squares	Mean Square	F-Value	Pr > F
Model	103	5506.06	53.46	4.04	<.0001
Sample	4	551.18	137.79	10.42	<.0001
Panelists	99	4954.88	50.05	3.78	<.0001
Error	396	5237.74	13.23		
Corrected Total	499	10743.80			
Intensity					
Source	DF	Sum of Squares	Mean Square	F-Value	Pr > F
Model	103	3484.93	33.83	3.34	<.0001
Sample	4	873.52	218.38	21.54	<.0001
Panelists	99	2611.42	26.38	2.60	<.0001
Error	396	4014.27	10.14		
Corrected Total	499	7499.20			

As shown in Table 4.6, P-values for the samples (treatments) in analyzing for both the acceptability and intensity were below α (= 0.05), indicating there were significant differences among the mean acceptability scores, as well as among the mean intensity scores, of the sensed odor of the five HDPE film samples.

Table 4.7 summarizes the pair-wise comparison of the average acceptability and intensity scores of the odor of the samples perceived by the panelists based on the Tukey-Kramer test (see Appendix 5).

HSD (Honestly Significant Difference) base on a RCBD can be calculated as:

$$HSD = \frac{q_{a,dfe,\alpha=0.05}}{\sqrt{2}} se\left(\bar{y}_{.j} - \bar{y}_{.j'}\right)$$

where q is the critical statistic in the Studentized range distribution (Ott & Longnecker, 2001) for a total number of treatments α, a degree of freedom of error dfe, and $\alpha = 0.05$; and se ($y_{.j} - y_{.j'}$) is the standard error between any two average sensory scores based on treatments.

HSD for the acceptability and intensity scores was calculated as:

$$HSD_{\text{acceptability}} = \frac{3.86}{\sqrt{2}} \times 0.5143 = 1.40$$

$$HSD_{\text{intensity}} = \frac{3.86}{\sqrt{2}} \times 0.4503 = 1.23$$

The difference between two average sensory scores must be higher than its appropriate HSD value to be considered significant.

Table 4.7 Paired comparisons of the acceptability scores and the intensity scores of the sensed odors of the five HDPE films

	Control	PATA	PBTA	PBTB	PCTA
Acceptability	10.22 [a]	9.89 [a]	9.07 [a]	9.62 [a]	7.25 [b]
Intensity	3.01 [a]	2.91 [a]	3.80 [a]	2.93 [a]	6.36 [b]

Note: Based on the significance level of $\alpha = 0.05$. Different letters on the same row indicate significant statistical differences.

As shown in Table 4.7, any two scores carrying different superscripts indicate a significant difference between them. Otherwise, there was no proved significant difference between them.

There was no significant difference among the acceptability scores of CONT, PATA, PBTA, and PCTA but the acceptability score of PCTA was significantly lower than others.

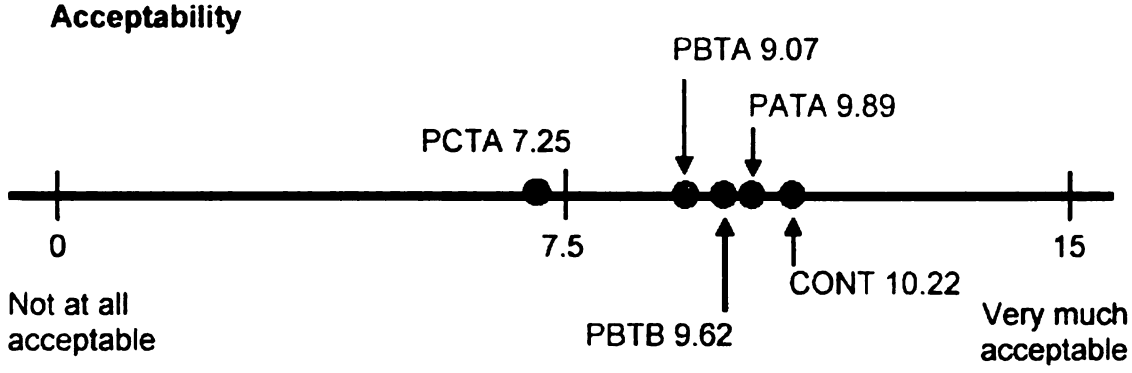


Figure 4.4 Average acceptability scores of the sensed odor of samples

As shown in Figure 4.4, four samples' average acceptability scores were found to be above the central point of the scale and in the range of 9.07 – 10.22. These four samples were CONT, PATA, PBTA, and PCTA. By comparing their

distance to the central point of the scale to the HSD value (1.40), their sensed odor profiles were considered "acceptable" by the panelists. On the other hand, the average acceptability score for sample PCTA was 7.25 and very close to the central point of the scale, indicating that opinions of the panelists to this sample's acceptability were neutral to slightly unacceptable. In other words, the panelists neither found the odor of PCTA acceptable or unacceptable.

Table 4.7 shows sample PCTA was perceived by the panelists as having a significantly more intense odor, which agreed with the conclusions drawn in the pairwise ranking test.

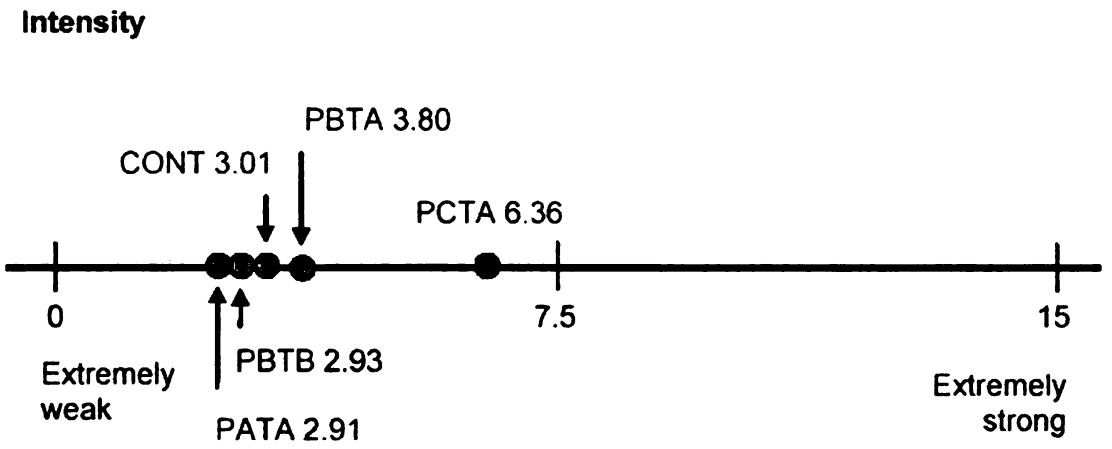


Figure 4.5 Average intensity scores of the sensed odor of samples

Moreover, Figure 4.5 shows that the average intensity scores for sample CONT, PATA, PBTA, and PBTB were determined to be in the lower range of the unstructured scale (2.91 – 3.80), while the average intensity score for sample

PCTA was 6.36. However, all five samples were perceived as having weak odor profiles by comparing their distance to the central point to the HSD value (1.23).

These results brought up a very interesting issue: the correlation between the intensity and the acceptability of the sensed odor.

The evaluations of "acceptability" and "intensity" of the sensed odor profiles of sample CONT, PATA, PBTA, and PBTB agreed with each other very well. Namely, the sensed odor was considered acceptable if its intensity was perceived as weak.

However, sample PCTA was different. It was evaluated as "has a weak intensity of the sensed odor" but the panel also evaluated it as "has neutral and slightly unacceptable sensed odor".

This further proved the two questions asked in the quantitative affective consumer test were appropriate and effective because it helped disclose information that was not addressed in the first sensory evaluation test, the pairwise ranking test.

In terms of odor analysis, the threshold values of various volatile compounds as well as the interactions among them all affect the perceived odor profile as a whole. It was suspected there was some volatile whose threshold

value was low in sample PCTA and thus the sample's odor profile was perceived as "neutral to slightly unacceptable" .

Effectiveness and Efficiency of RCBD

As shown in the SAS program output type II SS (Sum Square) in Appendix 5), the p-values of "judge" (panelists) were found to be < 0.0001, indicating using each panelist as one block in the RCBD was an effective way to control the variability. Again, this approach was employed because each panelist had different sensitivity and accuracy in evaluating the sensed odor of the HDPE film samples.

Moreover, the efficiency of the blocking can be measured by E, which was defined as (Ott & Longnecker, 2001) :

$$E = \frac{\sigma^2_{\text{CRD}}}{\sigma^2_{\text{RCBD}}}$$

where the two variance terms, σ_{CRD}^2 and σ_{RCBD}^2 are the residual variances from CRD (Complete Random Design) and RCBD (Randomized Complete Blocking Design), respectively. It is used to indicate how many more replicates would be needed for a CRD design to get the same standard error of any treatment mean difference as that in a RCBD (Ott & Longnecker, 2001). It can be estimated by using:

$$E = \frac{(n-1)MSBL + n(a-1)MSE}{(na-1)MSE}$$

in which n is the number of blocks; a is the number of treatments; MSBL is the mean square of blocks; and MSE is the mean square of errors.

Using the ANOVA table for the acceptability scores as one example, E of RCBD was estimated as (see Table 4.6 for the data):

$$E = \frac{(100-1)\times 50.05 + 100\times(5-1)\times 13.23}{(100\times 5-1)\times 13.23} \approx 2$$

indicating that about 2 times more replicates per treatment would have been required if CRD were used for this sensory evaluation test.

CHAPTER 5 ANALYSIS OF VOLATILES USING DH-TD COUPLED WITH GC-MS

INTRODUCTION AND OBJECTIVES

As discussed earlier, concentrations of volatile compounds in an odor system are usually too low to be detected directly with most analytical instruments, which makes a sample preparation step necessary to separate, purify, and concentrate analytes of interest from a sample matrix.

Many sample preparation techniques are available, among which dynamic headspace – thermal desorption (DH-TD) has been widely used to concentrate analytes from various sample matrices, including air, water, soil, food, pharmaceutical, and packaging materials.

Compared with other sample preparation techniques, DH-TD eliminated the use of organic solvents. Instead, a multi-bed adsorbent tube is used to adsorb volatiles released from the sample matrix, which is then heated to a high temperature very quickly to release the concentrated volatile compounds.

There have been many published papers about using Thermal Desorption coupled with GC-MS to identify volatile compounds. Esteban (1993) determined volatile compounds for different plants. Werkhoff and Bretschneider (1987a; 1987b) presented the principles and application, and the effect of sampling and

desorption parameters on recovery using dynamic headspace gas chromatography. All of them used the thermal desorption technique to collect and concentrate volatiles before GC-MS analysis. Sunesson et al (1992) used the multivariate optimization method to determine the experimental conditions for direct thermal desorption and gas chromatography.

Maneesin (2001) used the multi-bed adsorbent tube Carbotrap 400 from Supelco to collect volatiles from HDPE bottles and from water samples stored in the HDPE bottles for 6 months. In collecting volatiles released from HDPE bottles, 0.1g of HDPE flakes were incubated at 100°C for 2 minutes in a thermal stripper before the GC analysis. In studying water samples, 250 ml of water was heated at 100°C and flushed with pure helium gas at a flow rate of 100 ml/min for 10 minutes so that the volatiles were carried away and trapped by the desorption tube in the thermal stripper. Parameters mentioned in the sample preparation and introduction to the GC column included carrier gas, temperature, and time.

Kanavouras (2003) used two different desorption tubes, Tenax-TA and Carbotrap-300, in studying volatiles generated from fresh olive oil and oxidized olive oil samples. Investigated parameters in the sample preparation and analysis included temperature, time, carrier gas, and the ratio of headspace/sample in the sample vial.

In Chapter 3, it was found the odor profiles generated from five different HDPE film samples (one control and four coated with different adhesives) were differentiated by the electronic nose system. In Chapter 4, sensory evaluation determined that sample PCTA had significantly stronger odor compared with the other four HDPE samples.

This chapter reports use of the dynamic headspace – thermal desorption (DH – TD) technique to collect volatile compounds released from the HDPE film samples before the compounds were analyzed and identified by a GC-MS system.

MATERIALS AND METHODOLOGY

The same HDPE film samples used in Chapter 3 were used (see Table 3.1): the control sample HDPE film (CONT) and four film samples coated with different adhesives (PATA, PBTA, PBTB, and PCTA).

DIP (Direct Insertion Probe) Analysis

Two different film samples were studied: the control sample CONT (HDPE base film) and sample PCTA (the base film coated with adhesive PCTA). A sample of film measured 0.8 cm x 0.6 cm was cut and inserted into the heating tube in the DIP instrument located in the Mass Spectrometry Center at Michigan

State University. The film sample was heated (temperature started and stayed at 30°C for 2 minutes, increased at 16°C/min to 300°C, and was kept at 300°C for 2 minutes) to generate the odor system. The generated gaseous phase was ionized by the EI (Electron Impact) method, and ions were scanned and recorded by the mass spectrometer JEOL JMS-AX505 H (Jeol USA, Peabody, MA). The mass scan range was 45 to 750 Th (Thompsons, m/z ratio).

Selection of Thermal Desorption Tube

Two types of thermal desorption tubes were used: Carbotrap 400 and Carbotrap 300 (11.5 cm long and I.D. 4 mm) manufactured by Supelco (Bellefonte, PA) (see Figures 5.1 and 5.2).

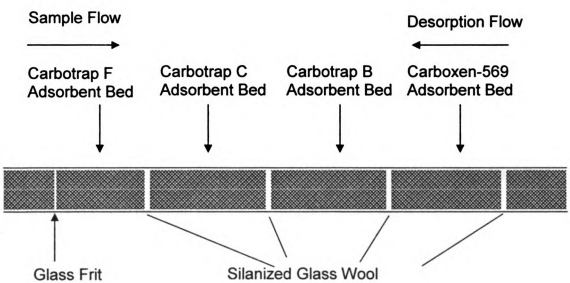


Figure 5.1 Carbotrap 400 multi-bed thermal desorption tube (Supelco, 1998a)

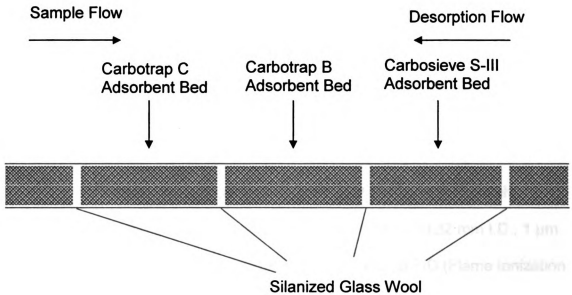


Figure 5.2 Carbotrap 300 multi-bed thermal desorption tube (Supelco, 1998a)

The thermal desorption (TD) tubes were pre-conditioned at 350°C under inert helium gas flow for 8 hours before use.

Two strips of 10" x 1" HDPE films were contained in a 9-ml stripping glass vial, which was then placed into a Dynamic Thermal Stripper Model-1000 (Dynatherm Analytical Instruments, Inc., Kelton, PA). Helium gas was flushed into the vial while the vial was heated. Volatile compounds were released and carried away by the helium gas and trapped in the thermal desorption tube that was attached to the exhaust port of the Stripper (see Figure 2.2).

Upon the completion of the stripping process, the TD tube was transferred to a Dynatherm Thermal Desorption Unit Model-890 (see Figure 2.4) (Dynatherm Analytical Instruments, Inc. Kelton, PA), where the tube was heated to a pre-

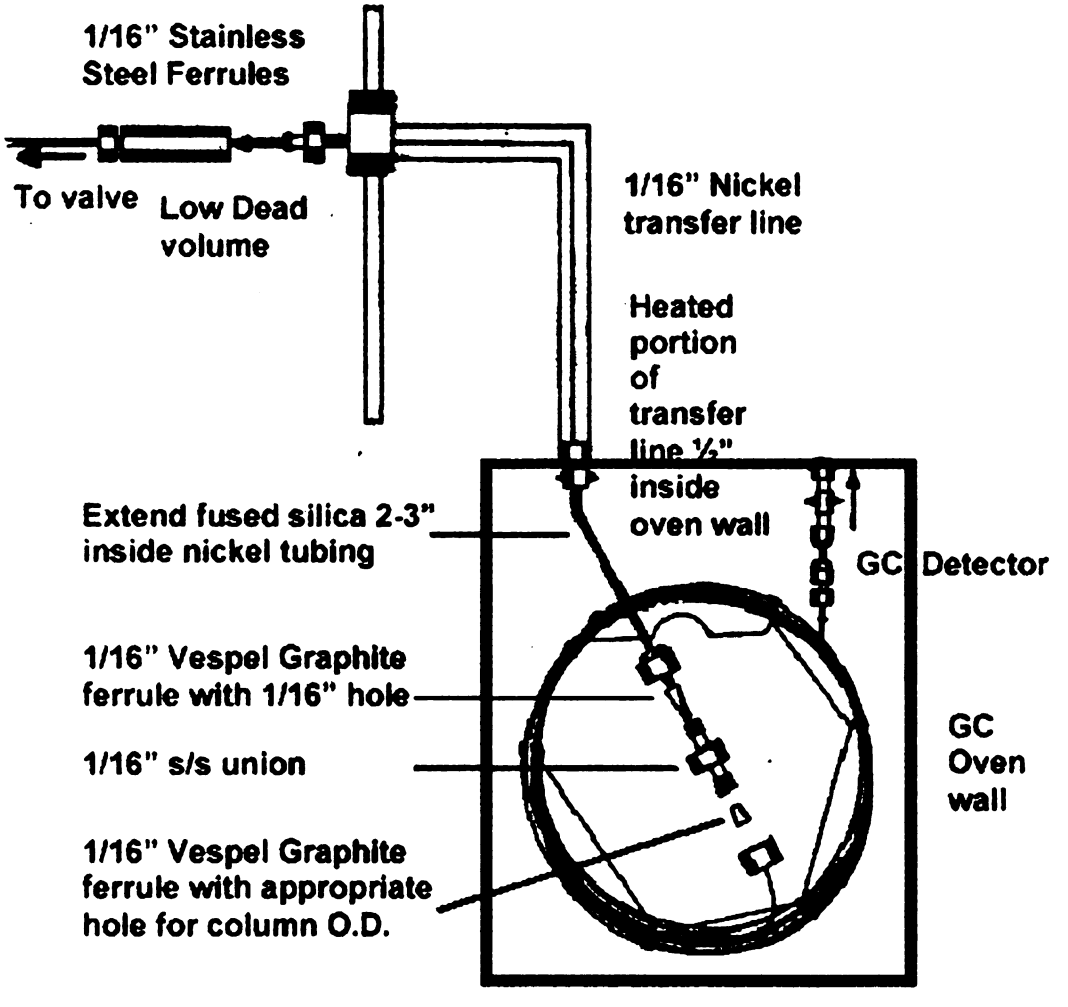
specified high temperature in a short period of time and volatile compounds were released to the GC column through a heated transfer line (1-meter long nickel tubing of 1/16" diameter that is connected to the fused silica capillary column via a 1/16" stainless steel union).

The GC was a HP 5890 Series II GC (Hewlett Packard, Philadelphia, PA) equipped with a fused silica capillary column SPB-5 (30 m x 0.32 mm I.D., 1 μm thickness of coating) from Supelco (Bellefonte, PA) and an FID (Flame Ionization Detector). Integration of the chromatography peaks was performed by an HP 3395 integrator (Hewlett Packard, Philadelphia, PA).

DH-TD GC/MS

The same procedures described in the previous section were followed to collect and concentrate volatile compounds released from the HDPE film samples using a Carbotrap 300 TD tube from Supelco (Bellefonte, PA). Trapped volatiles were separated and identified by a GC/MS system, HP G1800B GCD Plus system equipped with a fused capillary column HP-5 (30 m x 0.25 mm I.D., 1 μm thickness of coating) and an EI mass quadrapole detector and software GCD Plus ChemStation (G1074B version A.01.00) from Agilent Technologies, Inc. (Palo Alto, CA) in the Department of Chemistry at Michigan State University.

Ideally the TDU (Thermal Desorption Unit) and the GC system with a capillary fused silica column should be connected through a nickel transfer line, which enters the GC oven through an accessing opening on the top of the oven instead of through the injector (see Figure 5.3). The transfer line outside the TDU valve compartment should be wrapped by a heating jacket. Moreover, the heated portion of the transfer line should extend inside the GC oven by ¼" – ½" to avoid cold spots along the line (Dynatherm, 1989).



Reprinted from (Dynatherm, 1989)

Figure 5.3 Connecting nickel transfer line of TDU to GC

Due to the logistics of the GCD system, this connection was not allowed. A modified connection had to be adopted to connect the TDU with the GCD system.

The solution was to insert one deactivated fused silica tubing inside the nickel transfer line of the TDU. One end of the silica tubing was connected to the valve behind the thermal desorption tube inside the TDU valve compartment and the other end was connected to a custom-made aluminum connector tube (1/8" O.D.) which had a flat skirt on one end and sat on the top of the septum inside the injection port. This custom-made aluminum connector was then screwed tight and held in place by the septum retainer nut (Agilent catalog# 18740-60835). The extended part of the connector tubing was connected to one end of a Swagelock union and locked in place by a ferrule, and the other end of the union was connected to the transfer line's heating jacket and locked in place by another ferrule. The fused silica line inside the heating jacket and nickel tubing went all the way through the Swagelock union, the custom-made connector tube and GC injection port, and then extended another 7-8" into the injector (see Figure 5.4).

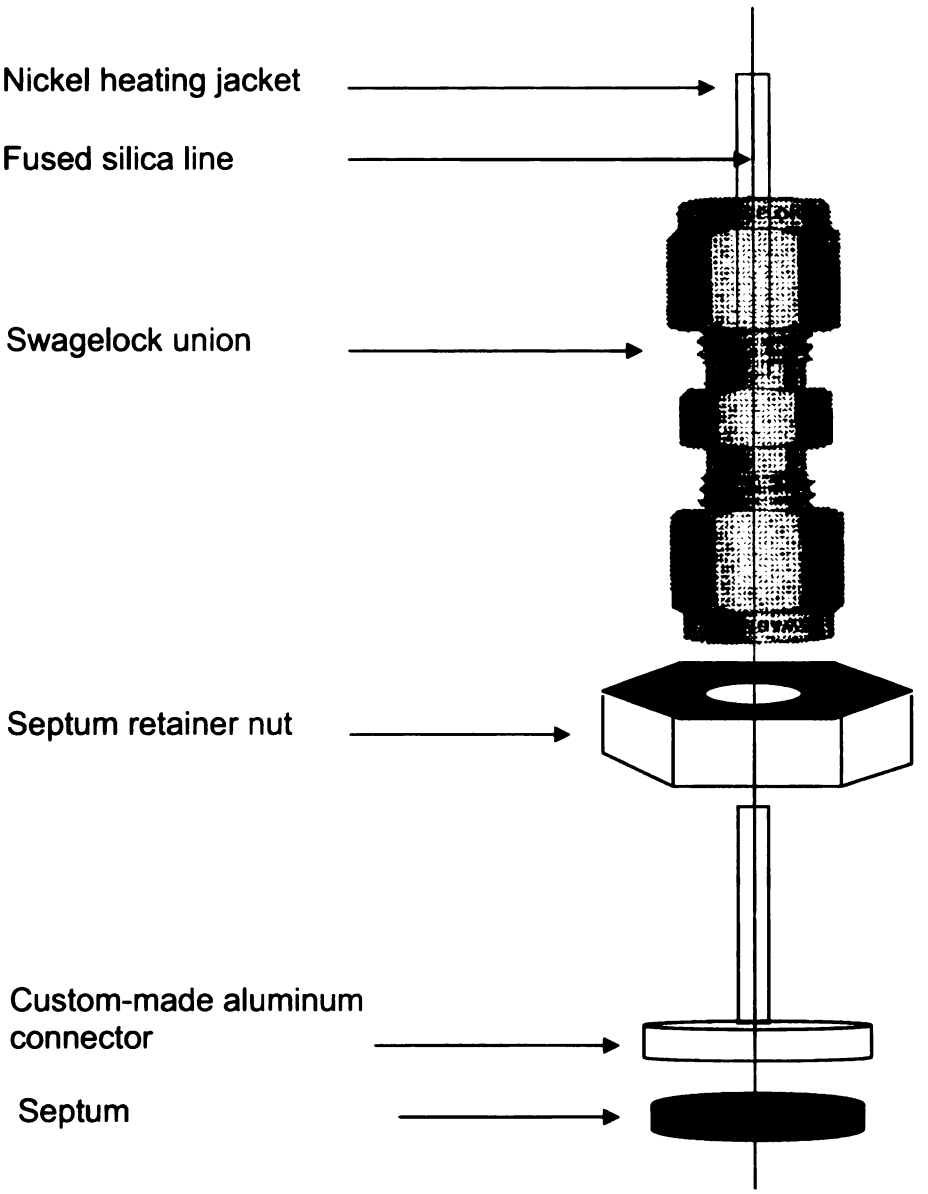


Figure 5.4 Modified connection between TDU and GCD system

RESULTS AND DISCUSSION

DIP (Direct Insertion Probe) Analysis

DIP analysis was used as a quick and simple technique to compare the odor systems generated from sample CONT and PCTA.

Figure 5.5 shows how the data was collected to get the IIC (Individual Ion Current) chromatogram and RTIC (Reconstructed Total Ion Current) chromatogram.

Based on the RTIC chromatograms of the control sample CONT and sample PCTA (see Figure 5.6 and 5.7), it was concluded that different odor systems were generated from the two film samples. More volatiles were released from sample PCTA because the average abundance of TIC (Total Ion Current) was much higher than that of the control sample. Moreover, maximum abundance of the control sample happened around RT (Retention Time) 1 – 3 min (corresponding to scan number 100 – 300), while for sample PCTA, maximum abundance happened around RT 5 – 8 min (corresponding to scan number 300 – 500), which further indicated the compositions of these two odor systems were different from each other.

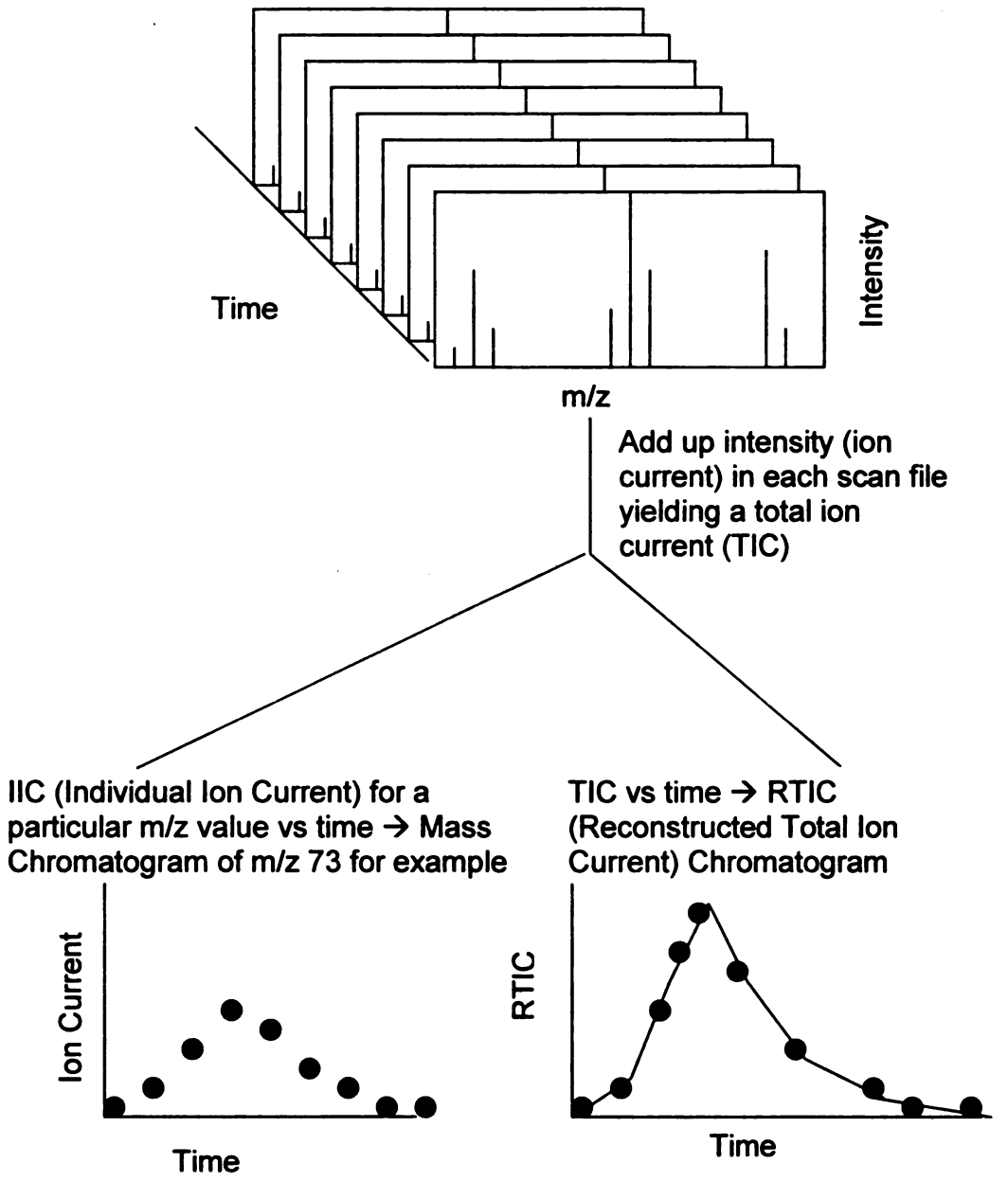


Figure 5.5 TIC and RTIC chromatogram from mass spectrometer

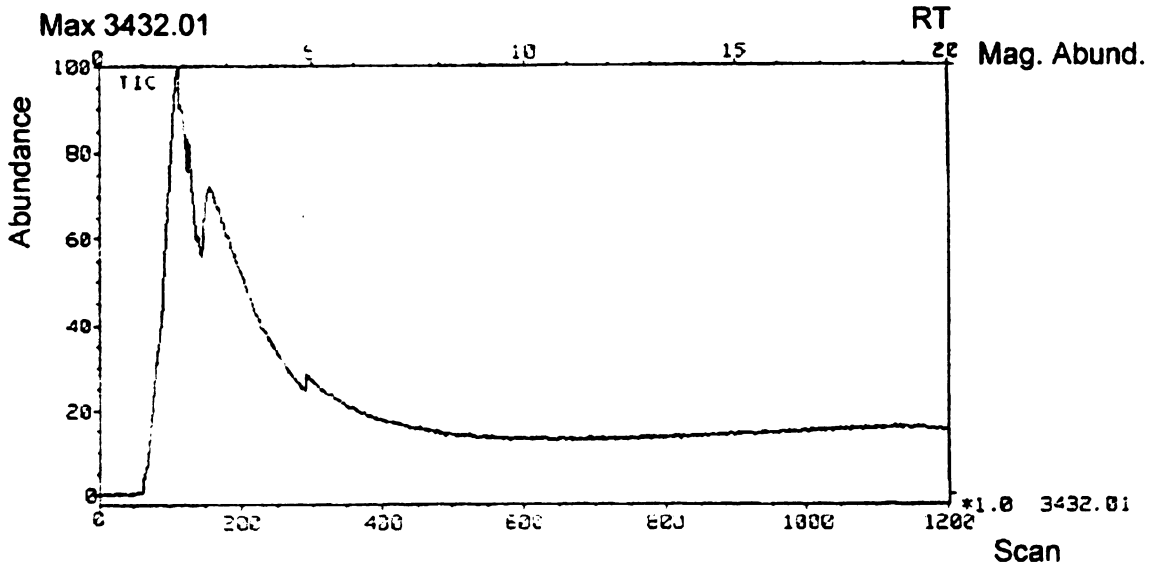


Figure 5.6 RTIC chromatogram of sample CONT in DIP analysis

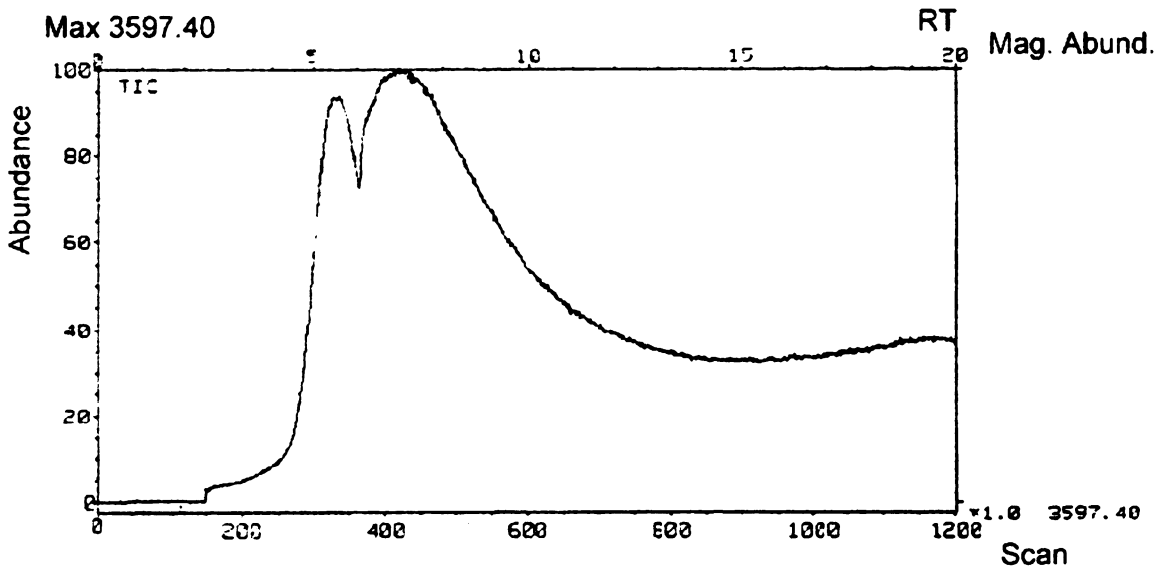


Figure 5.7 RTIC chromatogram of sample PCTA in DIP analysis

Two sets of IIC chromatograms were obtained. One included mass charge ratios (m/z) of 57, 71, 60, 210, 237, 310, 338 and 450 (see Figure 5.8 and 5.9), and the other featured m/z of 173, 239, 266, 357, 620, 634 and 659 (see Appendix 6 and 7). The high m/z values could be the result of high temperatures

the samples were exposed to during the analysis (up to 300°C). More attention should be given to the lower m/z values because they originated from volatile compounds. Direct comparison of the scan numbers cannot be made due to the difference of the starting time in the two scans in Figures 5.6 and 5.7.

