This is to certify that the

thesis entitled

THE PATTERN OF VOLATILE CHANGES DURING THE MODIFIED ATMOSPHERE PACKAGING (MAP) STORAGE OF APPLE

presented by

Weimin Deng

has been accepted towards fulfillment
of the requirements for

Masters degree in Horticulture

Major professor

Date 5-11-99

THE PATTERN OF VOLATILE CHANGES DURING THE MODIFIED ATMOSPHERE PACKAGING (MAP) STORAGE OF APPLE

By

Weimin Deng

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Horticulture

1999

ABSTRACT

PATTERN OF VOLATILE CHANGES DURING THE MODIFIED ATMOSPHERE PACKAGING (MAP) STORAGE OF APPLE

By

Weimin Deng

A simple sampling method was developed to measure volatiles produced by apple (*Malus xdomestica Borkh* cv. Golden Delicious) fruit in modified atmosphere packages. The sampling system incorporated GC column segments installed in gas tight syringes and used as needles for volatile analysis. The number of times the syringe plunger was pumped required to maximize GC response for each needle/volatile combination was determined. The pump number for maximal GC response was greatest (~40) for the larger more lipophilic molecules and lowest (~5-10) for the smaller, more polar molecules that did not readily partition into the column coating. A capillary needle resulted in an increase in GC response of 3- to 15-fold relative to an uncoated stainless steel needle. By using the capillary needle, at least 10 volatile compounds and their permeabilities to be measured under the real storage condition. This methodology was used to determine the dynamic relationship between flavor volatile synthesis and package atmosphere for packaged apple.

Dedicated to my father and mother

ACKNOWLEDGMENTS

I would like to express my sincere appreciation to Dr. Randolph M. Beaudry for his guidance, patient training, continued encouragement, help and support throughout my years of graduate study. The opportunity to study under his tutelage has been enlightening and gratifying.

I would like to express my special thanks to the member of my guidance committee: Dr. Art Cameron, Dr. Jerry N. Cash and Dr. Ruben Hernandez for their valuable advice, help, and critical review of this thesis.

I wish to thank Drs. David R. Dilley, Frank Dennis, Robert C. Herner, Muraleedharan G. Nair, John Everard, Zhenyong Wang, Jun Song, Nazir Mir, Dina Kadyrzhanova for their assistance and encouragement.

I also wish to thank Hongying Jiang; Yali Zhu, Cathy S. Roth, Silvanda Silva, Jennifer Dwyer, Stacie Wee, Jessica Townsend, Rujida Leepipattanawit, Rufino Perez, Michael Wendorf, Costas Vlachonasios, TJ McCully and all colleagues in the post-harvest lab for their help, friendship and encouragement.

I am grateful to Joyce Jackson, Lorri K. Busick, Sherry Mulvaney and all the people in the Department Office for their assistance.

Finally, I express my deep love and appreciation to my wife Yuan Dong, daughter Lu Deng, brothers and sisters for their love, understanding, support and encouragement.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 2

CHAPTER 3

CHAPTER 4

CHAPTER I: INTRODUCTION

The methodologies for organic volatile compounds analysis include solvent extraction, distillation, and headspace sampling. For the analysis of odorous volatile compounds of apple fruits, headspace analysis is considered the most suitable and widely used technique "since it reveals the identity and the concentration in the vapor phase of those compounds that are directly responsible for the odor of the product" (Maarse, 1991). Headspace sampling involves taking the sample from vapor phase above the food. This technique offers the possibility of volatile isolation without sample destruction or artifact formation (Land, 1975; Olafsdottir et al., 1985). Therefore, the volatiles that are measured by headspace analysis are more representation to the odor in the concentration and ratios presented to the human nose if compared with other analysis techniques (Bemelmans, 1978).

Of the various headspace techniques, direct or static headspace analysis is the simplest and fastest for studying the aroma properties and quality of food materials (Weurman, 1961; Martin, 1969; Guadagni et al., 1971; Yamashita et al., 1977; Mcnally and Grob, 1985). Direct headspace analysis is that in which a volume of the atmosphere containing the volatiles of interest is removed from the system and directly introduced into a gas chromatograph (GC) or other detection device. However, aroma volatiles are often produced at very low levels by almost all produce and therefore accumulate to low concentrations in most sampling systems. Direct headspace sampling often does not deliver enough of the molecules of interest to permit detection by flame ionization or mass spectrometry following separation by gas chromatograph (Reineccious, 1983).

The lack of sufficient analyte in the gas phase has lead to techniques that increase sample amount by concentrating volatiles using absorptive and adsorptive surfaces or devices (Bertsch et al., 1975; Buckholz et al., 1980; Farwell et al., 1979; Murray, 1977; Olafsdottir et al., 1985). In many cases, volatile compounds are removed from the headspace of a sampling chamber by carrier (N_2) gas purging and trapped onto a porous polymer (Hirvi and Honkanen, 1982; Shamaila et al., 1992; Song and Bangerth, 1994), hence the term 'purge-and-trap' is used to refer to this approach. The volatile compounds are extracted from the trapping material by heating (Dirinck et al., 1981; Mattheis et al., 1991a, 1991b) or by various liquid extraction techniques (Clark and Cronin, 1975; Guadagni et al., 1971; Olafsdottir, 1985; Shamaila, et al., 1992; Song and Bangerth, 1994). Tenax-GC (2,6-diphenyl-p-phenylene oxide polymer) and Tenax-TA are two popular and effective sorbants that have been widely used for the adsorption of a variety of aroma volatiles from foods. The purge-and-trap technique in which a gas is used to sweep volatiles onto a sorptive surface is specifically termed the dynamic headspace technique (Dirinck et al., 1981; Mattheis et al., 1991a, 1991b; Olafsdottir et al., 1985; Shamaila et al., 1992; Song, 1994; Song and Bangerth, 1994).

Dynamic headspace sampling is arguably the most suitable method for volatile analysis for apple fruit after CA storage or during ripening. However, it is incompatible with low-volume sealed systems such as packages or small vials. In addition, these kinds of methods are generally time and labor intensive. In many cases, research often requires the analysis of only a few of the more

plentiful volatiles but from many samples of food. The relationship between aroma biosynthesis and aroma accumulation in packages of produce for some major volatile compounds is not well understood. It would be useful, therefore, to have a sampling technique that is simple, fast, and accurate specifically for this system.

Modified-atmosphere packing (MAP) "has the potential to provide low O_2/high CO_2 regimes similar to those of CA storage" (Beaudry et al., 1992). It is a widely used technique for storage fresh and lightly processed fruits and vegetables. However, the loss or change of flavors by packing material during storage are "the greatest problems in providing high quality food" (Matsui et al., 1994). The flavor profile of apple in MAP can be affected by the production rate, which is related to O_2 and CO_2 concentration in the packaging and storage temperature and also by the interaction of flavor compounds with the package polymer (Debeaufort and Voilley, 1994; Mason et al., 1992; Matsui et al., 1994; Nielsen and Giacin, 1994; Osajima and Matsui, 1993). Loss of flavor constituents or changes their profile may due to sorption into packaging material or permeation through the package to the environment (Nielsen and Giacin, 1994) or due to the acquisition of flavor volatiles directly from the packing or indirectly by permeation from the environment through the package to the food (Mason et al., 1992).

Low-density polyethylene (LDPE) is a commonly used polymer film for the construction of MAP for fresh and lightly processed produce. Its high permeabilities to O_2 and CO_2 (Beaudry et al., 1992; Beaudry and Dube, 1994)

and water vapor (Debeaufort and Voilley, 1994) are well studied and make LDPE especially amenable to packaging of fresh produce. However, only a few studies on the permeability of LDPE film to aroma volatiles have been published (Delassus et al., 1988; Fukamachi et al., 1994).

Aroma volatile biosynthesis by apple fruit is well studied under various conditions, including following CA storage (Brackmann et al., 1993; Mattheis et al., 1991a; Song and Bangerth, 1994; Willaert et al., 1983) and during ripening (Song and Bangerth, 1994; Seifabad, 1988). To our knowledge, however, aroma volatile changes during MAP storage have not been studied.

Apple volatiles are produced in minute quantities, making study difficult and requiring the use of specialized equipment to concentrate, separate and detect these compounds in the atmosphere. Under actual conditions of apple storage such as low O_2, high CO_2, and low temperature, volatile emanation is reduced even further making *in situ* studies even more problematic. These are some of the reasons why most investigators have chosen to removed the fruit from the storage environment and place them under conditions of higher temperature and O_2. Studies employing these practices likely have masked the responses to the modified atmospheres. In order to understand the influence of the storage environment on the physiology and the biochemistry of aroma production, it is important to study the aroma biosynthesis of apples within their storage environment.

The purpose of this investigation was three-fold: 1. to develop a simple, rapid, and accurate system for measuring volatiles; 2. employ a package system

for the simultaneous measurement of volatile production by plant organs and the permeability of the packaging film to those volatiles; and 3. measure the production of volatiles as affected by package O_2 and storage duration.

Literature Cited:

Beaudry, R. M., A. C. Cameron, A. Shirazi and D. Dostal Lange. 1992. Modified-atmosphere packaging of blueberry fruit: Effect of temperature on package oxygen and carbon dioxide. J. Amer. Soc. Hort. Sci. 117

Beaudry, R. M. and N. Dube. 1994. A permeable membrane respirometer. HortScience 29 (5):132 (Abstr.).

Brackmann, A., J. Streif and F. Bangerth. 1993. Relationship between reduced aroma production and lipid metabolism of apples after long-term controlled-atmosphere storage. J. Amer. Soc. Hort. Sci. 118 (2): 243-247.

Clark, R. G. and D. A. Cronin. 1975. A new technique for trapping and sensory evaluation of flavor volatiles. J. Sci. Food Agric. 26: 1009.

Debeaufort, F. and a. Voilley. 1994. Aroma compounds and water vapor permeability of edible films and polymeric packaging. J. Agric. Food Chem. 42:2871-2875.

DeLassus P. T., J. C. Tou, M. A. Babinec, D. C. Rulf, B. K. Karp, and B. A. Howell. 1988. Transport of apple aromas in polymer films. In Food and Packaging Interactions, J. H. Hotchkiss,(ed.)ACS SymposiumSeries No. 365.

Dirinck, P. J., H. L. De Pooter, G. A. Willeart and N. M. Schamp. 1981. Flavor quality of cultivated strawberries: The role of the sulfur compounds. J. Agric. Food Chem. 29:319.

Fukamachi, M., T. Matsui, M. Shimoda, and Y. Osajima. 1994. Derivation of thermodynamic sorption equation of flavors with packaging films. 2. J. Agric. Food Chem. 1994 42:2893-2895.

Guadagni, D. G., J. L. Bomben and J. S. Hudson. 1971. Factors influencing the development of aroma in apple peels. J. Sci. Food Agric. 22:110-115.

Hirvi, T. and E. Honkanen. 1982. The volatiles of two new strawberry cultivars, "Annelie" and "Alaska Pioneer," obtained by backcrossing of cultivated strawberries with wild strawberries, *Fragaria vesca*, Rugen and *Fragaria virginana*. Z. Lebensm. Unters. Forsch. 175:113-116.

Land, D. G. 1975. Techniques for assessing odour: uses and limitations. Proc. Int. Symp. Aroma Research. Pudoc., Wageningen, The Netherlands.

Martin, J. M. 1969. Gas chromatographic qualitative and semiqualitative analysis of apple aroma by means of retention indexes. J. Anal. Chem. Acta. 48:169.

Mason R. C., P. T. Delassus, G. Strandburg, and B. A. Howell. 1992. Diffusion of flavors in polymers: effect of permeant size and shape. Tappi Journal. 75 (6):163-168.

Matsui, T., M. Fukamachi, M. Shimoda, and Y. Osajima. 1994. Derivation of thermodynamic sorption equation of flavors with packaging films. 1. J. Agric. Food Chem. 42(12):2889-2892.

Mattheis, J. P., D. A. Buchanan, and J. K. Fellman. 1991a. Change in apple fruit volatiles after storage in atmospheres inducing anaerobic metabolism. J. Agri. Food Chem. 39:1602-1605.

Mattheis, J. P., J. K. Fellman, P. Chen, and M. Patterson. 1991b. Changes in headspace volatiles during physiological development of bisbee delicious apple fruit. J. Agric. Food Chem. 39:1902-1906

McNally, M. E. and R. L. Grob. 1985. Current application of static and dynamic headspace analysis. A review. Part II: non environmental applications. J. Amer. Lab. Feb:106.

Nielsen, T. G. and J. R. Giacin. 1994. The sorption of Limonene/ethyl acetate binary vapor mixture by a biaxially oriented polypropylene film. Packaging technol. and Sci. 7:247-256.

Olafsdottir, G., J. A. Steinke and R.C. Lindsay. 1985. Quantitative performance of a simple Tenax-GC adsorption method for use in the analysis of aroma volatiles. J. Food Sci. 50:1431-1436

Osajima, Y. and T. Matsui. 1993. Dynamic Approach to the Sorption of flavors into a food packaging film. Anal. Sci. 9:753-763.

Reineccious, G. 1983. Determination of flavor components. 43th Annual Meeting Inst. Food Technologists, New Orleans, LA.

Seifabad, T. C. 1988. Effect of harvest date and ethylene concentration in controlled atmosphere storage on the quality of empire apple. PhD. Thesis, Michigan State Univ.

Shamaila, M., W. D. Powrie and B. J. Skura. 1992. Analysis of volatile compounds from strawberry fruit stored under modified atmosphere packaging (MAP). J. Food Sci. 57:1173-1176.

Song J. and F. Bangerth. 1994. Production and development of volatile aroma compounds of apple fruits at different times of maturity. Act Horticulturae. 357.

Weurman, C. 1961 Gas-liquid chromatographic studies on the enzymatic formation of volatile compounds in respberries. J. Food Technol. 15:531.

Willaert, Dirinck, De Pooter, and Schamp. 1983. Objective measurement of aroma quality of golden delicious apple as a function of controlled-atmosphere storage time. J. Agric. Food Chem. 31:809-813.

Yamashita, I., Y. Nemoto and S. Yoshikawa. 1977. Studies on flavor development in strawberries. 4. Biosynthesis of volatile alcohol and esters from aldehyde during ripening. J. Agric. Food Chem. 25: 1165.

CHAPTER II: LITERATURE REVIEW

"Biogenesis is the natural process by which flavor develops with progressive ripening, reaches an optimum value corresponding to the perfect ripening stage and subsequently exhibits a generalized rapid decay" (Feranoli, 1973).

IMPORTANCE OF VOLATILE COMPOUNDS IN FOOD

Volatile compounds are often present in small amounts in food. The concentration of the major volatile compounds varies from 10^{-1} to 10^{-3} ppm, while those of the trace constituents may be as low as 10^{-7} ppm (Bemelmans, 1978). Despite these low content levels, the flavor characteristics of a food are derived mainly from its volatile components. The degree to which volatiles contribute to the flavor is dependent on their threshold value and their concentration (Mulders, 1973). The concentration, ratio, and mixture of volatiles make the aroma specific to each type of food. Aroma as a component of flavor is one of the most important quality factors of food. It has been estimated that aroma comprises 70% of what we perceive of as flavor. Product re-purchase is highly dependent upon possession of acceptable flavor. In some cases, consumers are attracted to test a particular food solely from its aroma (Seifabad, 1998).

Volatiles have properties other than aroma that may confer value to their presence. For instance, some plant volatiles have been identified having antimicrobial activity. Red raspberries and strawberries possess some antifungal volatile compounds, which when applied at high concentrations, can completely inhibit postharvest decay fungi (Fries, 1973; Farag et al., 1989; Vaughn et al., 1993; Wilson et al., 1987). Davis and Smoot (1972) found *Penicillium digitatum* was inhibited by volatile C_5-C_9 aldehydes in mature citrus fruit. Hexanal was reported to inhibit fungal growth on apple slices and artificial media (Song et al., 1996). Importantly, this antifungal volatile is actively converted to hexylacetate by the apple itself such that no residues of the active compounds remain only

hours after application.

IMPORTANCE OF AROMA VOLATILES OF APPLE FRUIT

Aroma comprises the major component of flavor and directly affects the organoleptic quality of apple fruit. The amount and kinds of volatiles are affected by many factors including culture (Brown et al., 1968; Cunningham et al., 1986; Dimick and Hoskin, 1983; Paillard, 1981; Smock and Neubert, 1950; Yahia, 1986; Yahia et al., 1991), orchard environment (Brown et al., 1968; Somogyi et al., 1964; Yahia, 1994; Zerbinni et al., 1980), harvest date (Brown et al., 1966; Sapers, 1977; Seifabad, 1988; Song, 1994), stage of fruit development and storage conditions (Song, 1994; Willaert et al., 1983; Yahia, 1986; Yahia et al., 1991; Yahia, 1991a). Changes in the amount and kinds of volatiles occur during storage, thereby changing the apple's aroma and flavor. Understanding and controlling the pattern of volatile changes during storage is an important challenge for the apple industry.

PROGRESS IN APPLE VOLATILE RESEARCH

Interest in apple volatiles and essences began in this century. Power and Chestnut (1920) identified a number of compounds they believed responsible for aroma. White (1950) enlarged the list of compounds that contribute to apple aroma. He reported that the volatiles contained eight alcohols, four carbonyls, and numerous esters. Huelin (1952) identified several aldehydes and ketones. In the meantime, he found acetaldehyde as a major constituent. Due to the application of more powerful analytical tools such as the combined gas chromatography-mass spectrometry (GC-MS) (Holmes and Morrel, 1957) and

the use of more sensitive detectors such as the flame ionization detector (FID), detection of volatile compounds at the part per billion (ppb) level became possible in the early 1960's. Since then, the number of volatile compounds identified from apple has increased rapidly (Fig. 1). Flath et al. (1967) identified 56 compounds, of which, twenty were previously unreported. They found that cis or trans-2-hexenal and ethyl 2-methyl-butyrate had an apple or apple-like aroma; the former two were described as having a green apple odor. Koch et al. (1976), demonstrated the importance of (E)-2-hexenal in the odor of commercial apple essence. Williams et al. (1980) reported 88 identified components in which 55 were not found previously. To date, over 300 organic volatile compounds have been identified from apples or apple products (Dimick and Hoskin, 1983; Maarse, 1991), but only 20-40 volatiles contribute to apple aroma (Table 1, Fig. 1). Ethyl 2-methyl butanoate, ethyl butanoate, (E)-2-hexenal, (E)-2-hexenol, hexyl acetate, hexyl butanoate, hexyl hexanoate, hexanol, 1-hexanol, 1-butanol, (Z)-3-hexenal,(Z)-3-hexenol, (E)-hexenyl acetate, -damascenone, ethyl hexanoate, propyl 2-methyl butanoate, 4-methylallyl benzene, and other unidentified volatiles are reported to contribute to typical apple aromas and are considered to be character impact compounds (Berger, 1991). Aldehydes are the most abundant group of volatiles in immature apples (Depooter et al., 1987), but when fruit ripening begins, the major volatiles are esters and aliphatic alcohols (Flath et al., 1967; Mattheis et al., 1991b; Song, 1994; Song and Bangerth, 1994). Volatiles, including the above character-impact volatile compounds of apple do not contribute equally to apple aroma. For example,

ethyl 2-methylbutyrate was 30-fold lower than hexanol in commercial apple juices, but it was 30 times more important to juice aroma than hexanol (Lea and Ford, 1991). Jepsen (1978) reported that apple juice flavor quality was positively correlated with ethyl 2-methylbutyrate, (E)-2-hexanal and butanol, but negatively correlated with ethanol and ethyl acetate.