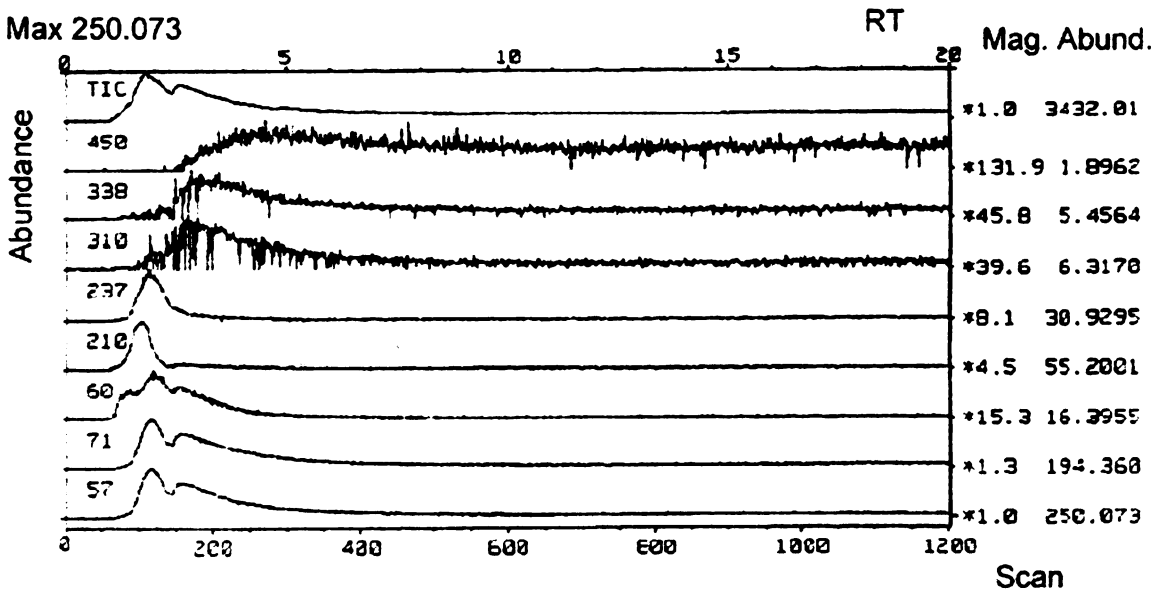


Figure 5.8 IIC chromatogram of sample CONT in DIP analysis (set 1)

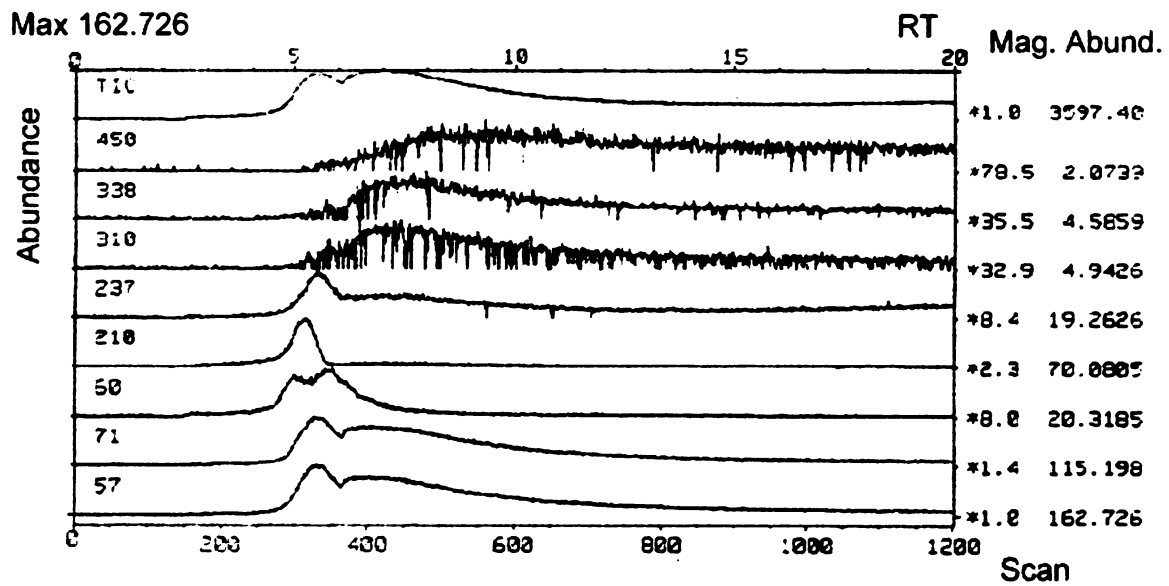


Figure 5.9 IIC chromatogram of sample PCTA in DIP analysis (set 1)

Comparing the first set of IIC chromatograms of samples CONT and PCTA, it was noticed that the elution time of individual m/z value was generally shorter for CONT. For both samples CONT and PCTA, the IIC profile of m/z 60 was different from those of m/z 57 and 71, indicating that m/z 60 might have originated from different compound(s) while m/z 57 and 71 could be fragment ions from the same source compound(s). This was true for m/z 210 and 237 as well, indicating these two fragment ions were from different source compounds.

All these indicated that compositions of the odor systems generated from sample CONT and PCTA were different from each other.

The presence of alkanes with various chain lengths in the odor system from sample CONT was confirmed in the mass spectra of scans number 117, 155, and 181 (see Figure 5.10, 5.11, and 5.12). These mass spectra all showed low mass ion clusters of m/z 57, 71, 85, and 99, which were separated from each other by a mass of CH_2 group (m/z 14) and among which the base peak happened at m/z 57 ($C_4H_9^+$). Lots of molecular ions (even m/z ions) were detected. For example, m/z 310 in the mass spectrum of scan number 155 could be the $C_{22}H_{46}^+$ molecular ion. The presence of ion m/z 210 in the mass spectrum of scan number 117 was considered unusual because it could not be a molecular ion of alkane, which indicated that secondary fragmentation could have occured.

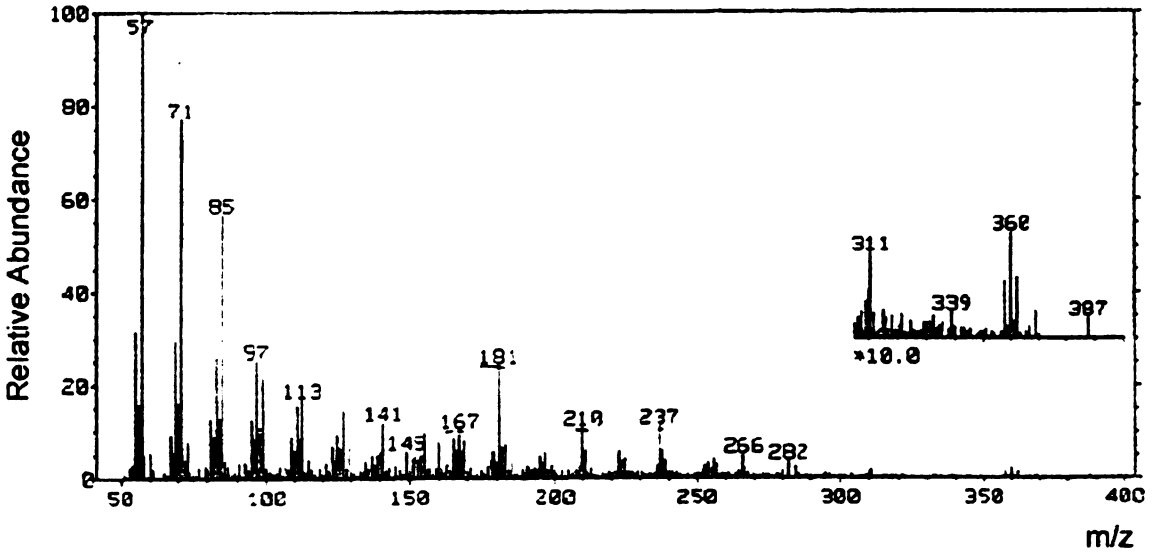


Figure 5.10 Mass spectrum of scan #117 of sample CONT in DIP analysis

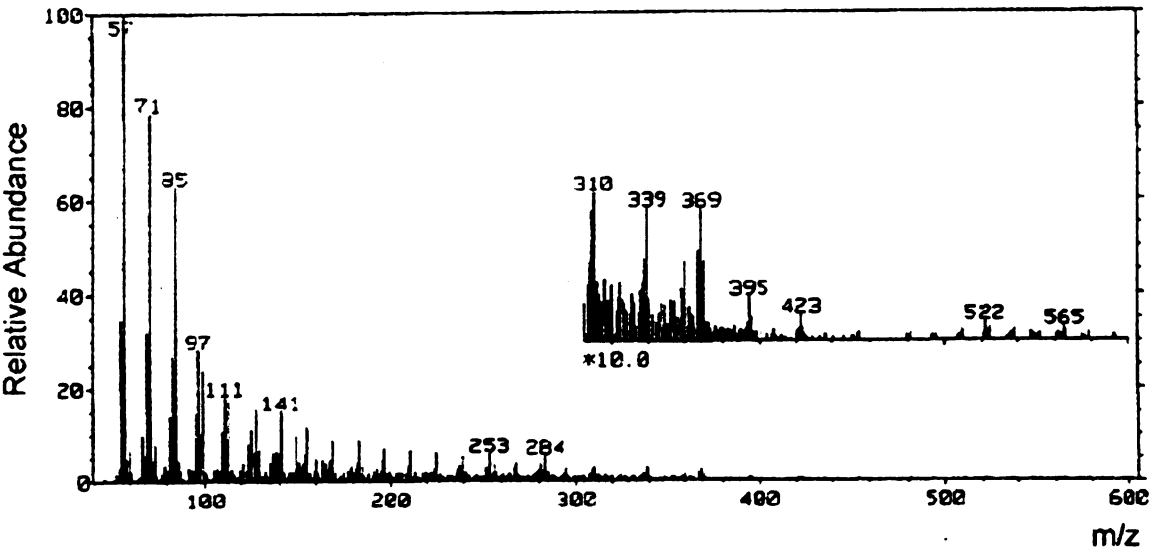


Figure 5.11 Mass spectrum of scan #155 of sample CONT in DIP analysis

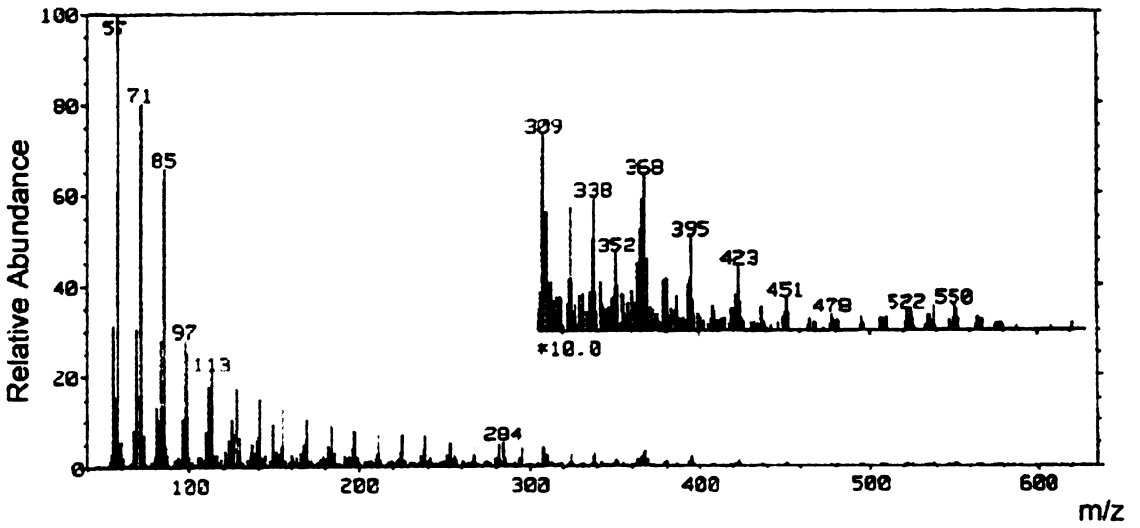


Figure 5.12 Mass spectrum of scan #181 of sample CONT in DIP analysis

For sample PCTA, the mass spectrum of scan number 196 (see Figure 5.13) had a base peak at m/z 181 while the mass spetra of both scan number 916 and 1163 (see Figure 5.14 and 5.15) had a base peak at m/z 256 and all other mass spectra had a base peak at m/z 57, indicating that these fragment ions originated from non-alkane volatile compounds. In the mass spectra of scan number 613, 660, 916 and 1163 (see Figure 5.16 and 5.17), the abundances of m/z 239 and m/z 256 were unusually high, which were not considered characteristic of the mass spectra of alkanes either.

All these observations pointed out that the volatiles released from sample PCTA were different from those from sample CONT in terms of both composition and concentration, resulting in different characteristic fragment ions in the mass spectra in the DIP analysis.

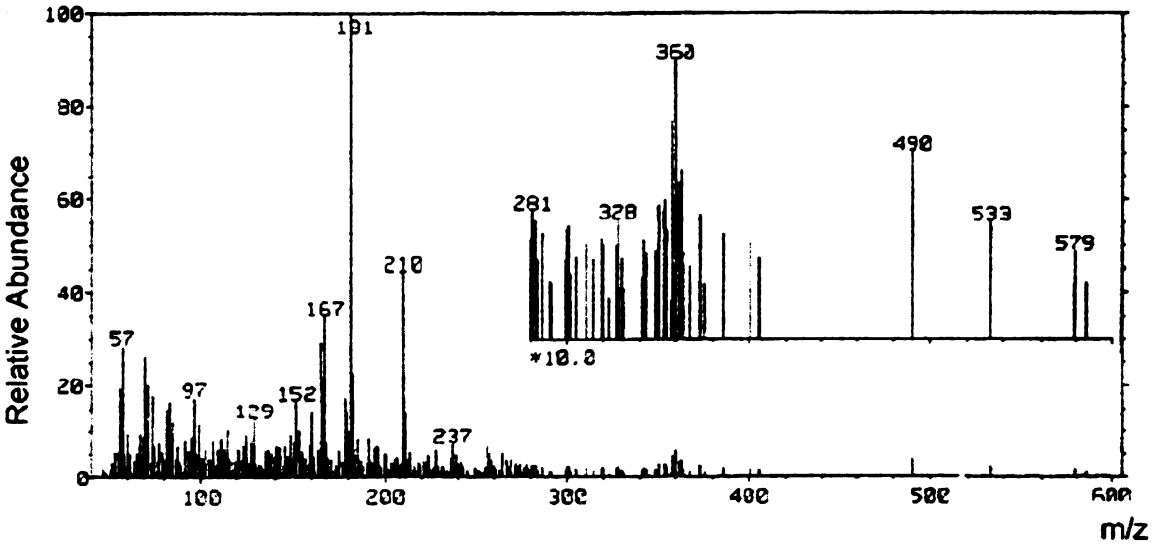


Figure 5.13 Mass spectrum of scan # 196 of sample PCTA in DIP analysis

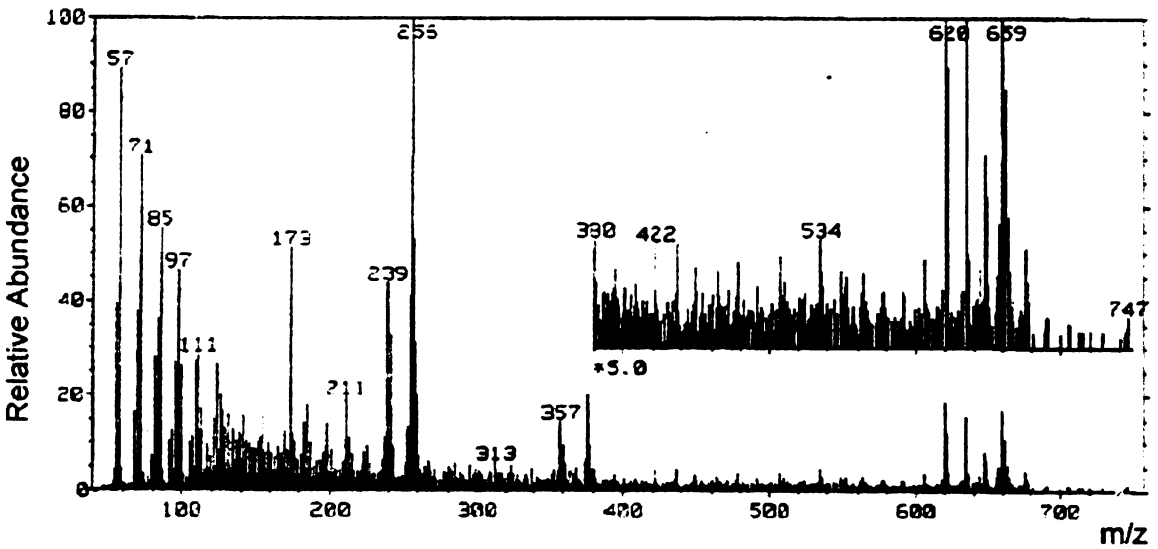


Figure 5.14 Mass spectrum of scan # 916 of sample PCTA in DIP analysis

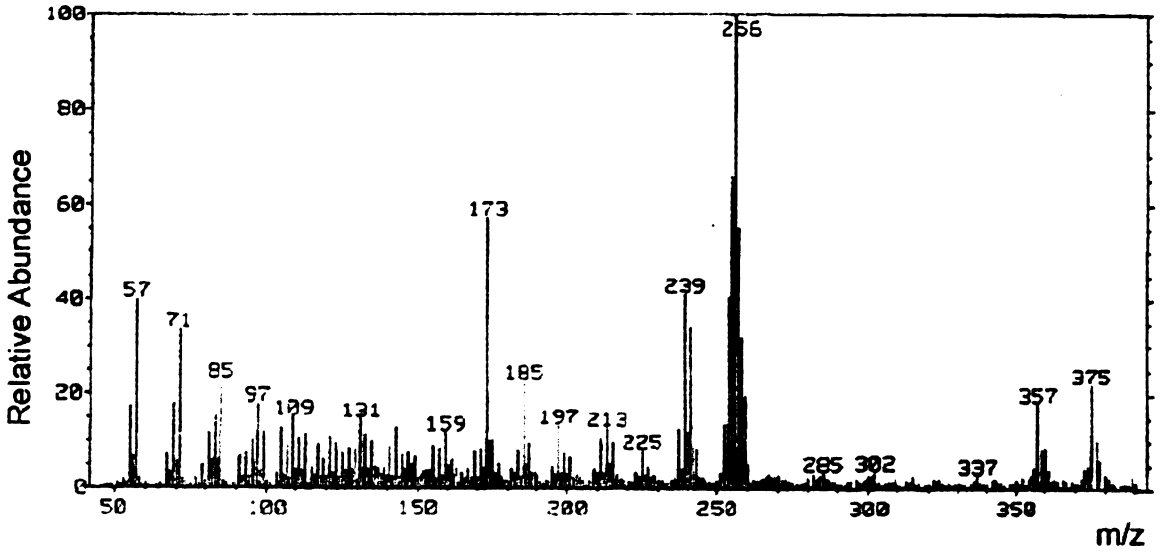


Figure 5.15 Mass spectrum of scan # 1163 of sample PCTA in DIP analysis

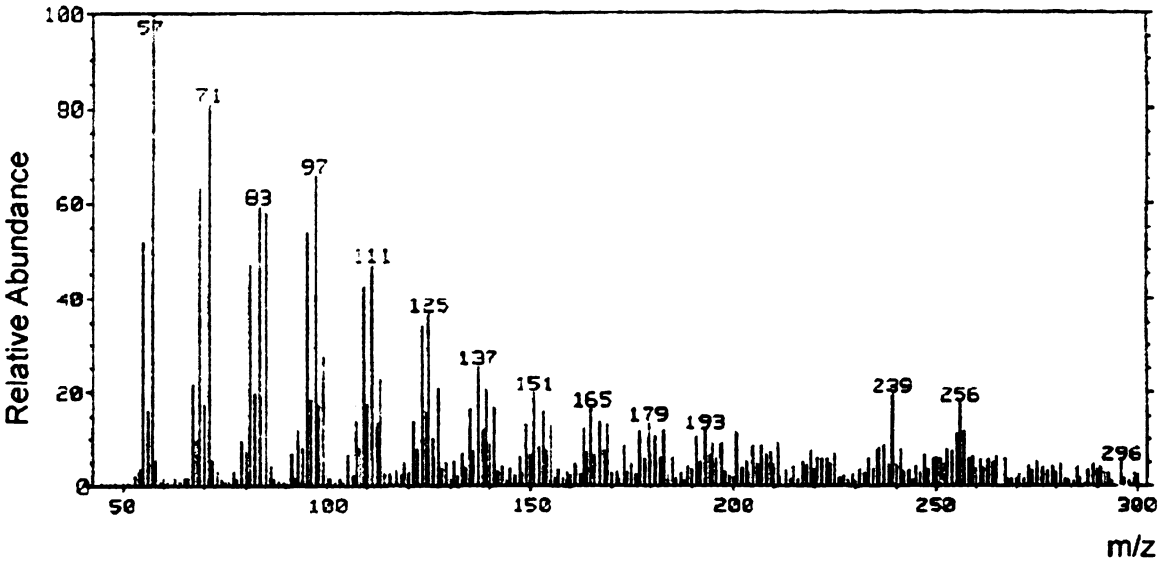


Figure 5.16 Mass spectrum of scan # 613 of sample PCTA in DIP analysis

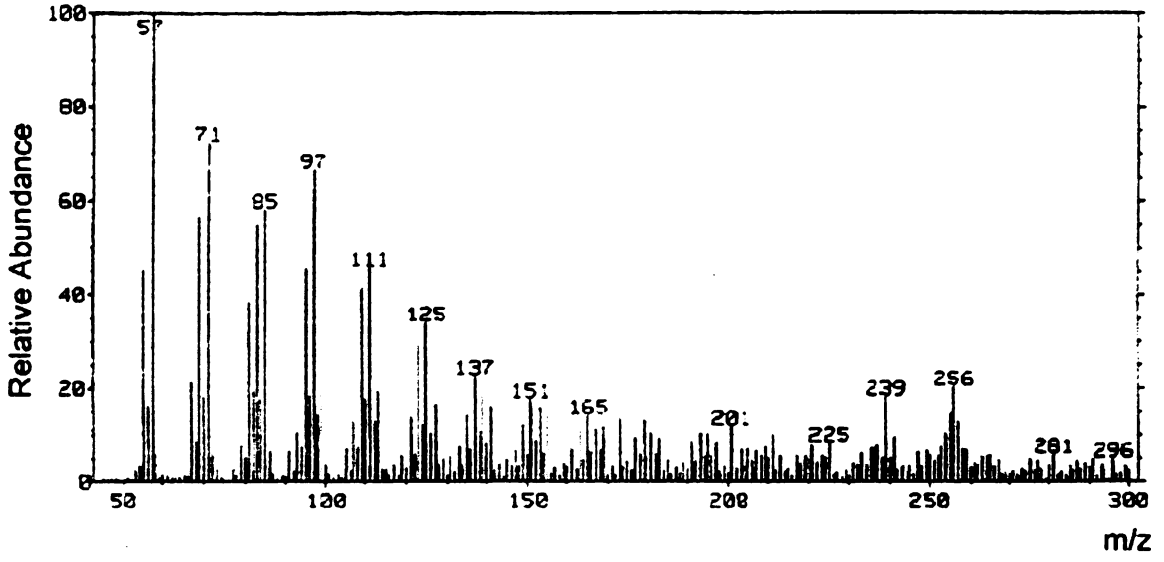


Figure 5.17 Mass spectrum of scan # 660 of sample PCTA in DIP analysis

Selection of Thermal Desorption Tube

Two types of thermal desorption tubes, Carbotrap 400 and Carbotrap 300, were used to collect and concentrate volatile compounds in the headspace of the HDPE film samples. Based on the comparisons of their performance and the repeatability of the results, one of the two tubes would be selected for the subsequent analysis.

Table 5.1 lists the parameters used in the DH-TD and GC analysis in order to choose the appropriate thermal desorption tube.

Table 5.1 DH-TD and GC analysis parameters

Dynamic headspace preparation	
Helium flow rate	40 cc/min
Temperature and time	100^{0}C for 20 min
Sample size	2 strips of 10" x 1" HDPE films
Thermal desorption (path B)	
Helium flow rate	6 cc/min
Temperature and time (for Carbotrap 400)	310^{0}C for 8 min
Temperature and time (for Carbotrap 300)	260^{0}C for 6 min
Desorption tube recovery (path A)	
Helium flow rate	2.5 cc/min
Temperature and time (for Carbotrap 400)	320^{0}C for 25 min
Temperature and time (for Carbotrap 300)	350^{0}C for 60 min
Other temperatures	
Transfer line	290^{0}C
Valve	250^{0}C
GC	
Helium flow rate	1.0 cc/min
Detector (FID)	300^{0}C
Temperature (for Carbotrap 400)	40^{0}C for 5 min, increased at 5^{0}C/min to 220^{0}C and then kept for 10 min
Temperature (for Carbotrap 300)	40^{0}C for 8 min, increase at 10^{0}C/min to 100^{0}C and then at 6^{0}C/min to 220^{0}C, kept for 5 min

Table 5.2 lists the results of the DH-TD GC analysis when Carbotrap 400 was used to collect volatile compounds generated from analyzing triplicates of sample CONT.

Table 5.2 Major peaks identified for sample CONT in the DH-TD GC analysis with Carbotrap 400

Replicate	Total number of identified peaks	RT (min) of major peaks*	Area (%) of the peaks
1	77	12.216	1.79
		13.774	1.89
		16.707	20.06
		19.169	31.69
		21.565	2.44
2	83	18.877	2.03
		24.559	20.97
		29.585	32.45
		33.950	6.72
3	74	18.851	2.46
		24.483	18.13
		29.500	29.34
		33.785	2.52

* Peaks whose percentage of area was more than 1%.

Results in Table 5.2 showed a low repeatability in terms of the distribution and intensity of peaks obtained in the gas chromatography analyses. Repeatability is important in the DH-TD GC analysis because it is the first step and the basis of the separation and identification of volatile compounds.

Many factors affect the results of DH-TD GC analysis, one of which is the selection of the appropriate thermal desorption tube.

Carbotrap 400 is usually suitable for analyzing aqueous samples while Carbotrap 300 is for gaseous samples (Supelco, 1998a). They consist of different adsorbent materials with different physical characteristics such as mesh

size, surface area, and pore size (see Figure 5.1 and 5.2). Carbotrap F in Carbotrap 400 has a larger pore size and thus a smaller surface area, which makes it suitable for trapping larger molecules. Similarly, the Carboxen-569 used in Carbotrap 400 has a larger mesh size and thus smaller particle size than the Carbosieve used in Carbotrap 300, making the former more suitable for applications involving larger molecules. The use of adsorbents designed to capture higher carbon-chain analytes, along with the fact that aqueous samples are more likely to have higher concentrations of the larger compounds than air samples, makes Carbotrap 400 better suited for aqueous samples.

Volatile compounds in the headspace generated from HDPE film samples were to be studied and thus Carbotrap 300 seemed a better candidate for the thermal desorption process.

Tables 5.3 to 5.7 list the major peaks and their area percentages for sample CONT, PATA, PBTA, PBTB, and PCTA when Carbotrap 300 was used.

Table 5.3 Main peaks and their area percentages of sample CONT in DH-TD analysis using Carbotrap 300

Component	Replicate 1		Replicate 2		Replicate 3	
	RT (min)	Area (%)	RT (min)	Area (%)	RT (min)	Area (%)
1	6.635	1.06	6.834	0.29	6.495	0.46
2	10.730	0.67	10.803	0.33	10.688	0.26
3	12.525	0.11	12.557	0.09	12.507	1.10
4	13.211	0.98	13.233	0.63	13.195	0.61
5	14.535	0.21	14.555	0.17	14.536	0.20
6	15.182	2.17	15.186	1.09	15.169	0.63
7	16.479	0.19	16.472	0.14	16.467	0.09
8	17.141	3.87	17.140	3.58	17.130	3.25
9	21.282	5.98	21.282	5.47	21.281	5.93
10	25.341	2.81	25.344	3.07	25.344	3.07
11	29.134	0.26	29.132	0.32	29.129	0.34
12	32.706	0.16	32.675	0.17	32.683	0.22

Table 5.4 Main peaks and their area percentages of sample PATA in DH-TD analysis using Carbotrap 300

Component	Replicate 1		Replicate 2		Replicate 3	
	RT (min)	Area (%)	RT (min)	Area (%)	RT (min)	Area (%)
1	5.898	1.46	6.016	0.27	6.010	1.26
2	10.491	0.85	10.522	0.67	10.524	0.58
3	12.420	0.21	12.429	0.19	12.429	0.18
4	13.127	1.84	13.134	1.90	13.089	1.04
5	14.496	0.33	14.498	0.32	14.499	0.29
6	15.135	1.50	15.134	1.28	15.135	0.84
7	16.514	0.23	16.561	0.49	16.578	0.56
8	17.105	3.13	17.100	3.67	17.097	2.77
9	21.264	4.80	21.259	5.58	21.259	5.40
10	22.919	1.28	22.911	0.82	22.915	1.49
11	23.554	6.29	23.542	2.24	23.592	2.95
12	25.329	3.04	25.327	4.53	25.335	5.93
13	29.119	0.52	29.116	1.03	29.125	1.75
14	32.696	0.47	32.680	0.47	32.690	0.58

Table 5.5 Main peaks and their area percentages of sample PBTA in DH-TD analysis using Carbotrap 300

Component	Replicate 1		Replicate 2		Replicate 3	
	RT (min)	Area (%)	RT (min)	Area (%)	RT (min)	Area (%)
1	6.299	0.36	6.026	0.93	6.015	1.03
2	10.617	0.53	10.539	0.46	10.535	0.43
3	12.473	0.14	12.440	0.15	12.439	0.12
4	13.094	0.72	13.092	0.89	13.109	1.11
5	13.166	0.76	13.145	0.69	13.146	0.67
6	14.518	0.27	14.506	0.26	14.505	0.23
7	14.748	0.33	14.760	0.54	14.790	0.57
8	15.153	0.91	15.146	0.86	15.147	0.98
9	16.544	0.36	16.599	0.63	16.647	0.84
10	17.107	2.50	17.112	2.25	17.113	3.05
11	21.271	6.68	21.255	4.42	21.254	3.70
12	22.917	0.67	22.910	0.60	22.910	0.35
13	23.597	2.50	23.592	3.04	23.583	0.75
14	25.341	6.61	25.320	2.93	25.319	2.73
15	29.126	1.60	29.116	0.51	29.119	0.58
16	32.701	0.59	32.679	0.34	32.705	0.27

Table 5.6 Main peaks and their area percentages of sample PBTB in DH-TD analysis using Carbotrap 300

Component	Replicate 1		Replicate 2		Replicate 3	
	RT (min)	Area (%)	RT (min)	Area (%)	RT (min)	Area (%)
1	5.990	0.69	5.957	0.06	5.805	0.16
2	10.525	0.35	10.508	0.31	10.470	0.25
3	12.427	0.09	12.421	0.09	12.407	0.08
4	13.010	0.57	12.996	0.58	13.002	0.63
5	13.135	0.56	13.131	0.51	13.125	0.44
6	14.495	0.16	14.490	0.13	14.489	0.11
7	14.716	0.30	14.710	0.37	14.737	0.39
8	15.133	0.39	15.129	0.36	15.130	0.38
9	16.547	0.34	16.535	0.53	16.597	0.66
10	16.926	0.06	16.943	0.25	16.944	0.24
11	17.098	1.72	17.095	1.64	17.100	1.51
12	21.240	3.06	21.236	3.01	21.240	2.39
13	22.898	0.32	22.894	0.26	22.901	0.17
14	23.587	4.03	23.575	2.43	23.580	1.50
15	25.307	2.37	25.309	3.09	25.316	2.86
16	27.473	2.06	27.474	1.95	27.480	1.63
17	27.720	0.62	27.718	0.59	27.739	0.50
18	27.886	0.66	27.886	0.60	27.890	0.53
19	28.025	1.05	28.015	1.09	28.020	0.92
20	28.289	3.70	28.285	3.61	28.291	3.29
21	28.429	1.27	28.427	1.21	28.441	1.06
22	28.575	1.65	28.573	1.66	28.580	1.49
23	28.728	2.38	28.722	2.37	28.725	2.17
24	28.890	1.12	28.887	1.15	28.898	1.06
25	29.011	0.73	29.005	0.66	29.015	0.57
26	29.129	1.12	29.132	1.66	29.148	1.71
27	29.390	4.20	29.385	4.20	29.393	3.99
28	29.554	1.42	29.555	1.56	29.556	1.38
29	29.808	2.94	29.805	3.16	29.810	3.21
30	30.080	2.69	30.081	3.17	30.095	3.22
31	30.430	1.43	30.422	1.64	30.438	1.65
32	30.725	0.73	30.719	0.90	30.729	0.95
33	30.817	0.46	30.804	0.54	30.813	0.57
34	31.029	0.75	31.028	1.01	31.038	1.08
35	31.386	0.48	31.393	0.66	31.401	0.73
36	31.590	0.35	31.585	0.45	31.594	0.48
37	31.812	0.11	31.812	0.17	31.818	0.18
38	32.703	0.28	32.684	0.39	32.699	0.34

Table 5.7 Main peaks and their area percentages of sample PCTA in DH-TD analysis using Carbotrap 300

Component	Replicate 1		Replicate 2		Replicate 3	
	RT (min)	Area (%)	RT (min)	Area (%)	RT (min)	Area (%)
1	1.940	2.30	1.958	13.65	/	/
2	6.296	0.36	6.248	0.04	6.408	2.96
3	10.719	0.31	10.682	0.13	10.654	0.73
4	13.190	0.70	13.179	0.36	13.185	1.76
5	14.533	0.18	14.531	0.11	14.553	0.26
6	15.160	1.15	15.153	0.41	15.163	2.36
7	16.235	0.13	16.232	0.08	16.248	0.31
8	17.100	2.67	17.095	1.79	17.116	3.22
9	18.218	5.10	18.215	5.23	18.234	4.05
10	19.782	0.74	19.781	0.51	19.799	0.58
11	21.262	6.30	21.255	4.11	21.270	5.86
12	22.669	2.11	22.667	1.65	22.682	1.32
13	22.925	1.32	22.920	0.93	22.939	0.87
14	23.380	0.48	23.376	0.36	23.387	0.43
15	23.553	7.79	23.545	4.63	23.559	2.14
16	25.335	5.52	25.325	3.76	25.345	6.48
17	29.132	2.21	29.125	1.45	29.143	2.37
18	32.710	0.66	32.708	0.30	32.700	0.07

The chromatograms of the five HDPE film samples were different from one another in terms of retention times of the main peaks and their relative abundance expressed as area percentage. Interestingly, sample PBTB seemed have the most complex volatile profile, even though sample PCTA was perceived as having the strongest odor by the sensory panel.