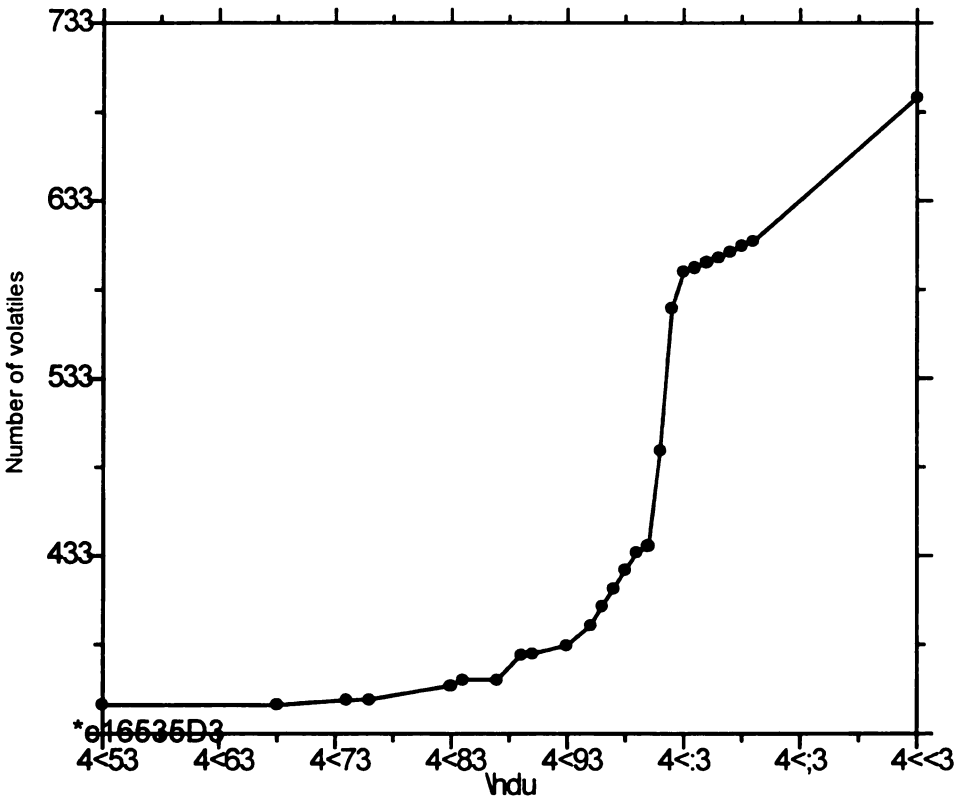


Fig. 1. Number of volatiles identified to be produced by apples since 1920. (from Yahia, 1994)

Table 1. Volatile components isolated from apple (Dimick and Hoskin, 1983; Maarse, 1991; Yahia, 1994). (Asterisk indicates important volatiles to apple flavor)

Alcohols:	
methanol	n-propanol
ethanol	isopropanol
l-propanol	n-butanol*
2-butanol	isobutanol
pentanol	2-methyl butanol
2-methyl butanol-1-ol	2-methyl-2-butanol
3-methyl butanol	2-pentanol
3-pentanol	4-pentanol
2-methyl-2-pentanol	3-methyl pentanol
4-methyl pentanol	hexanol *
(E)-2-hexenol	(E)-3-hexenol
1-hexen 3-ol	5-hexenol
(Z)-3-hexen-1-ol	(E)-hex-2-en-1-ol
(E)-2-hexen-1-ol	heptanol
octanol	(Z)-3-octen-1-ol
3-octanol	decanol
2-heptanol	2-octanol
E-thylhexanol	2-nonanol
2-phenethanol	1-nonanol
6-methyl-5-heptanol	(Z)-5-octenol
benzyl alcohol	terpinen-4-ol
isobareol	citronellol

Table 1 (Cont'd)

geraniol	eugenol
Esters:	
(formates):	
methyl formate	ethyl formate
propyl formate	butyl formate
2-methylbutyl formate	3-methyl butyl formate
pentyl formate	l-pentyl formate
hexyl formate	
(acetates):	
methyl acetate	ethyl acetate
propyl acetate *	butyl acetate*
isobutyl acetate *	t-butyl acetate
pentyl acetate *	2-methylbutyl acetate *
3-methylbutyl acetate *	hexyl acetate *
heptyl acetate	octyl acetate *
benzyl acetate	cis-3-hexenyl acetate
(E)-hex-3-enyl acetete	n-octyl acetate
trans-2-hexenyl acetate	2-phenylethyl acetate
nonyl acetate	decyl acetate
ethyl phenyl acetate	
(propionates):	
methyl n-propionate	ethyl propionate *
ethyl 2-methylpropionate	ethyl hydroxypropionate
propyl propionate *	isobutyl propionate *

Table 1 (Cont'd)

2-methylbutyl propionate *	3-methyl propionate
hexyl propionate	Butyl propionate
(butyrates):	
methyl butyrate	ethyl butyrate *
propyl butyrate *	isopropyl butyrate
butyl butyrate *	isobutyl butyrate *
pentyl butyrate	isopentyl butyrate *
hexyl butyrate *	cinnamyl butryate
ethyl crotonate	
(isobutyrates):	
methyl isobutyrate	ethyl isobutyrate
butyl isobutyrate	isobutyl-isobutyrate
pentyl isobutyrate	hexyl isobutyrate
(2-methyl butyrates):	
methyl 2-methyl butyrate	ethyl 2-methyl butyrate *
propyl 2-methyl butyrate	butyl 2-methyl butyrate *
isobutyl 2-methyl butyrate	pentyl 2-methyl butyrate
hexyl 2-methyl butyrate *	2-methylbutyl butyrate
3-methylbutyl butyrate	
(pentanoates):	
methyl pentanoate	ethyl pentanoate *
propyl pentanoate	butyl pentanoate
amyl pentanoate	isoamyl pentanoate
hexyl pentanoate	

Table 1 (Cont'd)

(isopentanoates):	
methyl isopentanoate	ethyl isopentanoate
isopentyl isopentanoate	
(hexanoates):	
methyl hexanoate *	ethyl hexanoate *
propyl hexanoate *	butyl hexanoate *
isobutyl hexanoate	pentyl hexanoate
2-methylbutyl hexanoate *	3-methylbutyl hexanoate *
hexyl hexanoate *	
(hexenoates):	
butyl trans-2-hexenoate	
(heptanoates):	
ethyl heptanoate	propyl heptanoate
butyl heptanoate	hexyl heptanoate
(octanoate):	
ethyl octanoate	propyl octanoate
butyl octanoate	isobutyl octanoate
pentyl octanoate	isopentyl octanoate
hexyl octanoate	
(nonanoates):	
ethyl nonanoate	
(decanoates):	
ethyl decanoate	butyl decanoate
isobutyl decanoate	pentyl decanoate

Table 1 (Cont'd)

isopentyl decanoate	hexyl decanoate
(dodecanoates):	
ethyl dodecanoate	butyl dodecanoate
hexyl dodecanoate	
(other):	
diethyl succinate	ethyl-2-phenyl acetate
dimethyl phthalate	dipropyl phthalate
diethyl phthalate	butyl (E)-hex-2-enoate
2-methylbutyl oxtanoate	
Aldehydes:	
formaldehyde	acetaldehyde
propanal	2-propenal
2-oxopropanal	butanal
isobutanal	2-methyl butanal
(E)-2-butenal	pentanal
isopentanal	hexanal
(E)-2-hexenal *	(Z)-3-hexenal
(E)-3-hexenal	heptanal
(E)-2-heptenal	octanal
nonanal	decanal
undecanal	dodecanal
benzaldehyde	phenylacetaldehyde
Ketones:	
2-propanone	2-butanone
3-hydroxybutan-2-one	2,3-butanedione

Table 1 (Cont'd)

2-pentanone	3-pentanone
4-methylpentan-2-one	2-hexanone
2-heptanone	3-heptanone
4-heptanone	2-octanone
7-methyloctan-4-one	6-methyl-5-hepten-2-one
acetophenone	gamma-undecalactone
Ethers:	
diethyl ether	methyl propyl ether
dibutyl ether	2-methyl butyl ether
3-methyl butyl ether	dihexyl ether
methyl phenyl ether	4-methoxyallyl benzene *
cis-linalool oxide	(E)-linalool oxide
Acids:	
formic acid	acetic acid
propanoic acid	butanoic acid
isobutanoic acid	2-methyl butanoic acid
3-methyl butanoic acid	pentanoic acid
pentenoic acid	4-methyl pentanoic acid
hexanoic acid	(E)-2-hexenoic acid
heptanoic acid	cis-3-heptenoic acid
octanoic acid	cis-octenoic acid
nonanoic acid	cis-3-nonenoic acid
decanoic acid	decenoic acid
undecanoic acid	undecenoic acid
dodecanoic acid	dodecenoic acid

Table 1 (Cont'd)

tridecanoic acid	tridecenoic acid
tetradecanoic acid	tetradecenoic acid
pentadecanoic acid	pentadecenoic acid
hexadecanoic acid	hexadecenoic acid
heptadecanoic acid	heptadecenoic acid
octadecenoic acid	9-octadecenoic acid
9, t2-octadecadienoic acid	9,12,15-octadecatrienoic acid
nonadecanoic acid	nonadecenoic acid
eicosanoic acid	benzoic acid
Bases:	
ethylamine	butylamine
isoamylamine	hexylamine
Acetals:	
diethyoxymethane	dibutoxymethane
dihexoxymethane	dihexoxyethane
1-ethoxy-1-propoxyethane	1,1-diisobutoxyethane
1-butoxy-1-ethoxyethane	1-ethoxy-1-hexoxyethane
1-ethoxy-1-octoxyethane	1,1-diethoxyethane
1-butoxy-1-2-methyl butoxy ethane	1,1-dibutoxyethane
1,1-di-2-methyl butoxy ethane	1-butoxy-1-hexoxy ethane
1,1-di-hexoxyethane	1,1-diethoxy propane
1,2-methyl butoxy-1-hexoxy ethane	1,1-dipthoxy pentantane
4-methoxyallyl benzene	furan
furfural	5-hydroxy methylfurfural
2,4,5-trimethyl-1,3-dioxolane	

Table 1 (Cont'd)

Hydrocarbons:	
ethane	thylene
farnesene	benzene
ethyl benzene	1-methylnaphthalene
2-methylnaphthalene	damascenone
pinene	

BIOGENESIS OF APPLE FLAVOR COMPONENTS

Apple flavor is composed of numerous classes of compounds, which include lipids, carbohydrates, proteins (Maga, 1975; Salunkhe and Do, 1976; Yahia, 1994) as well as some vitamins and minerals (Salunkhe and Do, 1976). The origin, formation, and metabolism of aroma volatiles is not completely understood (Goodenough and Entwistle, 1982; Yahia, 1994). Figure 2 summarizes possible diverse origins and mechanisms of biogenesis of the volatile compounds (Yahia, 1994).

A. Primary volatiles:

The aroma of apple originates primarily from carbohydrates, amino acids, and lipids (Salunkhe and Do, 1976; Yahia, 1994). Generally speaking, apple aromas are genetically controlled with genetic differences leading to distinct differences in flavor between cultivars (Kakiguchi et al., 1986). The primary, or natural, volatiles of apple fruit are those to be derived from enzymatically controlled lipid, terpene, amino acid, and phenyl propane metabolism (Schreier,

1984). Fatty acids are considered be the most important source of carbon skeletons needed for primary or natural odor volatiles biosynthesis in apple (Yahia, 1994). During fruit ripening,

some endogenous fatty acids are converted into esters, ketones, and alcohols (Tressel and Drawert, 1973; Yamashita et al., 1977). Drawert (1975) and Paillard (1979) reported that fatty acid metabolism was implicated in the formation a range of primary volatiles, which include the aliphatic esters, acids, alcohols, and carbonyls. Yahia (1994) reported that "alcohols were formed from the β-oxidation of aliphatic acids", for example, butanol and hexanol were produced by fatty acids with an even carbon number; propanol and pentanol however, were formed from those with an odd carbon number. Even though the pathway of ester synthesis is not fully understood (Goodenough and Entwistle, 1982), it seems clear that the formation of esters in fruit is largely dependent on the availability of precursor alcohols and acids (Bartley et al., 1985; Knee and Hatfield, 1981; Song et al., 1996; Yamashita et al., 1977).

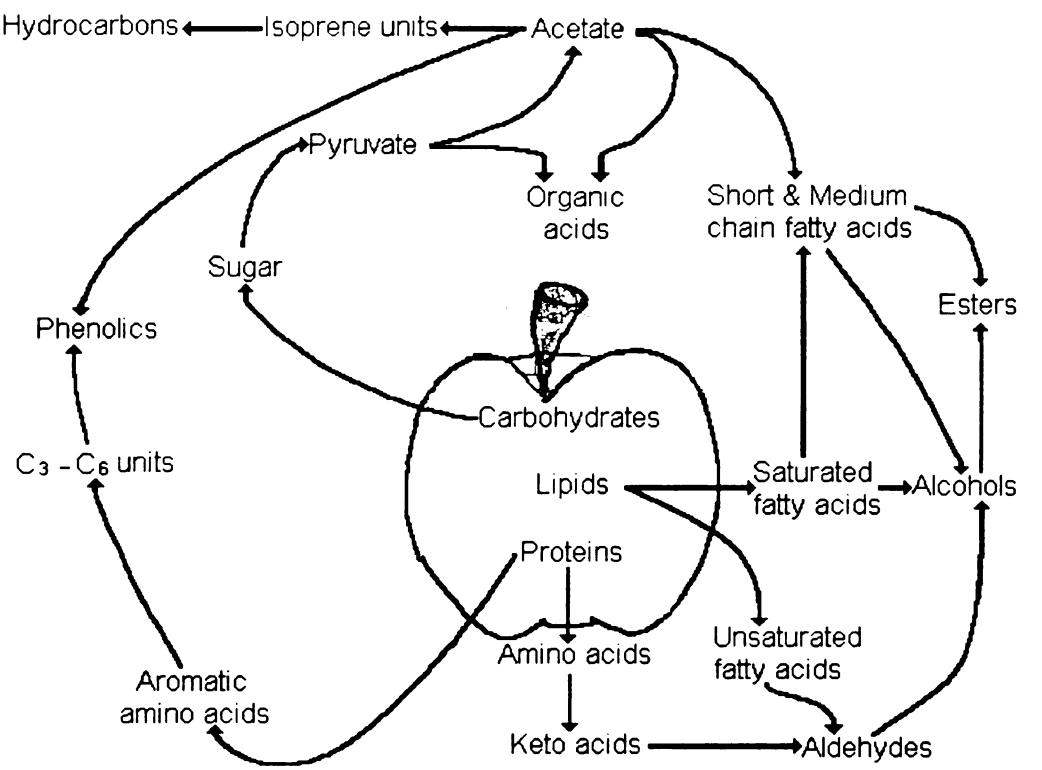


Fig. 2. Some possible routes for the synthesis of flavor compounds in apple. (From Yahia, 1994.)

The biosynthesis of esters in apple is frequently examined by supplying fruit tissues with short chain alcohols and/or carboxylic acids (Yahia,1994). For example, when hexanol was supplied as vapors to whole apple, the alcohol was incorporated into the "hexyl" portion of hexylacetate (Knee and Hatfield, 1981; Song et al., 1996). Bartley et al. (1985) reported that when short chain alcohols (C_2-C_8) and methyl esters of short chain fatty acids (C_4-C_8) were supplied as vapors to whole apples, the alcohols were converted to the corresponding acetate esters, while the methyl esters of short chain fatty acids were converted to esters with an alkyl group of C_2 or C_4.

B. Secondary volatiles:

Secondary volatiles are formed by various uncontrolled enzymatic reactions during fruit processing or chewing (Schreier, 1984). Therefore, the secondary volatiles play a important role in the flavor of processed products and of the fresh fruit after cutting or chewing (Yahia, 1994). Some secondary volatiles such as aldehydes (hexanal and (E)-2-hexenal) and alcohols (such as hexanol), were produced quickly after apple crushing and homogenizing (Drawert et al., 1968; Feys et al., 1980). The production of secondary volatile compounds from free fatty acids is very common in plant tissues and food systems when the tissues are crushed and exposed to oxygen (Croft et al., 1993; Drawert et al., 1973; Erikson, 1975; Galliard et al., 1976; Hatanaka,1983;). Gardner (1980) reported that the process of secondary volatile production is catalyzed by lipoxygenases (LOX), which catalyzes the peroxidation of polyunsaturated fatty acids with a *cis, cis,* 1, 4-pentadiene

structure to form conjugated hydroperoxides. The fatty acid hydroperoxides are unstable and their breakdown contributes significantly to the secondary volatile development (Crouzet et al., 1984; Galliard et al., 1976, 1977).

FACTORS AFFECTING VOLATILE PRODUCTION OF APPLE

A. Preharvest Factors. Many preharvest factors can affect volatile production of apple fruits. The genetic differences between cultivars also contribute to marked aroma differences and generally speaking, almost all fruits have their own characteristic aroma (Salunkhe and Do, 1976). The kinds and amount of volatiles and their patterns differ in the different cultivars of apples (Brown et al., 1968; Cunningham et al., 1986; Dimick and Hoskin, 1983; Paillard, 1981; Smock and Neubert,1950; Yahia, 1986; Yahia et al., 1991a). For example, 'Golden Delicious' and 'Jonagold' have higher butyl and hexyl acetate production than other cultivars (Song, 1994; Yahia, 1994). Butyl pentanoate, pentyl pentanoate, and isopentyl pentanoate were found in various cultivars, but were absent from 'McIntosh' and 'Cortland' apples (Yahia, 1994).

Fruit maturity at harvest also affects aroma (Song and Bangerth, 1994; Yahia, 1994). If a fruit is picked prior to full maturity volatile production is markedly reduced relative to mature fruits and it may never develop its characteristic flavor (Brown et al., 1966; Brackmann et al., 1993; Lidster et al., 1983; Paillard, 1981; Song and Bangerth, 1994). Hasen et al. (1992a) reported that early-picked 'Jonagold' fruit not only had a delayed onset of volatile production, but the production rate was lower compared to late-picked fruits.

However, if a fruit is picked too late, it also produces fewer volatiles compared to optimally harvested fruit and the types of volatiles differ.

Finally, culture and orchard environment are also important factors affecting aroma. For example, nutrients available in the soil can affect flavor production in many fruits. 'McIntosh' apples from trees receiving a heavy nitrogen fertilization treatment produced more volatile compounds than those from a low nitrogen fertilization treatment (Smock and Neubert, 1950; Somogyi et al., 1964). According to Brown et al. (1968), volatiles in freshly harvested 'Golden Delicious' fruit were increased by phosphorous fertilization. However, Forsyth and Webster (1971) found higher levels of phosphorous fertilizer treatment depressed the production of ethyl esters, acetaldehyde, and hexanol in 'McIntosh' fruit after cold storage.

B. Postharvest Factors:

Effect of temperature. Refrigeration is required for the long-term storage of apple fruits to minimize loss of external and internal quality. However, long-term storage of apples at 0 to 3°C can cause physiological disorders for some apple cultivars (Wills and Scott, 1975). Low temperatures affect not only the rate of metabolism of apple, but also the make-up of the metabolic products. If apples are susceptible to chilling, several physiological disorders may result, including low temperature breakdown, late storage corking and flavor loss. Wills and Scott (1975) reported that low temperature breakdown is a physiological disorder that causes a reduction in the level of volatile compounds during apple

cold storage. Fruit stored at 10 and 15°C produced more volatiles than those stored at 3°C (Grever and Doesburg, 1965). When storage temperature increased from –1°C to 10°C, 'Jonathan' fruit showed an increase in the emission of acetate esters (Wills and McGlasson, 1971). When CA stored 'McIntosh' and 'Cortland' apples were transferred to 20°C, the production of some odor-active volatiles was increased significantly. However, there was no significant increase when the apples were transferred to 3.3°C (Yahia et al., 1991). Insufficient aroma in long-term, low-temperature-stored fruits is a significant commercial problem, and this problem seems to have become more serious with the use of CA storage in conjunction with low temperature storage. The optimal temperature for aroma maintenance needs to be identified for important apple varieties to minimize the effect of chilling.

Effect of oxygen and carbon dioxide concentration. Controlled-atmosphere (CA) storage in conjunction with low temperature is highly effective in maintaining apple quality in terms of preserving texture and slowing ripening and senescence. However, suppression of aroma by low O_2 and high CO_2 is sometimes a problem (Brackman et al., 1993; Guadagni et al., 1971; Knee, 1981; Patterson et al., 1974). Importantly, suppression of fruit flavor can persist even when apples are removed to normal atmosphere (Hansen et al., 1992b; Knee and Sharples, 1981; Lidster et al., 1983; Liu 1985; Streif and Bagerth, 1988; Yahia et al., 1990a, 1991). The severity of CA storage suppression of flavor depends on the atmospheric composition and the duration of storage. Generally speaking, the lower the O_2 concentration, the higher the CO_2

concentration, and the longer the duration in CA storage, the greater the level of aroma suppression (Knee et al., 1981; Lidster et al., 1983; Patterson et al., 1974; Streif et al., 1988; Wills and Scott, 1975; Yahia et al., 1990).

The effect of low O_2 storage on aroma production is likely a function of retarded ripening. Patterson et al. (1974) found that 'Cox's Orange Pippin' fruit gradually lost their capacity to ripen normally when they were stored in 2 kPa oxygen at 3.5 °C. When 'Cox's Orange Pippin' fruit were stored in air or 5 kPa carbon dioxide in air, volatiles other than ethylene were produced in greater amounts than for fruit stored in 2 kPa oxygen. Storage of 'McIntosh' fruit in 1.5 kPa O_2 +1.5 kPa CO_2 or 1.0 kPa O_2 + 1.5 kPa CO_2 at 2.8 °C, acetaldehyde, ethanol, hexanol and ethyl butyrate were suppressed (Lidster et al., 1983). Streif and Bangerth (1988) reported that decreasing the O_2 partial pressure from 21 kPa to 3 kPa has little effect on volatile production, but if the partial pressure of O_2 decreases to 1 kPa, volatile production will be significantly reduced upon return of fruit on air. Brackmann et al. (1993) measured volatile production from 'Golden Delicious' fruit stored 8 months at 1 °C in air and under various controlled-atmospheres including ultra low oxygen (ULO) storage conditions (1 kPa O_2, 3 kPa CO_2 levels), their results show that all CA storage treatments suppress aroma production compared to fruit storage in air. The greatest reduction was found under ULO (1 kPa O_2) and high CO_2 (3 kPa) partial pressure.

Effect of ethylene. Ethylene, which is recognized as the ripening hormone, plays an important direct role in regulating the ripening of climacteric

fruits (Biale and Young, 1981; Rhodes, 1980; Yang, 1985). Ripening involves complex changes in metabolism of fruit in which both anabolic and catabolic processes are involved (Biale, 1975). Specific events correlated with ripening include increased respiration, autocatalytic ethylene production, chlorophyll degradation, carotenoid synthesis, conversion of starch to sugar, aroma volatile production, and solubilization of pectins and cellulose (Biale and Young, 1981; Grierson, 1985; Rhodes, 1980;). Reduced ethylene synthesis and/or action is a factor common to CA, and the suppression of aroma production after long-term CA storage may be caused by ethylene-related changes in physiology rather than the alteration in O_2 and CO_2 partial pressures (Bangerth and Streif, 1987). Yahia et al. (1991) reported that butanoates, 2-methyl butanoates, pentanoates and hexanoates were either severely or completely suppressed under low ethylene CA (LCA) storage. Because of the effect of ethylene concentration, "LCA storage would result in greater suppression of flavor than conventional CA storage" (Yahia et al., 1991)

Effect of storage duration. Having a ready supply of apple fruit year-round is important for meeting consumer needs for apple fruit availability. However, the fact that apple production is seasonal causes shippers and storage operators to desire extended apple storability and improve storage quality. The duration of apple storage has a close relationship with volatile production (Patterson et al., 1974; Willaert et al., 1993). Streif and Bangerth (1988) found that the effect of O_2 and CO_2 partial pressures on aroma production was clearly dependent on the duration of storage; the longer the storage duration, the

greater the suppression of flavor. Guadagni et al. (1971) reported that the rate of ethyl 2-methylbutyrate production steadily increased after storage for 1-4 months, at 1 °C, and dropped off severely after 4 and 6 months. 'Cox's Orange Pippin' fruit volatile ester biosynthesis declined 75% after 5 months storage in 2 kPa O_2 with or without 5 kPa CO_2 (Hatfield and Patterson, 1974). When 'McIntosh' apples were stored 1.5 kPa O_2 +1.5 kPa CO_2 or 1.0 kPa O_2 + 1.5 kPa CO_2 at 2.8 °C for 320 days, some volatiles were completely suppressed even after the apples were moved into air (Lidster et al., 1983).

Effect of packaging materials. Since modified-atmosphere packing (MAP) has the potential to provide low O_2/high CO_2 regimes similar to those of CA storage (Beaudry et al., 1992), it is a much researched technique for fruit and vegetable storage. However, loss or change of flavors by packing material during storage are potential problems (Matsui et al., 1994). The flavor profile of apple in MAP can be affected not only by the O_2 and CO_2 partial pressure in the packaging and storage temperature, but also by the interaction of flavor compounds with polymer (Debeaufort and Voilley, 1994; Mason et al., 1992; Matsui et al., 1994; Nielsen and Giacin, 1994; Osajima and Matsui, 1993;). Loss of flavor constituents or changes their profile may due to their sorption into packaging material or permeation through the package to the environment (Nielsen and Giacin, 1994) or due to picking up flavor directly from the packaging or indirectly by permeation from the environment through the package to the food (Mason et al., 1992). Low-density polyethylene (LDPE) is commonly used polymer for MAP of fresh and lightly processed produce and some of its

properties such as the permeabilities of O_2 and CO_2 (Beaudry et al., 1992; Beaudry and Dude, 1994) and water vapor (Debeaufort and Voilley, 1994) are well studied. However, only a few studies on LDPE film permeability to organic volatiles such as those produced by apple fruit have been published (Delassus et al., 1988; Fukamachi et al., 1994).

The loss of flavor by sorption into packaging material or permeation through the package to the environment commonly occurs in the packaged products. The permeation process for permeant is described by following equation:

$$P = D \times S$$

Where: P is permeability coefficient, which expresses the permeant transport rate at steady-state and where $P=P_0\ exp(-E_P/RT)$. D is the diffusion coefficient, which describes how fast the permeant molecules move in the film where $D=D_0\ exp(-E_D/RT)$. S is the solubility coefficient, which is a measure of the amount of a permeant that will be absorbed by polymer where $S=S_0\ exp(-H_S/RT)$. E_P = activation energy for permeation; E_D = activation energy for diffusion; H_s is the heat of solution.

Present studies show that the structure of both permeant and polymeric packaging material, measurement condition (such as relative humidity, temperature, etc.), permeant concentration and co-permeants are the major factors affecting volatile permeation.

METHODS OF VOLATILE COLLECTION, ISOLATION, AND ANALYSIS

There are many methods available for isolation, collection, and extraction of volatiles from food, including solvent extraction, distillation, and headspace (including equilibrium headspace and dynamic headspace) sampling (Bemelmans, 1978; Sugisawa, 1981).

Distillation and extraction. Distillation is the most widely used technique to isolate volatiles from apple and other fruits (Stevens et al., 1966; Flath et al., 1967, 1969). Since there is water present in the distillate, to use this technique, further solvent extraction is required (Heath and Reineccius, 1986). Extraction methods are used either to isolate volatiles directly from food or to recover them from diluted aqueous distillates. These extraction methods are based on the favorable distribution coefficient of the volatiles between solvent and food or the distillate (Seifabad, 1988). The first report on the distillation-extraction technique was made by Likens and Nickerson (1964). Since then, the modified and improved original Likens-Nickerson apparatus allows distillation and extraction simultaneously. Since the concentration of volatiles may not be high enough for GC analysis, it is sometimes necessary to concentrate the distillate which obtained from distillation-extraction techniques (Seifabad, 1988). Concentration is usually achieved by volatilizing the solvent with raised temperatures or purging with a gentle stream of nitrogen (Nursten and Woolfe, 1971; Seifabad, 1988; Shamaila, et al., 1992). The disadvantages of this method are moderately long isolation time and potential artifact formation (Alberola et al., 1978) and low percent recovery (Maarse, 1971). Land (1984) reported that the artifacts, which formed due to heat during distillation-extraction, are eliminated when this

apparati are operated under reduced pressure condition. Vacuum distillation procedure is commonly used to collect full flavor volatiles from fruits and fruit juices (Alberola et al., 1978; Kakiuchi et al., 1986; Seifabad, 1988).

Headspace analysis: Headspace sampling involves taking the sample from vapor phase above the product. This technique offers the possibility of volatile collection without sample destruction or artifact formation (Land, 1975; Olfsdottir, 1985). Headspace sampling can be performed in static hermetic environments or dynamic (flow-through) environments.

Compared with other headspace analysis methods, static headspace analysis is the simplest and fastest for studying the aroma properties and quality of food materials (Weurman, 1961; Martin, 1969; Guadagni et al., 1971; Yamashita et al., 1977; McNally and Grob, 1985). However, aroma volatiles are often present at very low concentration. Using this method, gas chromatography (GC) does not provide the sensitivity needed for trace analysis of volatiles (Reineccious, 1983).

In order to increase the concentration of sample, the dynamic headspace (purge and trap) analysis technique is widely used. In this method, volatile compounds are removed from headspace by purging the atmosphere around the product and trapping the volatiles onto a porous polymer (Hirvi and Honkanen, 1982; Shamaila et al., 1992; Song and Bangerth, 1994). The volatile compounds are either desorbed from the polymeric material by heating (Dirinck et al., 1981; Mattheis et al., 1991a, 1991b) or removed by various solvent extraction techniques (Clark and Cronin, 1975; Guadagni et al., 1971;

Olafsdottir, 1985; Shamaila, et al., 1992; Song and Bangerth, 1994).

Charcoal was widely used as a porous trap during 1950's and 1960's because of its high adsorption capacity and absence of involvement in artifact formation (Heinze et al., 1953; Turk et al., 1951; Tang and Jennings, 1967). Tenax-GC (2,6-diphenylparaphenylene oxide polymerhas) emerged as a widely-used porous polymer in 1970's (Jennings et al., 1972; Zlakis et al., 1973). Tenax-GC has a very low affinity for low molecular weight alcohols and water (Jennings et al., 1972; Kuo et al., 1977), but also avoids the production of artifacts characteristic of other porous polymers under certain operating conditions (Seifabad, 1988). Jennings and Filsoof (1977) found that Tenax-GC was a suitable porous polymer for the collection of volatiles from the headspace of samples by comparing its performance with other various porous polymers. Tenax-GC and the recently available Tenax-TA (MacLeod and Ames, 1986) are widely used and considered to be the most effective and broadly suitable sorbants today (Dirinck et al., 1981; Mattheis et al., 1991a, 1991b; Olafsdottir, 1985; Shamaila et al., 1992; Song and Bangerth, 1994).

The dynamic headspace analysis technique not only has high recovery rate and less variation, but also provides an inexpensive alternative to other methods for routine analysis of many intermediate molecular weight aroma volatiles in beverage and food (Olafsdottir, 1985). Therefore, dynamic headspace is one of the most suitable methods for volatile analysis for fruit and vegetables. However, compared with equilibrium headspace and direct headspace sampling techniques, the dynamic headspace is more complex, time

consuming and costly technique. There is a need for the creation of new simple, fast and accurate methods.

A relatively new sampling methodology, solid-phase extraction, is exposing a sorption surface in the headspace to collect and concentrate volatiles by virtue of its sorption characteristics. Unlike direct headspace and purge-and-trap sampling techniques, solid phase extraction does not require any volume of gas to be removed from the headspace.

Solid phase micro extraction (SPME) is a special form of solid phase extraction that uses a solid phase on an extendable fiber that is housed in a hollow needle. Approximately 2 to 12 min are required for absorption (Song et al., 1997). After absorption, the needle is inserted into the injection port of a GC. Desorption requires 30 seconds to 1 minute or more, which would lead unacceptable peak broadening. Thus cryofocussing of the desorbed compounds is required and the use of a capillary column is often necessary. Solid phase extraction does not require the removal of a volume of gas as in purge-and-trap, but still requires desorption and an additional concentration step.

The above methods are widely used for isolation, collection and extraction volatile compounds from apples. Since each technique has its advantages and limitations, it is difficult to say which one is suitable if the purpose of the analyses are not clearly designated defined. For example, the headspace methods are commonly used in aroma volatile studies because of volatile collection without sample destruction and artifact formation. However, the methods are not suitable for higher boiling point volatile compounds. More than one techniques

should probably be used when the purpose is to evaluate the complete flavor profile of apple fruits. Importantly, using different analysis techniques may yield quantitatively and qualitatively differing results (Kakiinshi et al., 1986), which will require additional work to reconcile the apparent inconsistencies to compare data from different analysis methods. What kind analysis techniques should be used must depend on the purpose, the food matrix, and the volatile compounds to be analyzed.

Literature Cited

Alberola, J., L. J. Izquierdo and V. Espana. 1978. The volatile fraction of orange juice. Methods for extraction and study of composition. *In* Flavor of Food and Beverages, G. Charalambous, and G. Inglet (eds.) Academic Press, London.

Bangerth, F. and J. Streif. 1987. Effect of aminoethoxyvinylglycine and low-pressure storage on the post-storage production of aroma volatiles by Golden Delicious apples. J. Sci. Food Agric. 41:351-360.

Bartley, I. M., P. G. Stoker, A. D. E. Martin and M. Knee. 1985. Synthesis of aroma compounds by apples supplied with alcohols and methyl esters of fatty acids. J. Sci. Food Agric. 36: 567-574.

Beaudry, R. M., A. C. Cameron, A. Shirazi and D. Dostal Lange. 1992. Modified-atmosphere packaging of blueberry fruit: Effect of temperature on package oxygen and carbon dioxide. J. Amer. Soc. Hort. Sci. 117

Beaudry, R. M. and N. Dube. 1994. A permeable membrane respirometer. HortScience 29 (5):132 (Abstr.).

Bemelmans, J. M. 1978. Review of isolation and concentration techniques, p 79-98. In: D. G. Land and H. E. Nursten (eds.). Progress in Flavour Research.

Berger, R. G. 1991. Fruits I. p.283-304. In: H. Maarse (ed), Volatile Compounds in Foods and Vegetables. Marcel Dekker, New York.

Biale, J. B. 1975. Post-harvest biology and handling of fruit and vegetables In: Haard, N. F. and D. K. Salunkhe (eds.). AVI Publishing Co., Westport, Conn.

Biale, J. B. and R. E. Young. 1981. Respiration and ripening in fruits-retrospect and prospect, In: Friend, J. and M. J. C. Rhodes (eds.). Recent Advances in the Biochemistry of Fruits and Vegetables. Academic Press, London.

Brackmann, A., J. Streif and F. Bangerth. 1993. Relationship between reduced aroma production and lipid metabolism of apples after long-term controlled-atmosphere storage. J. Amer. Soc. Hort. Sci. 118 (2): 243-247.

Brown, D. S., J. R. Buchanan and J. R. Hicks. 1966. Volatiles from apple fruits as related to variety, maturity, and ripening. J. Amer. Soc. Hort. Sci. 88:98-104.

Brown, D. S., J. R. Buchanan, J. R. Hicks, K. Uriu and T. Muraka. 1968. Volatile from apple fruits as effected by phosphorus fertilization. Proc. Amer. Soc. Hort. Sci. 93:705-715.

Clark, R. G. and D. A. Cronin. 1975. A new technique for trapping and sensory evaluation of flavor volatiles. J. Sci. Food Agric. 26: 1009.

Croft, K. P. C., F.Juttner, and A.J.Slusarenko. 1993. Volatile products of the lipoxygenase pathway evolved from *phaseolus vulgaris* (L.) leaves inoculated with Pseudomonas *syringae* pv *phaseolicola*. Plant Physiol. 101:13-24.

Crouzet, J., I. Nicholas, I. Molina, and G. Vallentin. 1984. Enzyme occurring in the formation of six-carbon aldehydes and alcohols in grapes. In: J. Adda (eds.). Progress in Flavor Research Proceedings of the 4th Weurman Flavor Research Symposium, Dourdan, France, Elsevier, Amsterdam. The Netherlands.

Cunningham, D. G.; T. A. Acree, J. Barnard, R. Butts and P. Braell. 1986. Analysis of apple volatiles. Food Chemistry. 19:137-147.

Davis, P. L. and J. J. Smoot. 1972. Germination of *Penicillium digitatum* spores as affected by solution of volatile compounds of citrus fruits. Phytopathology 62:448-489.

Debeaufort, F. and A. Voilley. 1994. Aroma compounds and water vapor permeability of edible films and polymeric packaging. J. Agric. Food Chem. 42:2871-2875.

DeLassus P. T., J. C. Tou, M. A. Babinec, D. C. Rulf, B. K. Karp, and B. A. Howell. 1988. Transport of apple aromas in polymer films. In: Food and Packaging Interactions, J. H. Hotchkiss,(eds.). ACS SymposiumSeries No. 365.

Dimick, P. S., and J. C. Hoskin. 1983. Review of apple flavor - state of the art. Crit. Rev. Food Sci. Nutr. 18:387-409.

Dirinck, P. J., H. L. De Pooter, G. A. Willeart and N. M. Schamp. 1981. Flavor quality of cultivated strawberries: The role of the sulfur compounds. J. Agric. Food Chem. 29:319.

Drawert, F., R. Tressl, W. Heinmann, R. Emberger, and M. Speck. 1973. On the biogenesis of aroma component in plants and fruits. XV. Enzymatic oxidative formation of C6 aldehydes and alcohols and their precursors in

apples and grapes. Chem. Mikrobiol. Tecknol. Lebensm. 2:10-22.

Drawert, F., W. Heinmann, R. Emberger, and R. Tressl. 1968. uber die biogenese von aromatoffen bei pflanzen and fruchten. III. Gas chromatographische bestandsanfnahme von apfel-aromastoffen. Phytochemistry 7:881-883.

Drawert, F. 1975. Biochemical formation of aroma components. In: H. Maarse and P. J. Groenen (eds.). Proc. Int. Symp. Aroma Res. Zeist, The Netherlands

Erickson, C. 1975. Aroma compounds derived from oxidized lipids. Some biochemical and analytical aspects. J. Agric. Food Chem. 23:126-128.

Farag, R. S., Z. Y. Daw, and S. H. Abo-Raya. 1989. Influnence of some spice essential oils on *Aspergillus parasiticus* growth and production of aflatoxins in a synthetic medium. J. Food Sci. 54 (1):74-76.

Fellman, J. K., D. S. Mattinson, B. C. Bostick, J. P. Mattheis, and M. E. Patterson. 1993. Ester biosynthesis in "Rome" apple subjected to low-oxygen atmosphere. Postharvest Biology and Technology. 3: 201-214.

Feranoli, G. 1973. "Feranoli's Handbook of Flavor Ingredients" T. E. Furia and Bellanca (eds.). Chemical Rubber Co., Cleveland, Ohio

Feys, M., P. Tibback, and E. Maes, 1980. Volatiles of apples (var. 'Schone van Boskoop'): Isolation and identification. J. Food Technol. 15: 485-492.

Flath, R. A., D. R. Black, D. G. Guadagni, W. H. McFadden and T. H. Schultz. 1967. Identification and organoleptic evaluation of compounds in delicious apple essence. J. Agric. Food Chem. 15: 29-35.

Flath, R. A., D. R. Black, R. R. Forrey, G. M. McDonald, T. R. Mon and R. Teranishi. 1969. Volatiles in gravenstein apple essence identified by GC-MS. J. Chromatogr. Sci. 7:508.

Forsyth, F. R., and D. H. Webster. 1971. Volatiles from 'McIntosh' apple fruits as affected by phosphorous nutrition. J. Amer. Soc. Hort. Sci. 96:259-263.

Fries, N. 1973. Effect of volatile organic compounds on the growth and development of fungi. Trns. Brit. Mycol. Soc. 60 (1):1-21.

Fukamachi, M., T. Matsui, M. Shimoda, and Y. Osajima. 1994. Derivation of thermodynamic sorption equation of flavors with packaging films. J. Agric.

Food Chem. 1994 42:2893-2895.

Galliard, T., D. R. Phillips, and J. Reynolds. 1976. The formation of cis-3-nonenal, trans-2-nonenal and hexanal from linoleic acid hydroperoxide isomers by a hydroperoxide cleavage enzyme system in cucumber (*Cucumis sativus*) fruits. Biochem. Biophys. Acta 441:181-192.

Galliard, T., J. A. Mathew, A. J. Wright, and M. J. Fisfwick. 1977. The enzymic breakdown of lipids to volatile and non-volatile carbonyl fragments in disrupted tomato fruits. J. Sci. Food Agric. 28:863-868.

Gardner, H. W. 1980. Lipid enzymes: lipases, lipoxygenases and hydroperoxidase. In: Simic, M. G. and M. Karel (eds.). Autoxidation in Food and Biological Systems. Plenum, New York.

Goodenough, P. W. and T. Entwistle. 1982. The hydrodynamic properties and kinetic constants with natural substrates of the esterase from Malus pumila fruit. Eur. J. Biochem. 127:145.

Grevers, G. and Doesburg, J. J. 1965. Volatiles of apples during storage and ripening. J. Food Sci. 30:412.

Grierson, D. 1985. Gene experession in ripening tomato fruit. Crit. Rev. PlantSci. 3:113-132.

Guadagni, D. G., J. L. Bomben and J. S. Hudson. 1971. Factors influencing the development of aroma in apple peels. J. Sci. Food Agric. 22:110-115.

Hansen,K., L. Poll, and M. J. Lewis. 1992a. The influence of picking time on postharvest volatile ester production of Jonagold apples. Lebensmittel-Wissenschaft unt-Technology. 25:451-455.

Hansen,K., L. Poll, C. E. Olsen, and M. J. Lewis. 1992b. The influence of oxygen concentration in storage atmospheres on the post-storage volatile ester production of 'Jonagold' apple. Lebensmittel-Wissenschaft unt-Technology. 25:457-461.

Hatanaka, A. 1983. Biosynthesis of leaf alcohol. Bull. Inst. Chem. Res. Kyoto University. 61 (2):180-192.

Heath, H. B. and G. Reineccius. 1986. Flavor and its study. In: Flavor Chemistry and Technology. AVI Publishing Co., Westport, CT.

Heinze, R. E., C. E. Baker and F. W. Quackenbush. 1953. The chemical composition of apple storage volatiles. I-acids, alcohol and esters. Proc.

Am, Soc. Hort. Sci. 61:237.

Hirvi, T. and E. Honkanen. 1982. The volatiles of two new strawberry cultivars, "Annelie" and "Alaska Pioneer," obtained by backcrossing of cultivated strawberries with wild strawberries, *Fragaria vesca*, Rugen and *Fragaria virginana*. Z. Lebensm. Unters. Forsch. 175:113-116.

Holmes, J. C. and F. A. Morrell. 1957. Appl. Spectr. 11:86.

Huelin, F. E. 1952. Volatile products of apple. III. Identification of aldehydes and ketones. Aust. J. Sci. Res. B. 5:328.

Jennings, W. G., R. Wohleb and M. J. Lewis. 1972. Gas chromatographic analysis of headspace volatiles of alcoholic beverages. J. Food Sci. 37:69.

Jepsen, O. 1978. Report International Fruit Juice Union Conference, Bern, pp. 49-362

Knee, M. and S. G. S. Hatfield. 1981. The metabolism of alcohols by apple fruit tissue. J. Sci. Food Agric. 32: 593-600.