By comparing the GC results with the sensory evaluation results, it proved that the complexity of a volatile system is not always directly correlated to the intensity of the perceived odor, probably because some volatile compounds were not odorous or their concentrations were below their threshold values.

DH-TD GCD (GC/MS) Analysis

Table 5.8 listed the parameters used in the dynamic headspace and thermal desorption coupled with gas chromatograph and mass spectrometry.

Table 5.8 DH-TD and GC-MS analysis parameters

Dynamic headspace preparation	
Thermal desorption tube	Supelco Carbotrap 300
Helium flow rate	40 cc/min
Temperature and time	100^{0}C for 20 min
Sample size	2 strips of 10" x 1" HDPE films
Thermal desorption (path B)	
Helium flow rate	6 cc/min at 60 psi
Temperature and time	260^{0}C for 6 min
Desorption tube recovery (path A)	
Helium flow rate	2.5 cc/min
Temperature and time (for Carbotrap 300)	350^{0}C for 60 min
Other temperatures	
Transfer line	290^{0}C
Valve	250^{0}C
GC	
Helium flow rate	1.0 cc/min at 7.1 psi
Split-less time	2 min
Injection port temperature	300^{0}C
Detector (EI mass quadrapole detector)	300^{0}C
Mass range	45 – 450 m/z
Temperature (for Carbotrap 300)	40^{0}C for 8 min, increase at 10^{0}C/min to 100^{0}C and then at 6^{0}C/min to 220^{0}C, kept for 5 min

Triplicates of each HDPE film were analyzed (CONT, PATA, PBTA, PBTB, and PCTA) with the DH-TD couple with the GCD system (GC/MS).

Repeatability of DH-TD/GCD Analyses

To investigate the repeatability of the DH-TD/GCD analyses, triplicates of sample PATA and of sample PBTA were analyzed with clean thermal desorption tubes (confirmed with blank runs to ensure the tubes were clean). The results are shown in Figures 5.18 and 5.19. Chromatograms showed that the TIC profiles of the triplicates were similar but the peak abundances varied significantly.

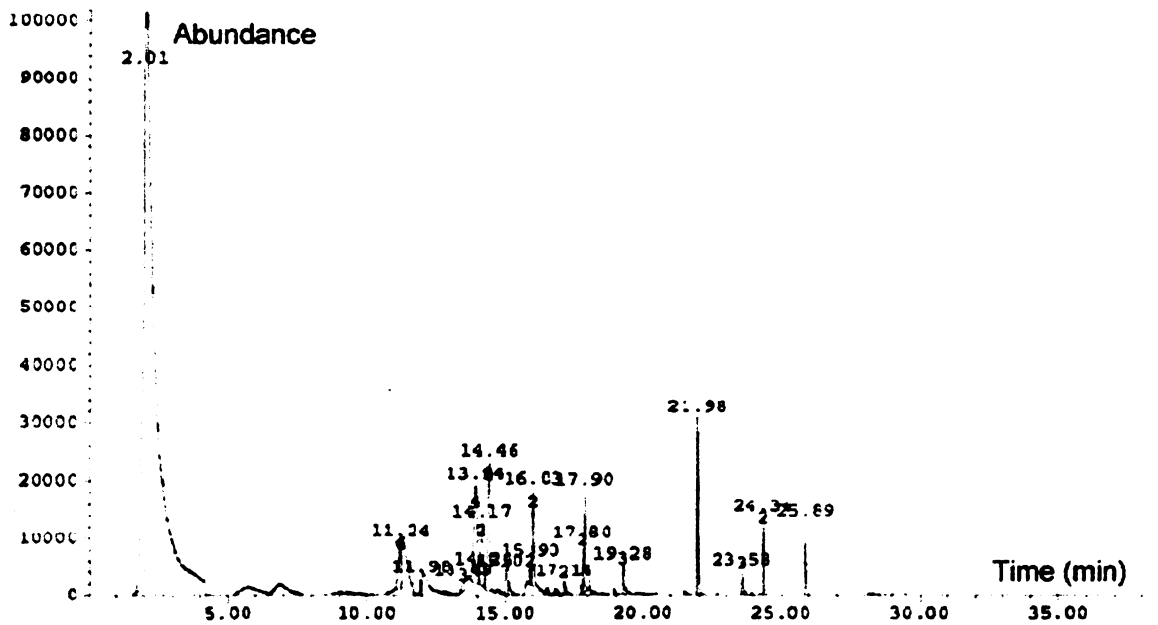
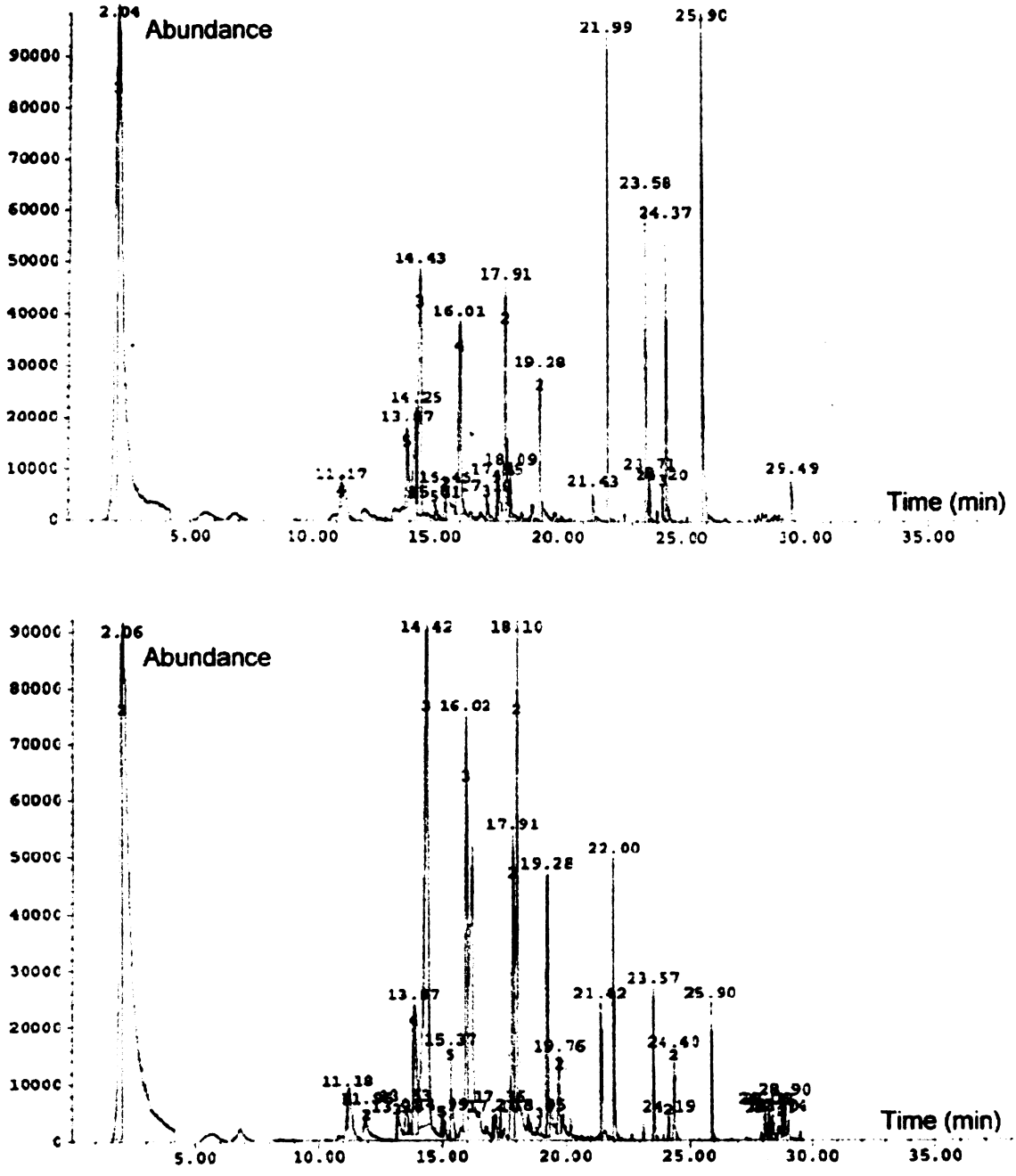


Figure 5.18 Triplicates of TIC chromatogram of sample PATA using DH-TD/GCD analysis

Figure 5.18 (Cont'd).



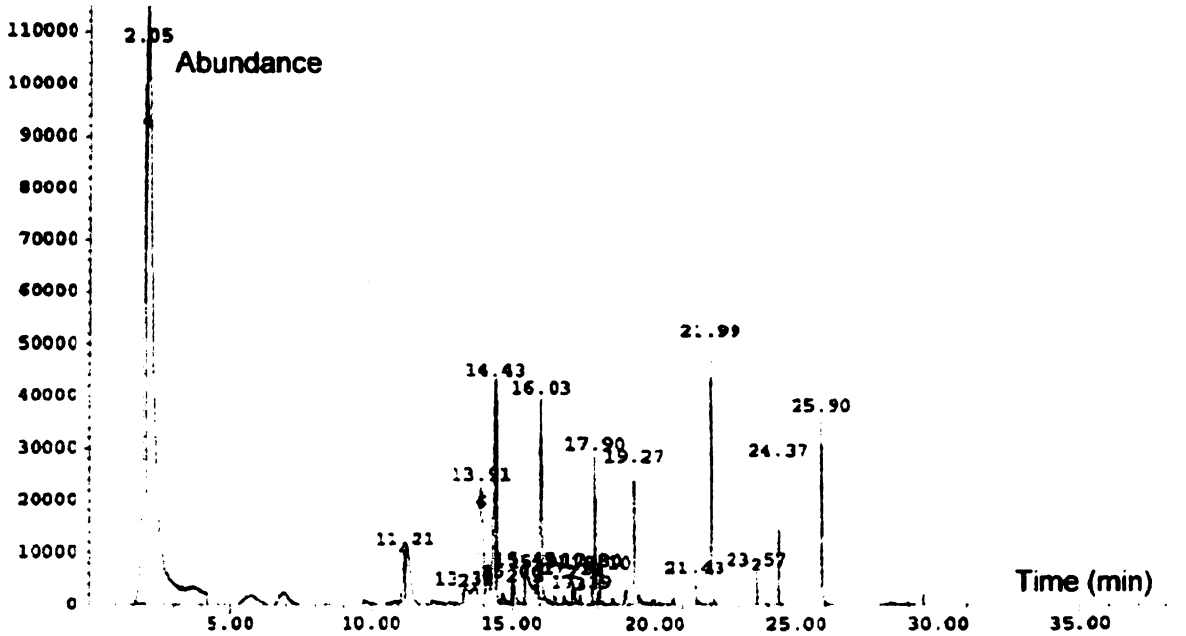


Figure 5.19 Triplicates of TIC chromatogram of sample PBTA using DH-TD/GCD analysis

Figure 5.19 (Cont'd).

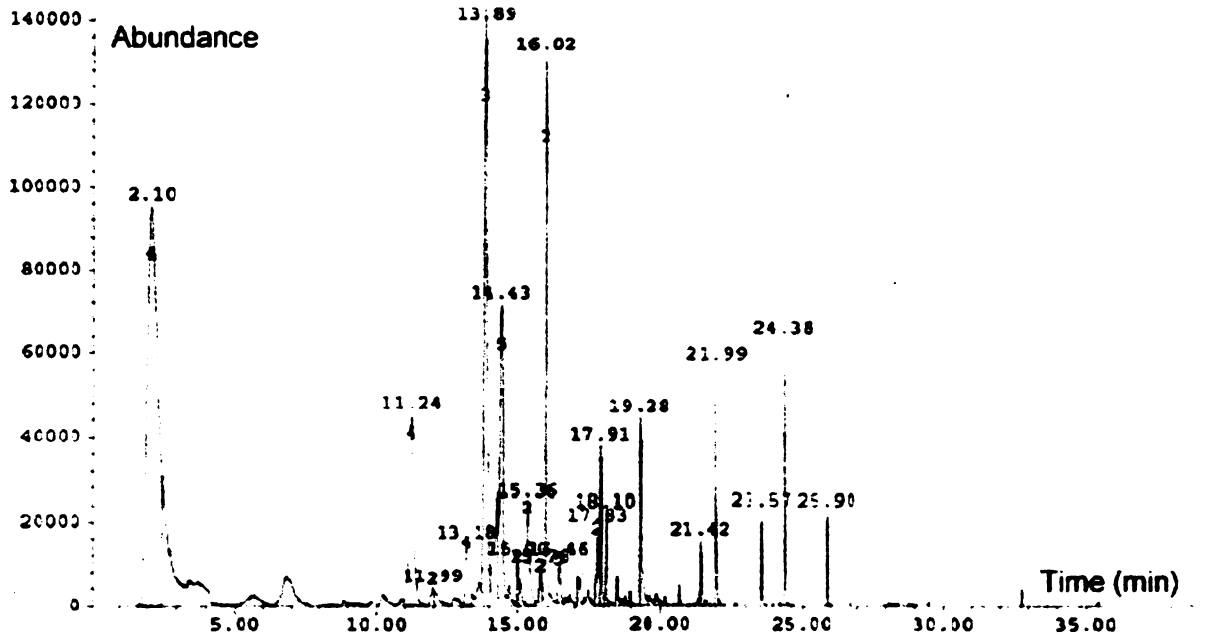
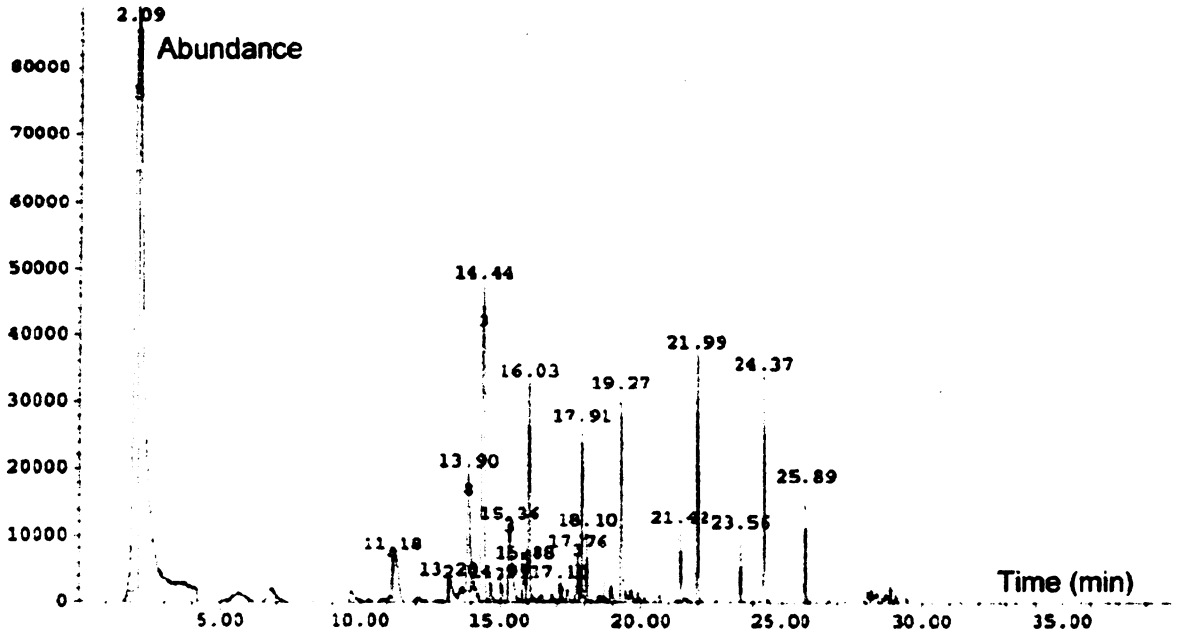


Figure 5.19 (Cont'd).



The low repeatability of the analysis happened for several possible reasons. The transfer line of the TDU was recommended to enter the GC oven via an access hole and be connected to the capillary column through the stainless steel union (see Figure 5.3). Making the transfer line go through the injection port was not suggested due to the dead volume of the injection port and poor heat transfer (Dynatherm, 1989).

Secondly, two carrier flows enter into the top of the capillary column with the modified connection instead of one single carrier gas source as displayed in Figure 2.5. In the modified connection, one flow came from the transfer line of the TDU, which went into the injection port of the GC through the custom-made connector (see Figure 5.4), and the other flow came from the carrier gas for the GC itself. The two carrier gas flows met and collided with each other inside the

injection port of the GC, which could have affected the consistency of the carrier gas flow and thus the transfer of volatile compounds to the GC column.

Moreover, the split-less injection mode on the GCD system might have affected the repeatability of the results as well. The split-less time is the time at the beginning of an injection when there is no flow out of the injection port vent, during which the entire injected sample is vaporized and allowed to flow onto the GC column. Because the transfer line entered the GC via the injection port and all thermally-desorbed volatiles were carried over to the GC column from the injection port, the split-less mode and its time setting took over the purge time of the TDU. In the other words, the injection port vent would be turned to open after the set split-less time (maximum 2 minutes), which was controlled by the ChemStation software solely and could not be changed by the control panel on the GC. Even though the purge time of the TDU was set to allow the desorption tube to be heated and flushed with carrier gas for 6 minutes, the effective purge time of the TDU was actually decreased from 6 minutes to 2 minutes. Volatile compounds that were not released from the thermal desorption tube or did not travel to the GC injection port within 2 minutes after the GCD system was started, would have been flushed out of the injection port vent and could not reach the GC column for analysis.

Figure 5.20 and 5.21 showed the effect of split-less time on the TIC chromatograms of sample CONT and PCTA.

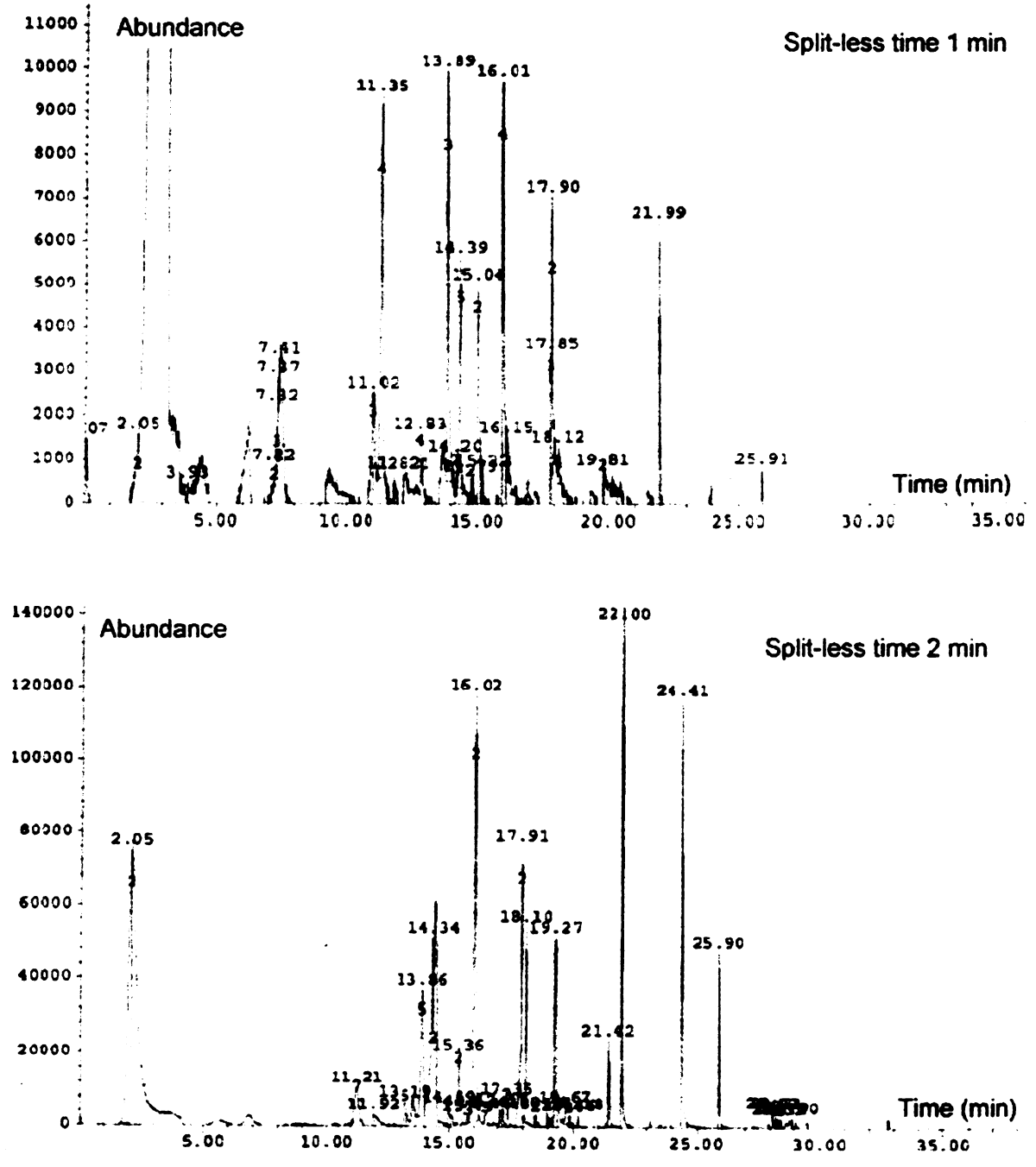


Figure 5.20 Effect of split-less time on TIC chromatogram profiles of sample CONT in DH-TD/GCD analysis

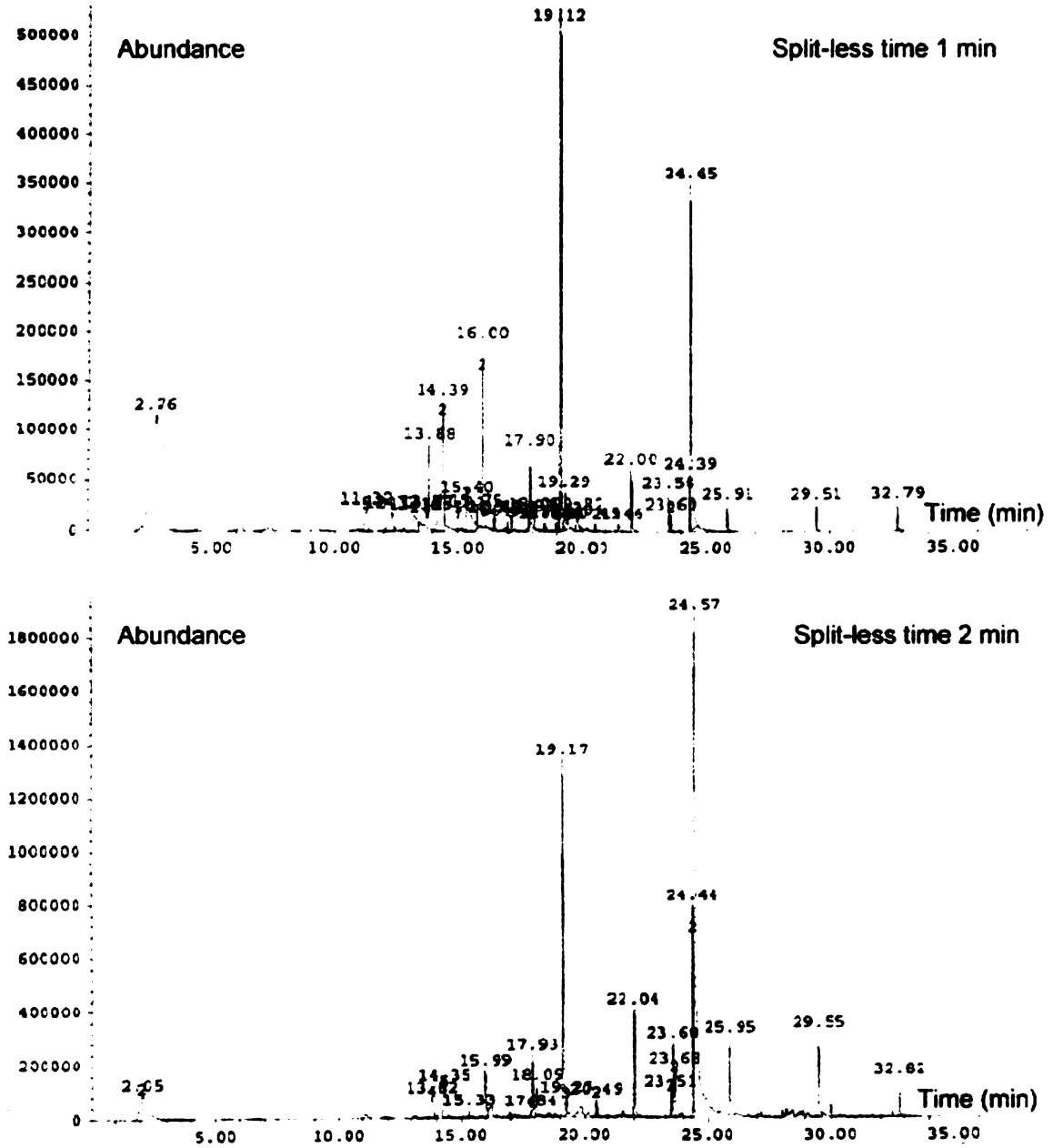


Figure 5.21 Effect of split-less time on TIC chromatogram profiles of sample PCTA in DH-TD/GCD analysis

Figure 5.22 was the TIC chromatogram of sample PBTB from the DH-TD/GCD analysis.

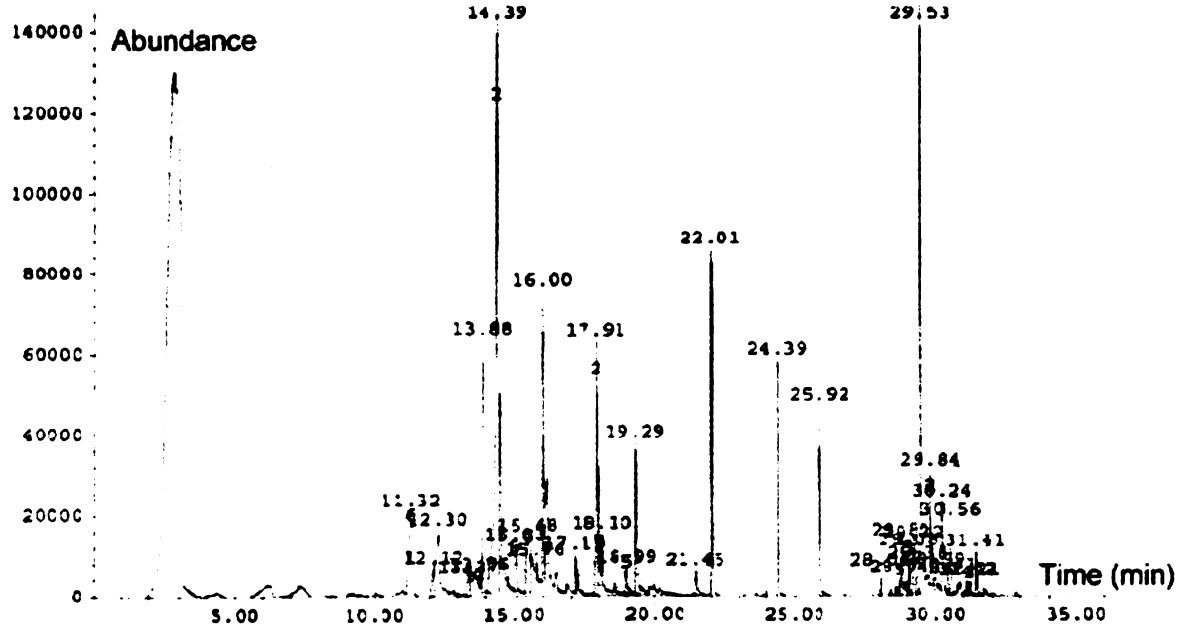


Figure 5.22 TIC chromatogram of sample PBTB from DH-TD/GCD analysis

Potential Identities of Volatile Compounds

Based on the chromatograms of the DH-TD/GCD analyses for sample CONT, PATA, PBTA, PBTB, and PCTA, identifications of detected peaks were made by matching the spectrum of each peak with the build-in mass spectra library. Several groups of aromatic and non-aromatic compounds, including aldehydes, alcohols, aromatics, and hydrocarbons were found for each sample. The results are listed in Table 5.9.

Table 5.9 Potential Identities of volatile compounds from the DH-TD/GCD analyses

Sample	CONT	PATA	PBTA	PBTB	PCTA
Aldehydes					
Heptanal	√	√	√	√	√
Butenal	×	×	×	√	×
Octanal	√	√	√	√	√
Nonanal	√	√	√	√	√
Decanal	√	√	√	√	√
Octadecanal	√	×	×	×	×
Alcohols					
Octanol	√	√	√	√	√
Aromatics					
Benzene, 1,3-bis(1,1-dimethylethyl)	×	×	×	×	√
Phenol, 2,4-bis(1,1-dimethylethyl)	√	√	×	×	√
Hydrocarbons					
Dodecane	√	√	√	√	√
Tetradecane	√	√	√	√	√
Hexadecane	√	√	×	√	×
Heptadecane	×	√	√	×	√

The compound benzene, 1,3-bis(1,1-dimethylethyl)benzene was only detected in sample PCTA. Earlier sensory tests found sample PCTA had a stronger odor compared to the other four samples. A hypothesis that this particular compound was responsible for the stronger odor of sample PCTA could thus be made.

However, caution must be taken when making such a hypothesis considering the low repeatability of the DH-TD/GCD analysis. Therefore, further investigation was necessary.

CHAPTER 6 ANALYSIS OF VOLATILES USING SPME COUPLED WITH GC-MS

INTRODUCTION AND OBJECTIVES

In Chapter 5, we used dynamic headspace and thermal desorption techniques to concentrate volatile compounds generated from the HDPE film samples. The parameters were optimized but the low repeatability problem could not be solved due to the connection between the thermal desorption unit and the GC-MS, and the split-less injection mode on the GC.

However, one particular compound, 1,3-bis(1,1-dimethylethyl)-benzene, was found in sample PCTA only, which was then hypothesized to be responsible for the stronger odor of the sample.

This chapter reports on utilization of another sample preparation technique, solid-phase micro-extraction, or SPME, to concentrate volatile compounds and improve the repeatability of the results.

Moreover, the human nose was to be utilized to detect and describe the odor profile generated from adhesive-coated HDPE film samples by using an ODO II sniffing unit. The ODO II unit works by splitting the eluted flow from the capillary column of a GC to two parts, one of which goes to a sniffing port, where

a human nose helps detect problematic odor, and another goes to mass spectrometry for identification.

The goal of this component of this research was to break down the odor system of the HDPE film sample into individual components with GC, and then investigate each component's contribution to the odor system with the ODO II sniffing unit, making it easier to determine the objectionable volatile compound(s).

MATERIALS AND METHODS

Three SPME fibers were considered: 100 µm PDMS, 65 µm PDMS/DVB, and 75 µm CAR/PDMS (Supelco, Bellefonte, PA). PDMS fibers are non-polar fibers and preferred for adsorbing non-polar compounds, while both PDMS/DVB and CAR/PDMS fibers are bi-polar fibers and suitable for polar volatiles. Kanavouras (2003) compared the effectiveness of the 100 µm PDMS fiber and the 65 µm PDMS/DVB fiber in his study of volatiles originating from virgin and oxidized olive oil samples. The PDMS/DVB fiber was determined to be able to adsorb more volatile compounds in his study. Chung (2004) compared the 65 µm PDMS/DVB and the 75 µm CAR/PDMS fibers in her study and concluded that the 75 µm CAR/PDMS fiber was a better candidate for her applications because it adsorbed more target volatiles at her chosen temperature. Our preliminary study showed the effectiveness of the PDMS/DVB and the CAR/PDMS fibers were comparable to each other, but the CAR/PDMS fiber

tended to have volatile carryover problems. Thus the 65 μm PDMS/DVB SPME fiber was chosen in our study.

Optimization of SPME Parameters

Two strips of HDPE film PCTA (10" x 1") were enclosed in a 20 ml screw top glass vial (Supelco, Bellefonte, PA), which was capped with a Mininert Valve (Supelco, Bellefonte, PA). The red-green pin on the valve can be easily pushed to close the valve during headspace preparation or to open the valve during the sampling process with SPME.

The enclosed glass vial with HDPE film samples was heated at 100^0C in an oven for 20 minutes. These conditions were consistent with the conditions used in the E-nose analysis to generate the headspace.

After 20 minutes of heating, the glass vial was taken out of the oven and transferred to and kept in an air-conditioned room (25^0C) or in a temperature-controlled water bath for 10 minutes, so that the temperature of the headspace was equilibrated with the environmental temperature.

After 10 minutes, the red-green pin on the Mininert Valve was pushed open and the SPME assembly was inserted into the glass vial and the fiber was exposed to the headspace for a pre-specified period of time.

Table 6.1 lists the parameters that were considered in optimizing the SPME method.

Table 6.1 Parameters tested in optimizing SPME method

Parameters	Settings
Headspace generation	100^{0}C for 20 min
Temperature equilibrium time before SPME sampling	10 min
SPME sampling temperature (^{0}C)	25, 35, 45, 60, 75
SPME sampling time (min)	5, 15, 30

Upon completion of the SPME sampling, the SPME assembly was transferred and inserted into the GC injection port, where the SPME fiber was exposed to the high temperature in the injection port to release the adsorbed compounds for 3 minutes. The released volatiles were carried by the helium gas to the top of the GC column, where liquid nitrogen was used to cryo-focus the volatiles before the temperature program of the GC was started.

The GC-MS equipment used in the study was located in the Postharvest Laboratory in the Department of Horticulture at Michigan State University. The gas chromatograph was Agilent 6980 equipped with a HP-5MS fused silica capillary column (30 m in length x 0.25 mm I.D., with a coating of 0.25 μm in thickness). The mass spectrometer was Leco Peasus II ChromaTOF (Time of Flight).

Table 6.2 lists the parameters used in the SPME GC-MS.

Table 6.2 Parameters used in the SPME/GC-MS with constant column gas flow

GC	
Injection mode	Splitless
Injector temperature	220^{0}C
Transfer line temperature	240^{0}C
Temperature program	40^{0}C for 0 min, increased to 240^{0}C at 40^0/min, stayed at 240^{0}C for 3 min.
Column flow	Constant flow at 1 ml/min
Purge flow	10 ml/min
Purge time	10 seconds
MS	
Solvent delay	100 seconds
Mass range	29 – 270 u
Acquisition rate	8 spectra/second

Comparisons of Gas Chromatogram Profiles

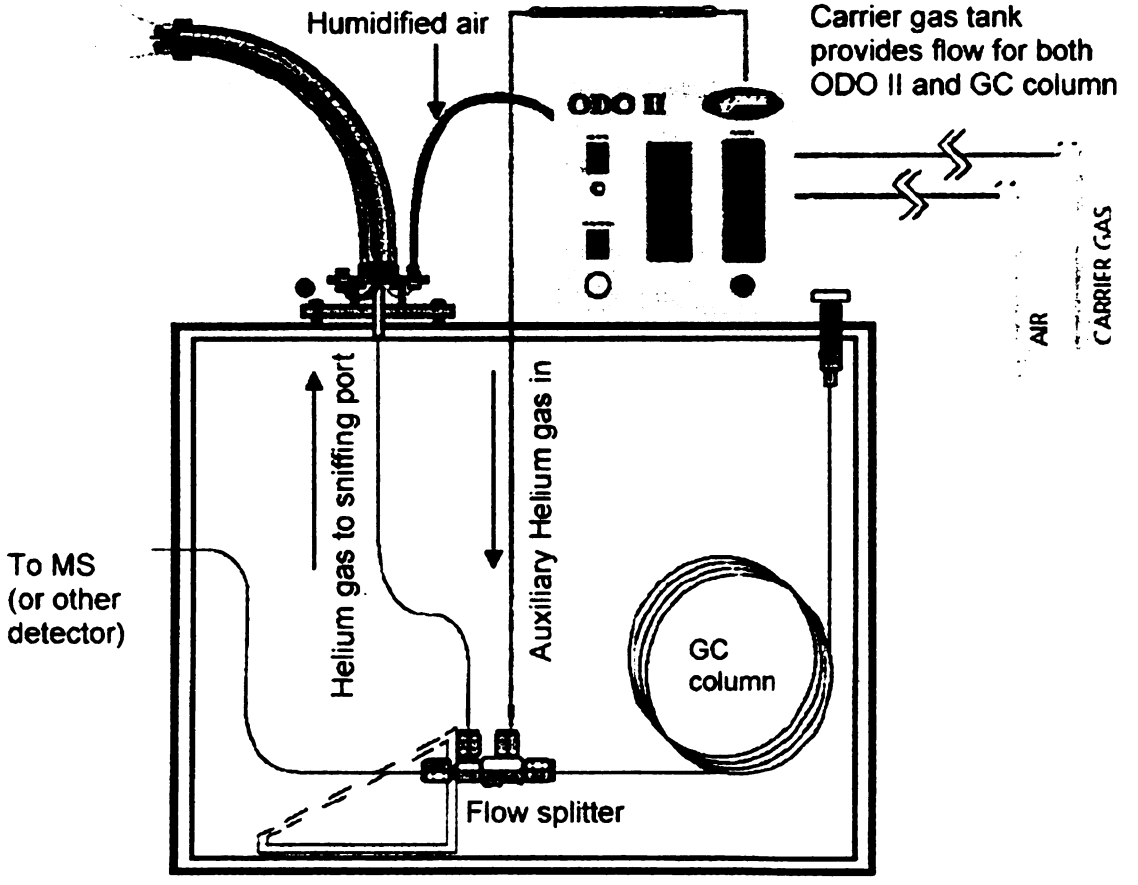
The gas chromatogram profiles of sample CONT, PATA, PBTA, PBTB, and PCTA were compared with one another to help identify the unique compound(s) which could potentially make sample PCTA have a stronger odor compared to other HDPE film samples (based on the results obtained in the sensory evaluation tests reported in Chapter 3).

The HDPE film samples were enclosed in the 20 ml glass vial capped with Supelco Mininert Valve and heated at 100^{0}C in an oven for 20 minutes to generate the volatiles. The enclosed glass vial was then transferred to and kept

in a water bath set up at 60^{0}C for 10 minutes so that the headspace reached the desired temperature. The SPME sampling time was 30 minutes. The SPME GC-MS parameters are listed in Table 6.2.

Description of Odor Profile with Sniffing Test

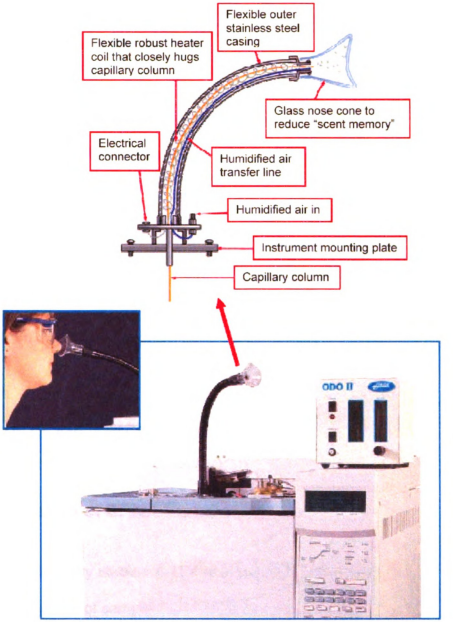
An ODO II sniffing system (SGE USA, Austin, TX) was connected to the GC/MS system. Figure 6.1 displays how the system was connected to the gas chromatograph.



Modified from (SGE, 2003)
Figure 6.1 ODO II module controls carrier gas to flow splitter and humidified air to sniffing nose cone

As shown in Figure 6.1, the ODO II module controls the auxiliary carrier gas flow from the module to the flow splitter, which provides make-up gas to the sniffing port. Volatile compounds elute out of the GC column and the flow is divided into two paths: one goes to the sniffing port and another goes to the mass spectrometer for identification. The ODO II module also regulates the flow of air, which is humidified before going to the heated transfer line that is connected to the glass sniffing nose cone. Humidified air is used to avoid drying of the nasal mucous membranes if an analysis runs for a long period of time.

Figure 6.2 shows a picture of a sniffing port that is connected to a gas chromatograph, as well as a detailed drawing of the heated transfer line that is connected to the glass sniffing nose cone.



Reprinted from (SGE, 2002)

Figure 6.2 Heated transfer line connected to sniffing nose cone in ODO II module

Two adhesive-coated HDPE films were studied. One was sample PCTA and another was PATA. Procedures described in the previous section

"comparison of gas chromatogram profiles" were used to prepare the odor system with 65 μm PDMS/DVB SPME.

The ODO II sniffing unit was turned on by turning on the heat and humidified air for the transfer line.

A timer was started simultaneously with the start of the GC/MS. The investigator then immediately put his nose by the glass sniffing nose cone. When an odor was perceived at the sniffing port, the investigator recorded the time from the timer and a description of the sensed odor.

RESULTS AND DISCUSSION

Optimization of SPME Parameters

Preliminary studies identified two of the peaks in the gas chromatogram of the odor system of sample PCTA as acetone and 1,3-bis(1,1-dimethylethyl)-benzene (data not shown, identified by the built-in library of the GC/MS). Acetone is a solvent commonly used in plastic processing and it carries a strong and unique "fruity" odor. On the other hand, 1,3-bis(1,1-dimethylethyl)-benzene (also called 1,3-Di-tert-butylbenzene) was found in the DH-TD/GC-MS analysis only from sample PCTA, which had been found to have stronger odor by a

sensory panel. Therefore, these two compounds were used as anchoring compounds in optimizing SPME parameters.

As explained earlier, it is more important to keep sampling parameters consistent than to reach a full equilibrium before taking samples in SPME analysis (see Figure 2.8). Extreme caution was taken in preparing the odor system of the HDPE film sample and using SPME to adsorb volatile compounds for the GC/MS analysis.

The purpose of optimizing SPME parameters was to adsorb as much of the chosen anchoring compounds as possible from the headspace to the SPME fiber.

Effect of SPME Sampling Time

Three SPME sampling times, 5 min, 15 min and 30 min, under a controlled sampling temperature of 35^0C were chosen to investigate the effect of time on the quantities of anchoring compounds adsorbed by the SPME fiber. Table 6.3 lists the areas of the peaks identified as acetone and 1,3-di-tert-butylbenzene in the selected ion current chromatograms. An m/z ratio of 58 was chosen for quantifying the peak for acetone and an m/z ratio of 175 was used for 1,3-di-tert-butylbenzene.

Table 6.3 Response areas for specific ions of volatile compounds in analyzing sample PCTA with SPME/GC-MS under different sampling times (sampling temperature 35^{0}C)

	Acetone (m/z 58)			1,3-Di-tert-butylbenzene (m/z 175)		
No.	5 min	15 min	30 min	5 min	15 min	30 min
1	1063757	696802	864362	205372	311186	921158
2	992068	509095	593618	32548	299098	644151
3	824543	498742	677928	199864	287495	486190
Ave.	960123	568213	711969	145928	299260	683833

Figure 6.3 shows the peak responses with SPME sampling time and the ratio of 1,3-di-tert-butylbenzene to acetone.

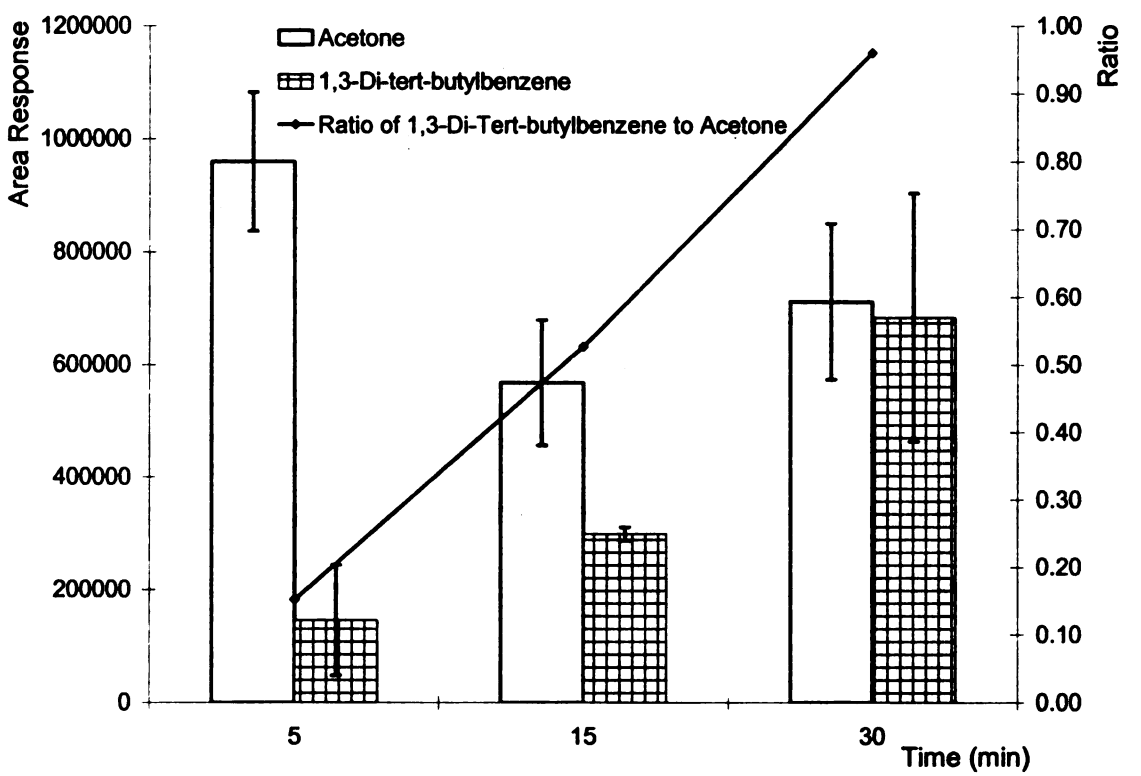


Figure 6.3 Average response areas of acetone and 1,3-di-tert-butylbenzene determined by SPME/GC-MS for sample PCTA versus SPME sampling time

As shown in Figure 6.3, a longer sampling time favored the extraction of 1,3-di-tert-butylbenzene by the SPME fiber but not necessarily for acetone. Even after 30 min of sampling, equilibrium of 1,3-di-tert-butylbenzene had not been established between the SPME fiber and the headspace inside the sampling vial. The data showed 5 min favored the extraction of acetone, probably because other volatile compounds were competing with acetone for the adsorbing sites on the SPME fiber with longer sampling times. The longer the sampling time, the more higher-boiling-point volatile compounds such as 1,3-di-tert-butylbenzene started to compete with lower-boiling-point volatiles compounds such as acetone. As a result, a slight decrease of peak response was observed with acetone from 5 min to 15 min. The increased quantities of adsorbed acetone between 15 min and 30 min along with 1,3-di-tert-butylbenzene might have been due to physical changes of the SPME fiber such as swelling due to the adsorbed volatile compounds.

Nevertheless, ratios of the two compounds kept increasing from the sampling time of 5 min to the sampling time of 30 min, indicating a longer sampling time was more favorable to the extraction of 1,3-di-tert-butylbenzne with the SPME fiber.

Considering 1,3-di-tert-butylbenzene was of more interest in the study, along with the need for an efficient SPME sampling process, 30 min was chosen as the sampling time for the subsequent SPME analysis.

Effect of SPME Sampling Temperature

After choosing the SPME sampling time (30 min), the effect of sampling temperature was investigated. Odor systems of sample PCTA were prepared with the 65 μm PDMS/DVB SPME fiber at five different sampling temperatures: 25°C, 35°C, 45°C, 60°C, and 75°C.

Tables 6.4 and 6.5 list the areas of peaks identified as acetone and 1,3-Di-tert-butylbenzene, respectively, in the selected ion current chromatograms. Again, an m/z ratio of 58 was chosen for quantifying the peak for acetone and an m/z ratio of 175 was used for 1,3-di-tert-butylbenzene.

Table 6.4 Peak response areas for acetone peak in sample PCTA with SPME/GC-MS at different sampling temperatures (sampling time 30 min)

Duplicate	25°C	35°C	45°C	60°C	75°C
1	1336234	864362	397145	358949	179310
2	992043	593618	385682	268136	112296
3	1105876	677928	540259	220958	113746
Average	1144718	711969	441029	282681	135117

Table 6.5 Peak response areas for 1,3-di-tert-butylbenzene peak in sample PCTA with SPME/GC-MS at different sampling temperatures (sampling time 30 min)

Duplicate	25°C	35°C	45°C	60°C	75°C
1	617073	921158	1683711	3084395	1934817
2	392890	644151	1518118	1496782	1451668
3	597791	486190	817489	2467235	1387441
Average	535918	683833	1339773	2349471	1591309

Figure 6.4 is the average peak response area versus sampling temperature for both acetone and 1,3-Di-tert-butylbenzene in sample PCTA with SPME/GC-MS.

As shown in Figure 6.4, the peak area for acetone decreased with SPME sampling temperature between 25^{0}C and 75^{0}C while the peak area for 1,3-Di-tert-butylbenzene increased with the sampling temperature from 25^{0}C to 60^{0}C, and then started to decrease after 60^{0}C.

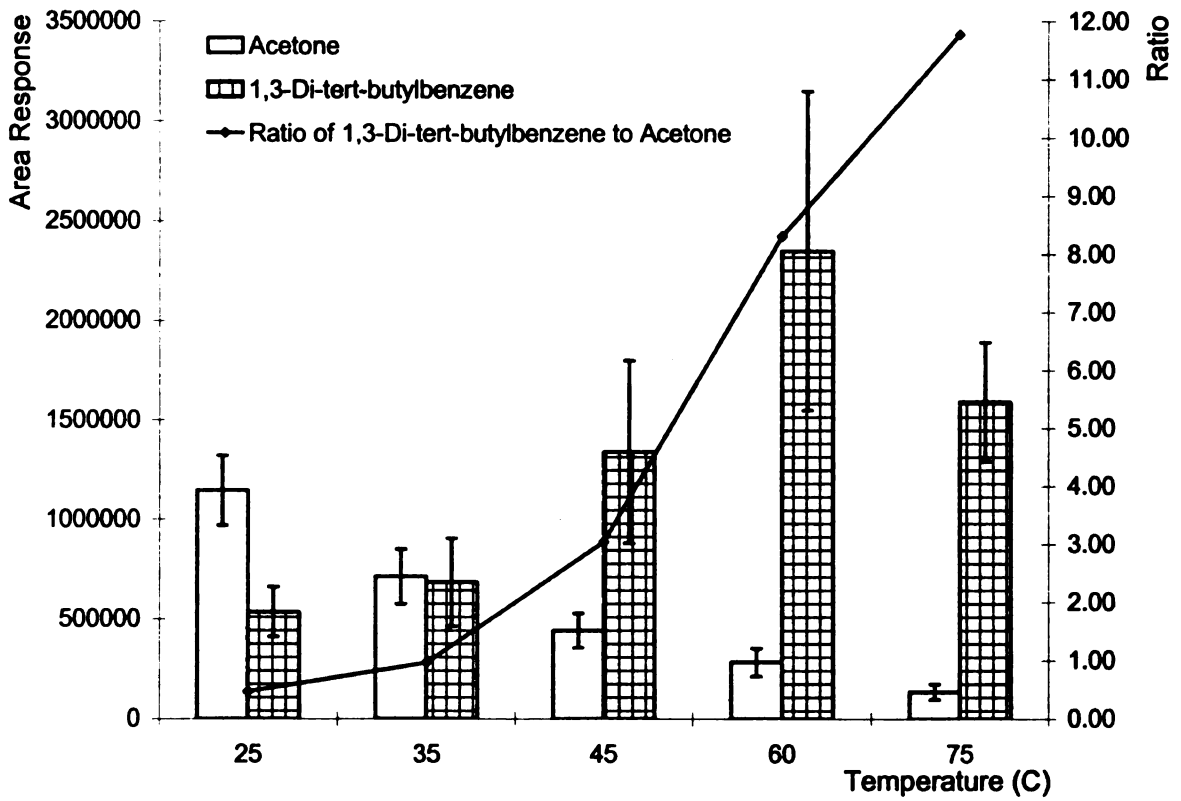


Figure 6.4 Average response areas of acetone and 1,3-di-tert-butylbenzene determined by SPME/GC-MS for sample PCTA versus SPME sampling temperature

The different trends observed for the two compounds in Figure 6.4 can be explained by the partition coefficient. The SPME sampling process is essentially a partition process of analytes between the coating on the fiber and the headspace. The amount of a particular compound that can be extracted by a SPME fiber is therefore proportional to its partition coefficient, which is affected by the sampling temperature (Yang & Peppard, 1994; Zhang & Pawliszyn, 1993; Zhang *et al.*, 1994). Partition coefficients for compounds usually increase with temperature at first, and then start to decrease after reaching an optimum temperature.

Ratios of the two compounds kept increasing from the sampling temperature of 25^0C to 75^0C, indicating a higher sampling temperature was more favorable to the extraction of 1,3-di-tert-butylbenzne. However, Figure 6.4 also shows that 1,3-di-tert-butylbenzene started to escape from the SPME fiber after the optimum temperature of 60^0C was passed. The higher ratio at 75^0C compared to that at 60^0C was probably because more acetone escaped from the SPME fiber than 1,3-Di-tert-butylbenzene did at the sampling temperature of 75^0C.

Because the compound 1,3-di-tert-butylbenzene was found in sample PCTA only, based on the DH-TD/GC-MS study in chapter 5, and sample PCTA was perceived as having a stronger odor compared to other adhesive-coated HDPE film samples, more attention was given to this particular compound. A

SPME sampling temperature of 60^{0}C was thus chosen for the subsequent investigations.

Comparisons of Gas Chromatogram Profiles

Odor systems of five different HDPE films were studied and compared with each other (samples CONT, PATA, PBTA, PBTB, and PCTA) using SPME/GC-MS analysis with a SPME sampling temperature of 60°C for 30 min.

Confirmation of 1,3-Di-tert-Butylbenzene

Studies in chapter 5 indicated that the compound 1,3-di-tert-butylbenzene was found in the odor profile of sample PCTA only. Figure 6.5 shows the mass spectrum of the standard compound (NIST, 2005).

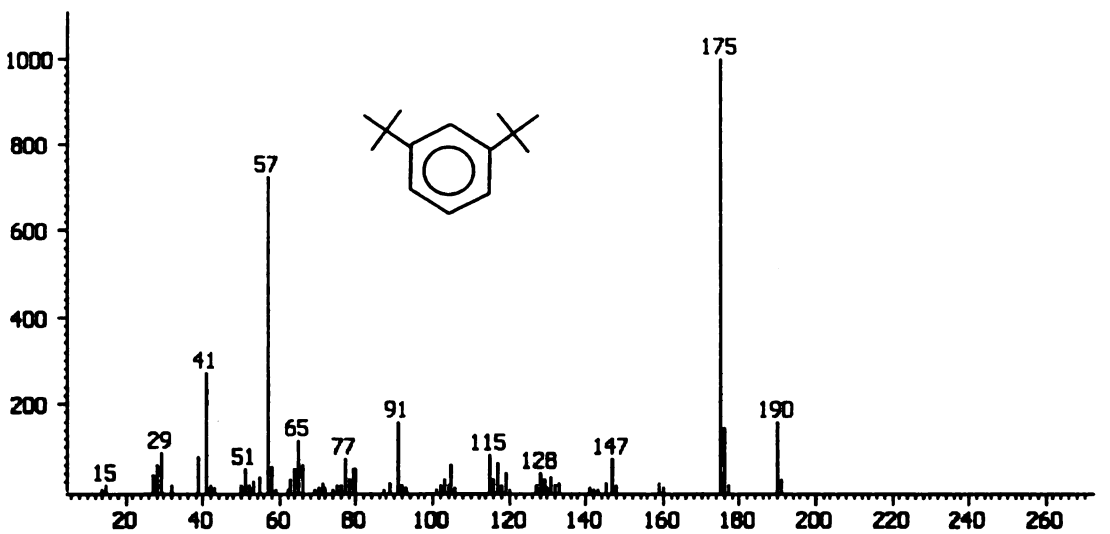


Figure 6.5 Mass spectrum of standard compound 1,3-Di-tert-butylbenzene

Figure 6.5 shows the m/z ratio of the base peak of 1,3-Di-tert-butylbenzene was 175, which was thus used as the fingerprint peak for the compound. The m/z 175 ion for 1,3-Di-tert-butylbenzne for sample PCTA was observed in the chromatogram at an RT of 271-272 seconds, which was used as the anchoring retention time to confirm whether the m/z 175 ion was observed in the chromatograms for the other samples (CONT, PATA, PBTA, and PBTB).

Table 6.6 lists response areas of the m/z 175 ion at the RT of 271-272 seconds if it was detected in the odor system of the HDPE film. Three duplicates were tested for each film and the results are displayed in Figure 6.6.

Table 6.6 Areas of m/z 175 ion at retention time 271 - 272 seconds

Duplicate	CONT	PATA	PBTA	PBTB	PCTA
1	9440	2227	2138	2269	3084395
2	5374	3494	2189	3759	1496782
3	4407	3395	2419	2420	2467235
Average	6407	3039	2249	2816	2349471

Obviously, compound 1,3-di-tert-butylbenzene exists in the odor systems of all HDPE film samples, but their quantities in samples CONT, PATA, PBTA, and PBTB were much lower than that of sample PCTA. Among the first four samples, the quantity of the compound in sample CONT was significantly higher than those of the other three samples.

The results proved the importance of using the fingerprint peak and the retention time of a particular compound in the chromatogram to detect the compound, which could otherwise not be detected due to its extremely low quantity.

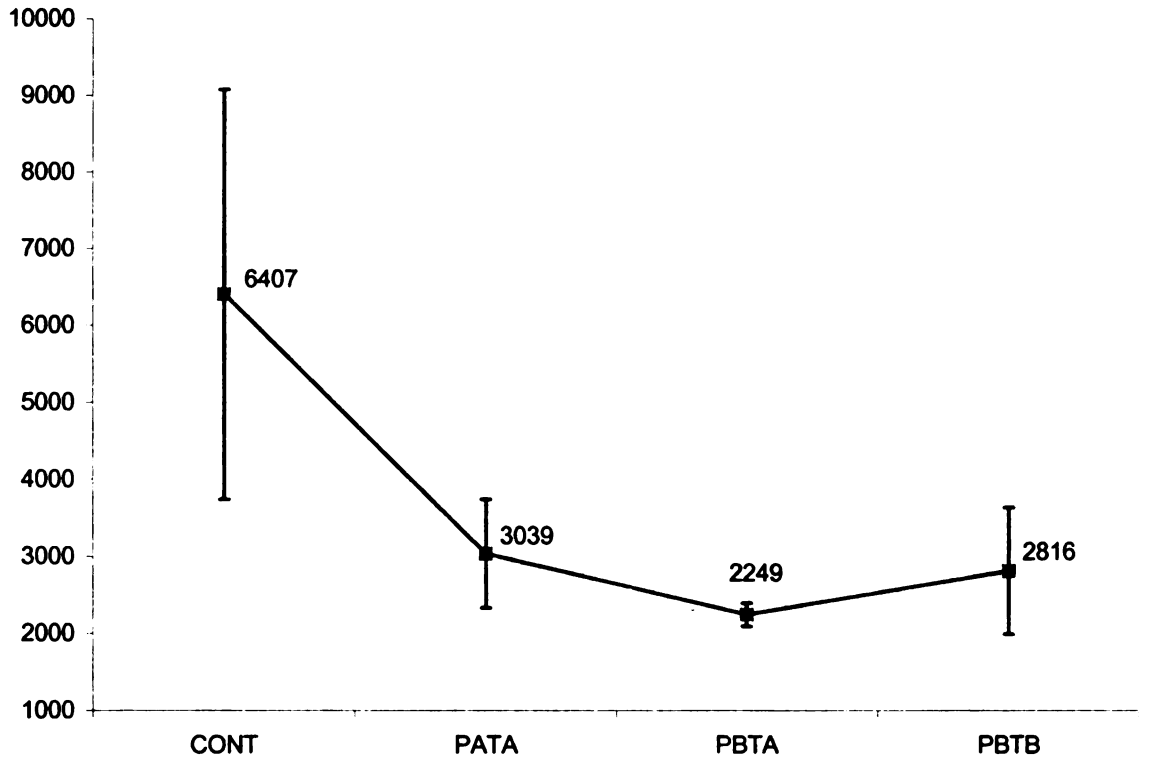


Figure 6.6 Average area of m/z 175 ion detected in the headspaces of HDPE films except sample PCTA using SPME/GC-MS

Origin of 1,3-Di-tert-Butylbenzene

Some researchers have found the compound 1,3-di-tert-butylbenzene in polyolefins including LDPE, HDPE and PP after they were exposed to gamma-irradiation (Demertzis *et al.*, 1999; Jeon *et al.*, 2004; Krzymien *et al.*, 2001; Lee *et al.*, 2004).

Their studies concluded that the additive Irgafos 168, tris(2,4-Di-tert-butylphenyl)phosphite, which is commonly used as a stabilizer in polyolefin processing, decomposed during the gamma irradiation and resulted in volatile compounds including 1,3-di-tert-butylbenzene and 2,4-di-tert-butylphenol.

Lee (2004) attributed the differentiation of odor profiles of red pepper powder by the E-nose system after different levels of gamma irradiation (0, 3, 5, and 7 kGy) to the formation of 1,3-di-tert-butylbenzene.

Based on their work, it is reasonable to believe the additive Irgafos 168 was used in preparing our HDPE film samples, which decomposed during the odor preparation process. We hypothesized that the odor profile of sample PCTA was perceived as stronger by the sensory panelists because it contained a much higher concentration of 1,3-di-tert-butylbenzene. However, this hypothesis needed to be tested with the sniffing test that is discussed in a subsequent section.

Potential Identities of Volatile Compounds

The identity of a particular compound can be confirmed by comparing its RT and mass spectrum with those of the standard. As one example, Figure 6.7 shows the mass spectrum of the compound detected at RT 271-272 seconds in

analyzing the odor profile of sample PCTA with SPME/GC-MS, which was almost identical to the mass spectrum of its standard (Figure 6.5).