Knee, M. and R. O. Sharples. 1981. The influence of controlled atmosphere storage on the ripening of apples in relation to quality. In: Quality in Stored and Processed Vegetables and Fruits. Goodenough and P. K. Atkins (eds.). Academic Press, London & New York.

Kakiuchi, N., S. Moriguchi, H. Fucuda, N. Ichimura, Y. Kato and Y. Banba. 1986. Composition of volatile compounds of apple fruits in relation to cultivar. J. Japan. Soc. Hort. Sci. 55:280-289.

Koch, J. 1976. Zur Beurteilung Von Natuerlichem Fruchtsaftaroma. Kongress-Bericht XIV Internationaler Fruchtsaft-Kongress, Meran.

Kuo, P. P., E. S. Chain, F. B. De Walle and J. H. Kim. 1977. Gas stripping, sorption, and thermal desorption procedures for preconcentrating volatile polar water-soluble organizes from water samples for analysis by gas chromatography. J. Anal. Chem. 49:1023.

Land, D. G. 1975. Techniques for assessing odour: uses and limitations. Proc. Int. Symp. Aroma Research. Pudoc., Wageningen, The Netherlands.

Land, D. G. 1984. Flavour research in 1984-where now and where next ? In: Progress in Flavour Research, Adda, J. (eds.). Elsevier Science, Amsterdam.

Lea, A. G.H. and Ford, G. D. 1991. Fruit processing 1(3):29-32. (supplement to Fl ss. Obst. 58(3)).

Lidster, P. D., H. J. Lightfood, and K. B. Mcrae. 1983. Production and regeneration of principal volatiles in apple stored in modified atmospheres and Air. J. Food Sci. 48:400-410.

Likens, S. D. and G. B. Nickerson. 1964. Detection of certain hop oil constituents in brewing products. Proc. Amer. Soc. Brew. Chem. 5:13

Lillian M. Ingster and Manning Feinlieb. 1996. Cardiovascular Disease Epidemiology and prevention. the 36th Annual Conference of the American Heart Association's Conference.

Liu, F. W. 1985. Low ethylene controlled-atmosphere storage of McIntosh apples. P. 385-392. In: Roberts T. A. and G. A. Tucker (eds.). Ethylene and Plant Development. Butterworth, London.

Maarse, H. 1971. Samenstelling Van de vluchtige olie van onganum vulgane 1. ssp. vulgane gedurende de ontwikkeling van de plant. Ph.d. thesis, university of Gronongen, the Netherlands.

Maarse, H. 1991. Volatile Compounds in Foods and Beverages. Marcel, New York.

Maga, J. A. 1975 Thiazoles in foods. In: Fenaroli's Handbook of Flavor Ingredients. Volume I 2nd edition, CRC Press, Inc. 18901 Granwood Parkway, Cleveland, Ohio.

Martin, J. M. 1969. Gas chromatographic qualitative and semiqualitative analysis of apple aroma by means of retention indexes. J. Anal. Chem. Acta. 48:169.

Mason R. C., P. T. Delassus, G. Strandburg, and B. A. Howell. 1992. Diffusion of flavors in polymers: effect of permeant size and shape. Tappi Journal. 75 (6):163-168.

Matsui, T., M. Fukamachi, M. Shimoda, and Y. Osajima. 1994. Derivation of thermodynamic sorption equation of flavors with packaging films. J. Agric. Food Chem. 42(12):2889-2892.

Mattheis, J. P., D. A. Buchanan, and J. K. Fellman. 1991a. Change in apple fruit volatiles after storage in atmospheres inducing anaerobic metabolism. J.

Agri. Food Chem. 39:1602-1605.

Mattheis, J. P., J. K. Fellman, P. Chen, and M. Patterson. 1991b. Changes in headspace volatiles during physiological development of bisbee delicious apple fruit. J. Agric. Food Chem. 39:1902-1906

McNally, M. E. and R. L. Grob. 1985. Current application of static and dynamic headspace analysis. A review. Part II: non environmental applications. J. Amer. Lab. Feb:106.

Mulders, E. G. 1973. The odour of white bread. IV. Quantitative determination of constituents in the vapor and their odour values. Z. Lebens. unters. Forsch. 151:310

Nielsen, T. G. and J. R. Giacin. 1994. The sorption of Limonene/ethyl acetate binary vapor mixture by a biaxially oriented polypropylene film. Packaging technol. and Sci. 7:247-256.

Nursten, H. E. and M. L. Woofe, 1971. An examination of the volatile compounds present in cooked Bramley's seedling apples and the changes they undergo on processing. J. Sci. food Agric. 23:803.

Olafsdottir, G., J. A. Steinke and R.C. Lindsay. 1985. Quantitative performance of a simple Tenax-GC adsorption method for use in the analysis of aroma volatiles. J. Food Sci. 50:1431-1436

Osajima, Y. and T. Matsui. 1993. Dynamic approach to the sorption of flavors into a food packaging film. Anal. Sci. 9:753-763.

Patterson and K. Hartfield. 1974. Residual effect of controlled atmosphere storage on the production of volatile compounds by two varieties of apple. J. Sci. Food Agric. 25:843-849.

Paillard, N. 1979. Biosynthesis desproduits volatils de la pomme: Formation des alcohols et des esters a partir des acides gras. Phytochemistry 18:1165-1171.

Paillard, N. 1981. Factors influencing flavor formation in fruit. In: P. Schrier (Eds.). Flavour "81". Walter de Gruyter: New York.

Pooter, D., V. Acker, and Schamp. 1987. Aldehyde metabolism and the aroma quality of stored golden delicious apples. phytochemistry 26: 89-92.

Power, F. B. and V. K. Chesnut. 1920. The odorous constituents of apples. Estimation of acetaldehyde from the ripe fruit. J. Amer. Chem. Soc.

42:1509-1525.

Reineccious, G. 1983. Determination of flavor components. 43th Annual Meeting Inst. of Food Technologists, New Orleans, LA.

Rhodes, M. J. C. 1980. The maturation and ripening of fruits. In: K. V. Thiamnn (eds.). Senescence in Plants. CRC Press, Florida.

Salunkhe, D. K. and J. Y. Do. 1976. Biogenesis of aroma constituents of fruits and vegetables. Cric. Rev. Food Sci. Nutr. 8:161-190.

Sapers, G. M., J. Abbott, D. Massie, A. Watada, and E. E. Finney. 1977. Volatile composition of McIntosh apple juice as a function of maturity and ripeness indices. J. Food Sci. 42: 44-47

Schreier, P. 1984. Chromatographic studies of biogenesis plant volatiles. Huthing, Germany.

Seifabad, T. C. 1988. Effect of harvest date and ethylene concentration in controlled atmosphere storage on the quality of empire apple. PhD. Thesis, Michigan State Univ.

Shamaila, M., W. D. Powrie and B. J. Skura. 1992. Analysis of volatile compounds from strawberry fruit stored under modified atmosphere packaging (MAP). J. Food Sci. 57:1173-1176.

Smock, R. M. and A. M. Neubert. 1950. Apples and apple products. Interscience Publ., New York.

Somogyi, L. P., N. F. Childers and S. S. Chang. 1964. Volatile constituents of apple fruits as influenced by fertilizer treatment. Proc. Am. Soc. Hort. Sci. 84:51-58.

Song J. and F. Bangerth. 1994. Production and development of volatile aroma compounds of apple fruits at different times of maturity. Act Horticulturae. 357.

Song J. 1994. Einflu verschiedenber erntezeitpunkte auf die fruchtreife under besonderer ber cksichtigung der Aromabildung bei pfeln, tomaten und erdbeeren. Verlag Ulrich E. Grauer. Stuttgart. 1994.

Song J., R. Leepipattwit, W. Deng and R. M. Beaudry. 1996. Hexanal vapor is a natural, metabolizable fungicide: Inhibition of fungal activity and enhancement of aroma biosynthesis in apple slices. J. Amer. Soc. Hort. Sci. 121:937-942.

Song J, B. D. Gardner, J. F. Holland and R. M. Beaudry. 1977. Rapid analysis of volatile flavor compounds in apple fruit using SPME and GC/Time-of-Flight Mass Spectrometry. J. Agric. Food Chem. 45 (5): 1801-1807.

Stevens, K. L., Bomben, J., Lee, A. and McFedden W. H. 1966. Volatiles from grapes, Muscat of Alexandria. J. Agric. Food Chem. 14: 249.

Streif, J. and F. Bangerth. 1988. Production of volatile aroma substances by golden delicious apple fruits after storage for various times in different CO_2 and O_2 concentrations. J. Hort. Sci. 63 (2):193-199.

Sugisawa, H. 1981. Sample preparation: Isolation and concentration. In: R. Teranishi, R. A. Flath and H. Sugisawa (eds.). Flavor Research Recent Advances. Marcel Dekker, New York.

Tang, C. S. and W. G. Jennings. 1967. Volatile components of apricot. J. Agric. Food Chem. 15:24.

Tressel, R. and F. Drawert. 1973. Biogenesis of banana volatiles. J. Agric. Food Chem. 21:560-565.

Turk, A., R. M. Smock and T. I. Taylor. 1951. Mass and infrared spectra of apple vapor. J. Food Technol. 5:58.

Vaughn, S. F., G. F. Spencer, and B. S. Shasha. 1993. Volatile compounds from raspberry and strawberry fruit inhibit postharvest decay fungi. J. Food Sci. 58:1-4.

Weurman, C. 1961 Gas-liquid chromatographic studies on the enzymatic formation of volatile compounds in respberries. J. Food Technol. 15:531.

White, J. W. 1950. Composition of volatile fraction of apple. Food Res. 15:68-78.

Willaert, Dirinck, Depooter, and Schamp. 1983. Objective measurement of aroma quality of golden delicious apple as a function of controlled-atmosphere storage time. J. Agric. Food Chem. 31:809-813.

Wills, R. B. H. and W. B. McGlasson. 1971. Effect of storage temperature on apple volatiles associated with low temperature breakdown. J. Hort. Sci. 46:115-120.

Wills, R. B. H. and K. J. Scott. 1975. Effect of geraniol on levels of volatile

terpenoid compounds produced by apple during cool storage. J. Hort. Sci. 50:475-480.

Williams, A. A., M. J. Lewis, and O. G. Tucknott. 1980. The neutral volatile components of cider apple juices. Food Chem. 6:139

Wilson, C. L., J. D. Franklin, and B. E. Otto. 1987. Fruit volatiles inhibitory to *Monilinia fructicola* and *Botrytis cinerea*. Plant Dis. 71(4):316-319.

Yahia, E. M. 1986. Changes in the flavor components in apple during maturation ripening and storage. PhD thesis, Cornell Univ. Ithaca, NY.

Yahia E. M., F. W. Liu and T. E. Acree. 1990a. Changes of some odor-active volatiles in controlled atmosphere-stored apples. J. Food Quality 13:185-202.

Yahia E. M., F. W. Liu and T. E. Acree. 1990b. The evolution of some odor-active volatiles during the maturation and ripening of apples on the tree. Lebens. Wiss. u-Technol. 23: 488-493.

Yahia, E. M. 1991a. Production of some odor-active volatiles by McIntosh apples following low-ethylene controlled atmosphere storage. HortScience 26: 1183-1185.

Yahia, E. M. 1991b. Changes in some odor-active volatiles in apple during storage in air and in simulated low-ethylene controlled atmosphere. Turrialba 42(2): 160-165.

Yahia E. M., F. W. Liu and T. E. Acree. 1991. Changes of some odour- active volatiles in low-ethylene controlled atmosphere stored apples. Lebensmittel-Wissenschaft und technologie. 24: 145- 151.

Yahia E. M. 1994. Apple flavor. Hort. Rev. 197-234.

Yamashita, I., Y. Nemoto and S. Yoshikawa. 1975. Formation of volatile esters in strawberries. J. Agric. Biol. Chem. 29:(12): 2303

Yamashita, I., Y. Nemoto and S. Yoshikawa. 1976. Formation of volatile alcohols and esters from aldehydes in strawberries. J. Phytochem. 15:1633.

Yamashita, I., Y. Nemoto and S. Yoshikawa. 1977. Studies on flavor development in strawberries. 4. Biosynthesis of volatile alcohol and esters from aldehyde during ripening. J. Agric. Food Chem. 25: 1165.

Yang, S. F. 1985. Biosynthesis and action of ethylene. HortScience. 20: 41-45.

Zerbini, P. C., T. Eccher and F. L. Gorin. 1980. Determination of quality indices of apples. II. Quality parameters of 'Golden Delicious' apples from orchards at different altitudes. Freddo 34:101-115.

Zlakis, A., H. A. Lichtenstein and A. Tishbee. 1973. Solid adsorbent. J. Chromatogr.

CHAPTER III:

OVERCOMING GAS SAMPLING PROBLEMS: ANALYSIS OF VOLATILES USING CAPILLARY COLUMN NEEDLES

ABSTRACT

In an attempt to develop a simple, rapid sampling method, segments of capillary gas chromatography (GC) columns were installed in gas-tight syringes and used as needles for volatile analysis. Results were compared to a standard stainless steel needle. Volatiles tested included hexyl acetate, ethyl 2-methylbutyrate, 6-methyl-5-hepten-2-one and butanol. GC response increased as the number of times the syringe was pumped (syringe pump number) increased. As pump number continued to increase, the rate of increase in the GC response diminished until saturation occurred. The pump number required to achieve response saturation was determined for each volatile and each needle type. The use of a capillary needle resulted in an increase in GC response in the range of 3- to 15-fold relative to a stainless steel needle. Injection volume affected GC response in a needle- and volatile-dependent manner. When injection volume was regressed against GC response, it did not extrapolate through the origin for any of the needle types. The GC response for capillary column needles increased as temperature decreased. The capillary column needle may be a useful tool for rapid and simple analysis of volatiles that readily partition into the column coating. This sample collection technique allowed the use of a packed-column GC system under isothermal conditions for volatile measurements.

INTRODUCTION

Analysis of aroma-related volatiles requires extraction and, often, concentration of the volatiles prior to detection. Extraction techniques include use of solvents, distillation, and headspace sampling. For the analysis of odor-active volatile compounds of apple fruit, headspace sampling is considered the most suitable and widely used since it should measure the concentration of those compounds in the vapor phase that are directly responsible for the odor of the product (Maarse, 1991). Headspace sampling is accomplished without sample destruction or the formation of artifact volatiles resulting from heat, tissue maceration, etc. (Land, 1975; Olfsdottir, 1985). Headspace analysis also permits detection of odor-active compounds closer to the concentrations and ratios perceived by human nose if compared with other analysis techniques (Bemelmans, 1978).

Techniques for headspace sampling include direct headspace (static headspace), purge-and-trap (dynamic headspace), and solid phase extraction. Direct headspace sampling is the taking of a gas volume directly from the vapor phase above the food. Purge-and-trap sampling involves purging the headspace into a device that absorbs the volatiles to concentrate volatile compounds from the headspace. Solid-phase extraction sampling involves exposing an absorptive substance in the headspace to collect and concentrate volatiles by virtue of its sorption characteristics. Unlike direct headspace and purge-and-trap sampling techniques, solid phase extraction does not remove any significant volume of gas from the headspace.

Compared with other headspace sampling techniques, direct sampling is the simplest and fastest (Guadagni et al., 1971; Martin, 1969; McNally and Grob,1985; Weurman, 1961; Yamashita et al., 1977). However, aroma volatiles are often present at very low concentrations and gas chromatography (GC) using a flame ionization detector (FID) often does not have sufficient sensitivity for detection of aroma volatiles present in trace amounts (Reineccious, 1983).

In purge-and-trap analysis, volatile compounds are desorbed from the trapping material by heating (Dirinck et al., 1981; Mattheis et al., 1991a, 1991b) or by various solvent extraction techniques (Clark and Cronin, 1975; Guadsgni et al., 1971; Olafsdottir, 1985; Shamaila, et al., 1992; Song and Bangerth, 1994). Purge-and-trap techniques require complex and costly equipment employing a variety of adsorption, desorption and cryofocussing devices (Bertsch et al., 1975.; Buckholz et al., 1980; Farwell et al., 1979; Murray, 1977; Olafsdottir et al., 1985). Tenax-GC (2,6-diphenyl-p-phenylene oxide polymer) and Tenax-TA (2,6-diphenyl-p-phenylene oxide chemistry) have been widely used for the absorption and adsorption of a variety of aroma volatiles from foods in purge- and-trap analysis (Dirinck et al., 1981; Mattheis et al., 1991a, 1991b; Olafsdottir, 1985; Shamaila et al., 1992; Song and Bangerth, 1994; Song, 1994). Although purge-and-trap headspace sampling is one of the most suitable methods for the analysis of apple volatiles, it is complicated, and both time and labor intensive.

While solid phase extraction does not require the removal of a volume of gas as in purge-and-trap, but still requires desorption and an additional concentration step such as cryofocussing. A relatively new approach, solid

phase micro extraction (SPME) uses a solid phase on an extendable fiber that is housed in a hollow needle. SPME has the potential to reduce the time investment in sampling and providing a linear response to concentrations of many volatile compounds (Gardner et al., 1995; Song et al., 1997). However, this simple and fast sampling approach must work in combination with rapid separation and detection equipment.

In many cases, research requires the analysis of individual or a few major volatiles whose concentration is not extremely low, yet too low for direct headspace sampling. It would be useful to find a simple, rapid method for standard packed-column GC's where sample preparation is at a minimum. This would be valuable for monitoring applications.

The purpose of this investigation was to develop a simple and fast method for analysis of volatiles produced in relatively high amounts by apple fruit using an isothermal packed-column GC. Needles used had absorptive linings to concentrate volatiles while attached to standard low-volume (50 to 100 µL) gas-tight glass syringes. Thus, their use combined aspects of direct headspace, purge-and-trap, and solid phase sampling techniques. Factors that could affect linearity, variation and the magnitude of GC response such as the number of times the syringe plunger was pumped (syringe pump number), injection volume, needle type, sampling temperature, compound polarity and boiling point were investigated.

MATERIALS AND METHODS

Needle preparation: Steel-jacketed (steel) and glass (glass) capillary column needles were created for comparison to standard unlined stainless-steel (stainless) needles. Steel and glass capillary needles were made by cutting steel and glass capillary columns. The steel capillary column lining (0.28 mm id; 1.0 µm film thickness) was crossbonded 5% diphenyl and 95% polydimethylsiloxane. The glass capillary column (0.32 mm id; 1.0 µm film thickness) was bonded with 100% polydimethylsiloxane. After cutting to the appropriate length, the column segments were installed in gas-tight syringe (1710 RN syringe, Hamilton Co., Reno, Nevada) and used as needles for volatile analysis. A standard 3.8-cm long, 26-gauge stainless steel needle was used as control.

Preparation of volatile standard: Volatile standards were generated by evaporating authenticated pure liquids of the volatiles of interest into a specially-designed 4.3-L glass jar having a ground glass stopper fitted with a Mininert valves (Supelco Inc., Supelco Park, Bellefonte, Penn.) The liquids were allowed to completely volatilize. The concentrations of volatiles were calculated based on their molecular weight, density, purity, and the volume of standard jars. The concentrations were 16, 16.5, 18.5, and 30.5 $\mu L.L^{-1}$ for ethyl 2-methylbutyrate ($C_7H_{14}O_2$), hexyl acetate ($C_8H_{16}O_2$), 6-methyl-5-hepten-2-one ($C_8H_{14}O$), and butanol ($C_4H_{10}O$), respectively. These concentrations were <2% of saturation for each compound at room temperature, so reduced temperatures should not have

affected the amount of material in the headspace of container.

Volatile analysis: Volatile samples were directly injected into a GC (Carle AGE Series 400, Hach Company, Loveland, Colo.) equipped with a flame ionization detector. The column (3mm O.D., 3.3 m long) was packed with 10% DEGS-PS, 80/100 mesh Supelcoport. The column was maintained isothermally at 140°C. Purified helium (99.9%) was used as carrier gas. The flow rate for the helium, air, and hydrogen were 60 $mL \cdot min^{-1}$, 200 $mL \cdot min^{-1}$, and 20 $mL \cdot min^{-1}$ respectively. In order to desorb the volatiles, the needle was held in the GC injector for 3 seconds prior to injection, then held for an additional 2 seconds before removal.

Effect of syringe pumping: The effect of the number of times a syringe was pumped (syringe pump number) on GC/FID response was studied by varying syringe pump number from 1 to 40. This was done for each volatile (hexyl acetate, 6-methyl-5-hepten-2-one, and butanol) and the three needle types. Injection volume was 50 µL. The first replication was taken starting with 1 rep and then increasing incrementally to forty pumps. The next replication was done in reverse order (40 pumps to 1 pump), and so on for 15 replications.

Needle Length: The effect of needle length on GC response was studied by using five different length of needles (2.5, 3.5, 4.5, 5.5, and 6.5 cm). After cutting, the needles were installed in the gas-tight syringe. The injection volume was 50 µL, and syringe pump number was 20.

Injection volume: The effect of injection volume on GC response was determined by using 10, 20, 30, 40, 50, 60, 70, and 80 µL injection volumes.

The syringe pump number was 20 and three volatile compounds (hexyl acetate, ethyl 2-methylbutyrate, butanol) and three needle types (capillary steel, capillary glass and standard stainless steel) were used.

Environment temperature and volatile partial pressure: The effect of temperature and concentration on GC response was determined by using two sample collection temperatures (0°C and 25°C) and three partial pressures of the volatile compound (1.77, 3.53, and 5.31 Pa). At each temperature and concentration, more than 10 measurements were made; a capillary steel needle syringe was used and the syringe pump number was 20.

RESULTS AND DISCUSSION

Effect of syringe pump number and needle type on GC response: When syringe pump number was increased from 1 to 10, the GC response increased for all tested volatile and needle types although the pumps number required to reach saturation differed for each volatile/needle combination (Table 1, Fig. 2, 3, 4). The maximal GC response for hexyl acetate using standard stainless steel, capillary glass, and capillary steel needles required approximately 10, 20, and 30 pumps of the syringe plunger, respectively (Fig. 2). 6-Methyl-5-hepten-2-one required 20 pumps for capillary glass needle and 10 pumps for standard steel and capillary steel needles (Fig. 3). For butanol measurement however, the required syringe pump number was 5 to 10 for all needle types (Fig. 4). The data indicate that needles with highly non-polar

sorptive linings had a significant absorption potential for lager more lipophilic molecules such as hehyl acetate and 6-methyl-5-hepten-2-one but not for polar and highly volatile molecules such as butanol.

Maximal GC response was dependent on the chemical nature of the volatiles. Once the needle was saturated with hexyl acetate, use of capillary glass and capillary steel needles resulted in 7- and 14-fold increases in signal response relative to the stainless steel needle. For 6-methyl-5-hepten-2-one, capillary glass and capillary steel needles yielded a 2.5- and 3.5-fold enhancement in signal relative to the stainless steel needle. For butanol, however, needle type had no effect on GC response.

The interaction between needle coating, volatile chemistry, and GC response probably reflects the sorption potential of the different needle coating materials for the different volatiles. Since the needle coating materials were highly non-polar, polar, highly volatile molecules such as butanol would not be expected to be retained appreciably. One would assume, therefore, that if a needle coating was composed of more polar materials, better retention of polar compounds could have been achieved and GC response would have been improved.

Effect of injection volume on GC response: The injection volume directly affects the amount of material delivered to the GC and therefore markedly influences GC response. Generally speaking, increasing injection volume should increase GC response linearly for almost all uncoated commercial steel needles. One would also expect that if the relationship is

linear, GC response should be near zero when the injection volume is zero. While this can not be tested directly, fitting a line to data for a range of injection volumes permits extrapolation. A non-zero Y-axis intercept should be indicative of needle effects, for example, as when some of the volatile material adheres to the inner walls of the needle.

When injection volume increased from 10 to 80 µL, butanol showed a linear increase for all three needle types (Fig. 5). For the capillary steel needle, ethyl 2-methylbutyrate and butanol increased linearly with injection volume (Fig. 6). Interestingly, GC response did not vary significantly with injection volume for hexyl acetate. Importantly, the three compounds differed markedly in their zero-extrapolated injection volume, indicating marked differences in needle retention of volatiles. This finding is consistent with the effect of needle type on GC response noted earlier. Evidently, the capillary steel needle had very high adsorption potential for hexyl acetate. The lack of a typical injection-volume/GC response relationship for hexyl acetate on the capillary steel needle may be evidence for losing some of the volatile material to the cooler portion of the needle that resides in the syringe barrel during injection or adsorption to the glass syringe barrel itself. Butanol had only minor needle retention (Figs. 5 and 6); the volatile material in the needle may be only lightly adsorbed and/or may represent the material in the needle volume, rather than adsorbed to the needle lining. One would calculate that roughly 50% of the needle volume would be expressed just by heating to 250°C. The needle volume in this case was approximately 10 µL. The increase in response to 5-10 µL additional volume can

be calculated from the data in Figs. 5 and 6. The zero extrapolation is many fold higher than the contribution expected by the needle volume. Therefore, a significant contribution to GC response is evidently made by adsorption. The response to injection volume tended to be non-linear below 40 µL (Figs. 5 and 6). This may be the result of the requirement for a minimal volume of gas to sweep the volatile molecules from the needle into the GC.

Effect of needle length on GC response: If the needle is responsible for retention of a significant portion of the volatile molecules, then needle length should affect GC response. The increase of the needle length not only increases the adsorption potential of needle but also the volume of injection. Since needles had inside diameters for capillary steel and capillary glass were 0.28 and 0.32 mm, the effect of length on volume was approximately only 9.85 and12.86 µL, respectively, when needle length was increased from 250 to 650 mm. In addition, because the concentration of tested volatile was as low as 16 ppm, the effect of needle length-related volume on GC response should be minimal relative to sorption effects. Therefore, the effect of needle length on GC response should be primarily from the increase of absorption potential when needle length is increased.

When the length of capillary glass, and capillary steel needles was increased from 250 to 650 mm (a 2.3-fold increase), GC response for 6-methyl-5-hepten-2-one increased 2.25- and 2.14-fold, respectively. However, there was no increase for standard stainless steel (Fig. 7). Compared with the stainless steel needle, capillary glass and capillary steel needles had a greater increase in

GC response due to their higher sorption potential.

Effect of injection volume and needle length on GC response variation: Injection volumes influenced the coefficient of variation (CV) of GC response. Increasing injection volume from 10 to 50 µL, reduced CV 2.5-, 3.7-, and 6.7-fold when standard stainless steel, capillary glass, and capillary steel needles were used, respectively (Fig. 8).

As needle length increased, a decline in the CV of response was also found for lined capillary needles, but not for the stainless steel needles. For example, when capillary glass and capillary steel needles were used in the measurement of 6-methyl-5-hepten-2-one, the CV declined 3.7- and 4.9-fold, respectively, when needle length was increased from 2.5 to 6.5 cm (Fig. 9), achieving a low of 1 to 1.5 %.

Effect of environmental temperature on GC response: It is well-known that temperature affects the sorption process. When the sample was taken in a lower temperature environment (0°C), the needle absorbed more molecules than when the sample was taken at higher temperature (25°C), thereby increasing GC response (Fig. 10). With the technique used here, the process of sorption is used to collect volatiles in syringe needles. Factors that could affect volatile absorption and desorption from the needle coating should therefore be well controlled to achieve consistent and accurate results.

CONCLUSIONS

Our study suggests needles lined with sorptive material have a number of attributes that may be helpful for volatile analysis. Using a capillary column needle resulted in an increase in GC response of nearly 15-fold relative to a standard stainless steel needle for lipophilic materials. Plunger pump number was an important factor affecting GC response. Up to 35 plunger pumps were required to achieve equilibrium with the sample headspace. This factor, more than any other, determined rapidity of obtaining a sample. It took roughly one minute to obtain a gas sample, when the plunger pump number was 40. Injection volume and needle length affected GC response in a needle- and volatile-dependent manner. The GC response for capillary column needles decreased when sample temperature increased. Thus, use of lined needles requires placement of the standard in the same temperature regime as the sample. Because capillary GC column needle can work with both flow through and low volume sealed system such as modified atmosphere packaging (MAP), it permits volatile measurement for packaged products under its storage environment. Therefore, sorptive needles may be a useful tool for determining the dynamic relationship between flavor volatile synthesis and package atmosphere for packaged produce.

Since capillary GC column needles improved GC response for volatiles that readily partition into the column coating, by changing the lining material of column needle coating, needles conferring better GC response and a broader range of applicability could, in theory, be constructed. The use of this sample

collection technique allows use of a simple GC system such as packed-column and isothermal heating, instead of the more complex, time-consuming, and expensive capillary GC system.

Literature Cited

Bemelmans, J. M. 1978. Review of Isolation and Concentration Techniques. In "Progress in Flavour Research" D. G. Land and H. E. Nursten (ed.) P 79-98.

Bertsch, W., E. Anderson, and G. Holzer. 1975. Trace analysis of organic volatiles in water by gas chromatography-mass spectrometry with glass capillary columns. J. Chromat. 112: 701

Buckholz, L. L. J., D. A. Withycombe, and H. Daun. 1980. Application and characteristics of polymer adsorption method used to analyze flavor volatiles from peanuts. J. Agric. Food Chem. 28: 760.

Clark, R. G. and D. A. Cronin. 1975. A new technique for trapping and sensory evaluation of flavor volatiles. J. Sci. Food agri. 26: 1009.

Dirinck, P. J., H. L. De Pooter, G. A. Willeart and N. M. Schamp. 1981. Flavor quality of cultivated strawberries: The role of the sulfur compounds. J. Agric. Food Chem. 29: 319.

Gardner, B. D., Johnson, J. A., Artaev, V. B., Sparkman, O. D., Holland, J. F. 1995. Rapid sampling for rapid analysis: A new method fo VOCs in drinking water. Proceedings of the 43th ASMS conference on Mass Spectrometry and allied topics, Atlanta, GA, May 21-26, 1995

Farwell, S. O., S. J. Gluck, W. L. Bamesberger, T. M. Schutte, and D. F. Adams. 1979. Determination of sulfur-containing gases by a deactivated cryogenic enrichment and capillary gas chromatographic system. Anal. Chem. 51: 609-615

Guadagni, D. G., J. L. Bomben and J. S. Hudson. 1971. Factors influencing the development of aroma in apple peels. J. Sci. Food Agric. 22: 110-115.

Hirvi, T. and E. Honkanen. 1982. The volatiles of two new strawberry cultivars, "Annelie" and "Alaska Pioneer," obtained by backcrossing of cultivated strawberries with wild strawberries, *Fragaria vesca*, Rugen and *Fragaria virginana*. Z. Lebensm. Unters. Forsch. 175: 113-116.

Land, D. G. 1975. Techniques for assessing odor: uses and limitations. Proc. Int. Symp. Aroma Research. Pudoc., Wageningen, The Netherlands.

Maarse, H. 1991. Volatile compounds in foods and beverages. Marcel, New York.

Martin, J. M. 1969. Gas chromatographic qualitative and semiqualitative analysis of apple aroma by means of retention indexes. J. Anal. Chem. Acta. 48:

Mattheis, J. P., D. A. Buchanan, and J. K. Fellman. 1991a. Change in apple fruit volatiles after storage in atmospheres inducing anaerobic metabolism. J. Agri. Food Chem. 39: 1602-1605.

Mattheis, J. P., J. K. Fellman, P. Chen, and M. Patterson. 1991b. Changes in headspace volatiles during physiological development of bisbee delicious apple fruit. J. Agric. Food Chem. 39: 1902-1906

McNally, M. E. and R. L. Grob. 1985. Current application of static and dynamic headspace analysis. A review. Part II: non environmental applications. J. Amer. Lab. Feb: 106.

Murray, K. E. 1977. Concentration of headspace, airborne and aqueous volatiles in chromosorb 105 for examination by gas chromatography and gas chromatography-mass spectrometry. J. Chromat. 135: 49.

Olafsdottir, G., J. A. Steinke and R.C. Lindsay. 1985. Quantitative performance of a simple Tenax-GC adsorption method for use in the analysis of aroma volatiles. J. Food Sci. 50: 1431-1436

Reineccious, G. 1983. Determination of flavor components. Presented at 43th Annual Meeting Inst. of Food Technologists, New Orleans, LA.

Seifabad, T. C. 1988. Effect of harvest date and ethylene concentration in controlled atmosphere storage on the quality of empire apple. Ph. D. thesis Department of Food Science, Michigan State University.

Shamaila, M., W. D. Powrie and B. J. Skura. 1992. Analysis of volatile compounds from strawberry fruit stored under modified atmosphere packaging (MAP). J. Food Sci. 57: 1173-1176.

Song, Jun, B. D. Gardner, J. F. Holland, and R. M. Beaudry. 1997. Rapid analysis of volatile flavor compounds in apple fruit using SPME and GC/time-of flight mass spectrometry. J. Agric. Food Chem. 45: 1801-1807.

Song, Jun and F. Bangerth. 1994. The production and changes of volatile compounds from apple fruits of maturities. Acta Hort. 368: 150-159.

Song, Jun 1994. Einfluß verschiedenber erntezeitpunkte auf die fruchtreife under besonderer berücksichtigung der Aromabildung bei äpfeln, tomaten und erdbeeren. Verlag Ulrich E. Grauer. Stuttgart, Germany

Weurman, C. 1961 Gas-liquid chromatographic studies on the enzymatic formation of volatile compounds in raspberries. J. Food Technol. 15: 531.

Yamashita, I., Y. Nemoto and S. Yoshikawa. 1977. Studies on flavor development in strawberries. 4. Biosynthesis of volatile alcohol and esters from aldehyde during ripening. J. Agric. Food Chem. 25: 1165.

Table 1. The effect of pump number, needle type, and volatile compound on GC response.

Volatiles	Syringe Pump number	Needle type: Cap. Steel Needle		Cap. Glass Needle		Standard Steel Needle	
		GC response					
Hexyl acetate	1	585	a*	52	a	200	a
	10	3302	b	1089	b	329	ab
	20	4659	c	2240	c	373	ab
	30	5324	d	2304	c	368	ab
	40	5568	d	2304	c	388	ab
6-methyl-5-hepten-2-one	1	316	a	30	a	150	a
	10	1443	b	576	a	393	b
	20	1472	b	976	c	406	b
	30	1440	b	1014	c	408	b
	40	1440	b	1000	c	408	b
Butanol	1	329	a	311	a	291	a
	10	490	ab	475	ab	473	ab
	20	498	ab	495	ab	479	ab
	30	498	ab	500	ab	492	ab
	40	505	ab	504	ab	494	ab

* n= 10 to 20; In a column of each syringe plunger pump number, means of GC response followed by different letter are significantly different at 5% level.

Figure 1. Standard Jar: The jar was specially-designed glass chamber with a volume of 4-L, fitted with a Mininert value. The standard concentration was generated by evaporating sub-micro liter amount of the liquid of each of the volatile compounds of interest.

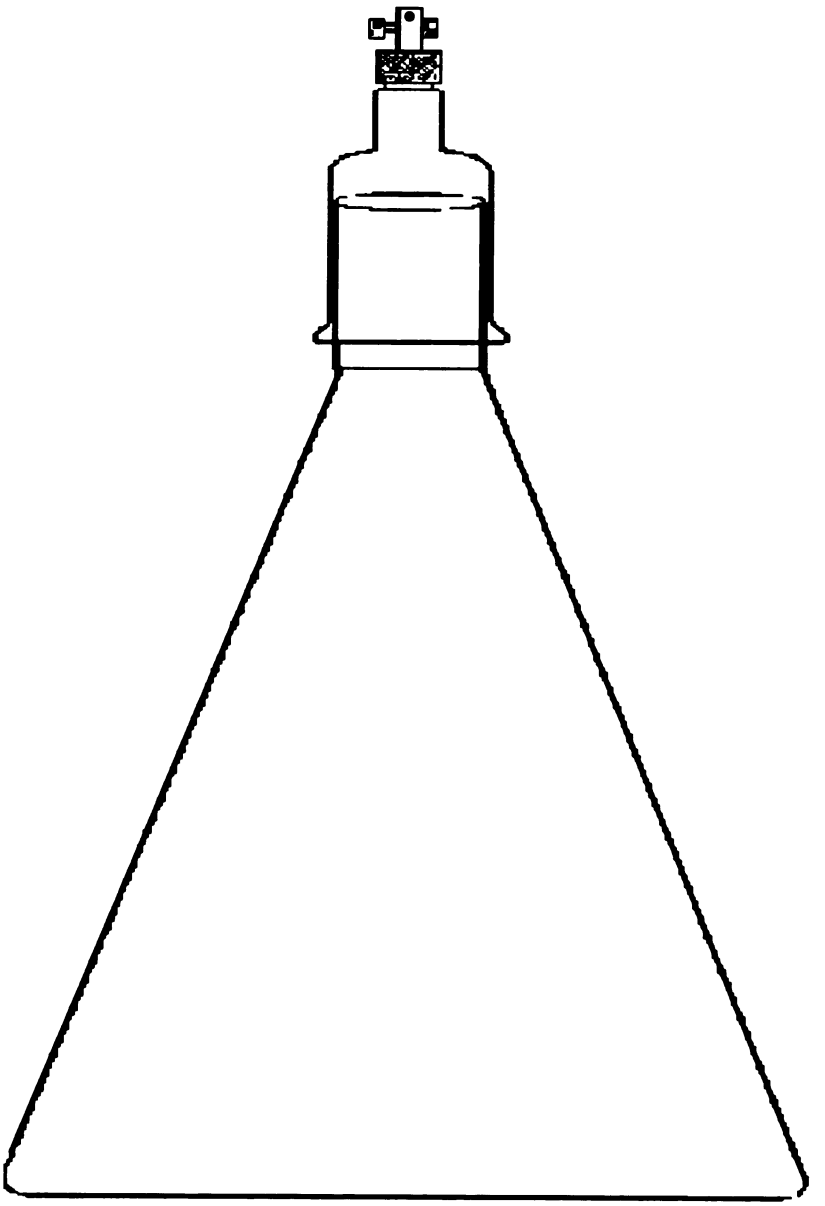


Figure 2. GC response as a function of number of times a syringe plunger is pumped for 50 µL injections of hexyl acetate (1.673 Pa) from glass, gas-tight syringes fitted with a stainless steel needle and needles made from capillary column (glass and steel) segments. Arrows denote estimated saturation point. Bars represent SE of more than 10 randomized replicates. Each data point represent mean of 10 to 20 injections of a single standard.

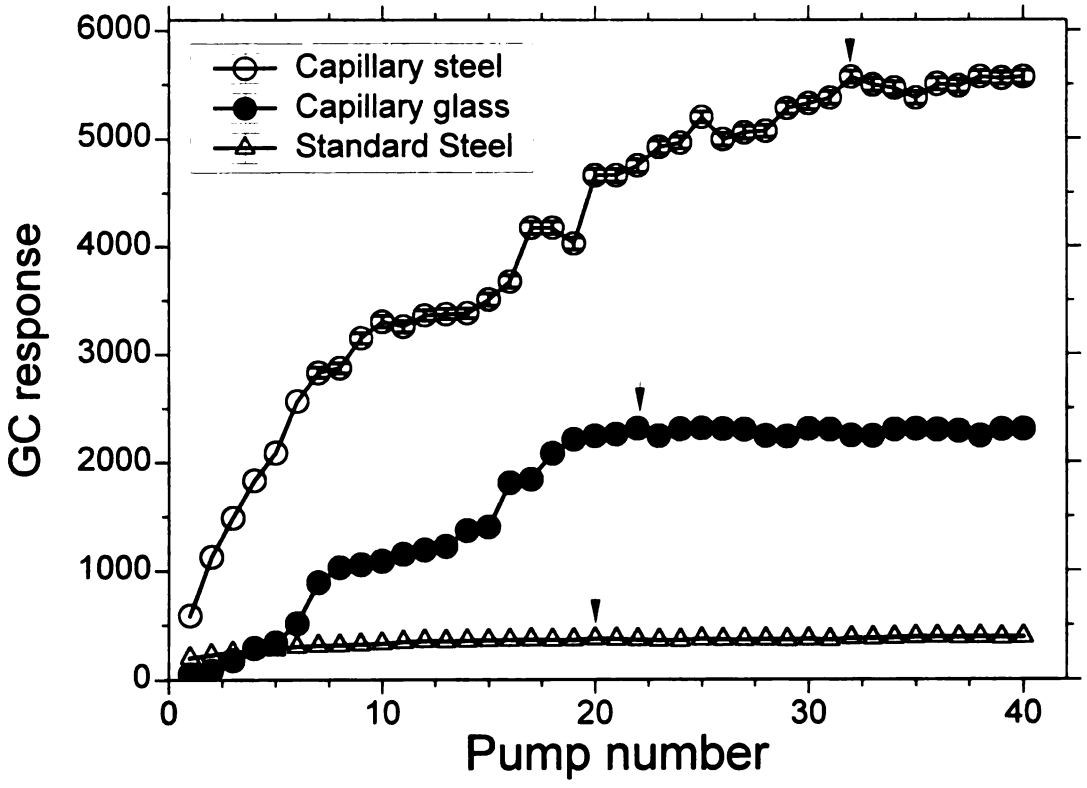

Capillary steel
Capillary glass
Standard Steel
GC response
Pump number
0
1000
2000
3000
4000
5000
6000
0
10
20
30
40

Figure 3. GC response as a function of number of times a syringe plunger is pumped for 50 µL injections of 6-methyl-5-hepten-2-one (1.847 Pa) from glass, gas-tight syringes fitted with a stainless steel needle and needles made from capillary column (glass and steel) segments. Arrows denote estimated saturation point. Bars represent SE of more than 10 randomized replicates. Each data point represent mean of 10 to 20 injections of a single standard.