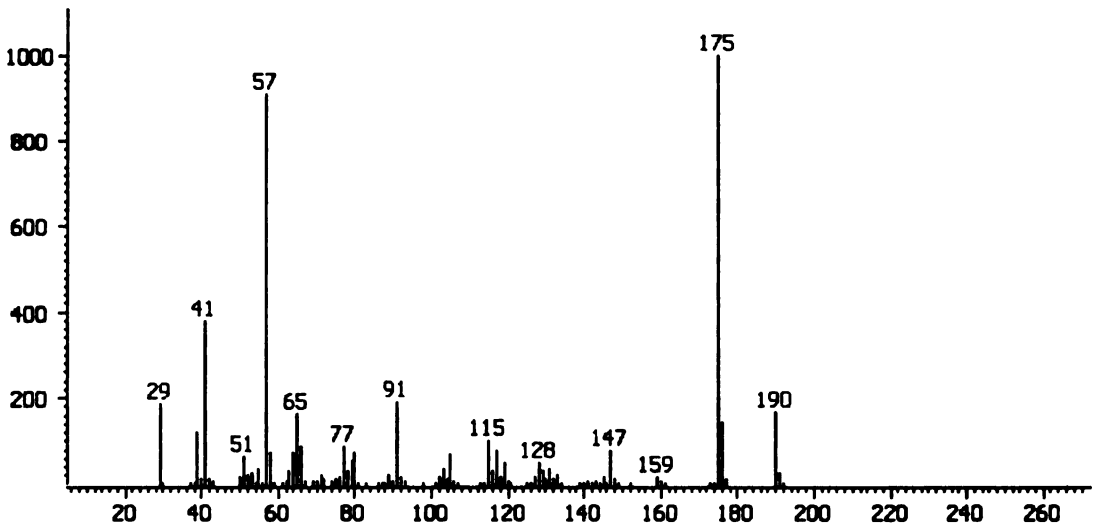


Figure 6.7 Mass spectrum of volatile compound detected at RT 271-272 seconds in SPME/GC-MS analysis

Table 6.7 lists the retention time of several compounds detected in the odor profiles of the HDPE film samples.

Table 6.7 Confirmation of volatile compounds by comparing their retention times with those of standards

	Acetone[1]		Hexanal[1]		Benzaldehyde[2]		1014-60-4[2,3]	
	Std[4]	Run[5]	Std[4]	Run[5]	Std[4]	Run[5]	Std[4]	Run[5]
1	119.64	121.52	164.97	165.77	205.02	206.02	272.02	271.27
2	118.52	120.89	165.39	165.64	205.89	206.02	272.77	271.89
3	117.59	122.14	166.39	166.39	204.39	206.64	271.89	272.02
Aver	118.58	121.52	165.58	165.93	205.10	206.23	272.23	271.72
Diff[6]		2.48		0.21		0.55		-0.18

Note: All retention times were in seconds and three duplicates were tested;
1 – The standard compound was tested individually;
2 – The compound was tested in a standard mixture including acetone, ethyl acetate, propyl acetate, butyl acetate, hexyl acetate, hexanal, benzaldehyde, and 1,3-Di-tert-butylbenzene;

3 – CAS registration number for 1,3-Di-tert-butylbenzene;
4 – Retention time of the standard compound;
5 – Retention time of the compound detected in the odor profile of sample PCTA in the SPME/GC-MS analysis (sampling temperature/time were 60^0C for 30 min);
6 – % Difference was calculated as (Run RT – Std RT)/Std RT * 100%.

Table 6.8 lists volatile compounds detected in the odor profiles originated from different HDPE film samples. The TIC chromatograms with the tentative identities of compounds are listed in Appendix 8.

Table 6.8 Volatile compounds tentatively identified from the HDPE film samples using SPME/GC-MS analysis

Sample	CONT	PATA	PBTA	PBTB	PCTA
Ketones					
Acetone	√	√	√	√	√
Aldehydes					
Hexanal	√	√	√	√	√
Heptanal	√	√	√	√	√
Octanal	√	√	√	√	√
Nonanal	√	√	√	√	√
Decanal	√	√	√	√	√
Aromatics					
Benzaldehyde	√	√	√	√	√
Benzene, 1,3-bis(1,1-dimethylethyl)	√	√	√	√	√
Phenol, 2,4-bis(1,1-dimethylethyl)	√	√	√	√	√
Hydrocarbons					
Decane	√	√	√	√	√
Dodecane	√	√	√	√	√
Tetradecane	√	√	√	√	√
Hexadecane	√	√	√	√	√
Nonadecane	√	√	√	√	√

Data in Table 6.8 indicates that the headspaces of the five different HDPE films had similar major components but differed from each other in terms of their concentrations. The result again confirmed the conclusion in the previous section "Confirmation of 1,3-Di-tert-Butylbenzene", that this compound existed in the odor profiles of all the HDPE films.

Description of Odor Profile with Sniffing Test

Comparison of Two Retention Times

Several standard compounds were tested using the SPME/GC-MS analysis with the sniffing port attached. The purpose of the study was to confirm that the time the odor of one compound was detected at the sniffing port was the same as the time the compound was detected by the mass spectrometer.

Preliminary studies found that the flow of the auxiliary carrier gas to the sniffing port affected the amount of compounds going to the mass spectrometer after they eluted out of the GC column and split into two portions at the flow splitter of the ODO II module. The higher the flow rate of the auxiliary carrier gas, the lower was the amount of the eluted compounds that went to the MS detector. Therefore, the flow control for the auxiliary carrier gas on the ODO II module was adjusted to a scale reading "5" so that the majority of the eluted volatile

compounds went to the sniffing port for the investigator to describe detected odors, but sufficient material went to the MS so that they could be easily detected.

Moreover, the GC capillary column flow program had to be adjusted as well due to the modified flow scheme at the flow splitter (see Figure 6.1). Preliminary studies found the GC could not maintain a constant carrier gas flow in the capillary column and would shut itself down after the inlet reached a certain pressure. As the result, a ramped pressure program was used to replace the constant flow column mode (see Table 6.9).

Table 6.10 lists the times the standard compounds were detected by the GC/MS and at the sniffing port of the ODO II module.

Table 6.9 New parameters used in the SPME/GC-MS with ramped pressure program

GC	
Injection mode	Splitless
Injector temperature	220^{0}C
Transfer line temperature	240^{0}C
Temperature program	40^{0}C for 0 min, increased to 240^{0}C at 40^0/min, stayed at 240^{0}C for 3 min.
Column flow	Ramped pressure program (initial pressure 21.4 psi, increased to 30.4 psi at 1.8 psi/min, and stayed at 30.4 psi for 3 min).
Purge flow	10 ml/min
Purge time	10 seconds
MS	
Solvent delay	0 seconds
Mass range	29 – 270 u
Acquisition rate	8 spectra/second

Table 6.10 Comparison of retention times of volatiles determined by the two detectors using SPME/GC-MS and ODO II sniffing port

	Duplicate	1	2	3
Ethyl acetate	GC RT (second)	115.41	115.53	115.03
	ODO II RT (second)	120	115	115
Butyl acetate	GC RT (second)	149.66	149.91	149.03
	ODO II RT (second)	149	147	148
Hexanal	GC RT (second)	146.78	150.28	150.28
	ODO II RT (second)	146	144	146
Benzaldehyde	GC RT (second)	190.78	190.91	190.53
	ODO II RT (second)	191	191	193
Hexyl acetate	GC RT (second)	198.91	199.16	198.78
	ODO II RT (second)	196	196	197
1,3-Di-tert-butylbenzene	GC RT (second)	258.53	258.78	258.41
	ODO II RT (second)	260	258	257

Data in Table 6.10 confirmed the claim made by the manufacturer of the ODO II sniffing module, SGE Analytical Science, that it took a similar time for the compounds to reach both the sniffing port and the MS by introducing the make-up gas at the exact point where the column flow is split between the two detectors so as to ensure the flow to the olfactory detector traveled at the same speed as the flow to the MS (SGE, 2002).

The matching of the two retention times to each other is considered crucial in investigating the contribution by each component compound to the odor profile,

because otherwise the identity of the compound cannot be determined when its associated odor is perceived at the sniffing port.

Descriptions of Odor Profiles

Odor profiles from sample PATA and PCTA were studied. The investigator was trained first with the standard 1,3-di-tert-butylbenzene to get familiar with its characteristic odor, after which the two odor profiles were presented in a randomly chosen order. Three sets of samples were tested by three investigators and each set of samples (standard, sample PATA, and sample PCTA) was tested by each investigator on the same day to ensure a consistent olfactory sensitivity. The side-by-side comparison of odor profiles of samples PATA and PCTA described by each investigator is listed in Appendix 9.

Because the majority of the eluted volatile compounds was split to the sniffing port, the area responses detected by the MS were relatively low. To construct the profiles, the summation of selected ions was used, including m/z ratios of 41, 43, 57, 58, 60, 84, 85, 89, 70, 71, 73, 99, 147, 175, and 191. The resultant chromatograms are shown in Figures 6.8 and 6.9.

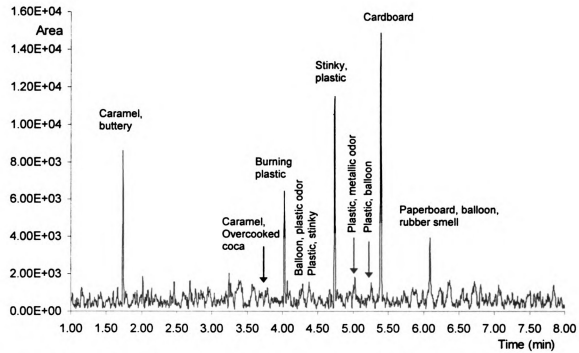


Figure 6.8 Odor profile of sample PATA detected in the sniffing test

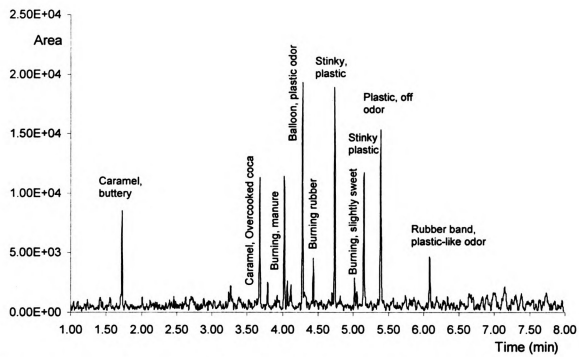


Figure 6.9 Odor profile of sample PCTA detected in the sniffing test

As shown in Figures 6.8 and 6.9, the odor profiles of sample PATA and PCTA were similar to each other. However, it must be mentioned that different terms might have been used by the investigators to describe the odor of the same volatile compound because they were neither pre-screened nor trained to improve their olfactory sensing sensitivity and judging precision. As a result, the same odor might be described one way by one person but differently by another, depending on the individual's preference and sensitivity to the particular compound.

The intensity of the odor of compound 1,3-di-tert-butylbenzene was perceived as "very weak" even though it did carry a plastic or a burning fabric odor, which was confirmed in the training process with the standard. However, a strong "balloon-like, plastic-like, and stinky" odor was perceived at nearly the same retention time as that 1,3-di-tert-butylbenzene (see Table 6.10) in the odor profile of sample PATA, whose quantity in the odor profile of sample PATA had been determined to be very low compared to that of sample PCTA (see Table 6.6).

The strong "balloon-like, plastic-like, and stinky" odor perceived at the retention time 260 seconds (4 min 20 seconds) was more likely from some other volatile compound that was not detected by the GC/MS while the ODO II was attached. Even though its concentration was low, its threshold value was

probably very low as well, which made it a significant contributor to the odor profiles of the HDPE film samples.

These observations indicated that this particular compound, 1,3-di-tert-butylbenzene, was not the crucial compound that made the perceived odor of sample PCTA by the sensory panelists stronger than those of the other HDPE film samples.

Instead, it is more likely the odor profile of the HDPE film was the result of the interactions of two or more odorous compounds existing in the system. Each odorous compound contributed to the odor profile, depending on its concentration and threshold value. In the other words, sample PCTA had a stronger odor probably because its odor profile contained higher concentrations of two or more of these yet unidentified odorous compounds.

CHAPTER 7 CORRELATION OF ANALYSES OF E-NOSE, SENSORY EVALUATION, AND GC-MS

INTRODUCTION AND OBJECTIVES

As mentioned earlier, the objective of the study was to investigate the potential of utilizing the electronic nose system as a quality control tool. One way to reach that goal is to use the E-nose to predict the sensory scores of odor profiles of HDPE film samples, or to predict the quantities of crucial volatile compound(s).

This chapter is devoted to discussion of the strengths and the weakness of three analytical techniques, along with the correlation among them (see Figure 2.14).

E-NOSE AND SENSORY EVALUATION

Instrument Sensitivity versus Human Nose Sensitivity

The PCA module of the E-nose system was capable of differentiating the headspaces of the five HDPE films (see Figure 3.9). On the other hand, sensory panels said there was no significant difference in terms of "Acceptability" and

"Intensity" of the perceived odor among samples CONT, PATA, PBTA, and PBTB (see Table 4.7, Figure 4.4, and Figure 4.5).

The E-nose system seemed more discriminating than the noses of the untrained panelists in our application. However, whether the higher discriminating capability of the E-nose system was significant from a practical standpoint requires further scrutiny because the odor profiles of sample CONT, PATA, PBTA, and PBTB were all grouped as "acceptable" by the sensory panelists even though the E-nose system can differentiate the headspaces.

Subjective versus Objective Judgment

In analyzing the headspaces of the HDPE film samples with the electronic nose system, it was concluded that the PCA (Principle Component Analysis) module of the system was capable of differentiating those profiles from each other. Moreover, the DFA (Discriminative Function Analysis) module of the E-nose system proved capable of identifying correctly an unknown sample as one of the training groups.

However, both modules only gave objective judgments. In order to get subjective information such as acceptability, preference, and good or bad, sensory evaluation was still necessary.

Sensory panels in both the pair-wise ranking test and the quantitative affective consumer test found sample PCTA had a stronger perceived odor profile. However, when the panelists in the consumer test were asked to evaluate the intensity and the acceptability of the odor profile of sample PCTA, they indicated a neutral response to both questions.

The information obtained from the sensory evaluation could not be offered from the E-nose analysis, which proved the importance of both analyses and showcased how they can complement each other to help the investigator to study the odor profiles of HDPE film samples.

One more interesting observation that was made in the study and is worthy of mentioning was the comparison of the sensor responses of the E-nose system and the responses from the sensory panel.

As shown in Figure 7.1, it was found that the odor profile of sample PBTA, rather than sample PCTA, generated the highest sensor responses in the E-nose analysis, even though the latter was perceived by the sensory panels as having a stronger odor profile than any other tested samples.

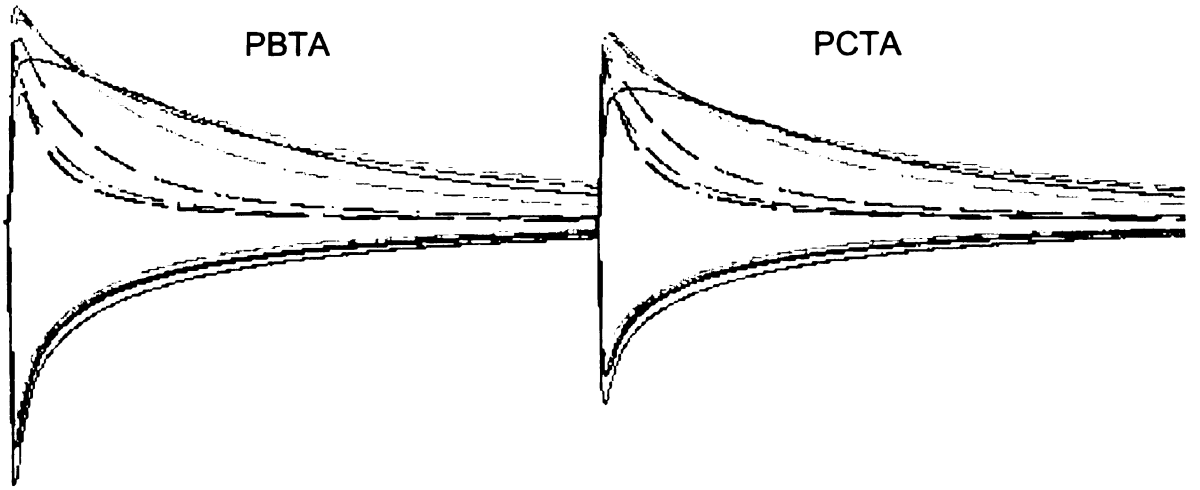


Figure 7.1 Side-by-side comparison of E-nose sensor responses to headspaces of sample PBTA and PCTA

A very likely reason could be the complexity of the volatile system, which was composed of hundreds of volatile compounds at different concentrations. In the E-nose analysis, all volatile compounds existing in the odor profile interacted with the sensors and thus influenced the sensor responses. On the other hand, only those that were odorous and whose concentrations were above their threshold values interacted with human noses in the sensory evaluation. Even though the odor system of sample PBTA generated the highest sensor responses in the E-nose system, some of the volatile compounds in the system might not carry any odors, or their concentrations may have been below their thresholds. On the other hand, the odor system of sample PCTA might have more odorous compounds that were perceived by the sensory panelists.

The comparison again proved the importance of looking at the data from both the E-nose analysis and the sensory evaluation, in order to get an accurate and thorough understanding of the odor profiles.

Partial Least Squares Using Sensory Scores

The potential of the E-nose system as a quality control tool in a manufacturing environment can be investigated by the PLS (Partial Least Square) module.

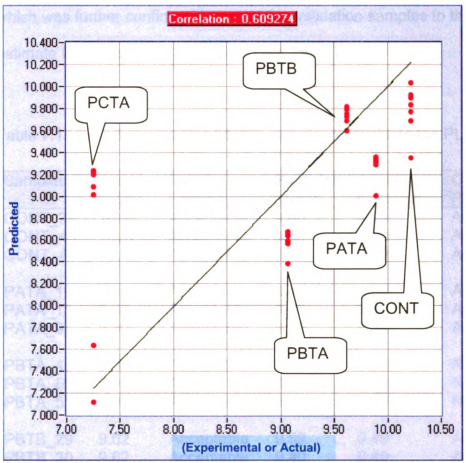
If a correlation between sensory scores and E-nose analyses could be developed, a few obstacles associated with sensory evaluation could be avoided. These obstacles include the high cost, the time-consuming training process, and the susceptibility of results to panelists' health and mood.

PLS Based on Five Groups of Samples

Figure 7.2 is the PLS plot of the "Acceptability" scores of the odor profiles based on the results listed in Table 4.7. Five groups of samples and 7 duplicates in each group were used to build the PLS model. The x-axis of the plot represents the experimental (or actual) values while the y-axis is the predicted values. The predicted sensory scores were calculated from the PLS model built

by the E-nose software, which correlated sensor responses of the E-nose with the experimental (or actual) sensory scores.

Each solid dot on the plot represents one duplicate of the analyzed samples. The straight line is the line on which the predicted values equal the experimental values. The closer a dot is to the straight line, the closer its predicted value was to its experimental value.



(Original data were generated on 10/18/06)

Figure 7.2 PLS plot of "Acceptability" scores of odor profiles of 5 groups of samples with training data for the E-nose shown only

Ideally, each cluster of dots should be next to or on the straight line when the PLS model fits the data perfectly. However, variations from one duplicate to another always induce some spread of data along the Y-axis. As shown in Figure 7.2, a bigger variation among the duplicates of sample PCTA was observed than in the other samples. The PLS model under-estimated the "Acceptability" scores of odor profiles of sample CONT, PATA, and PBTA but over-estimated significantly that of sample PCTA. A correlation coefficient of 0.61 shown in Figure 7.2 also indicated the PLS was not robust or effective, which was further confirmed by projecting validation samples to the PLS model to estimate their "Acceptability" scores (see Table 7.1).

Table 7.1 Using 5 groups of validation samples to evaluate the PLS model

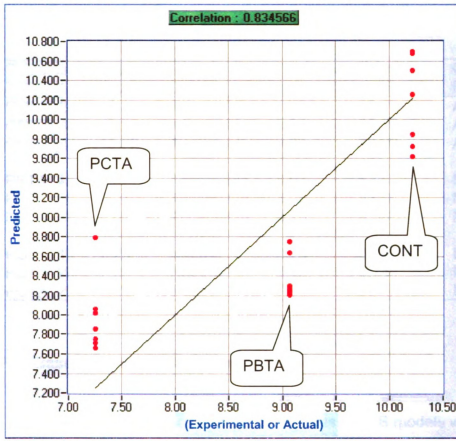
Samples	Actual scores	Panel Opinion	Predicted scores	Average predicted	Opinion from PLS model
CONT_39	10.22	Acceptable	9.80	9.69	Acceptable
CONT_40	10.22	Acceptable	9.60	9.69	Acceptable
CONT_41	10.22	Acceptable	9.67	9.69	Acceptable
PATA_10	9.89	Acceptable	9.41	9.36	Acceptable
PATA_11	9.89	Acceptable	9.28	9.36	Acceptable
PATA_9	9.89	Acceptable	9.39	9.36	Acceptable
PBTA_49	9.07	Acceptable	8.57	8.59	Neutral
PBTA_50	9.07	Acceptable	8.62	8.59	Neutral
PBTA_51	9.07	Acceptable	8.58	8.59	Neutral
PBTB_29	9.62	Acceptable	9.89	9.49	Acceptable
PBTB_30	9.62	Acceptable	9.40	9.49	Acceptable
PBTB_31	9.62	Acceptable	9.19	9.49	Acceptable
PCTA_19	7.25	Neutral	9.08	9.06	Acceptable
PCTA_20	7.25	Neutral	9.03	9.06	Acceptable
PCTA_21	7.25	Neutral	9.07	9.06	Acceptable

In Chapter 4, the HSD (Honestly Significant Difference) for "Acceptability" was determined as 1.4 and the neutral point of the unstructured scale carried a value of 7.5. As the result, any odor with an "Acceptability" score between 6.1 and 8.9 would be considered as neutral.

In the sensory evaluation, the panel perceived the odor of sample PCTA as neutral and that of sample PBTA as acceptable. However, the PLS model over-estimated the scores for sample PCTA, based on which an "acceptable" judgment would have been made, and under-estimated the scores for sample PBTA, based on which the odor would have been considered as "neutral".

PLS Based on Three Groups of Samples

Next, three groups of samples (CONT, PBTA, and PCTA) were selected to build the PLS model in the E-nose (see Figure 7.3).



(Original data were generated on 10/18/06)

Figure 7.3 PLS plot of "Acceptability" scores of odor profiles of 3 groups of samples with training data for the E-nose shown only

The correlation coefficient increased to 0.83. Again, validation data was used to evaluate the robustness of the model (see Table 7.2). The data showed that the PLS model under-estimated the sensory scores for the odor profile of sample PBTA, which lead to an incorrect "neutral" evaluation of the odor.

Table 7.2 Using 3 groups of validation samples to evaluate the PLS model

Samples	Actual scores	Panel Opinion	Predicted scores	Average predicted	Opinion from PLS model
CONT_39	10.22	Acceptable	11.29	10.20	Acceptable
CONT_40	10.22	Acceptable	9.94	10.20	Acceptable
CONT_41	10.22	Acceptable	9.37	10.20	Acceptable
PBTA_49	9.07	Acceptable	8.03	8.08	Neutral
PBTA_50	9.07	Acceptable	8.05	8.08	Neutral
PBTA_51	9.07	Acceptable	8.16	8.08	Neutral
PCTA_19	7.25	Neutral	9.79	8.74	Neutral
PCTA_20	7.25	Neutral	8.46	8.74	Neutral
PCTA_21	7.25	Neutral	7.96	8.74	Neutral

PLS Based on Pairs of Samples

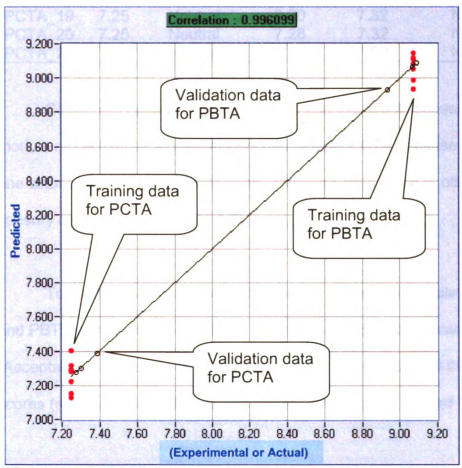
Table 7.3 lists the correlation coefficients of PLS models when different pairs of samples were used to build the PLS model in the E-nose system, which showed improvements compared to the PLS model based on three groups of samples.

Table 7.3 Correlation coefficients of PLS models based on different pairs of samples

Samples	CONT	PATA	PBTA	PBTB	PCTA
CONT	/	0.996773	0.996001	0.979335	0.921467
PATA		/	0.987000	0.998566	0.984701
PBTA			/	0.997945	0.996099
PBTB				/	0.991600
PCTA					/

The correlation coefficient was considered important in evaluating the robustness and effectiveness of the PLS model. However, it was equally important to validate the model with test data (validation data), as shown in Table 7.1 and 7.2.

As one example, Figure 7.4 is the PLS plot based on samples PCTA and PBTA. Each cluster of solid dots (in total 7 points) represents one group of training data. The empty dots are the validation data (3 duplicates).



(Original data were generated on 10/18/06)

Figure 7.4 PLS plot of "Acceptability" scores of odor profiles of sample PCTA and PBTA with both training data and validation data

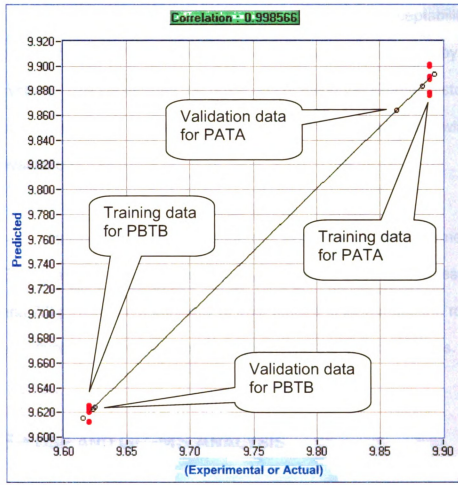
Table 7.4 lists the predicted "Acceptability" scores based on the PLS model shown in Figure 7.4. More PLS models based on pairs of samples involving sample PCTA and their validation results are listed in Appendix 10.

Table 7.4 Validate the PLS model based on sample PCTA and PBTA

Samples	Actual scores	Panel Opinion	Predicted scores	Average predicted	Opinion from PLS model
PBTA_49	9.07	Acceptable	8.93	9.03	Acceptable
PBTA_50	9.07	Acceptable	9.09	9.03	Acceptable
PBTA_51	9.07	Acceptable	9.06	9.03	Acceptable
PCTA_19	7.25	Neutral	7.30	7.32	Neutral
PCTA_20	7.25	Neutral	7.28	7.32	Neutral
PCTA_21	7.25	Neutral	7.39	7.32	Neutral

So far every pair of samples used to test the effectiveness of the PLS module of the E-nose system always had sample PCTA because it was the only one whose odor was found significantly stronger than those of others in the sensory evaluation (see Table 4.7).

To further investigate the effectiveness of the PLS module, samples PATA and PBTB were paired to build and validate the PLS model because their "Acceptability" scores were the closest to each other among all the pairs of scores (see Table 4.7). The results are shown in Figure 7.5 and Table 7.5.



(Original data were generated on 10/18/06)

Figure 7.5 PLS plot of "Acceptability" scores of odor profiles of sample PATA and PBTB with both training data and validation data

Table 7.5 Validate the PLS model based on sample PATA and PBTB

Samples	Actual scores	Panel Opinion	Predicted scores	Average predicted	Opinion from PLS model
PATA_10	9.89	Acceptable	9.88	9.88	Acceptable
PATA_11	9.89	Acceptable	9.86	9.88	Acceptable
PATA_9	9.89	Acceptable	9.89	9.88	Acceptable
PBTB_29	9.62	Acceptable	9.62	9.62	Acceptable
PBTB_30	9.62	Acceptable	9.62	9.62	Acceptable
PBTB_31	9.62	Acceptable	9.62	9.62	Acceptable

Correct predictions were made to evaluate the acceptability of the odor profile of the HDPE film sample based on the PLS model built by the E-nose system. This showcases the possibility of using the E-nose system to make correct subjective judgments which were initially only possible with costly sensory evaluation.

However, it would be dangerous to generalize the statement by saying the E-nose system could be used in any application because the results in Figure 7.2 and 7.3 proved the complexity of data tended to jeopardize the robustness and effectiveness of the PLS model in predicting the sensory scores.

E-NOSE AND GC-MS ANALYSIS

In Chapter 6, major potential component volatiles in the odor profiles of the HDPE film samples were identified, among which acetone and nonanal had previously been mentioned as important contributors to the off odor of HDPE-based packaging materials (Maneesin, 2001).

Table 7.6 lists the response areas of acetone and nonanal detected in the odor profiles of five HDPE film samples with the SPME/GC-MS analysis. Appendix 11 explains how the original data from GC-MS was processed to get the data listed in Table 7.6.

Table 7.6 Average response areas of acetone and nonanal detected in the odor profiles of different HDPE film samples in SPME/GC-MS analysis

Sample	CONT	PATA	PBTA	PBTB	PCTA
Acetone	72929	837784	1980094	97014	1784602
Nonanal	6017466	1851147	1394606	1080479	13931678

Data in Table 7.6 was then used to build PLS models to predict the response areas of acetone and nonanal in the odor profiles.

PLS Based on Five Groups of Samples

All five groups of samples, CONT, PATA, PBTA, PBTB, and PCTA were used to build the PLS model, which was then used to predict the response areas of acetone and nonanal in the odor profiles of validation samples based on their E-nose sensor responses.

Table 7.7 lists the predicted response areas and the percentages of difference of the predictions from the actual areas when all five groups of samples were used to build the PLS model in the E-nose system.

Correlation coefficients of 0.96 and 0.42 were obtained in the PLS model for acetone and nonanal, respectively. However, the models proved ineffective, as can be seen in Table 7.7, in predicting the response areas for either compound in the odor profiles of the HDPE film samples.

Table 7.7 Predicted response areas of acetone and nonanal based on the PLS model of five groups of samples

Sample	Acetone Prediction	Ave.	% Diff[1]	Nonanal Prediction	Ave.	%Diff[1]
CONT_32	118371			1558786		
CONT_33	68283	85305	17	4578414	4126995	-31
CONT_34	69261			6243785		
PATA_2	1530306			3226480		
PATA_3	1389259	1427785	70	3272042	3187805	72
PATA_4	1363788			3064894		
PBTA_42	2030657			2544512		
PBTA_43	1772624	1826275	-8	2192277	2283498	64
PBTA_44	1675545			2113705		
PBTB_22	133502			1920658		
PBTB_23	111549	120412	24	2168431	2136555	98
PBTB_24	116186			2320577		
PCTA_12	3160451			27168847		
PCTA_13	746504	1547853	13	1888663	10408647	-25
PCTA_14	736603			2168433		

1. The percentage of the difference between the prediction and the actual value (see Table 7.6) divided by the actual value.

PLS Based on Three Groups of Samples

Three groups of samples, CONT, PBTA, and PCTA, were used to build the PLS model to predict the response areas of acetone and nonanal in their odor profiles.

Correlation coefficients of the models were improved to 0.99 and 0.75 for acetone and nonanal, respectively. Table 7.8 lists the predicted response areas

of acetone and nonanal in the odor profiles of validation samples based on their E-nose sensor responses and the PLS model.

Table 7.8 Predicted response areas of acetone and nonanal based on the PLS model of three groups of samples

Sample	Acetone Prediction	Ave.	% Diff[1]	Nonanal Prediction	Ave.	%Diff[1]
CONT_32	88244			516817		
CONT_33	80153	87947	21	8843125	10441686	74
CONT_34	95444			21965115		
PBTA_42	2726726			3614981		
PBTA_43	2521001	2527948	28	3543651	3425828	146
PBTA_44	2336117			3118853		
PCTA_12	1092304			1513611		
PCTA_13	1135062	1192056	-33	3980545	4524301	-68
PCTA_14	1348803			8078748		

1. The percentage of the difference between the prediction and the actual value (see Table 7.6) divided by the actual value.

Obviously, the PLS model was still not effective.

PLS Based on Pairs of Samples

Tables 7.9 to Table 7.12 list the predicted response areas of acetone and nonanal when pairs of samples were used to build the PLS model, which was then used to predict the areas for the validation samples based on their E-nose responses.

Table 7.9 Predicted response areas of acetone and nonanal based on the PLS model of samples CONT and PCTA

Sample	Acetone Prediction	Ave.	% Diff[1]	Nonanal Prediction	Ave.	%Diff[1]
CONT_39	118512			6835595		
CONT_40	59451	77069	6	5703156	6026371	0.15
CONT_41	53242			5540361		
PCTA_19	938422			11768314		
PCTA_20	1263152	1182694	-34	12723262	12476705	10
PCTA_21	1346509			12938539		

1. The percentage of the difference between the prediction and the actual value (see Table 7.6) divided by the actual value.

Table 7.10 Predicted response areas of acetone and nonanal based on the PLS model of samples PATA and PCTA

Sample	Acetone Prediction	Ave.	% Diff[1]	Nonanal Prediction	Ave.	%Diff[1]
PATA_10	856445			1963260		
PATA_11	1056522	907127	8	3438313	2361511	28
PATA_9	808414			1682961		
PCTA_19	1724812			12720433		
PCTA_20	1557396	161230	-10	9686047	10679749	23
PCTA_21	1554181			9632768		

1. The percentage of the difference between the prediction and the actual value (see Table 7.6) divided by the actual value.

Table 7.11 Predicted response areas of acetone and nonanal based on the PLS model of samples PBTA and PCTA

Sample	Acetone Prediction	Ave.	% Diff[1]	Nonanal Prediction	Ave.	%Diff[1]
PBTA_49	1964731			1657115		
PBTA_50	1981943	1969365	-0.5	1366089	1476115	6
PBTA_51	1979422			1405141		
PCTA_19	1789727			13074536		
PCTA_20	1787198	1791832	0.4	13490362	12762543	-8
PCTA_21	1798571			11722730		

1. The percentage of the difference between the prediction and the actual value (see Table 7.6) divided by the actual value.

Table 7.12 Predicted response areas of acetone and nonanal based on the PLS model of samples PBTB and PCTA

Sample	Acetone Prediction	Ave.	% Diff[1]	Nonanal Prediction	Ave.	%Diff[1]
PBTB_29	74869			860631		
PBTB_30	166111	162090	67	1732529	1677560	55
PBTB_31	245289			2439519		
PCTA_19	1572515			12466997		
PCTA_20	2000872	1840920	3	15403524	14308466	3
PCTA_21	1949372			15054878		

1. The percentage of the difference between the prediction and the actual value (see Table 7.6) divided by the actual value.

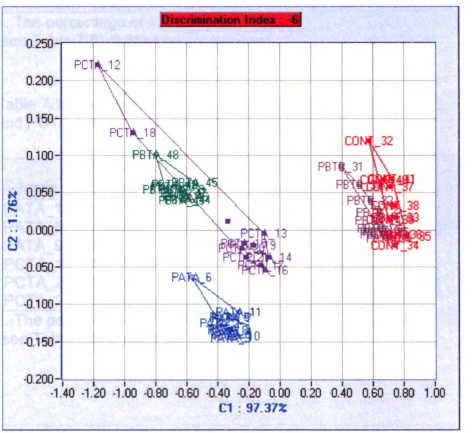
Data in Tables 7.9 to Table 7.12 show the percentages of difference varied from 0.15% to 67%, indicating the PLS models were not robust. The effectiveness of the model was affected by which pair of films and which volatile compound were studied. For example, the PLS model based on sample PBTA and PCTA predicted the response areas of both acetone and nonanal satisfactorily (see Table 7.11). The model in Table 7.9 was more effective in predicting the response areas of nonanal, while the model in Table 7.11 was better in predicting the areas of acetone.

PLS Based on Transformed Data

A close observation of the response areas of acetone and nonanal in Table 7.6 indicated a wide range among different samples (10E4 to 10E6 for acetone and 10E6 to 10E7 for nonanal). A Log_{10} transformation was therefore

made to the data to make them more uniform to see if it would improve the robustness and effectiveness of the PLS models. However, no significant improvements were obtained from the data transformation (see Appendix 12).

Yuzay (2004) proposed using PCA and sensor responses in the E-nose system to detect outliers and eliminate them before building PLS models. Figure 7.6 is the PCA plot of all the training and validation data, based on which CONT_32, PATA_6, PATA_11, PBTA_48, PBTB_30, PBTB_31, PCTA_12, and PCTA_18 were eliminated from the model training and validation process.



(Original data were generated on 10/03/06)

Figure 7.6 Using PCA to detect outliers before building PLS models

Tables 7.13 to 7.16 list the predicted response areas of acetone and nonanal based on the PLS models without the outliers.

Table 7.13 Predicted response areas of acetone and nonanal of samples CONT and PCTA based on the PLS model without outliers

Sample	Acetone Prediction	Ave.	% Diff[1]	Nonanal Prediction	Ave.	%Diff[1]
CONT_39	79590			6157144		
CONT_40	97414	86662	19	6492655	6291594	5
CONT_41	82982			6224982		
PCTA_19	1792154			13947142		
PCTA_20	1761395	1848053	4	13883892	14056036	0.9
PCTA_21	1990611			14337073		

1. The percentage of the difference between the prediction and the actual value (see Table 7.6) divided by the actual value.

Table 7.14 Predicted response areas of acetone and nonanal of samples PATA and PCTA based on the PLS model without outliers

Sample	Acetone Prediction	Ave.	% Diff[1]	Nonanal Prediction	Ave.	%Diff[1]
PATA_10	817074			1731523		
PATA_8	766912	792834	-5	1462124	1600164	-14
PATA_9	794515			1606845		
PCTA_19	1597429			10364863		
PCTA_20	1600201	1626113	-9	10412890	10882783	-22
PCTA_21	1680708			11870598		

1. The percentage of the difference between the prediction and the actual value (see Table 7.6) divided by the actual value.

Table 7.15 Predicted response areas of acetone and nonanal of samples PBTA and PCTA based on the PLS model without outliers

Sample	Acetone Prediction	Ave.	% Diff[1]	Nonanal Prediction	Ave.	%Diff[1]
PBTA_49	1960186			1744306		
PBTA_50	1974515	1973515	-0.3	1484527	1512235	8
PBTA_51	1985846			1307872		
PCTA_19	1796569			12015296		
PCTA_20	1817475	1798605	0.8	9300128	11915184	-14
PCTA_21	1781771			14430127		

1. The percentage of the difference between the prediction and the actual value (see Table 7.6) divided by the actual value.

Table 7.16 Predicted response areas of acetone and nonanal of samples PBTB and PCTA based on the PLS model without outliers

Sample	Acetone Prediction	Ave.	% Diff[1]	Nonanal Prediction	Ave.	%Diff[1]
PBTB_27	94416			1054854		
PBTB_28	101191	89140	-8	1121001	1001821	7
PBTB_29	71814			829608		
PCTA_19	1512940			12054699		
PCTA_20	1904134	1766242	-1	14752592	13802131	-0.9
PCTA_21	1881651			14599103		

1. The percentage of the difference between the prediction and the actual value (see Table 7.6) divided by the actual value.

Table 7.17 compares the percentages of difference before and after the outliers were eliminated.

Table 7.17 Comparison of percentages of difference before and after outliers were eliminated from PLS models

Acetone	CONT/PCTA	PATA/PCTA	PBTA/PCTA	PBTB/PCTA
Before	6, -34	8, -10	-0.5, 0.4	67, 3
After	19, 4	-5, -9	-0.3, 0.8	-8, -1
Nonanal	**CONT/PCTA**	**PATA/PCTA**	**PBTA/PCTA**	**PBTB/PCTA**
Before	0.15, 10	28, 23	6, -8	55, 3
After	5, 0.9	-14, -22	8, -14	7, -0.9

Eliminating outliers did improve the effectiveness of the PLS model based on the pair of samples PBTB and PCTA. However, the percentage of difference increased in predicting the area of acetone in the pair of samples CONT and PCTA.

The results further confirmed that the robustness and effectiveness of the PLS models were affected by which pair of samples and which volatile compound were investigated. Moreover, improvement by eliminating outliers was not obtained in all the PLS models.

CHAPTER 8 SUMMARY AND CONCLUSIONS

ELECTRONIC NOSE

Settings of major data acquisition parameters, including incubation time, temperature, acquisition time, delay, and sample size, in the Alpha MOS Fox 3000 E-nose analysis for HDPE films were investigated and optimized. The headspace of the HDPE film was generated from 2 strips of 10" x 1" HDPE film samples crimp-closed in a 10 mil glass vial, which was heated at 100^{0}C for 20 minutes. Sensor responses were recorded during the ten minutes (600 seconds) of the acquisition period and a fifteen minute (900 seconds) delay was used so that the sensor responses could go back to their baselines.

The E-nose system was proved capable of differentiating the headspaces of the five different HDPE film samples (sample CONT, PATA, PBTA, PBTB, and PCTA) with a discrimination index of 87 in the PCA (Principle Component Analysis) module.

A DFA (Discriminative Function Analysis) model was built during the training process, which had a percentage of recognition of 94. The model was then validated successfully by correctly identifying unknown samples as one of the training samples.

SENSORY EVALUATIONS

The pairwise ranking test was used to differentiate the odor profiles of the HDPE film samples by an untrained panel with 50 panelists. Friedman analysis was used to analyze the data and calculate rank sums as well as the HSD (Honestly Significant Difference) value.

The odor profile of sample PCTA was perceived significantly stronger than those of other samples. No significant difference was found among the odor profiles of sample CONT, PATA, PBTA, and PBTB.

In the quantitative affective consumer test, the "Acceptability" and "Intensity" of the odor profiles were evaluated by 100 panelists using unstructured scales. The RCBD (Random Complete Block Design) was used in the experimental design, which proved effective and efficient.

Sensory scores of both "Acceptability" and "Intensity" were determined and their HSD values were calculated. Pairwise comparison of the sensory scores indicated a "neutral" opinion by the panel for the odor profile of sample PCTA and "acceptable" opinions for the odor profiles of all other samples. In terms of the intensity, the odor profile of sample PCTA was found significantly stronger than those of others, which confirmed the result of the pairwise ranking test. However, the position of its intensity score on the unstructured intensity

scale indicated the odor profile of sample PCTA was perceived by the panel as "weak" to "neutral".

GC-MS ANALYSIS

DIP (Direct Insertion Probe) analysis was used to pre-study the headspaces of sample CONT and PCTA. Mass spectra indicated the existence of hydrocarbons in the headspaces of both samples. Moreover, results indicated a more complex volatile system from sample PCTA and the presence of compounds other than hydrocarbons.

Two techniques, DH-TD (Dynamic Headspace and Thermal Desorption) and SPME (Solid-Phase Microextraction) were used to collect and concentrate volatile compounds in the headspace generated from the HDPE film samples before analysis with GC-MS (Gas Chromatography – Mass Spectrometry).

In DH-TD, a Carbotrap 300 multi-bed thermal desorption tube was selected to collect and concentrate volatile compounds. However, the modified connection between the transfer line and the GC column in the DH-TD/GC-MS analysis proved not effective and thus a low repeatability was observed. Data from the DH-TD/GC-MS indicated compound 1,3-di-tert-butylbenzene only existed in the headspace of sample PCTA, which was then assumed to be the

objectionable volatile compound that was responsible for the stronger odor of the sample.

The 65 μm PDMS/DVB SPME fiber was used in the SPME/GC-MS analysis. The SPME sampling temperature and time were optimized as 60^0C and 30 minutes. Comparison of gas chromatograms indicated same groups of volatile compounds existed in the odor profiles of all the HDPE film samples, including ketones, aldhydes, aromatics, and hydrocarbons, among which acetone, hexanal, benzaldehyde, and 1,3-di-tert-butylbenzene were confirmed by comparing their retention times and mass spectra with those of standard compounds.

More volatile compounds might have been collected in the DH-TD overall because the extraction of volatile compounds by the SPME fiber was affected by the SPME sampling temperature and time but the extraction with the DH-TD was not. However, the DH-TD analysis required much more complicated instrument setup and a longer time to recover the thermal desorption tube (60 minutes). On the other hand, SPME analysis proved effective and efficient in collecting volatile compounds of interest. A much simpler instrument setup and fewer steps were involved in the SPME procedure. Moreover, no recovery time was needed in the SPME analysis because the fiber was cleaned at the same time as the extracted volatiles were desorbed to the GC column.

An ODO II sniffing unit was connected to the GC/MS instrument to split the eluted flow from the GC column so that odors of the volatile compounds could be detected at the same time as they were detected by the mass spectrometer. The efficiency of the sniffing unit was confirmed by comparing the two retention times (RT at the sniffing port and RT at the mass spectrometer) of several standard compounds, including ethyl acetate, butyl acetate, hexanal, benzaldehyde, hexyl acetate, and 1,3-di-tert-butylbenzene.

The odor profiles of samples PATA and PCTA were described in the sniffing test. Compound 1,3-di-tert-butylbenzene was found existing in the odor profiles of all the HDPE film samples, contradicting the results in the DH-TD/GC-MS analysis. Its odor was determined "weak" in the sniffing test. It was concluded that the compound was not responsible for the stronger odor profile of sample PCTA. Instead, it was more likely various odorous compounds affected the perceived odor profiles of the HDPE film samples, depending on their characteristic odors and respective threshold values.

CORRELATION OF E-NOSE, SENSORY EVALUATION AND GC-MS ANALYSIS

The E-nose system was capable of making objective judgments about the odor profiles. In addition, the E-nose system seemed more discriminating than the noses of the untrained panelists in detecting the difference among the odor

profiles of the HDPE films. However, sensory evaluation was necessary when subjective judgments were wanted.

Correlation coefficients in the range of 0.92 to 0.99 were obtained in the PLS (Partial Least Square) models in the E-nose system when the sensory scores of pairs of samples were used. The models were proved effective and robust in predicting the "Acceptability" sensory scores, based on which correct subjective judgments were made.

The successful correlation between the E-nose system and the sensory evaluation proved the potential of the E-nose system as a quick and accurate tool to replace costly and time-consuming sensory evaluations to predict the acceptability of odor profiles of the HDPE film samples, if the E-nose system was trained first with the results from the sensory evaluation.

Response areas of acetone and nonanal from the SPME/GC-MS analysis were used to correlate the E-nose system and the GC/MS by building PLS models. Unlike those for predicting sensory scores, the PLS models for response areas were not effective or robust.

Log_{10} transformation of the original data did not improve the effectiveness or the robustness of the PLS models significantly. However, eliminating outliers by using the PCA module in the E-nose seemed to improve the effectiveness of

some PLS models, depending on which pairs of samples and which volatile compound were studied in the correlation between the E-nose and GC/MS analysis.

The correlation between the E-nose system, sensory evaluation, and GC-MS analysis also proved it was important to complement the three techniques with one another.

RECOMMENDATIONS FOR FUTURE WORK

Using a trained panel in the quantitative descriptive analysis might help improve the accuracy of the "Acceptability" and "Intensity" sensory scores when the unstructured scale is used. A training process would help the panelists reach consensus on the correlation of a sensory score and its appropriate position on the scale, which in turn would help avoid the skewness of the resultant data. An alternative to using the unstructured scale would be using a structured scale. It would also be interesting to investigate the correlation between the opinion of the trained panel and the opinions of untrained consumers.

Moreover, descriptive tests by a pre-screened and trained sensory panel could be used to describe the characteristic odors of the HDPE film samples, which might help identify the major volatile contributors to the odor system.

More work in the SPME/GC-MS analysis might be worthwhile in order to get a more accurate characterization of the volatile profile. A slower temperature program would help better separate volatile compounds from each other, which in turn would help the detection of the odors at the sniffing port.

If the investigators in the sniffing test were pre-screened and trained, improved consistency and accuracy would be obtained. As a result, similar sensitivity would be maintained among the investigators and the same terminology would be used in describing the sensed odors.

The correlation between the E-nose and sensory evaluation was successful but that between the E-nose and the GC-MS was not. One possible reason might be that the sensory scores represented the overall evaluation of the perceived odor profiles by the sensory panel while the PLS models were built to predict the amount of one particular compound in the very complex odor system which contained hundreds of volatile compounds.

It would thus make more sense to get a value to represent the overall amount of the major odorous compounds in the odor system, such as a weighted average of ratios of their concentrations to their corresponding threshold values, which would then be used to build the PLS model to correlate the E-nose system and the GC/MS analysis.

APPENDICES

APPENDIX 1 WORKSHEET USED IN THE PAIRWISE RANKING TEST

5	1	16	7	10	18	17	5	20	18
9	16	11	15	17	7	20	10	11	20
6	2	19	3	1	11	10	3	8	5
1	10	12	8	18	14	18	15	15	8
4	5	2	16	11	5	2	18	4	11
16	11	20	17	4	9	12	6	16	19
3	12	7	6	13	3	15	8	3	15
13	7	3	1	14	17	14	4	12	2
20	13	5	11	8	4	6	14	10	4
12	4	8	4	9	2	7	1	6	6
19	9	1	12	3	16	9	7	5	12
2	14	6	13	7	13	5	17	7	10
17	3	19	10	5	12	8	11	14	16
11	8	13	14	16	15	1	12	2	1
15	6	15	9	19	1	4	16	17	14
10	19	17	18	2	10	13	2	13	9
18	15	10	20	12	6	3	19	9	3
8	20	4	5	6	19	16	9	18	7
14	18	14	2	20	8	19	20	1	17
7	17	9	19	15	20	11	13	19	13

APPENDIX 2 WORKSHEET AND DATA IN THE QUANTITATIVE AFFECTIVE CONSUMER TEST

Judge	Sample*	Accept	Intensity	Judge	Sample*	Accept	Intensity
1	5	0.6	0.3	8	5	6	0.1
	2	14.8	0.1		3	6.4	0.1
	3	13.9	0.2		1	1.2	0.3
	1	11.1	0.8		4	6.2	0.2
	4	14	0.6		2	7	0.3
2	4	15	0	9	2	8.5	0.9
	3	15	0		1	2	4.3
	5	10	5		5	8	7.3
	2	14	1		3	2.6	1.3
	1	12	2		4	7.6	0.6
3	1	1.8	1.1	10	4	0.9	0.6
	5	4.2	1.7		5	8	0.5
	4	6.2	0.3		2	15	0
	3	7.5	0.3		1	15	0
	2	10.5	0.4		3	3.9	0.5
4	2	11.2	0.2	11	1	5.6	0.8
	4	10.5	0.1		2	2.8	0.3
	1	10.1	0.1		5	1.2	8
	5	3	6		3	3	6.9
	3	5.7	5.1		4	6.3	4.4
5	3	3.1	0.9	12	4	10.7	0.3
	1	4.4	1.3		5	7.4	4.3
	2	0.6	1.1		1	2.1	3.5
	4	7	3.3		2	2.3	6.7
	5	1.5	0.4		3	6.7	0.5
6	3	10.6	3.4	13	3	7.5	0
	2	7.1	3.6		1	7.5	0
	4	8.2	4.6		4	6	7.5
	5	7.9	6.5		5	1	12
	1	7.5	3.6		2	7.5	0
7	1	14.8	0	14	2	10	0.1
	4	3.6	0.1		4	8	4
	3	11.6	3.4		3	7.1	2
	2	5.6	5.9		1	6.8	3
	5	0	9		5	4	9

* 1 – CONT, 2 – PATA, 3 – PBTA, 4 – PBTB, 5 – PCTA.

Judge	Sample*	Accept	Intensity	Judge	Sample*	Accept	Intensity
15	5	7.5	9.9	23	4	15	0
	3	14.1	0.4		3	15	0
	2	14.4	0.1		1	10.7	2.3
	4	14.5	0.1		2	11	2.2
	1	0.4	14.5		5	12.9	4
16	2	14.8	0	24	5	12	4
	5	14.8	0.1		1	12.5	2
	3	14.9	0.2		4	11.5	1
	1	14.9	0		3	8	4.5
	4	14.9	0		2	11.5	2
17	4	9	1	25	2	14.5	0.2
	3	12	5		4	10.9	2.4
	2	11	5		5	4.5	9.8
	5	4	12		1	12.1	1.5
	1	11	3.5		3	7.5	7.3
18	1	12.9	2.2	26	3	12.4	0.2
	2	12.1	1.5		1	7	1.3
	4	12	5.4		4	14.7	0.3
	3	6.6	7.4		5	9.6	0.2
	5	8.1	6.7		2	13.9	0.1
19	5	6.7	5.7	27	2	14.6	0.1
	4	12.7	2.4		4	14.9	0.3
	1	11.8	3.3		3	13.7	3.2
	2	10	4.2		1	14.7	2
	3	2.7	12.1		5	14.2	2
20	3	8.1	4.3	28	5	13.6	3.9
	1	2.2	2		3	8.1	7.9
	5	12.5	12		2	7.3	4.7
	4	13	3.6		4	14	1.9
	2	8.5	3.9		1	7.6	6.7
21	3	10.4	6.1	29	1	15	0.1
	5	6.8	11.3		2	15	0.7
	2	6.9	8.5		5	5	13.5
	4	5.4	10.3		3	15	0.1
	1	5.3	10		4	0.1	5
22	1	9.5	0.8	30	4	15	0.1
	2	11	1.2		5	14.1	0.1
	3	0.2	12.9		1	14.2	0.1
	5	0.2	14.1		2	13.7	0.3
	4	8	7.2		3	14.3	0.2

* 1 – CONT, 2 – PATA, 3 – PBTA, 4 – PBTB, 5 – PCTA.

Judge	Sample*	Accept	Intensity	Judge	Sample*	Accept	Intensity
31	2	10.3	3.4	39	2	15	1
	4	14.4	0.9		1	14.1	1.5
	5	14.5	0.5		3	15	0.2
	1	12.8	1.4		5	13.1	4
	3	13.3	0.6		4	15	0
32	3	0	0	40	4	12.5	0.5
	5	0	6.4		3	11.3	1.8
	2	15	0		2	4.9	6.1
	4	14.9	0		1	14.6	0.7
	1	14.8	0		5	1.4	14.2
33	1	14.6	0.4	41	5	2.2	6.4
	2	14.3	0.5		4	2.6	2
	3	13.4	2		3	7.6	2
	5	0.7	13.5		2	4.2	2.5
	4	14.8	0.3		1	5.6	2
34	4	12.4	1.7	42	1	13.8	0.2
	3	11.6	1.2		3	13.4	0.1
	1	11.4	4.8		5	0.6	13.4
	2	11.5	5		4	0.4	13.4
	5	4.8	8.8		2	4.6	10.8
35	5	3.2	0.2	43	2	7.5	0.1
	1	1	0.1		5	0.8	5.4
	4	0.1	0		1	13.7	0.2
	3	3.8	0.5		3	5.7	9.4
	2	1	1		4	9.5	0.2
36	5	14.9	0.6	44	4	4	2
	2	13	3.7		1	4	2
	4	14.9	4		2	3	1.5
	3	14.9	0.2		5	0.5	10
	1	14.5	1.9		3	8	10
37	1	12.7	2.2	45	3	11	3.9
	4	3.8	7.5		2	11.7	2.2
	5	1.2	9.7		4	3.6	8.1
	2	1.4	7.6		1	5.1	5.9
	3	1.9	8.5		5	6.7	6.7
38	3	13.9	0.7	46	2	14.3	0.7
	5	9.8	6.5		3	10.3	4.9
	1	10.9	3.2		5	7.7	9.9
	4	12.3	2.9		1	14.3	0.3
	2	9.3	6.2		4	7.4	11.4

* 1 – CONT, 2 – PATA, 3 – PBTA, 4 – PBTB, 5 – PCTA.

Judge	Sample*	Accept	Intensity	Judge	Sample*	Accept	Intensity
47	4	15	0.1	55	2	14	2
	5	4	12		3	10	6
	2	10	8.5		5	9	9
	3	4	12		4	7	11
	1	13	7.5		1	4	13
48	1	13.8	2	56	3	7.2	0.8
	2	7.8	6.7		1	6.7	2.4
	4	10.2	7.7		4	4	2.6
	5	5	9.8		2	10.9	5.6
	3	10.8	8.2		5	1.8	8.9
49	3	5.7	9.2	57	5	5.5	4.3
	4	8.8	4.3		4	8.3	8.8
	1	6.6	3.8		3	9.6	7.5
	2	4.8	8.5		1	12.3	5.9
	5	3.1	10.5		2	11	7.6
50	5	0	0	58	2	4.3	7.3
	1	12.8	0.3		3	4.2	9
	3	6.7	5.3		5	4.3	4
	4	12.5	1		4	10	2
	2	5.8	10.2		1	12.4	1
51	1	14.2	7.8	59	1	4.5	0.2
	5	8.7	13.7		5	0.6	4.2
	2	14.9	5.4		2	0.6	4.8
	3	2.4	2.9		3	2.3	0.3
	4	14.9	0.3		4	1.8	1
52	4	5	5	60	4	15	0
	2	12	5		2	13.7	0.2
	1	8	9.9		1	7.2	7.4
	5	13.6	4		5	0.1	14.8
	3	4.8	7		3	13.4	1
53	3	4.8	0.6	61	2	11.2	1.9
	1	4.9	0.8		3	8.7	4.9
	4	6.9	0.5		4	8.3	6.5
	2	7.2	0.1		1	8	5.4
	5	2	9.1		5	4	12.3
54	5	10.8	0.7	62	5	14.8	0.3
	4	13	0.5		4	14.9	1.2
	3	12.8	0.5		2	12.8	2.2
	1	12.1	1.7		3	11.4	4.2
	2	12.8	0.2		1	14.8	1.8

* 1 – CONT, 2 – PATA, 3 – PBTA, 4 – PBTB, 5 – PCTA.

Judge	Sample*	Accept	Intensity	Judge	Sample*	Accept	Intensity
63	1	9.6	1.9	71	5	6	6.5
	2	11	3.7		3	14.8	1
	5	5.9	5.1		4	14	1.5
	4	3.1	5.8		1	14.6	1.1
	3	1.6	7.9		2	13.7	1.1
64	3	15	0	72	2	7.5	4.6
	5	10	6		4	15	0
	1	5	10		5	15	0.1
	2	8	7		3	2.5	1.5
	4	7	8		1	14	2
65	4	14	1	73	1	11	2.3
	1	13	2		5	3.5	11.3
	3	9	10		2	12.1	2.2
	5	7	14		4	12	3.7
	2	10	8		3	11.6	4
66	2	7.7	0.1	74	3	15	0
	3	3.9	4		2	15	0
	4	5.5	4.4		1	15	0
	1	2.3	9.9		5	15	15
	5	4.2	4.5		4	15	0
67	5	14.1	0.2	75	4	7.9	0.6
	4	3.9	3.4		1	8.8	3.8
	2	8	0.5		3	8.6	4.3
	3	0.1	11		2	8.3	6.9
	1	12	15		5	4.3	11
68	1	14.9	0.2	76	5	15	0.1
	2	14.3	0.2		4	14.9	0
	5	12.6	5.3		1	11.1	2
	4	10	7.6		3	9	4
	3	0.1	14		2	13.7	1.3
69	3	15	0.3	77	2	10	1
	5	14.9	0.3		1	7	5.5
	1	14.8	1		5	4	8
	2	14.9	0.3		4	3	5
	4	14.8	0.2		3	2	9.5
70	4	15	5.5	78	3	12.8	0.9
	1	2.4	10.6		5	10.4	0.9
	3	13.9	0.2		2	12.5	0.3
	5	0.7	11.3		1	12.4	0.4
	2	7.3	6.8		4	6.7	1.3

* 1 – CONT, 2 – PATA, 3 – PBTA, 4 – PBTB, 5 – PCTA.

Judge	Sample*	Accept	Intensity	Judge	Sample*	Accept	Intensity
79	4	12.6	0.2	87	1	15	0.5
	2	11.5	2.1		4	14.4	0.5
	3	10.4	3.2		5	1	13
	5	9.9	4.4		2	13	1
	1	12.9	0.5		3	13	1
80	1	14.2	2.5	88	3	13	2
	3	12.5	3		5	10	5
	4	0.8	10.8		1	12.5	3.7
	2	14.3	0.6		4	13	4
	5	11.5	12		2	12.2	4.6
81	2	8.5	7.1	89	2	14.9	0
	5	5.5	7.3		1	14.8	0.1
	1	10.1	8.6		3	14.2	0.3
	4	2.7	4.7		5	14.9	0.2
	3	13.1	4.3		4	14.5	0.4
82	3	10.3	7	90	4	7	0.5
	1	14.1	3.5		3	8.6	3.1
	2	8	8.2		2	8.3	4.3
	5	7.7	9		1	4.9	2.5
	4	8.1	8.2		5	10.7	7.2
83	4	14.8	0.1	91	3	14.9	0.1
	2	12.3	1.7		2	14.9	0
	3	14.6	2.3		1	15	0
	1	12.5	8.7		5	15	0
	5	8.1	11.5		4	8.6	2.9
84	5	4.1	0.5	92	4	9.7	3.8
	3	2.2	0.7		1	9.9	4.7
	4	3	0.1		3	3	9.8
	2	3.9	2		2	10.2	2.6
	1	2.8	3.2		5	6.5	6.4
85	1	14.9	0.2	93	5	4.4	4.4
	4	14.7	0.3		3	5.8	3.3
	5	10.7	4.3		4	9.7	1
	3	13	3.1		1	6.6	1
	2	8.3	10.1		2	6.9	1
86	5	6.6	8	94	2	1.4	1.4
	2	13.1	2.4		4	1.4	1.3
	4	4.2	14.3		5	14.4	6.9
	3	2.2	14.1		3	14.8	4.9
	1	13.9	3.1		1	14.2	6.8

* 1 – CONT, 2 – PATA, 3 – PBTA, 4 – PBTB, 5 – PCTA.

Judge	Sample*	Accept	Intensity	Judge	Sample*	Accept	Intensity
95	1	6.8	2.1	98	1	12	3.3
	5	5.3	6.6		5	15	0.1
	2	5	8.2		4	15	0.4
	4	7.1	8		3	6.1	7.7
	3	2.7	10.7		2	7.6	1.3
96	5	7.5	1.3	99	2	15	0
	2	0	0.2		4	8.7	7.6
	3	14.8	0.3		1	14.1	0.1
	1	14.8	0.2		5	12.9	3.9
	4	6.7	0.8		3	13	3.4
97	4	14.5	0.5	100	3	13.9	0
	3	14	1		1	14.8	0
	5	9	4		2	15	0
	2	9	4		4	15	0
	1	6	7		5	12.9	7.3

* 1 – CONT, 2 – PATA, 3 – PBTA, 4 – PBTB, 5 – PCTA.

APPENDIX 3 SCORESHEET USED IN THE PAIRWISE RANKING TEST

MULTIPLE PAIRED COMPARISONS TEST

Name: _____ Date: _____

Sample: Plastic films

Difference: Odor

Instructions:

1. Receive the sample tray and note each sample code below according to its position on the tray.
2. Open and smell the odor in each sample pair from left to right and note **which sample has a stronger odor** (stronger smell). Indicate by placing an "X" next to the code. Samples should be smelled in order and re-smell is not allowed.
3. Continue until all 4 pairs have been evaluated. Do not fatigue your olfactory system by sniffing too long or too often. Take a break as necessary.

Pair number	Left sample	Right sample
4	_____ ☐	_____ ☐
3	_____ ☐	_____ ☐
2	_____ ☐	_____ ☐
1	_____ ☐	_____ ☐

If you perceive no difference, please make a best guess.

("No difference" response is not permitted.)

APPENDIX 4 SCORESHEET USED IN THE QUANTITATIVE AFFECTIVE CONSUMER TEST

QUESTIONNAIRE FOR UNSTRUCTURED SCALING TEST

Name: _____ Date: _____

Sample: Plastic films

Instructions:

1. There are FIVE vials of samples presented in this test. We are asking you to evaluate the acceptability and intensity of the odor of the sample. by marking a line at the appropriate point.
2. Please follow the sample ID shown below to evaluate your samples.
3. Open one vial each time and sniff the headspace immediately. Make a vertical line on the horizontal line to indicate your rating of the acceptability. then a vertical line on a 2nd horizontal line to indicate your rating of the intensity of odor. Rest as much as you want before you go ahead to the next sample.
4. Please try to evaluate each sample individually. Do not compare among them when you make your evaluation.
5. please do not go back to sniff evaluated samples for a 2nd time because the odor profile might have changed since your first sniffing.