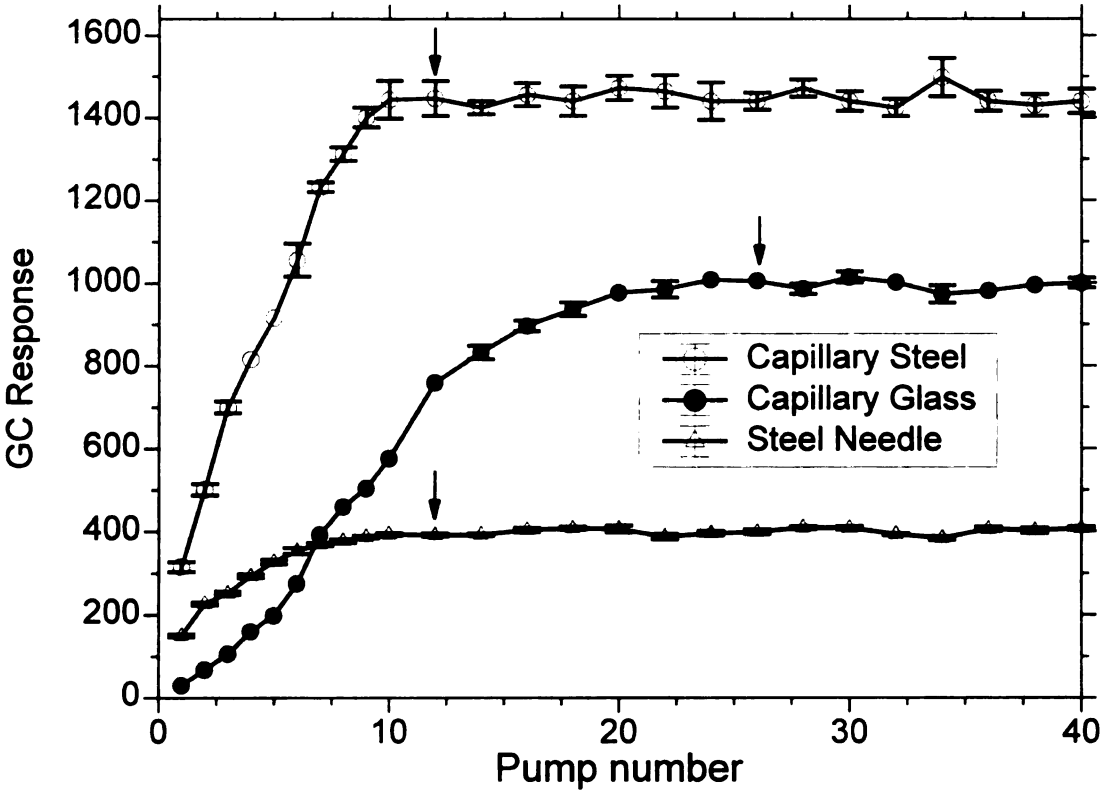

GC Response
Pump number
Capillary Steel
Capillary Glass
Steel Needle
0
200
400
600
800
1000
1200
1400
1600
0
10
20
30
40

Figure 4. GC response as a function of number of times a syringe plunger is pumped for 50 µL injections of butanol (3.051 Pa) from glass, gas-tight syringes fitted with a stainless steel needle and needles made from capillary column (glass and steel) segments. Arrows denote estimated saturation point. Bars represent SE of more than 10 randomized replicates. Each data point represent mean of 10 to 20 injections of a single standard.

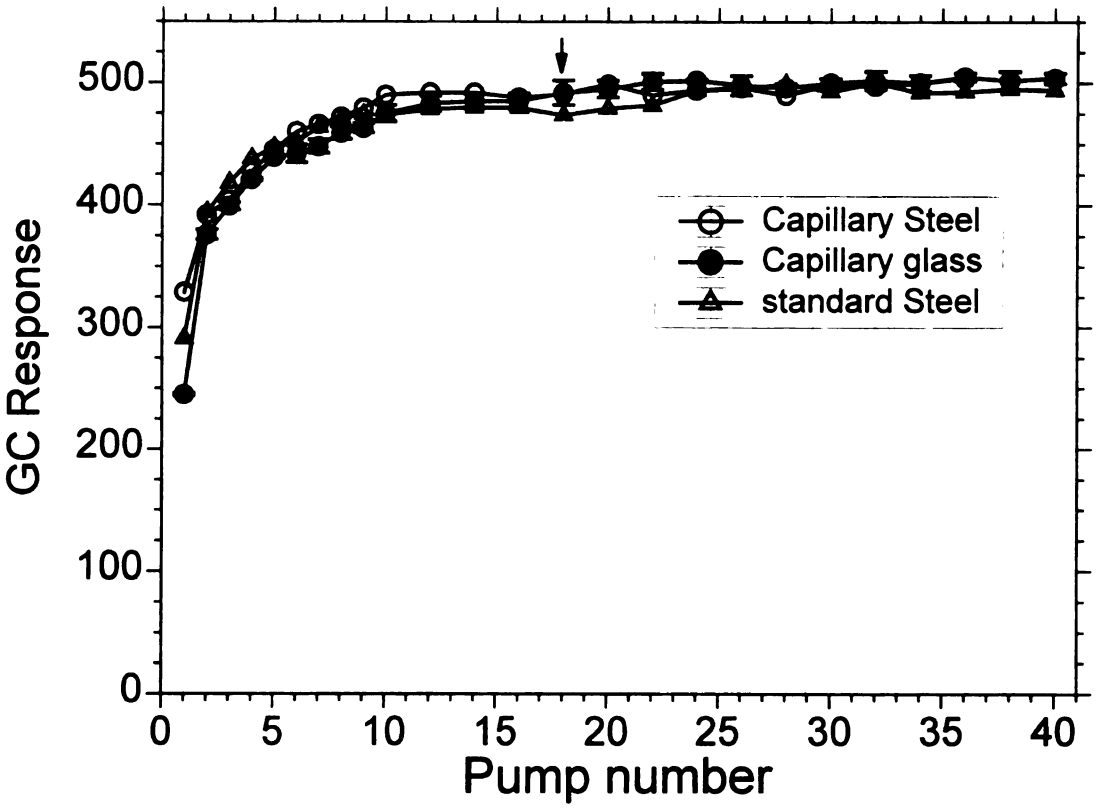

GC Response
Pump number
Capillary Steel
Capillary glass
standard Steel
0
100
200
300
400
500
0
5
10
15
20
25
30
35
40

Figure 5. GC response to injection volume of butanol using a gas-tight syringes fitted with a stainless steel needle and needles made from capillary column (glass and steel) segments. Note the fitted line dose not extrapolate through the origin. Dotted line segment represents extrapolation to zero injection volume (reflects contribution of needle volume, material adhering to the interior of the needle and effect of pressure of carrier gas). Syringe pump number: 20; Needle length: 5.7 cm; Temperature: 22°C.

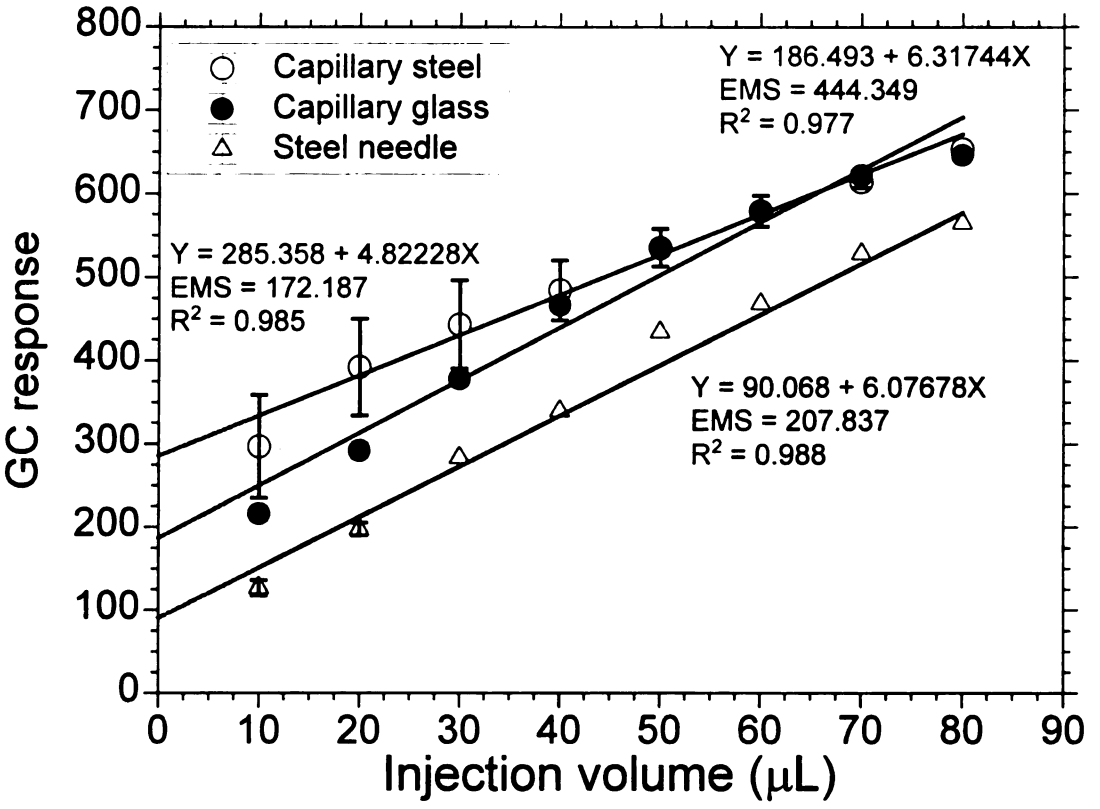

Capillary steel
Capillary glass
Steel needle
Y = 186.493 + 6.31744X
EMS = 444.349
R2 = 0.977
Y = 285.358 + 4.82228X
EMS = 172.187
R2 = 0.985
Y = 90.068 + 6.07678X
EMS = 207.837
R2 = 0.988
GC response
Injection volume (μL)
0
100
200
300
400
500
600
700
800
0
10
20
30
40
50
60
70
80
90

Figure 6. GC response to injection volume of hexyl acetate, ethyl 2-methylbutyrate, and butanol using a gas-tight syringes fitted with a needle made from steel capillary column segments. Note the fitted line dose not extrapolate through the origin.

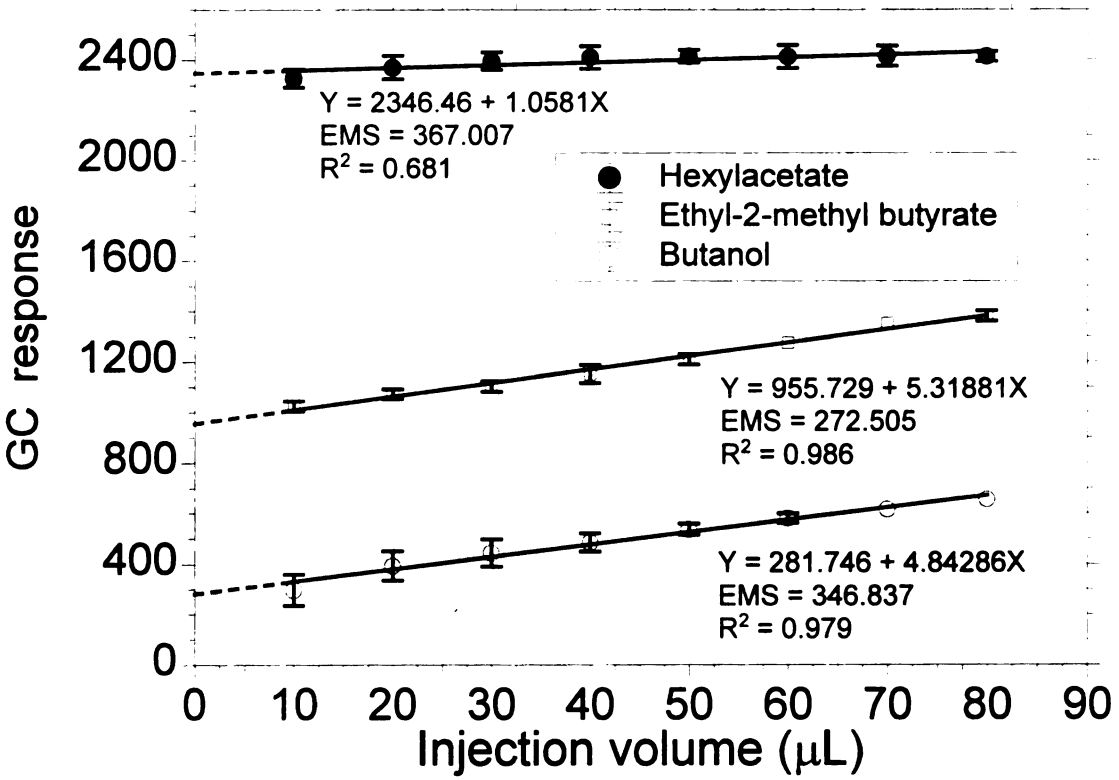

2400
2000
1600
1200
800
400
0
GC response
0
10
20
30
40
50
60
70
80
90
Injection volume (µL)
Y = 2346.46 + 1.0581X
EMS = 367.007
R2 = 0.681
Hexylacetate
Ethyl-2-methyl butyrate
Butanol
Y = 955.729 + 5.31881X
EMS = 272.505
R2 = 0.986
Y = 281.746 + 4.84286X
EMS = 346.837
R2 = 0.979

Figure 7. Effect of needle length on GC response to 50 µL injections of 6-methyl-5-hepten-2-one from glass, gas-tight syringes fitted with a stainless steel needle and needles made from capillary column (glass and steel) segments.

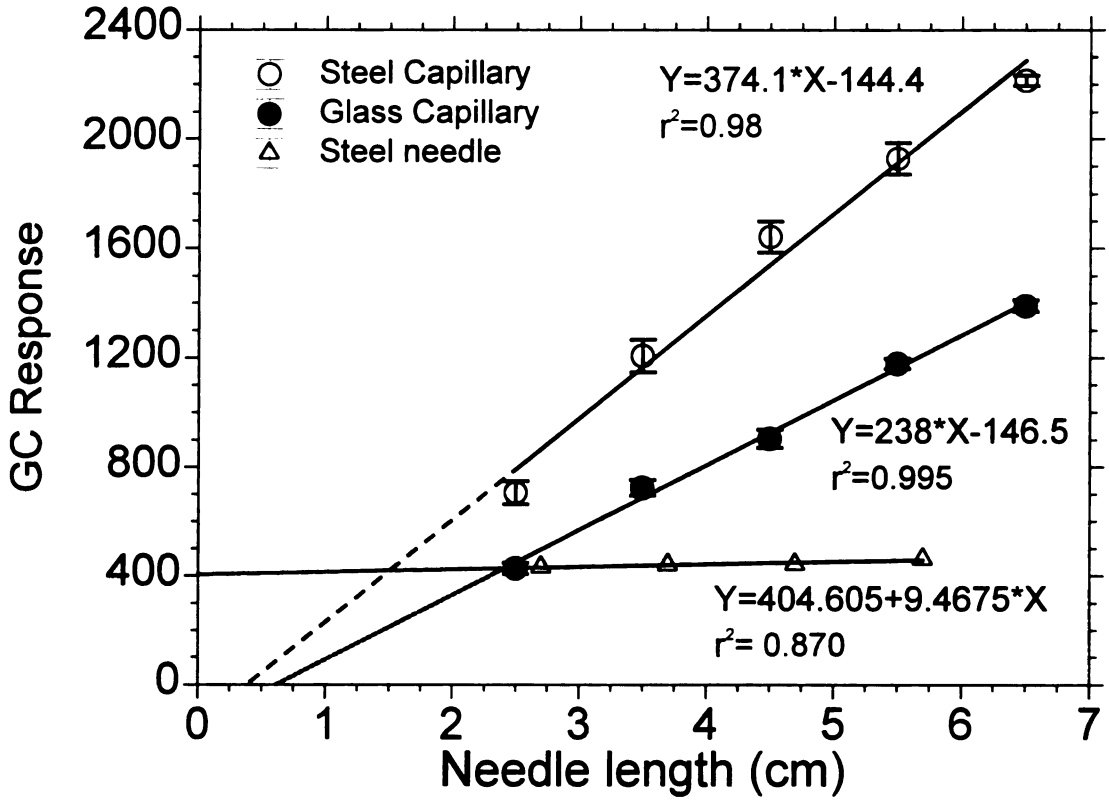

Steel Capillary
Glass Capillary
Steel needle
Y=374.1*X-144.4
r²=0.98
Y=238*X-146.5
r²=0.995
Y=404.605+9.4675*X
r²= 0.870
GC Response
Needle length (cm)
2400
2000
1600
1200
800
400
0
0
1
2
3
4
5
6
7

Figure 8. Coefficient of variation for injection volume of butanol using a gas-tight syringes fitted with a stainless steel needle and needles made from capillary column (glass and steel) segments.

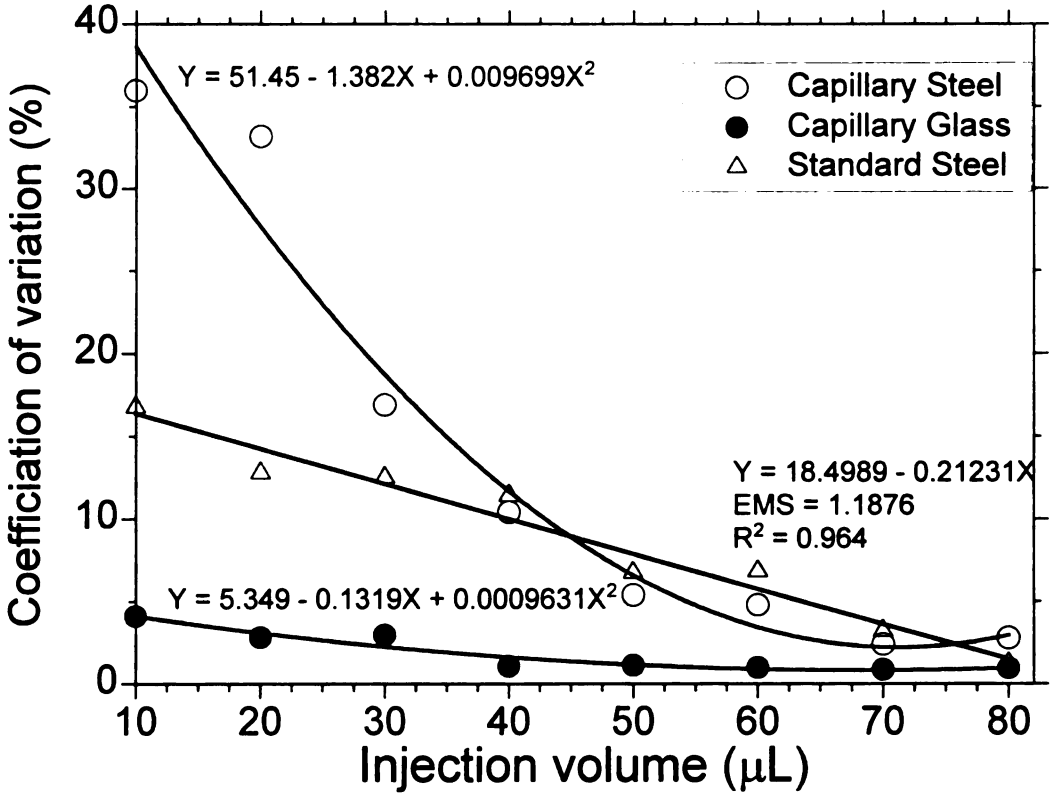

Y = 51.45 - 1.382X + 0.009699X²
Capillary Steel
Capillary Glass
Standard Steel
Y = 18.4989 - 0.21231X
EMS = 1.1876
R² = 0.964
Y = 5.349 - 0.1319X + 0.0009631X²
Coefficiation of variation (%)
Injection volume (µL)
0
10
20
30
40
10
20
30
40
50
60
70
80

Figure 9. Effect of needle length on Coefficient of variation to 50 µL injections of 6-methyl-5-hepten-2-one from glass, gas-tight syringes fitted with needles made from capillary column (glass and steel) segments.