Please sniff the samples in the following order:

1. _____ 2. _____ 3. _____ 4. _____ 5. _____

Overall opinion of the odor

Not at all acceptable — Very much acceptable

Intensity of the odor

Extremely weak — Extremely strong

APPENDIX 5 SAS PROGRAM AND ITS OUTPUT

SAS Program

```
data sensory;
  input panelist$ sample$ acceptability intensity @@;
  cards;
  /*data set emitted in the program*/

Title 'Produce Residuals Dataset in Analyzing Acceptability Using GLM';
proc glm data=sensory;
  class panelist sample;
  model acceptability = panelist sample;
  random panelist;
  output out=predict r=resid p=pred;
run;

proc univariate normal plot data=predict;
  var resid;
run;

symbol value=dot interpol=none color=blue height=1;
Title 'Residuals Diagnostic';
proc gplot data=predict;
  Title 'Figure 4.2: Residual versus sample ID in analyzing
acceptability';
  plot resid*sample/vref=0;
run;

Title 'Analysis of Acceptability Using Mixed';
proc mixed data=sensory;
  class panelist sample;
  model acceptability = sample;
  random panelist;
  lsmeans sample/adjust=tueky diffs;
run;
quit;
```

Output for Analyzing Acceptability

Produce Residuals Dataset in Analyzing Acceptability Using GLM

The GLM Procedure

Class Level Information

Class	Levels	Values
panelist	100	1 10 100 11 12 13 14 15 16 17 18 19 2 20 21 22 23 24 25 26 27 28 29 3 30 31 32 33 34 35 36 37 38 39 4 40 41 42 43 44 45 46 47 48 49 5 50 51 52 53 54 55 56 57 58 59 6 60 61 62 63 64 65 66 67 68 69 7 70 71 72 73 74 75 76 77 78 79 8 80 81 82 83 84 85 86 87 88 89 9 90 91 92 93 94 95 96 97 98 99
sample	5	1 2 3 4 5

Number of Observations Read	500
Number of Observations Used	500

Produce Residuals Dataset in Analyzing Acceptability Using GLM

The GLM Procedure

Dependent Variable: acceptability

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	103	5506.06004	53.45689	4.04	<.0001
Error	396	5237.74468	13.22663		
Corrected Total	499	10743.80472			

R-Square	Coeff Var	Root MSE	acceptability Mean
0.512487	39.49484	3.636843	9.208400

Source	DF	Type I SS	Mean Square	F Value	Pr > F
panelist	99	4954.880720	50.049300	3.78	<.0001
sample	4	551.179320	137.794830	10.42	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
panelist	99	4954.880720	50.049300	3.78	<.0001
sample	4	551.179320	137.794830	10.42	<.0001

Produce Residuals Dataset in Analyzing Acceptability Using GLM

The GLM Procedure

Source	Type III Expected Mean Square
panelist	Var(Error) + 5 Var(panelist)
sample	Var(Error) + Q(sample)

Produce Residuals Dataset in Analyzing Acceptability Using GLM

The UNIVARIATE Procedure
Variable: resid

Moments

N	500	Sum Weights	500
Mean	0	Sum Observations	0
Std Deviation	3.23982751	Variance	10.4964823
Skewness	-0.5228333	Kurtosis	0.64147781
Uncorrected SS	5237.74468	Corrected SS	5237.74468
Coeff Variation	.	Std Error Mean	0.14488949

Basic Statistical Measures

Location		Variability	
Mean	0.00000	Std Deviation	3.23983
Median	0.19790	Variance	10.49648
Mode	-0.81160	Range	19.22700
		Interquartile Range	3.71800

NOTE: The mode displayed is the smallest of 4 modes with a count of 2.

Tests for Location: Mu0=0

Test	-Statistic-		-----p Value------	
Student's t	t	0	Pr > |t|	1.0000
Sign	M	20	Pr >= |M|	0.0810
Signed Rank	S	3456	Pr >= |S|	0.2854

Tests for Normality

Test	--Statistic---		-----p Value------	
Shapiro-Wilk	W	0.98054	Pr < W	<0.0001
Kolmogorov-Smirnov	D	0.06855	Pr > D	<0.0100
Cramer-von Mises	W-Sq	0.431538	Pr > W-Sq	<0.0050
Anderson-Darling	A-Sq	2.514278	Pr > A-Sq	<0.0050

Quantiles (Definition 5)

Quantile	Estimate
100% Max	8.4394
99%	6.8039
95%	4.9624
90%	3.9124

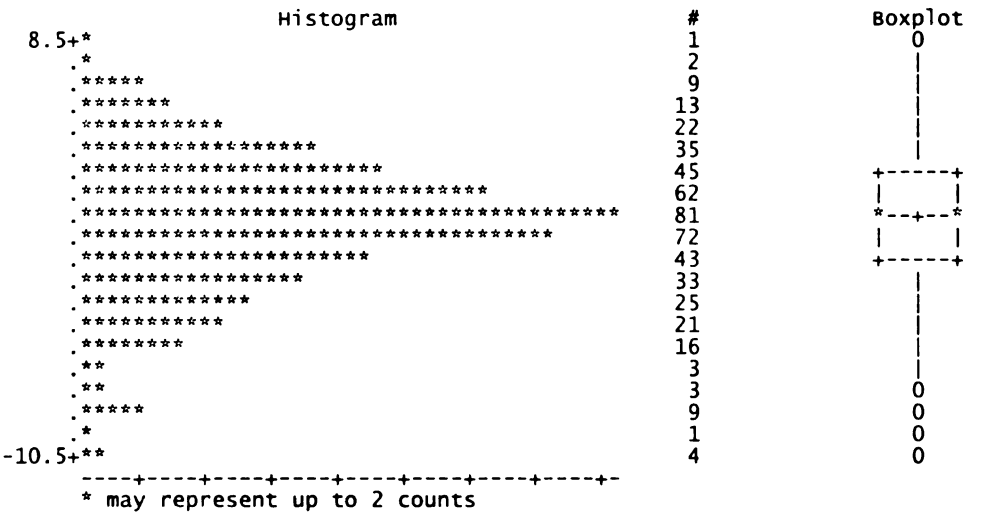
The UNIVARIATE Procedure
Variable: resid

Quantiles (Definition 5)

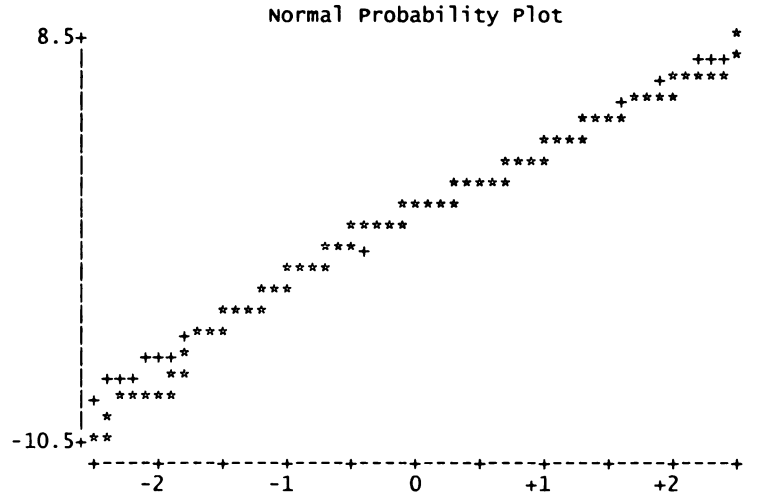
Quantile	Estimate
75% Q3	2.0444
50% Median	0.1979
25% Q1	-1.6736
10%	-4.3276
5%	-5.5906
1%	-9.1721
0% Min	-10.7876

Extreme Observations

------Lowest-----		-----Highest----	
Value	Obs	Value	Obs
-10.7876	75	6.8794	259
-10.3316	145	6.9834	207
-10.2716	398	7.1194	468
-10.1366	340	7.4924	181
-9.4436	477	8.4394	331



The UNIVARIATE Procedure
Variable: resid



Analysis of Acceptability Using Mixed

The Mixed Procedure

Model Information

Data Set	WORK.SENSORY
Dependent Variable	acceptability
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information

Class	Levels	Values
panelist	100	1 10 100 11 12 13 14 15 16 17 18 19 2 20 21 22 23 24 25 26 27 28 29 3 30 31 32 33 34 35 36 37 38 39 4 40 41 42 43 44 45 46 47 48 49 5 50 51 52 53 54 55 56 57 58 59 6 60 61 62 63 64 65 66 67 68 69 7 70 71 72 73 74 75 76 77 78 79 8 80 81 82 83 84 85 86 87 88 89 9 90 91 92 93 94 95 96 97 98 99
sample	5	1 2 3 4 5

Dimensions

Covariance Parameters	2
Columns in X	6
Columns in Z	100
Subjects	1
Max Obs Per Subject	500

Number of Observations

Number of Observations Read	500
Number of Observations Used	500
Number of Observations Not Used	0

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	2925.08167653	

Analysis of Acceptability Using Mixed

The Mixed Procedure

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
1	1	2837.72674190	0.00000000

Convergence criteria met.

Covariance Parameter Estimates

Cov Parm	Estimate
panelist	7.3645
Residual	13.2266

Fit Statistics

-2 Res Log Likelihood	2837.7
AIC (smaller is better)	2841.7
AICC (smaller is better)	2841.8
BIC (smaller is better)	2846.9

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
sample	4	396	10.42	<.0001

Least Squares Means

Effect	sample	Estimate	Standard Error	DF	t Value	Pr > |t|
sample	1	10.2160	0.4538	396	22.51	<.0001
sample	2	9.8920	0.4538	396	21.80	<.0001
sample	3	9.0650	0.4538	396	19.98	<.0001
sample	4	9.6200	0.4538	396	21.20	<.0001
sample	5	7.2490	0.4538	396	15.97	<.0001

Analysis of Acceptability Using Mixed

The Mixed Procedure

Differences of Least Squares Means

Effect	sample	_sample	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
sample	1	2	0.3240	0.5143	396	0.63	0.5291	Tukey-Kramer	0.9702
sample	1	3	1.1510	0.5143	396	2.24	0.0258	Tukey-Kramer	0.1680
sample	1	4	0.5960	0.5143	396	1.16	0.2472	Tukey-Kramer	0.7748
sample	1	5	2.9670	0.5143	396	5.77	<.0001	Tukey-Kramer	<.0001
sample	2	3	0.8270	0.5143	396	1.61	0.1086	Tukey-Kramer	0.4932
sample	2	4	0.2720	0.5143	396	0.53	0.5972	Tukey-Kramer	0.9844
sample	2	5	2.6430	0.5143	396	5.14	<.0001	Tukey-Kramer	<.0001
sample	3	4	-0.5550	0.5143	396	-1.08	0.2812	Tukey-Kramer	0.8173
sample	3	5	1.8160	0.5143	396	3.53	0.0005	Tukey-Kramer	0.0042
sample	4	5	2.3710	0.5143	396	4.61	<.0001	Tukey-Kramer	<.0001

Output for Analyzing Intensity

Produce Residuals Dataset in Analyzing intensity Using GLM

The GLM Procedure

Class Level Information

Class	Levels	Values
panelist	100	1 10 100 11 12 13 14 15 16 17 18 19 2 20 21 22 23 24 25 26 27 28 29 3 30 31 32 33 34 35 36 37 38 39 4 40 41 42 43 44 45 46 47 48 49 5 50 51 52 53 54 55 56 57 58 59 6 60 61 62 63 64 65 66 67 68 69 7 70 71 72 73 74 75 76 77 78 79 8 80 81 82 83 84 85 86 87 88 89 9 90 91 92 93 94 95 96 97 98 99
sample	5	1 2 3 4 5

Number of Observations Read	500
Number of Observations Used	500

Produce Residuals Dataset in Analyzing intensity Using GLM

The GLM Procedure

Dependent Variable: intensity

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	103	3484.933160	33.834303	3.34	<.0001
Error	396	4014.266120	10.137036		
Corrected Total	499	7499.199280			

R-Square	Coeff Var	Root MSE	intensity Mean
0.464707	83.75963	3.183871	3.801200

Source	DF	Type I SS	Mean Square	F Value	Pr > F
panelist	99	2611.415280	26.377932	2.60	<.0001
sample	4	873.517880	218.379470	21.54	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
panelist	99	2611.415280	26.377932	2.60	<.0001
sample	4	873.517880	218.379470	21.54	<.0001

Produce Residuals Dataset in Analyzing intensity Using GLM

The GLM Procedure

Source	Type III Expected Mean Square
panelist	Var(Error) + 5 Var(panelist)
sample	Var(Error) + Q(sample)

Produce Residuals Dataset in Analyzing intensity Using GLM

The UNIVARIATE Procedure
Variable: resid

Moments

N	500	Sum Weights	500
Mean	0	Sum Observations	0
Std Deviation	2.83630419	Variance	8.04462148
Skewness	0.47144594	Kurtosis	0.78466027
Uncorrected SS	4014.26612	Corrected SS	4014.26612
Coeff Variation	.	Std Error Mean	0.12684338

Basic Statistical Measures

Location		Variability	
Mean	0.00000	Std Deviation	2.83630
Median	-0.18130	Variance	8.04462
Mode	-3.58480	Range	18.67300
		Interquartile Range	3.56300

NOTE: The mode displayed is the smallest of 7 modes with a count of 2.

Tests for Location: Mu0=0

Test	-Statistic-		-----p Value------	
Student's t	t	0	Pr > |t|	1.0000
Sign	M	-9	Pr >= |M|	0.4471
Signed Rank	S	-2858	Pr >= |S|	0.3771

Tests for Normality

Test	--Statistic---		-----p Value------	
Shapiro-Wilk	W	0.983963	Pr < W	<0.0001
Kolmogorov-Smirnov	D	0.056283	Pr > D	<0.0100
Cramer-von Mises	W-Sq	0.301602	Pr > W-Sq	<0.0050
Anderson-Darling	A-Sq	1.990163	Pr > A-Sq	<0.0050

Quantiles (Definition 5)

Quantile	Estimate
100% Max	10.2932
99%	7.6677
95%	5.0522
90%	3.6012

Produce Residuals Dataset in Analyzing intensity Using GLM

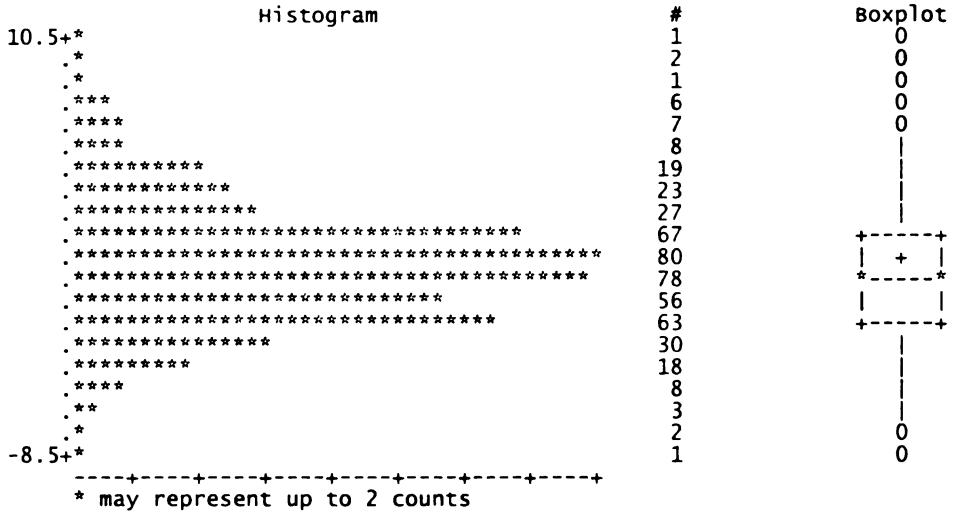
The UNIVARIATE Procedure
Variable: resid

Quantiles (Definition 5)

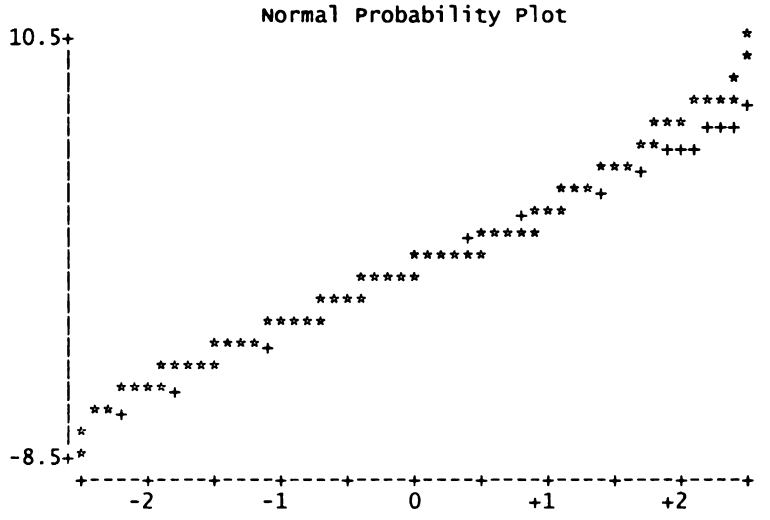
Quantile	Estimate
75% Q3	1.5342
50% Median	-0.1813
25% Q1	-2.0288
10%	-3.2743
5%	-4.2033
1%	-6.3913
0% Min	-8.3798

Extreme Observations

-----Lowest-----		-----Highest-----	
Value	Obs	Value	Obs
-8.3798	331	7.7352	250
-7.4758	207	8.5442	340
-7.0528	231	9.4402	369
-6.6758	348	9.7732	335
-6.5868	206	10.2932	75



The UNIVARIATE Procedure
Variable: resid



Analysis of intensity Using Mixed

The Mixed Procedure

Model Information

Data Set	WORK.SENSORY
Dependent Variable	intensity
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information

Class	Levels	Values
panelist	100	1 10 100 11 12 13 14 15 16 17 18 19 2 20 21 22 23 24 25 26 27 28 29 3 30 31 32 33 34 35 36 37 38 39 4 40 41 42 43 44 45 46 47 48 49 5 50 51 52 53 54 55 56 57 58 59 6 60 61 62 63 64 65 66 67 68 69 7 70 71 72 73 74 75 76 77 78 79 8 80 81 82 83 84 85 86 87 88 89 9 90 91 92 93 94 95 96 97 98 99
sample	5	1 2 3 4 5

Dimensions

Covariance Parameters	2
Columns in X	6
Columns in Z	100
Subjects	1
Max Obs Per Subject	500

Number of Observations

Number of Observations Read	500
Number of Observations Used	500
Number of Observations Not Used	0

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	2711.87961304	

Analysis of intensity Using Mixed

The Mixed Procedure

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
1	1	2668.96870974	0.00000000

Convergence criteria met.

Covariance Parameter Estimates

Cov Parm	Estimate
panelist	3.2482
Residual	10.1370

Fit Statistics

-2 Res Log Likelihood	2669.0
AIC (smaller is better)	2673.0
AICC (smaller is better)	2673.0
BIC (smaller is better)	2678.2

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
sample	4	396	21.54	<.0001

Least Squares Means

Effect	sample	Estimate	Standard Error	DF	t Value	Pr > |t|
sample	1	3.0080	0.3659	396	8.22	<.0001
sample	2	2.9060	0.3659	396	7.94	<.0001
sample	3	3.7970	0.3659	396	10.38	<.0001
sample	4	2.9340	0.3659	396	8.02	<.0001
sample	5	6.3610	0.3659	396	17.39	<.0001

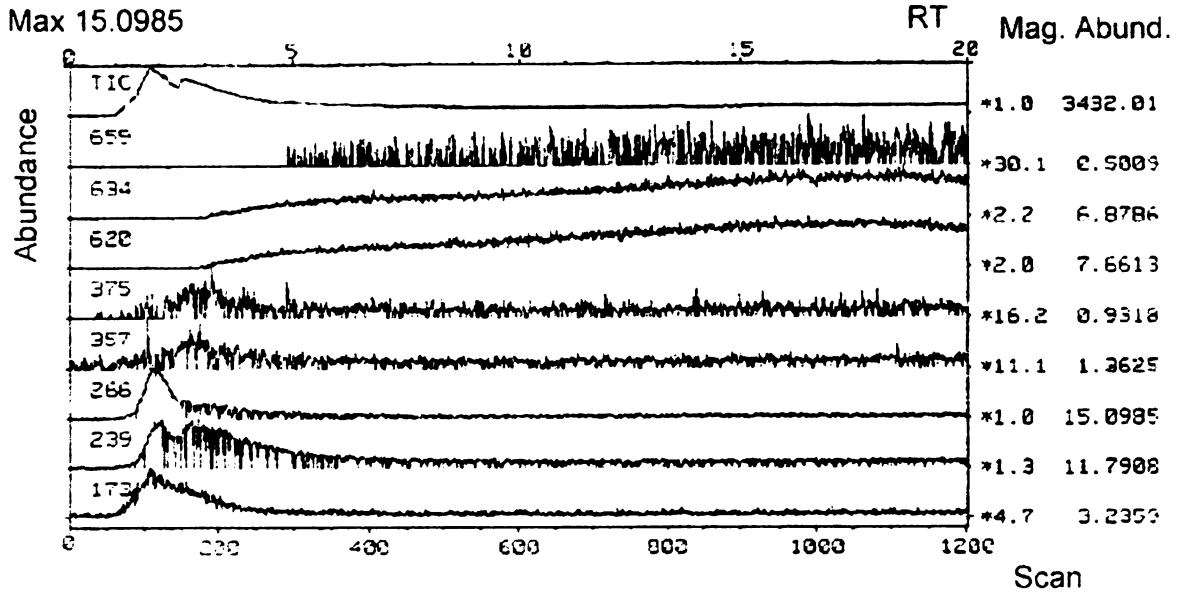
Analysis of intensity Using Mixed

The Mixed Procedure

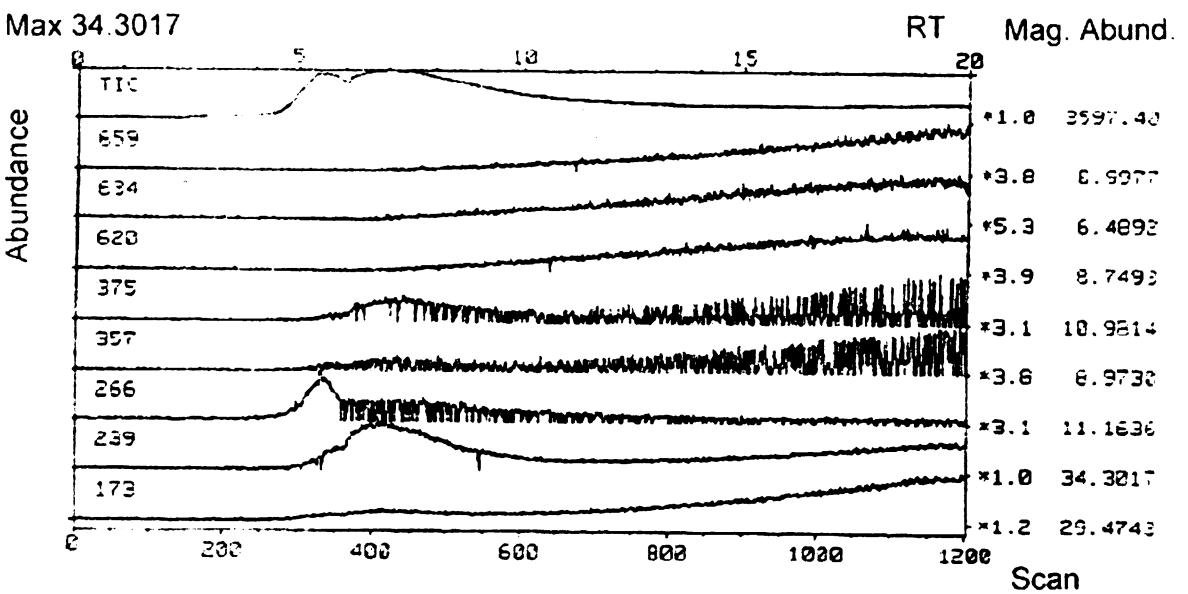
Differences of Least Squares Means

Effect	sample	_sample	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
sample	1	2	0.1020	0.4503	396	0.23	0.8209	Tukey-Kramer	0.9994
sample	1	3	-0.7890	0.4503	396	-1.75	0.0805	Tukey-Kramer	0.4032
sample	1	4	0.0740	0.4503	396	0.16	0.8695	Tukey-Kramer	0.9998
sample	1	5	-3.3530	0.4503	396	-7.45	<.0001	Tukey-Kramer	<.0001
sample	2	3	-0.8910	0.4503	396	-1.98	0.0485	Tukey-Kramer	0.2783
sample	2	4	-0.0280	0.4503	396	-0.06	0.9504	Tukey-Kramer	1.0000
sample	2	5	-3.4550	0.4503	396	-7.67	<.0001	Tukey-Kramer	<.0001
sample	3	4	0.8630	0.4503	396	1.92	0.0560	Tukey-Kramer	0.3101
sample	3	5	-2.5640	0.4503	396	-5.69	<.0001	Tukey-Kramer	<.0001
sample	4	5	-3.4270	0.4503	396	-7.61	<.0001	Tukey-Kramer	<.0001

APPENDIX 6 IIC CHROMATOGRAPH OF SAMPLE CONT IN DIP ANALYSIS (2ND SET)



APPENDIX 7 IIC CHROMATOGRAPH OF SAMPLE PCTA IN DIP ANALYSIS (2ND SET)



APPENDIX 8 TIC CHROMATOGRAMS OF ODOR PROFILES FROM HDPE FILMS ANALYZED WITH SPME/GC-MS

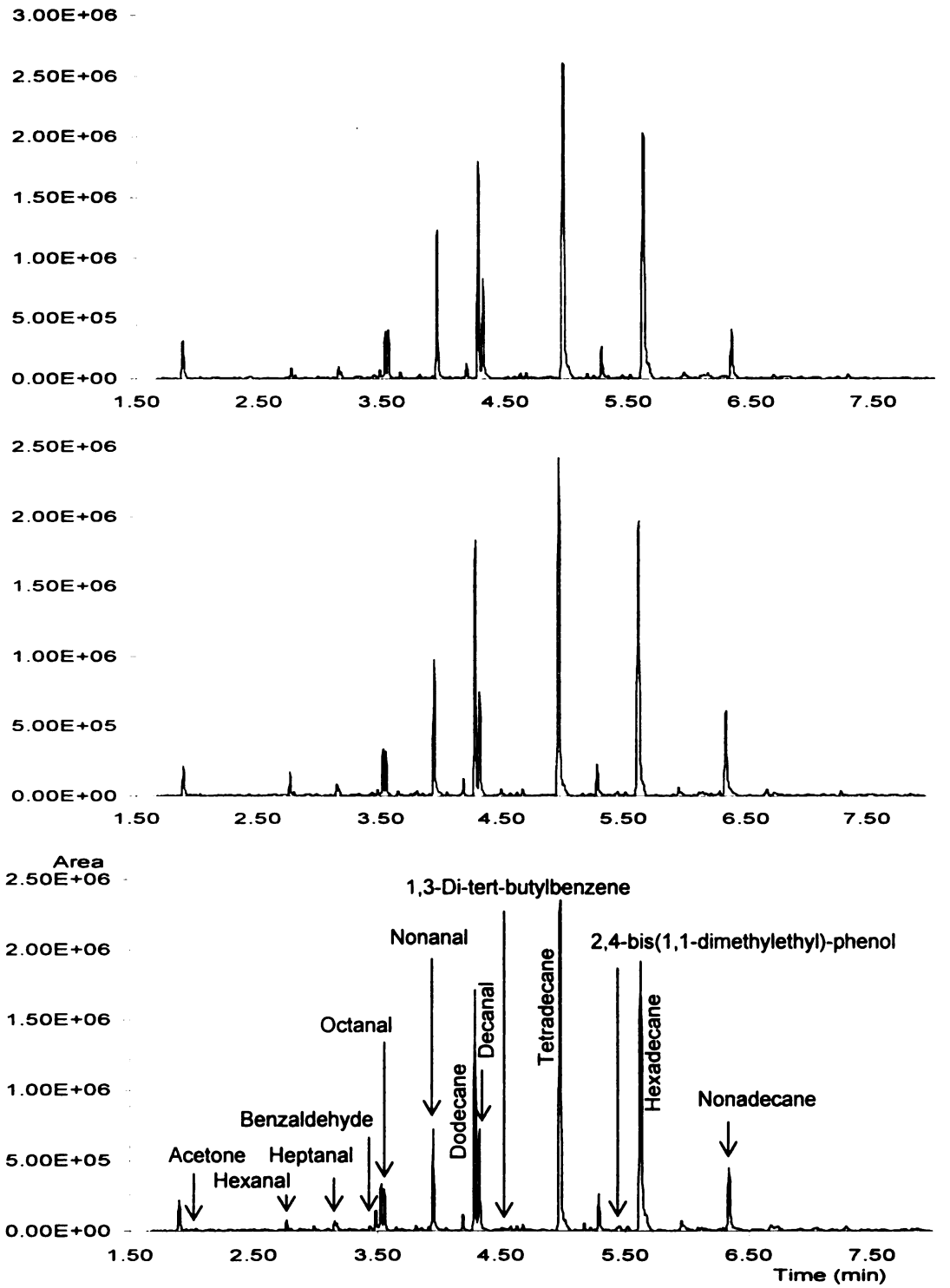


Figure A8.1 Total ion current chromatograms of odor profiles of triplicates of sample CONT

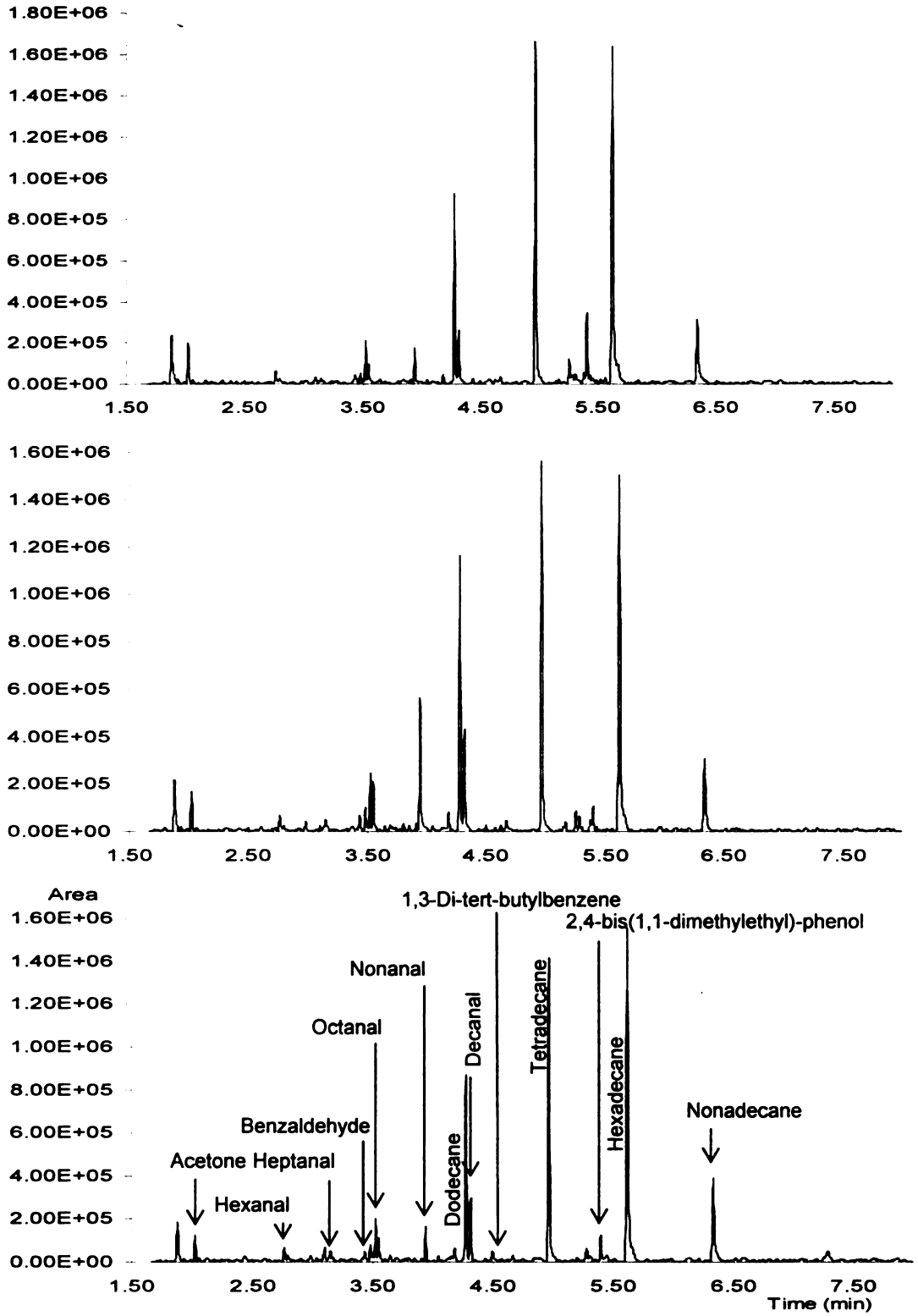


Figure A8.2 Total ion current chromatograms of odor profiles of triplicates of sample PATA