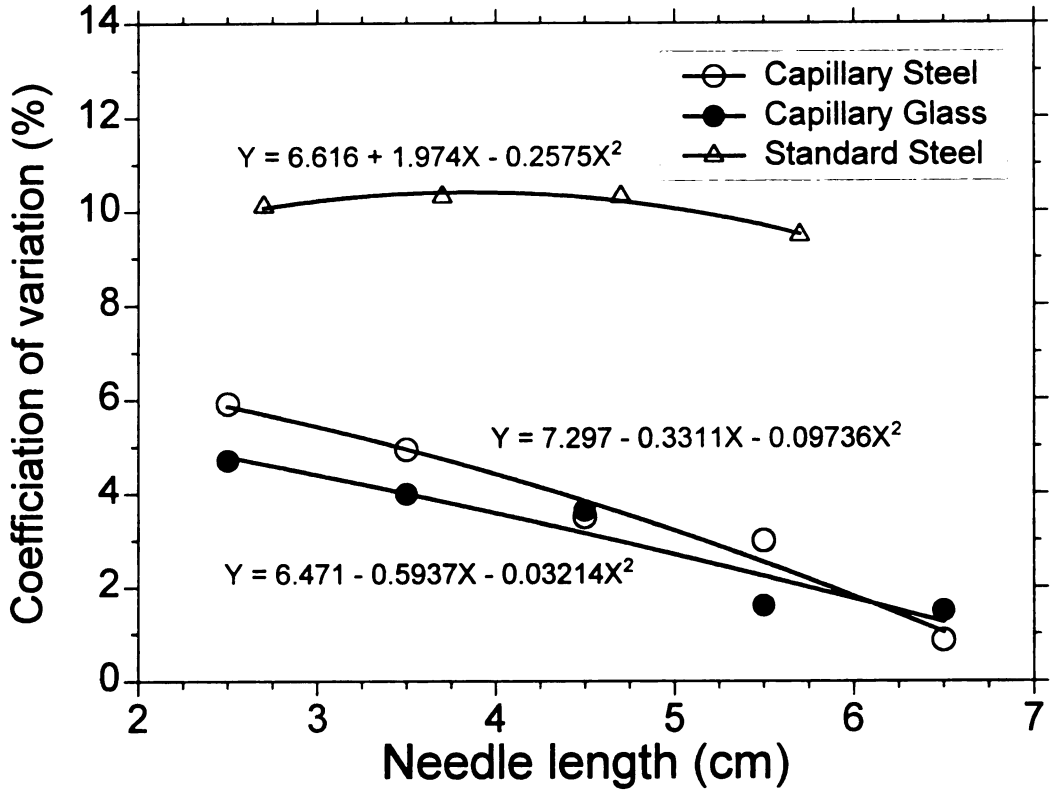
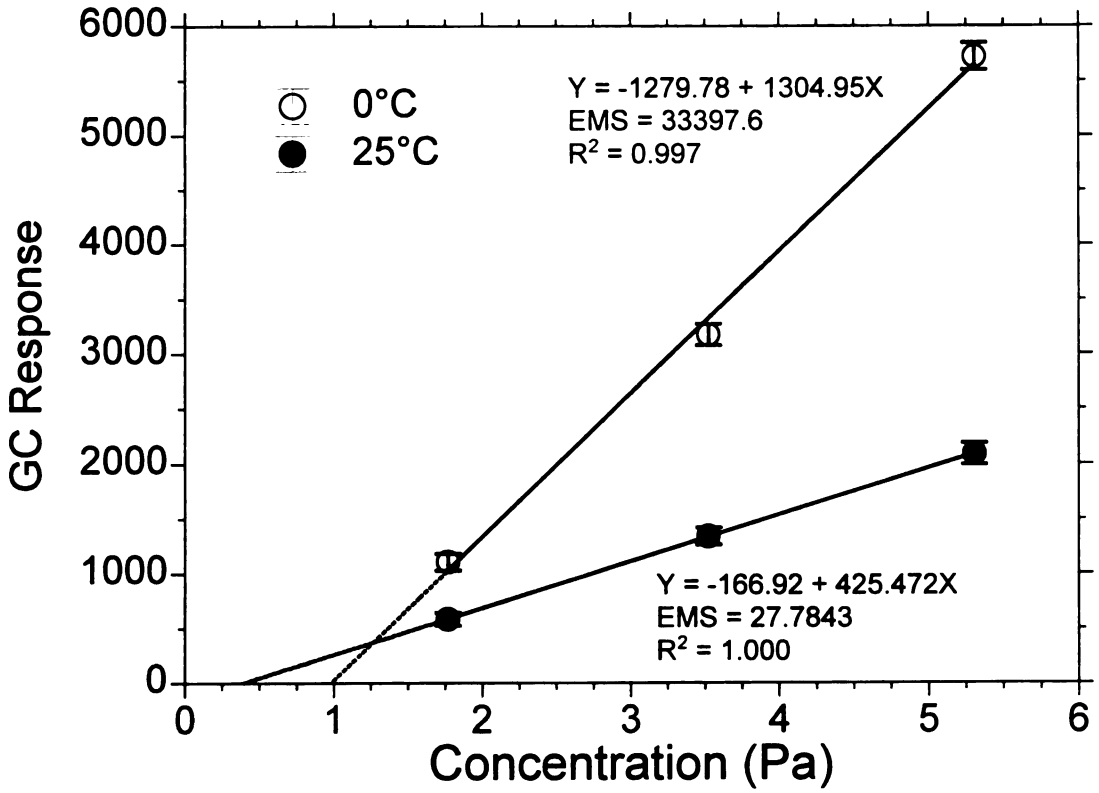

Capillary Steel
Capillary Glass
Standard Steel
Y = 6.616 + 1.974X - 0.2575X²
Y = 7.297 - 0.3311X - 0.09736X²
Y = 6.471 - 0.5937X - 0.03214X²
Coefficiation of variation (%)
Needle length (cm)
0
2
4
6
8
10
12
14
2
3
4
5
6
7

Figure 10. Effect of temperature and concentration of hexyl acetate vapors on GC response to 50 µL injections from glass, gas-tight syringes fitted with a needle made from steel capillary column segments (length: 5.7 cm).


6000
5000
4000
3000
2000
1000
0
GC Response
0
1
2
3
4
5
6
Concentration (Pa)
0°C
25°C
Y = -1279.78 + 1304.95X
EMS = 33397.6
R2 = 0.997
Y = -166.92 + 425.472X
EMS = 27.7843
R2 = 1.000

CHAPTER IV:

EVALUATION OF A PACKAGE SYSTEM FOR THE MEASUREMENT OF AROMA VOLATILE SYNTHESIS

EVALUATION OF A PACKAGE SYSTEM FOR THE MEASUREMENT OF AROMA VOLATILE SYNTHESIS

Abstract: Polymer film packages have been used to measure respiration, but their use to measure aroma volatile production has not been demonstrated. Apple (*Malus domestica* Borkh. cv. Golden Delicious) fruit were placed in packages, in glass containers, and in packages within glass containers to determine the feasibility of using the gradient of aroma volatiles across the film to calculate the rate of production by the product. The flux of CO_2, O_2, ethylene, hexyl acetate, butyl acetate, 2-methylbutyl acetate, and α-farnesene was determined as affected by temperature and O_2 level. At the same O_2 concentration, non-packaged fruit evolved greater amounts of the esters and α-farnesene than packaged fruit. The flux of α-farnesene, hexyl acetate and 2-methylbutyl acetate was approximately 27-, 1.7-, and 1.4-fold higher, respectively, for fruit in a glass container without packages than for fruit in packages within glass containers. The packaging material is suspected of absorbing α-farnesene and the esters, thereby altering the aroma profile of packaged produce relative to a flow-through system. When storage temperature increased from 0°C to 22°C the production rate of hexyl acetate and 2-methylbutyl acetate increased 11- and 17-fold, respectively. At 0 C, as O_2 decreased in concentration from 10% to 5% (v/v), hexyl acetate and butyl acetate declined significantly; however, 2-methylbutyl acetate production was not affected. This can be taken to indicate the production of the branched-chain

alcohols such as 2-methyl butanol is not as O_2 concentration-dependent as straight-chain alcohols such as hexanol and butanol.

INTRODUCTION

Modified-atmosphere packaging (MAP) "has the potential to provide low O_2/high CO_2 regimes similar to those of controlled-atmosphere (CA) storage" (Beaudry et al., 1992), and is widely used to store fresh and lightly-processed fruits and vegetables. However, the optimal atmospheric environment is difficult to maintain during MAP storage since many plant and environmental factors affect O_2 and CO_2 concentrations. In addition, loss or changes in flavors during MAP storage is one of the primary concerns in providing high quality food (Matsui et al., 1994). The aroma profile of packaged products can be affected by the interaction of aroma compounds with the packaging film (Debeaufort and Voilley, 1994; Matsui et al., 1994; Mason et al., 1992; Nielsen and Giacin, 1994; Osajima and Matsui, 1993). Changes in the aroma profile may due to sorption into packaging material or permeation through the package to the environment (Delassus, 1986; Nielsen and Giacin, 1994) or due to sorption of odor-active volatiles from the packing or the environment into the package (Mason et al., 1992). In addition to film effects, the package may alter the aroma profile through its effect on package O_2 and CO_2 levels and, subsequently, on aroma biosynthesis.

Low-density polyethylene (LDPE) is a commonly used polymer for MAP of fresh and lightly-processed produce. Some of its properties such as the permeabilities to O_2 and CO_2 (Beaudry et al., 1992; Cameron et al., 1993; Lakakul et al., 1994) and water vapor (Debeaufort and Voilley, 1994) are well

studied. However, few studies of the permeability of LDPE film to aroma volatiles have been reported (Delassus et al., 1988; Fukamachi et al., 1994).

Loss of flavor can occur by sorption into or permeation through the packaging film to the environment. The permeation process is described by following equation when sorption is Fickian in nature:

$$P = D \times S$$

Where *P* is permeability coefficient which expresses the permeant transport rate as a function of film thickness, film area, temperature and driving force. *D* is the diffusion coefficient it describes how fast the permeant molecules move in the film. *S* is the solubility (or sorption) coefficient, which is a measure of the amount of a permeant that will be absorbed by polymer. The steady state permeability is useful quantity for describing the rate of mass transfer through a polymer film at steady state. Based on Fick's Law the steady state permeability can be described by following equation:

$$\frac{\Delta M_x}{\Delta t} = \frac{P \bullet A \bullet \Delta p_x}{l}$$

Were: $\frac{\Delta Mx}{\Delta t}$ is the total amount of permeant *x* (mol) which permeates through a polymer film with a cross-sectional area *A* (m^2) a thickness *L (m)*, and a partial pressure gradient of Δp_x (Pa) during time *t* (s). Present studies show that the structure of both permeant and polymeric packaging material, measurement conditions (such as relative humidity, temperature etc.), permeant concentration and co-permeants are the major factors affecting volatile permeation.

Aroma volatile synthesis by fresh produce during ripening (Song and Bangerth 1994; Seifabad, 1988) and following CA storage (Brackmann et al., 1993; Mattheis et al., 1991a; Song and Bangerth, 1994; Willaert et al., 1983) has been much studied. Since apple volatiles are produced in minute quantities, especially under conditions of apple storage such as low O_2, high CO_2, and low temperature, investigators typically remove the fruit from the storage environment and warm them to room temperature for aroma biosynthesis measurements. Thus, little is known regarding the dynamic changes of volatile production during storage under modified atmospheres. The purpose of these studies was to test whether a simple packaging system could be used for measuring aroma volatile production by plant organs under storage conditions. Our hypothesis was that the aroma volatiles would attain steady-state in the package (in much the same way that O_2 and CO_2 reach steady-state) such that the flux of volatiles from the package was essentially the same as the flux from the fruit.

MATERIALS AND METHODS

In order to measure the rate of flux of aroma compounds from package, packages were enclosed in ventilated glass chambers. The rate of flux of volatiles from the packages was compared to that from fruit in glass chambers with no packages. The behavior of volatiles in the packages in chambers was further compared to fruit held in packages without chambers.

Fruit storage: Apples (CV. Golden Delicious) were harvested from the Michigan State University Clarksville Horticultural Experiment Station on 2, October 1995. Fruit were held 2 days at 0°C prior to use. Two experiments were conducted: one at 0°C and a second at 22°C. At 0°C, fruit were given four treatments (each with 4 replications): 1) apples were placed in a 2 L glass container ventilated with air, 2) apples were placed in a glass container ventilated with a modified atmosphere, 3) apples were first placed in a low density polyethylene (LDPE) bag which was then placed in a ventilated glass container (Beaudry et al., 1992) (Fig. 1), and 4) fruit were placed in packages alone. The three target O_2 levels (4-5, 7-8, and 10-11 kPa) in the package headspace were achieved by sealing different weights (110-240 g) of apple fruit in LDPE packages with area of 0.02 m^2 and film thickness of 0.00508 cm (2 mil). At 22°C, fruit were given three treatments (with 5 replications for each treatment): 1) packaged, 2) packaged and placed in a glass container (package-in-a-jar), and 3) unpackaged and placed in a glass container. The packaged treatments involved sealing the fruit in a 2 mil (.00508 cm) thick LDPE package with an area of 0.06-0.07 m . At 0 and 20°C, glass containers were ventilated with a low air flow (6-7 $mL \cdot min^{-1}$). Packages without containers were placed a storage shelf exposed to ambient atmosphere. For package-in-a-jar and jar only treatments, air or a modified atmosphere flow was slowly passed through the glass containers such that the O_2 level in the package was similar to that in glass containers with no package. The storage duration for 0°C and 22°C was 90 and 35 days, respectively.

Preparation of standard: A standard of known concentration was generated for hexyl acetate, butyl acetate, 6-methyl-5-hepten-2-one, ethyl 2-methylbutyrate, hexanal, butanol, and 2-methylbutyl acetate by evaporating sub-microliter amounts of the liquid of each compounds into a specially-designed 4.3-L glass chamber, fitted with a Mininert (Supelco) valve (Fig. 2). The concentrations of volatiles were calculated based on their molecular weight, density, purity, and the volume of standard jars. During measurement, the temperature of standard jars was carefully controlled.

The permeabilities of LDPE film: using a specially built permeation cell (Fig. 3) tested The permeability of LDPE to single aroma compounds. The cell was composed of two hemispheric glass chambers, each fitted with inlet and outlet ports. The donor and receiver chambers were divided by the film to be studied. The LDPE film thickness was 0.00508 cm (2-mil) and the area was 7.55 cm^2. On the donor side, the volatile of interest was metered into a humidified air stream free of aroma volatiles. The volatile partial pressure on the donor side was maintained in the range of 1 to 9 Pa (approximately 10 to 90 ppm). On the receiver side, humidified air stream free of aroma volatiles was metered at 1.5-1.6 mL min^{-1}. The total pressure in both chambers of the cell was maintained near atmospheric pressure (approx. 10^5 Pa). Receiver- and donor-side volatile concentrations were measured more than 10 times for each of two permeation cells. The analysis method was the same as described previously for volatile analysis. The standard jar was maintained at the same temperature as the permeability cell. Permeability was measured at 0, 5, 10, 15, 20, 25, and 30 C

±1°C by changing the room temperature. In order to assure that the system was at steady-state, the measurement was made 8 to 12 hours after the desired temperature was obtained. The film samples were changed with each temperature change. The steady state permeability was calculated by following equation:

$$P = \frac{Q \bullet L}{A \bullet t \bullet \Delta p}$$

Were *Q* is the total amount of permeant which has passed through a polymer film with an area *A* during time *t*. *L* is thickness of the film, and *Δp* is partial pressure gradient of the permeant. The permeability is expressed in $mol \cdot m \cdot m^{-2} \cdot s^{-1} \cdot Pa^{-1}$.

Measurement of aroma production: The partial pressure gradient across the film of the package ($P_j^{in} - P_j^{out}$) was measured and, using Fick's Law, permeability of LDPE to ethylene and major apple aroma volatiles was determined. Using these permeability measurements, the rate of production of aroma volatiles was also calculated for apple fruit enclosed in a package, but lacking the jar. Controls were apple fruit enclosed in a jar only and in a package only. For atmospheres were examined air, 10% O_2, 7 to 8% O_2 and 4% O_2.

Aroma volatile sampling and analysis: A steel capillary column segment (0.28 mm id; 1.0 µm phase thickness, 5% diphenyl and 95% dimethyl polysiloxane) was installed in a gas tight syringe (1710 RN, 100 µL syringe) and used as a needle for volatile analysis. Volatiles were pulled into the syringe

barrel by pumping the syringe plunger 30 times while the needle was in the headspace of the sample. Volatiles were absorbed by the needle coating.

The sampling protocol was validated as previously described (Chapter III). Gas samples were directly injected into the GC (Carle AGE series 400, Hach, Loveland, Colorado). In order to desorb the volatiles absorbed by the needle, the needle was held in the GC injection port maintained at 220°C for 3 seconds prior to injection. The GC was equipped with a flame ionization detector, and a 3.3 m long, 3mm ID packed column (10% DEGS-PS, 80/100 mesh Supelcoport) was used for volatile analysis. The column was maintained isothermally at 140°C. Purified helium (99.9%) was used as a carrier gas. The flow rates for the helium, air, and hydrogen were 60 $mL \cdot min^{-1}$, 200 $mL \cdot min^{-1}$, and 20 $mL \cdot min^{-1}$, respectively.

RESULTS AND DISCUSSION

Permeability of LDPE film to using permeation cell. With the exception of butanol, the permeability of LDPE film to all the tested volatiles increased with temperatures (Fig. 4). The permeability ranking at 20°C was hexyl acetate > 6-methyl-5-hepten-2-one >butyl acetate >ethyl 2-methylbutyrate >hexanal >butanol. Interestingly, determination of the permeability using this technique does not require the use of a standard if the response of the detection system is linear with concentration. This is because the standard would be used to determine both the rate of flux as well as the gradient.

The increase in permeability from 0 to 30°C for hexanal,

ethyl 2-methylbutyrate, butyl acetate, hexyl acetate, 6-methyl-5-hepten-2-one, and butanol were 10.21, 8.36, 2.63, 2.59, 1.64, and 0.59, respectively. In this study, the LDPE film was tested above its glass transition temperature (Tg) of -100°C. Tg is the temperature of which the polymer goes from a crystalline state to an amorphous state. The fact that measurements were made well above Tg, accounts for the observed temperature sensitivity of permeability to the volatiles examined. The different properties of the tested volatiles (such as size, shape, and polarities) could directly or indirectly affect their solubility and diffusivity in LDPE resulting in different permeability values.

LDPE permeability under actual storage conditions. Using the packaged fruit in a ventilated glass container, the permeability of the packaging film to some volatiles including ethylene was calculated using the package headspace and container headspace partial pressures and the measured rate of flux (Figs. 5 and 6). The permeability to hexyl acetate, 2-methylbutyl acetate, and α-farnesene was, respectively, 288, 130, and 172 times higher than to ethylene. Since many volatile compounds are simultaneously migrating through the film during the actual MAP storage, there is a possibility that significant matrix effects can result. In some instances, the permeant can change driving force and the permeation properties of packaging film by the interaction between permeant and polymer (e.g. via swelling of the polymer). Thereby the permeant may increase or decrease the permeability of the package to other co-permeants. At 0°C, for instance, the permeability of the MAP film to hexyl acetate and butyl

acetate measured by package-in-a-jar system were lower than for the permeation cell declined while ethyl 2-methylbutyrate was increased (Fig. 7). The effect of co-permeants on the permeability of packaging film can also differ with temperature. The package permeability to ethyl 2-methylbutyrate increased 4.4- and 0.4-fold respectively when the permeability was measured with co-permeants at 0°C and 22°C relative to measurements made with no co-permeants. Under real storage conditions, the permeability of LDPE film to some volatile may never reach steady-state (Fig. 5) since the dynamic change in both the amount and the kinds of co-permeants and other unknown organic compounds in the package.

MAP storage of apple. The O_2 and CO_2 partial pressures reached steady-state in approximately 5 to 18 days following sealing of the fruit in the package (Figs. 8, 9 and 10). The peak ethylene production declined with decreased package O_2 and increased CO_2 concentration (Fig. 11). The ethylene production rate of apple fruit was also dependent on the storage temperature. Increased storage temperature caused an increase in the rate of respiration (data not shown) and ethylene production (Fig. 12). The maximum ethylene production rate was approximately 2.5-fold higher at 22°C than at 0°C. The rate of increase to achieve the maximum rate also occurred sooner for the fruit held at 22°C.

The effect of O_2 and CO_2 partial pressures on volatile production rates during MAP storage of apple. At 0 °C, package atmospheric composition affected the production of aroma compounds (Fig. 13). The production of hexyl

acetate appeared to be the most sensitive. The production rate of hexyl acetate for the package with 7-8 kPa O_2 and 3 kPa CO_2 was ½ that of the package with 10-11 kPa O_2 and 2-2.5 kPa CO_2. The production of butyl acetate and 2-methylbutyl acetate did not appear to differ between these packages. The production rate of hexyl acetate and butyl acetate for the package with 4-5 kPa O_2 and 3-4 kPa CO_2 was reduced. The results suggest that relative to packages with higher O_2 and lower CO_2 levels the production rate of straight-chain volatile compounds such as hexyl acetate and butyl acetate were more sensitive to reduced O_2 levels and elevated CO_2 levels than branch-chain volatile compounds such as 2-methylbutyl acetate. This differential sensitivity is consistent with known differences in the biosynthetic pathways for straight and branched-chain volatiles (Tressl and Drawert, 1973). The precursors of straight-chain alcohols for ester synthesis were thought to be derived from the oxidation of fatty acid through the ß-oxidation pathway (Yahia, 1994; Gerhardt, 1983). Branched-chain esters however, were thought to be formed by the transamination and subsequent oxidative decarboxylation of the amino acid (Yahia, 1994; Tressl and Drawert, 1973).

Compared with 0°C, room temperature storage caused a higher volatile accumulation in the package (compare Figs. 14 and 15 with Figs. 8 and 9). The data for α-farnesene content for packages stored at 0°C are lacking due to the low amount of material present in packages at that temperature, however, -farnesene accumulated to significant amounts at 22°C (Fig. 16). The mean

production rate of 2-methylbutyl acetate and hexyl acetate were 6.49 X 10^{-8} and 5.45 X 10^{-8} mol·kg^{-1}·h^{-1}, respectively, when apples were stored at 22°C. However, under the same atmospheric composition (10 kPa O_2 and 2 kPa CO_2), the mean of production rate of these compounds was 3.58 X 10^{-9} and 4.44 X 10^{-9} mol·kg^{-1}· h^{-1}, respectively when storage temperature was 0°C. The difference in production with temperature reflects an increase in the production rate of hexyl acetate and 2-methylbutyl acetate of 11- and 17-fold, respectively, as storage temperature was increased 22°C.