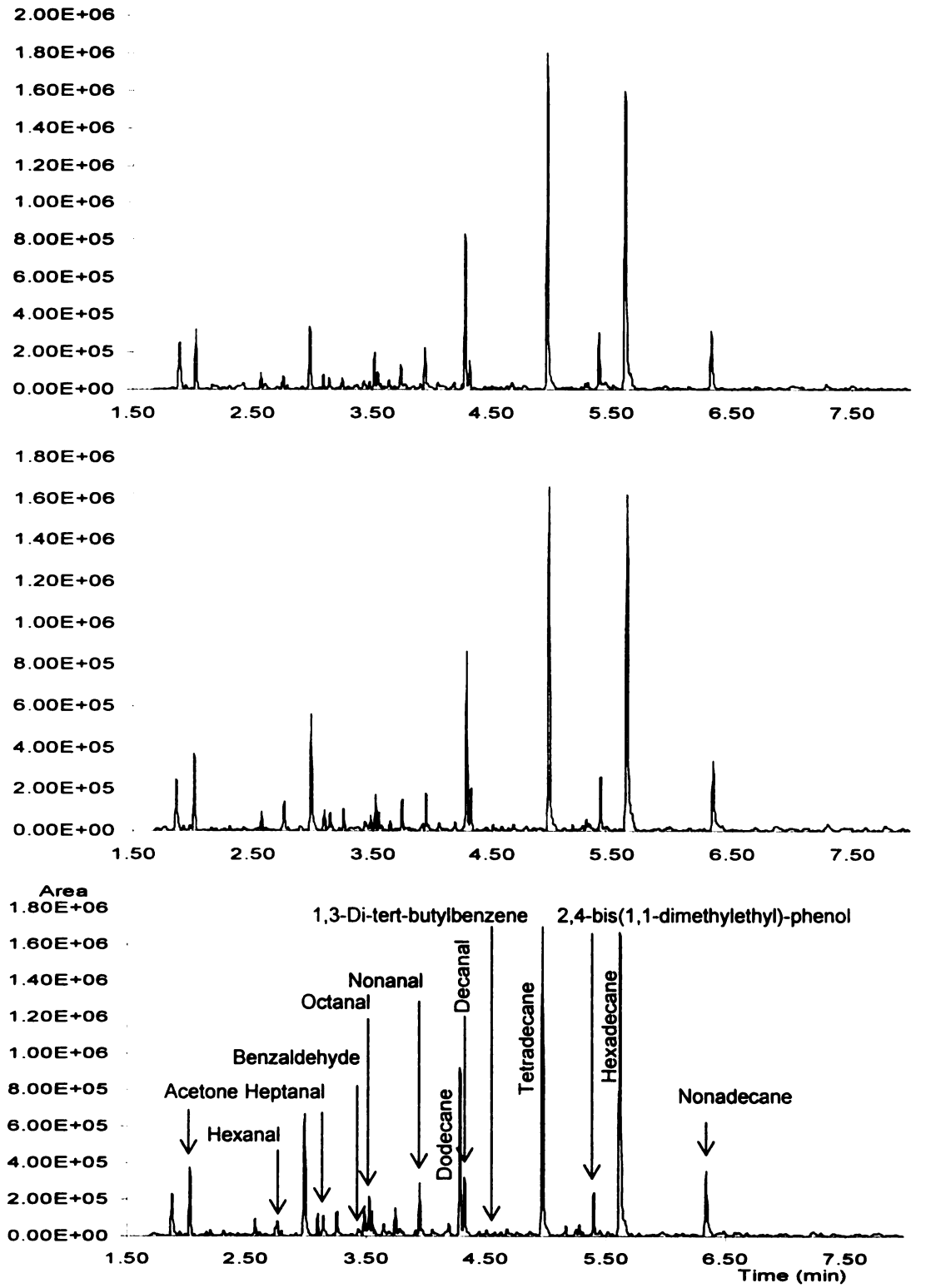


Figure A8.3 Total ion current chromatograms of odor profiles of triplicates of sample PBTA

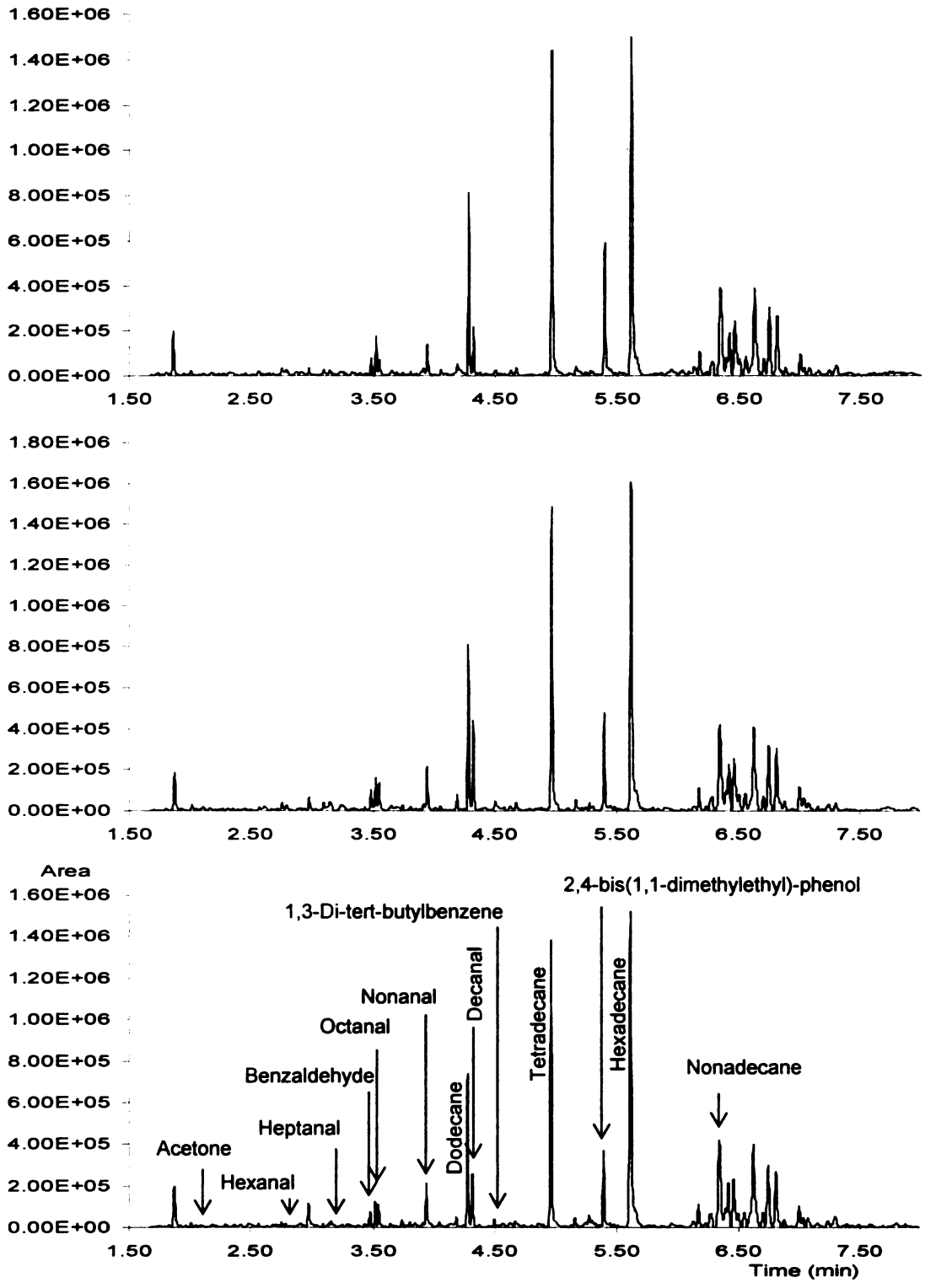


Figure A8.4 Total ion current chromatograms of odor profiles of triplicates of sample PBTB

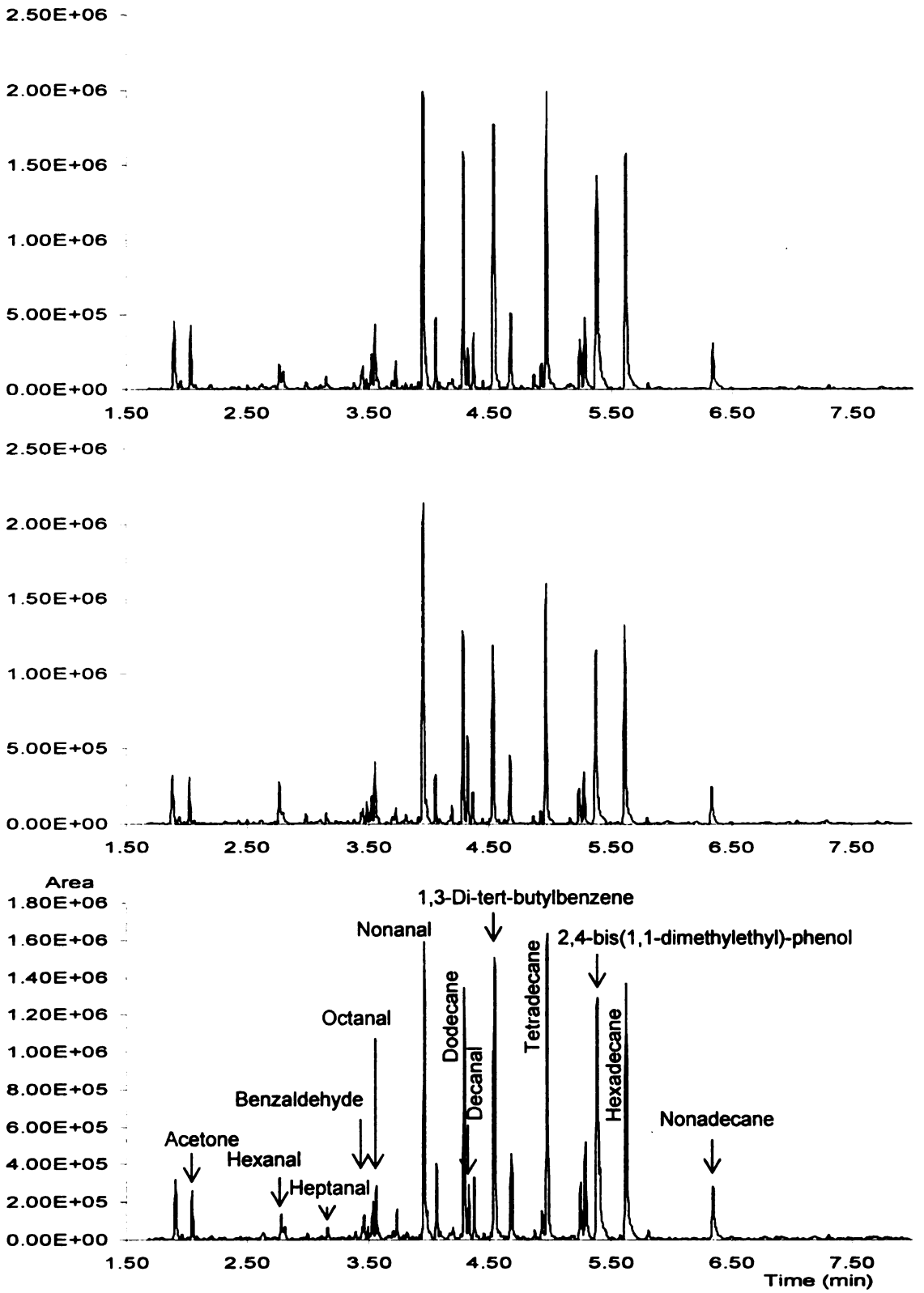


Figure A8.5 Total ion current chromatograms of odor profiles of triplicates of sample PCTA

APPENDIX 9 DESCRIPTION OF ODOR PROFILES USING GC/MS WITH ODO II SNIFFING PORT

Investigator 1

PATA		PCTA	
RT	Description	R.T.	Description
1:38	Something minty, apple-like	1:35	Plastic
1:56	Stinky		
2:20	Plastic		
2:25	Something odorous	2:25	Stinky
2:33	Sweet	2:30	Apple smell
2:37	Stinky	2:40	Stinky
2:44	Sweet	2:48	Sweet
2:54	Stinky	2:55	Something odorous
3:04	Plastic	3:02	Something odorous
3:12	Stinky		
3:17	Sweet	3:20	Something odorous
3:26	Plastic		
3:30	Sweet		
3:34	Plastic	3:34	Sweet
		3:39	Stinky, off odor
3:45	Caramel	3:45	Caramel
3:52	Plastic	3:56	Something odorous
4:06	Something odorous	4:04	Caramel
4:14	Plastic		
4:21	Sweet	4:21	Plastic
4:30	Something odorous	4:29	Something odorous
4:40	Plastic	4:44	Something odorous
4:54	Caramel	4:50	Candy, sweet
5:01	Something sweet	5:04	Detergent smell
5:10	Something sweet	5:12	Stinky
5:20	Plastic		
5:26	Detergent smell, fresh		
5:48	Something odorous		
5:56	Detergent smell		
6:13	Something odorous	6:17	Sweet

Investigator 2

PATA		PCTA	
RT	Description	R.T.	Description
1:33	Light odor		
		1:44	Light odor
1:53	Caramel (lightly)	1:54	Acid odor
2:04	Buttery		
2:10	Plastic-like odor		
2:16*	Plastic-like odor	2:17	Solvent, plastic
2:20	Plastic-like odor	2:25	Solvent-like odor
2:28	Stinky odor	2:35*	Strong odor
2:33	Solvent-like odor	2:40	Buttery, caramel
2:38*	Strong stinky odor	2:49	Buttery, caramel
2:45	Plastic, electric-like	2:54	Sweet odor
2:54	Plastic-like	3:04	Stinky
3:08	Metallic	3:10	Mushroom-like odor
3:15	Manure	3:15	Mushroom-like odor
3:20	Metallic	3:20	Something burning
3:30	Buttery	3:30	Sweet
3:35*	Strong odor	3:33	metallic
3:44	Buttery, caramel	3:41*	Stinky (strong), followed by caramel
3:54	Plastic	3:54	Stinky (strong), followed by caramel
3:58	Strong manure odor	3:59	Manure
4:06	Plastic	4:05	Plastic
4:16	Plastic-like odor	4:17	Plastic
4:21	Rubbery	4:20	Balloon
4:31	Plastic	4:37	Off odor
4:43	Cardboard	4:44	Stinky
		4:46	Cardboard
4:50*	Strong caramel	4:49	Stinky
4:55	Buttery		
5:08	Metallic	5:04	Slightly off odor
5:13	Balloon-like odor	5:11	Stinky, plastic-like
5:25	Slightly off odor	5:21	Off odor
5:34	Slightly off odor	5:47	Slightly off odor
5:48	Light odor		
6:09	Balloon, rubbery odor	6:10	Rubber band
7:27	Cardboard	7:20	Balloon
7:46	Plastic-like odor		

* The perceived odor was strong.

Investigator 3

PATA		PCTA	
RT	Description	R.T.	Description
0:56	Sweet		
1:28	Sweet	1:22	Light smell
1:37	Butter coca	1:41	Something odorous
1:54	Stinky	1:50	Something sweet
2:02	Stinky		
2:11	Plastic-like odor	2:09	Plastic-like odor
2:15*	Strong off odor	2:17	Plastic-like odor
2:21*	Strong stinky odor	2:21*	Perfume-like, plastic, stinky
2:25*	Strong plastic odor		
2:30	Fruity odor	2:30	Ripen fruit
2:37*	Stinky, strong	2:37*	Stinky, strong
2:42	Plastic-like odor	2:43	Perfume
		2:49	Stinky
2:52*	Strong plastic	2:56	Ripen fruit
3:04*	Burning plastic	3:02	Something burning
		3:08	Burning plastic
		3:12	Plastic
3:18*	Perfume	3:18	Perfume, fruity
3:29	Stinky	3:29	Burning wire
3:34	Stale	3:36*	Burning wire
3:40	Perfume		
3:45	Overcooked caramel	3:45	Burning coca butter
3:53*	Strong perfume	3:55	Something burning, sweet
4:06*	Cologne	4:09	Perfume, burning
4:17*	Balloon	4:15	Perfume, burning
4:26	Something odorous	4:20*	Plastic, strong
4:29*	Strong, stinky	4:30	Burning rubber
4:40	Plastic	4:39	Something odorous
4:45	Burning wire	4:45	Dry paper sheet, burning
4:51*	Perfume, followed by plastic	4:56	Something burning
5:10	plastic	5:05	Burning, slightly sweet
5:21	Cardboard	5:16	Light plastic scent
5:26	Sweet, cardboard	5:29	Plastic
5:35	Light smell	5:42	Plastic, smoky
5:50	Something odorous	5:59	Something odorous
6:00	Paperboard	6:12	Plastic, slightly sweet
6:48	Sweet	6:22	Plastic
7:01	Coca butter	7:10	Plastic
7:21	Plastic	7:19	Something odorous
7:58	Something burning, stinky	7:57	Something odorous

* The perceived odor was strong.

APPENDIX 10 PLS MODELS BASED ON PAIRS OF SAMPLES AND THEIR VALIDATION DATA

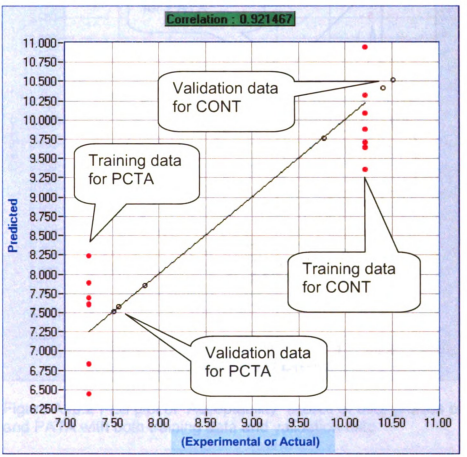


Figure A10.1 PLS plot of "Acceptability" scores of odor profiles of sample PCTA and CONT with both training data and validation data

Table A10.1 Validate the PLS model based on sample PCTA and CONT

Samples	Actual scores	Panel Opinion	Predicted scores	Average predicted	Opinion from PLS model
CONT_39	10.22	Acceptable	9.77	10.23	Acceptable
CONT_40	10.22	Acceptable	10.41	10.23	Acceptable
CONT_41	10.22	Acceptable	10.51	10.23	Acceptable
PCTA_19	7.25	Neutral	7.85	7.64	Neutral
PCTA_20	7.25	Neutral	7.57	7.64	Neutral
PCTA_21	7.25	Neutral	7.51	7.64	Neutral

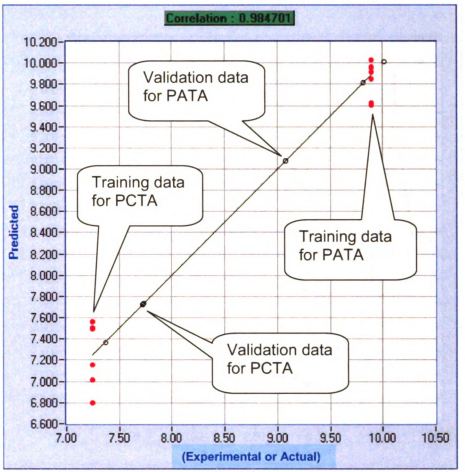


Figure A10.2 PLS plot of "Acceptability" scores of odor profiles of sample PCTA and PATA with both training data and validation data

Table A10.2 Validate the PLS model based on sample PCTA and PATA

Samples	Actual scores	Panel Opinion	Predicted scores	Average predicted	Opinion from PLS model
PATA_10	9.89	Acceptable	9.81	9.63	Acceptable
PATA_11	9.89	Acceptable	9.08	9.63	Acceptable
PATA_9	9.89	Acceptable	10.01	9.63	Acceptable
PCTA_19	7.25	Neutral	7.37	7.61	Neutral
PCTA_20	7.25	Neutral	7.73	7.61	Neutral
PCTA_21	7.25	Neutral	7.73	7.61	Neutral

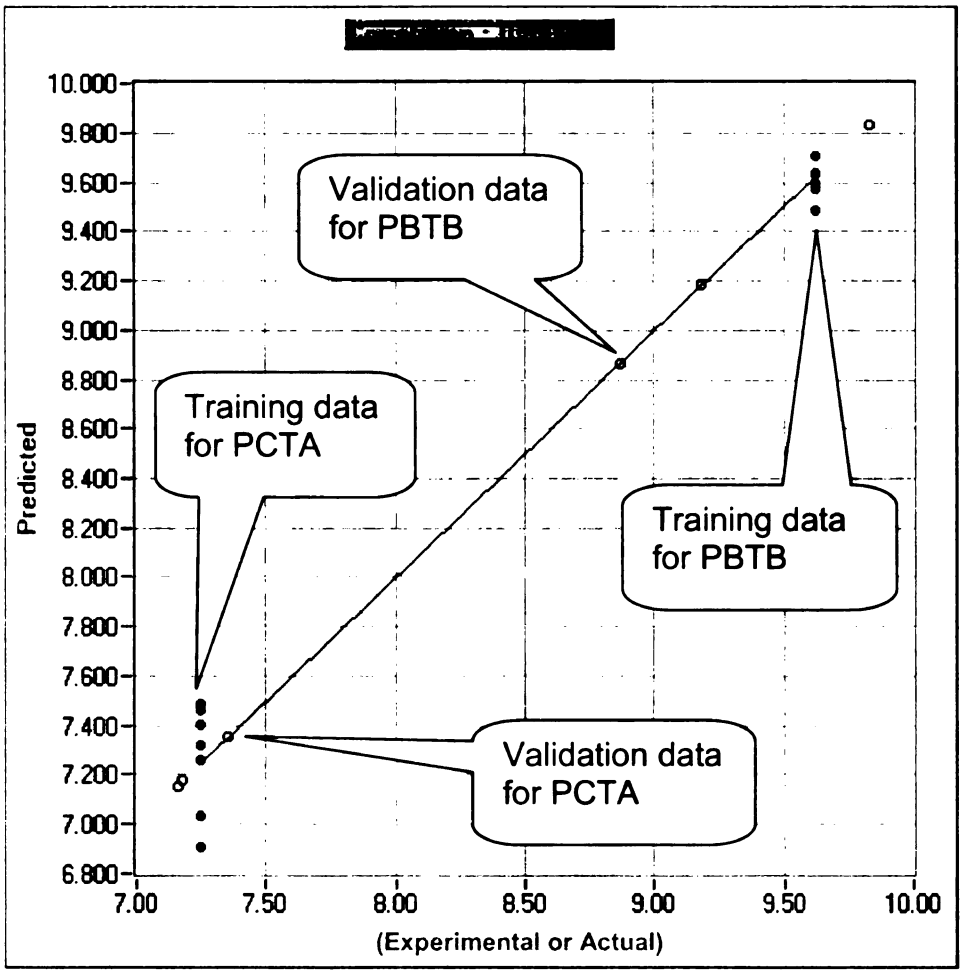


Figure A10.3 PLS plot of "Acceptability" scores of odor profiles of sample PCTA and PBTB with both training data and validation data

Table A10.3 Validate the PLS model based on sample PCTA and PBTB

Samples	Actual scores	Panel Opinion	Predicted scores	Average predicted	Opinion from PLS model
PBTB_29	9.62	Acceptable	9.83	9.29	Acceptable
PBTB_30	9.62	Acceptable	9.18	9.29	Acceptable
PBTB_31	9.62	Acceptable	8.87	9.29	Acceptable
PCTA_19	7.25	Neutral	7.35	7.23	Neutral
PCTA_20	7.25	Neutral	7.16	7.23	Neutral
PCTA_21	7.25	Neutral	7.18	7.23	Neutral

APPENDIX 11 RESPONSE AREAS OF ACETONE AND NONANAL BASED ON THE DATA FROM SPME/GC-MS ANALYSIS

Table A11.1 lists the response areas of the selected ion for acetone (m/z 58) and nonanal (m/z 57) in the SPME/GC-MS analysis.

Table A11.1 Response areas of ion m/z 58 for acetone and ion m/z 57 for nonanal detected in the odor profiles of different HDPE film samples in SPME/GC-MS analysis

Acetone (m/z 58)					
Duplicate	CONT	PATA	PBTA	PBTB	PCTA
1	12201	157529	293257	10832	358949
2	8210	137601	313017	18934	268136
3	14246	102985	334667	16335	220958
Average	11552	132705	313647	15367	282681
Nonanal (m/z 57)					
Duplicate	CONT	PATA	PBTA	PBTB	PCTA
1	895002	124454	156424	93162	1675261
2	689027	403363	132386	144804	1912029
3	524490	120826	199861	140635	1294369
Average	702840	216214	162890	126200	1627220

Figures A11.1 and A11.2 are the standard mass spectra of acetone and nonanal, respectively (NIST, 2005), based on which all the m/z ions and their intensities can be determined (NIST, 2005) and the results were listed in Table A11.2.

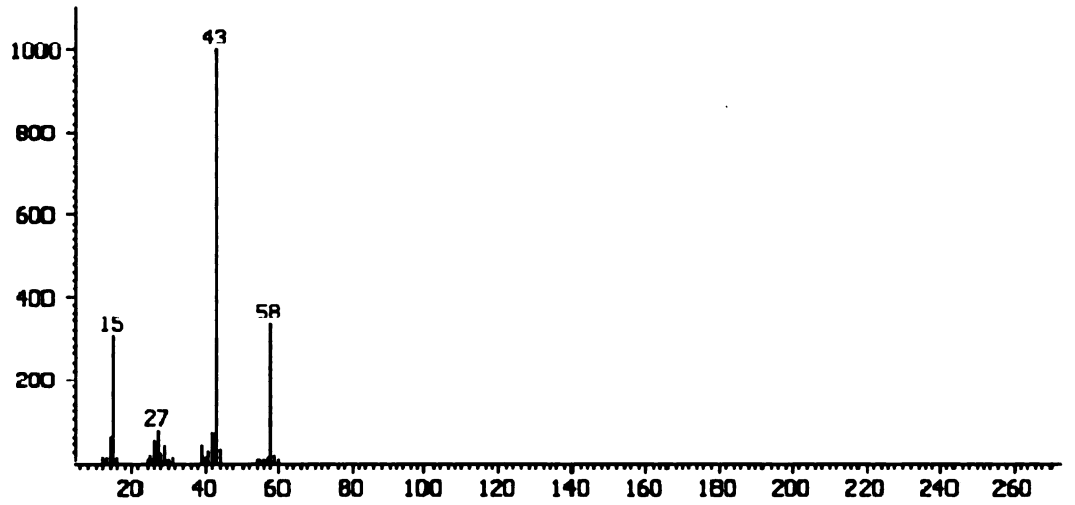


Figure A11.1 Standard mass spectrum of acetone (NIST, 2005)

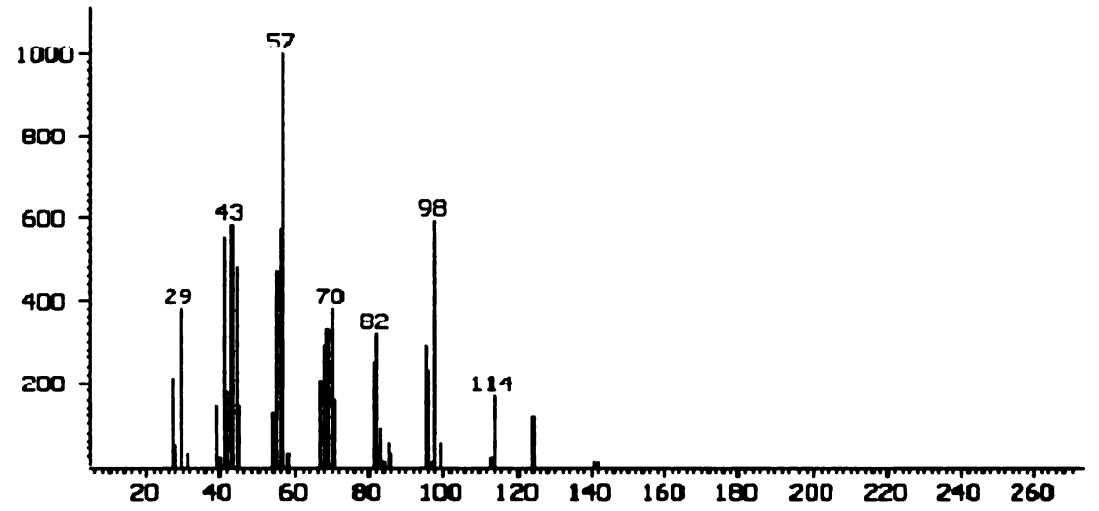


Figure A11.2 Standard mass spectrum of nonanal (NIST, 2005)

Table A11.2 Ions and their intensities in the mass spectra of acetone and nonanal (NIST, 2005)

Acetone

m/z	Intensity	m/z	Intensity	m/z	Intensity	m/z	Intensity	m/z	Intensity
12	5	25	10	31	5	44	24	59	13
13	5	26	49	39	34	54	1	60	1
14	59	27	75	40	7	55	3		
15	305	28	18	41	19	56	3		
16	6	29	37	42	68	57	9		
24	2	30	2	43	999	58	331		

Nonanal

m/z	Intensity	m/z	Intensity	m/z	Intensity	m/z	Intensity	m/z	Intensity
27	212	43	584	67	204	84	9	113	18
28	44	44	478	68	292	85	53	114	168
29	381	45	142	69	327	86	27	124	115
31	27	54	124	70	381	95	292	141	9
39	142	55	469	71	159	96	230	412	9
40	18	56	575	81	248	97	9		
41	549	57	999	82	319	98	593		
42	177	58	27	83	88	99	53		

Calculations were then made to calculate the percentage of the intensities of m/z 58 ion and m/z 57 in the total intensities of all the ions of acetone and nonanal, respectively.

Acetone

$$(m/z58)\% = \frac{331}{2090} \times 100\% = 15.84\%$$

Nonanal

$$(m/z57)\% = \frac{999}{8551} \times 100\% = 11.68\%$$

Based on the calculations shown above and the data in Table A11.1, the response areas of compound acetone and nonanal were determined (see Table A11.3).

Table A11.3 Average response areas of acetone and nonanal detected in the odor profiles of different HDPE film samples in SPME/GC-MS analysis

Sample	CONT	PATA	PBTA	PBTB	PCTA
Acetone	72929	837784	1980094	97014	1784602
Nonanal	6017466	1851147	1394606	1080479	13931678

APPENDIX 12 PLS MODELS BASED ON TRANSFORMED DATA OF PAIRS OF SAMPLES

Data in Table 7.6 was Log10 transformed first (see Table A12.1), which was then used to build the PLS models. The predicted values of the validation samples based on the models were then reverse-converted to the predicted response areas (see Tables A12.2 to A12.5).

Table A12.1 Log10 transformed average response areas of acetone and nonanal

Sample	CONT	PATA	PBTA	PBTB	PCTA
Acetone	4.86	5.92	6.30	4.99	6.25
Nonanal	6.78	6.27	6.14	6.03	7.14

Table A12.2 Predicted response areas of acetone and nonanal of sample CONT and PCTA by PLS model based on transformed data

Sample	Acetone Prediction[1]	Ave.[2]	% Diff	Nonanal Prediction[1]	Ave.[2]	%Diff
CONT_39	5.049226			6.833474		
CONT_40	4.782503	72051	-1	6.757622	6010206	-.01
CONT_41	4.741183			6.745572		
PCTA_19	5.941827			7.066130		
PCTA_20	6.082370	1111069	38	7.100187	12339096	-11
PCTA_21	6.113026			7.107533		

1. Predicted values of the Log10 transformed data.
2. Average response area after reverse-converting the average of predicted values listed in the previous column.

Table A12.3 Predicted response areas of acetone and nonanal of sample PATA and PCTA by PLS model based on transformed data

Sample	Acetone Prediction[1]	Ave.[2]	% Diff	Nonanal Prediction[1]	Ave.[2]	%Diff
PATA_10	5.929363			6.293779		
PATA_11	6.019338	893693	7	6.524975	2239450	21
PATA_9	5.904864			6.231670		
PCTA_19	6.234741			7.098314		
PCTA_20	6.189243	1599937	-10	6.974865	10355935	26
PCTA_21	6.188325			6.972389		

1. Predicted values of the Log10 transformed data.
2. Average response area after reverse-converting the average of predicted values listed in the previous column.

Table A12.4 Predicted response areas of acetone and nonanal of sample PBTA and PCTA by PLS model based on transformed data

Sample	Acetone Prediction[1]	Ave.[2]	% Diff	Nonanal Prediction[1]	Ave.[2]	%Diff
PBTA_49	6.296240			6.209817		
PBTA_50	6.300451	1989951	0.5	6.131689	1450481	4
PBTA_51	6.299836			6.143030		
PCTA_19	6.251375			7.110338		
PCTA_20	6.250697	1786226	0.1	7.124948	12540379	-10
PCTA_21	6.253737			7.059646		

1. Predicted values of the Log10 transformed data.
2. Average response area after reverse-converting the average of predicted values listed in the previous column.

Table A12.5 Predicted response areas of acetone and nonanal of sample PBTB and PCTA by PLS model based on transformed data

Sample	Acetone Prediction[1]	Ave.[2]	% Diff	Nonanal Prediction[1]	Ave.[2]	%Diff
PBTB_29	4.891031			5.940023		
PBTB_30	5.201855	141676	46	6.221129	1495753	38
PBTB_31	5.361001			6.363428		
PCTA_19	6.189163			7.087778		
PCTA_20	6.305519	1830209	3	7.187546	14147119	2
PCTA_21	6.292820			7.176680		

1. Predicted values of the Log10 transformed data.
2. Average response area after reverse-converting the average of predicted values listed in the previous column.

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