Effect of package film on volatile profile. When apple fruit were sealed in a glass container, the production of volatiles from the container was higher than for apples in packages in containers (Fig. 17). The flux of α-farnesene, hexyl acetate and 2-methylbutyl acetate was 26.6-, 1.7-, and 1.4-fold higher, respectively, for fruit in glass container than for those in package in a container. The sorption of α-farnesene and the other volatiles into LDPE film is evidently considerable such that a significant portion of the volatiles are not released even after several weeks. The result is that package presence alters the aroma profile of packaged produce relative to the more inert jar only system. Understanding the dynamic relationship between the composition of the package atmosphere, the volatile synthesis, and sorption or permeation of packaging film is important for quality improvement of packaged produce. Since packaging film can selectively absorb certain flavor constituents from food, changing the film type would likely alter the volatile profile. The relationship between volatile synthesis,

package atmosphere, and permeation or sorption of packaging film is dynamic, complex and of potential importance in perceived quality of packaged fruit.

Using a package-in-a-jar system we are able to simultaneously measure the volatile concentration and the permeability of the packaging film to those volatiles. The profile changes of CO_2, O_2, and volatile concentration, volatile production rate and permeability of LDPE film during storage suggest a permeation-based respirometry system was not suitable for the calculation of the flux of organic molecules. However, it may be a useful methodology for evaluating the effects of package atmosphere and other treatments for packaged produce.

LITERATURE CITED:

Beaudry, R. M., A. C. Cameron, A. Shirazi and D. Dostal Lange. 1992. Modified-atmosphere packaging of blueberry fruit: Effect of temperature on packaging oxygen and carbon dioxide. J. Amer. Soc. Hortic. Sci. 117

Brackmann, A., J. Streif and F. Bangerth. 1993. Relationship between reduced aroma production and lipid metabolism of apples after long-term controlled-atmosphere storage. J. Amer. Soc. Hort. Sci. 118 (2): 243-247.

Cameron, A. C., B. D. Patterson, P. C. Talasila, and D. W. Joles. 1993. Modeling the risk in modified-atmosphere packaging: A case for sense-and-respond packaging. Proceeding from the Sixth Intl. CA. Conf., Ithaca, NY., June 15-17. ARAES-71(1): 95-102.

Debeaufort, F. and A. Voilley. 1994. Aroma compounds and water vapor packaging. J. Ag. Food Chem. 42: 2871-2875.

Delassus P. T. 1986. Transport of unusual molecules in polymer films. Tappi Journal. 69 (12):

DeLassus P. T., J. C. Tou, M. A. Babinec, D. C. Rulf, B. K. Karp, and B. A. Howell. 1988. Transport of apple aromas in polymer films. In Food and Packaging Interactions J. H. Hotchkiss, (ed.) ACS Symposium Series No. 365.

Foster, R. H. 1978. Ethylene vinyl alcohol copolymer resins for better solvent, aroma and flavor barrier. Future-Pak 87. 5th International Conference on Packaging Innovations. Atlanta, GA Ryder Associates. Inc.

Fukamachi, M., T. Matsui, M. Shimoda, and Y. Osajima. 1994. Derivation of thermodynamic sorption equation of flavors with packaging films. 2. J. Ag. Food Chem. 1994 42: 2893-2895.

Gerhardt, B. 1983. Localization of ß-oxidation enzymes in peroxisomes isolated from non fatty plant tissues. Planta 159: 238-246.

Imai, T., B. R. Harte, and J. R. Giacin. 1990. Partition distribution of aroma volatiles from orange Juice into selected polymeric sealant films. J. Food Sci. 55: 158-161.

Lidster, P. D., H. J. Lightfoot, and K. B. Mcrae. 1983. Production and regeneration of principal vocatives in apple stored in modified atmospheres and air. J. Food Sci. 48: 400-410.

Marshall, M. R., J. P. Adams, and J. W. Williams. 1985. Flavor absorption by aseptic packaging materials. Aseptikpak 85. 3rd International Conference and Exhibition on Aseptic Packaging, Princeton, NJ.

Mason R. C., P. T. Delassus, G. Strandburg, and B. A. Howell. 1992. Diffusion of flavors in polymers: effect of permeant size and shape. Tappi Journal 75 (6): 163-168.

Matsui, T., M. Fukamachi, M. Shimoda, and Y. Osajima. 1994. Derivation of thermodynamic sorption equation of flavors with packaging films. 1. J. Agri. Food Chem. 42(12): 2889-2892.

Mattheis, J. P., D. A. Buchanan, and J. K. Fellman. 1991a. Change in apple fruit vocatives after storage in atmospheres inducing anaerobic metabolism. J. Agri. Food Chem. 39: 1602-1605.

McNally, M. E. and R. L. Grob. 1985. Current application of static and dynamic headspace analysis. A review. Part II: non environmental applications. J. Amer. Lab. Feb: 106.

Nielsen, T. G. and J. R. Giacin. 1994. The sorption of limonene/ethyl acetate binary vapor mixture by a biaxially oriented polypropylene film. Packaging Technol. and Sci. 7: 247-258.

Osajima, Y. and T. Matsui. 1993. Dynamic approach to the sorption of flavors into a food packaging film. Anal. Sci. 9: 753-763.

Patterson and K. Hartfield. 1974. Residual effect of controlled atmosphere storage on the production of volatile compounds by two varieties of apple. J. Sci. Food Agric. 25: 843-849.

Seifabad, T. C. 1988. Effect of harvest date and ethylene concentration in controlled atmosphere storage on the quality of Empire apple. Ph. D. thesis Department of Food Science, Michigan State University.

Song Jun and F. Bangerth. 1994. Production and development of volatile aroma compounds of apple fruits at different times of maturity. Acta Horticulturae. 357.

Tressl, R., and F. Drawert. 1973. Biogenesis of banana volatiles. J. Agr. Food Chem. 21: 560-565.

Willaert, Dirinck, de Pooter, and Schamp. 1983. Objective measurement of aroma quality of golden delicious apple as a function of controlled-atmosphere storage time. J. Agric. Food Chem. 31: 809-813.

Yahia, E. M. 1994. Apple flavor. Hort. Rev. 197-234.

Yahia, E. M. 1991a. Production of some odor-active vocatives by McIntosh apples following low-ethylene controlled atmosphere storage. HortScience 26: 1183-1185.

Yahia, E. M. 1991b. Changes in some odor-active vocatives in apple during storage in air and in simulated low-ethylene controlled atmosphere. Turrialba 42(2): 160-165.

Yahia E. M., F. W. Liu and T. E. Acree. 1991. Changes of some odour- active vocatives in low-ethylene controlled atmosphere stored apples. Lebensmittel-Wissenschaft und technologie. 24: 145-151.

Figure 1. Package-in-a-jar system. Apples (110-240 g) were sealed in a 2-mil (0.00508 cm) thick LDPE package with an area of 0.06-0.07 m^2. Packages were placed in a 2-L glass jar ventilated at 6-7 mL/min with air. The package and jar headspaces were sampled for aroma volatile analysis. The permeability of packaging film was calculated by the gradient of aroma volatiles across the film, the rate of production by product was simultaneously calculated based on the flux of volatile compounds.

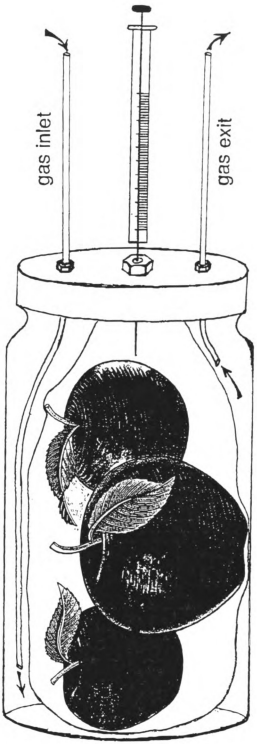

gas inlet
gas exit

Figure 2. The standard jar was a specially-designed, 4-L Erlenmeyer flask having a ground-glass stopper fitted with a Mininert valve. Volatile concentrations were generated by evaporating sub-microliter amounts of the liquid of each of the volatile compounds of interest.

Figure 3. Glass permeation cell used for measurement of steady-state permeability of plastic film to single volatile permeants.

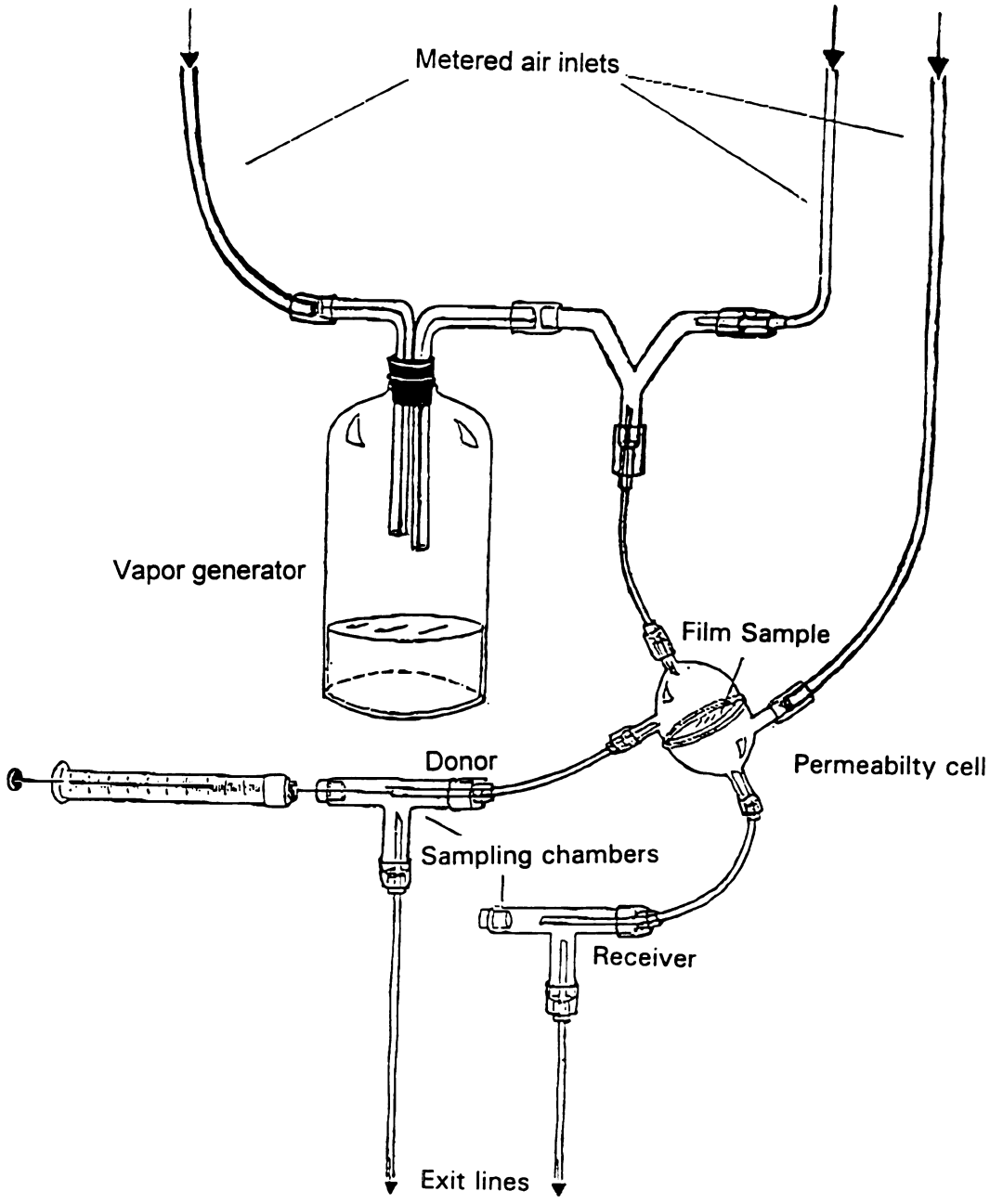

Metered air inlets
Vapor generator
Film Sample
Donor
Permeabilty cell
Sampling chambers
Receiver
Exit lines

Figure 4. Effect of temperature on the permeability of LDPE film to Hexyl acetate, 6-methyl-5-hepten-2-one, butyl acetate, Ethyl 2-methylbutyrate, hexanal, and butanol. Test film area was 7.55 cm^2 and thickness was 0.00508 cm (2-mil). Four replicates were performed and 10 measurements were made for each permeation cell.

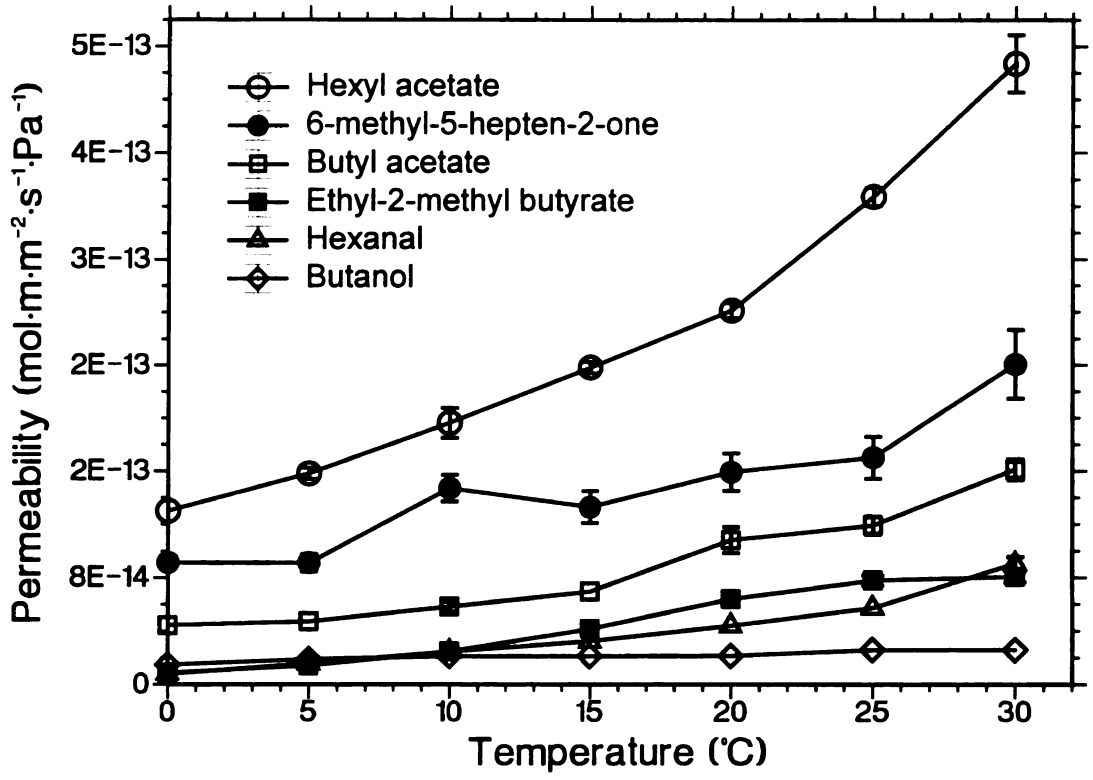

Permeability (mol·m·m⁻²·s⁻¹·Pa⁻¹)
5E-13
4E-13
3E-13
2E-13
2E-13
8E-14
0
0
5
10
15
20
25
30
Temperature (°C)
Hexyl acetate
6-methyl-5-hepten-2-one
Butyl acetate
Ethyl-2-methyl butyrate
Hexanal
Butanol

Figure 5. Effect of storage duration on the 'apparent' permeability of LDPE film to volatiles during MAP storage of apple at 22°C as measured by the package-in-a-jar system. Each symbol represents 5 replications ± SE

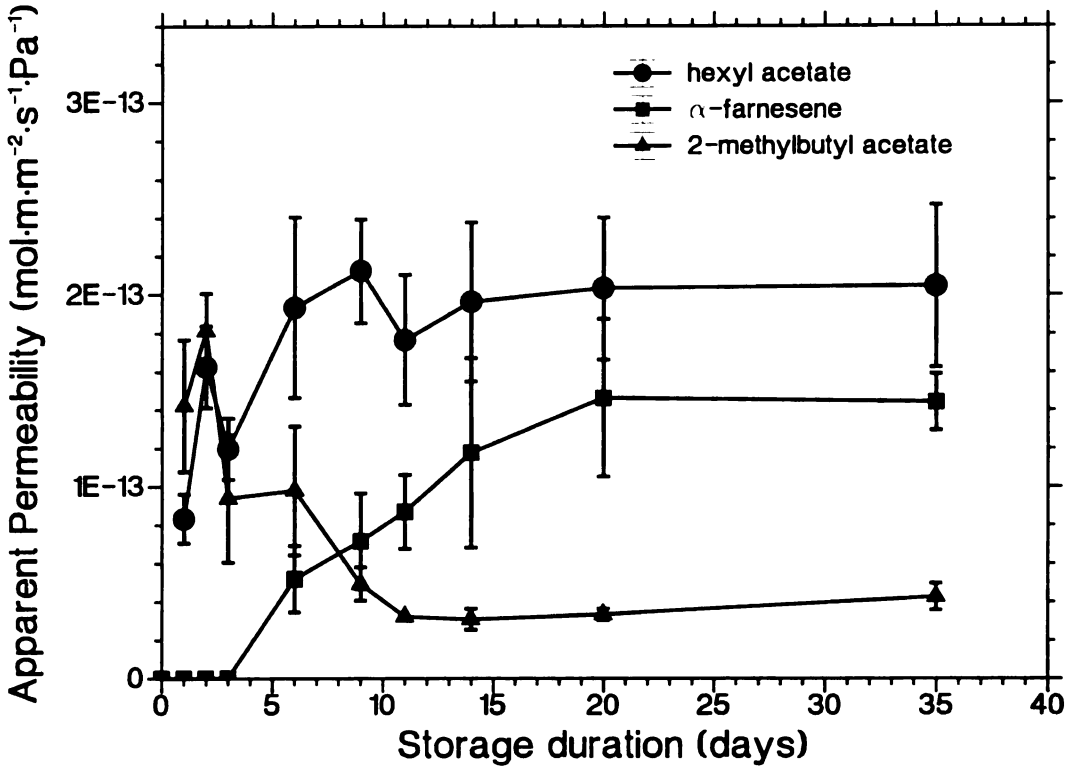

hexyl acetate
α-farnesene
2-methylbutyl acetate
Apparent Permeability (mol·m·m⁻²·s⁻¹·Pa⁻¹)
3E-13
2E-13
1E-13
0
0
5
10
15
20
25
30
35
40
Storage duration (days)

Figure 6. Permeability of LDPE to ethylene during MAP storage at 22°C as measured by the package-in-a-jar system. Each symbol represents 5 replications ± SE

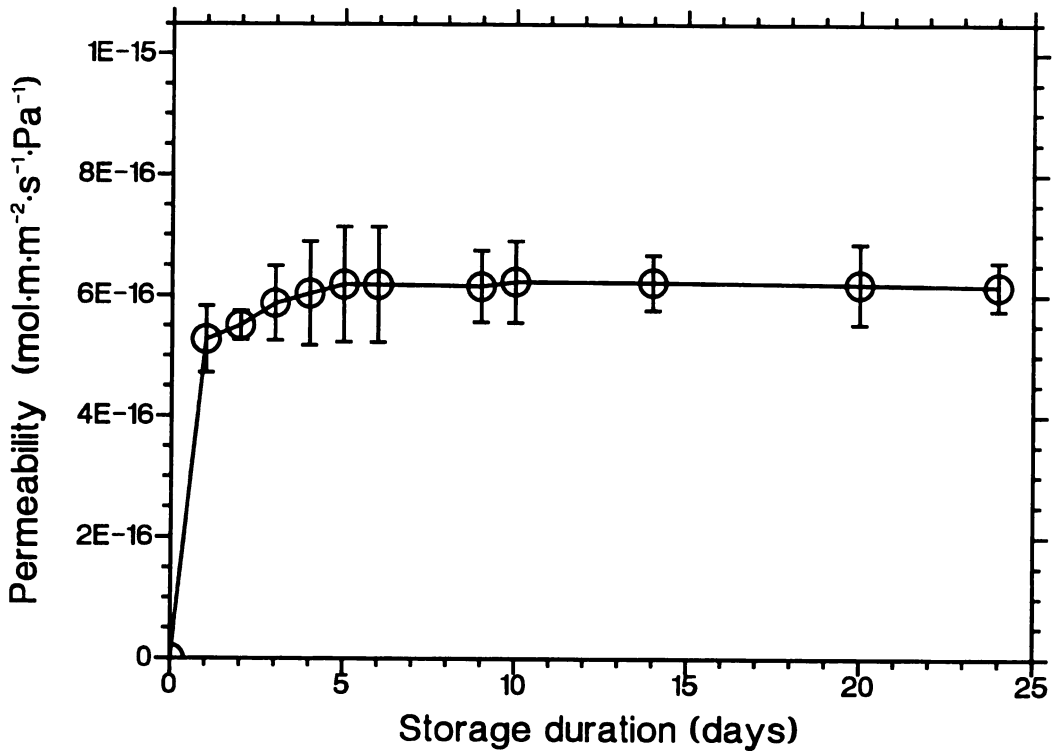
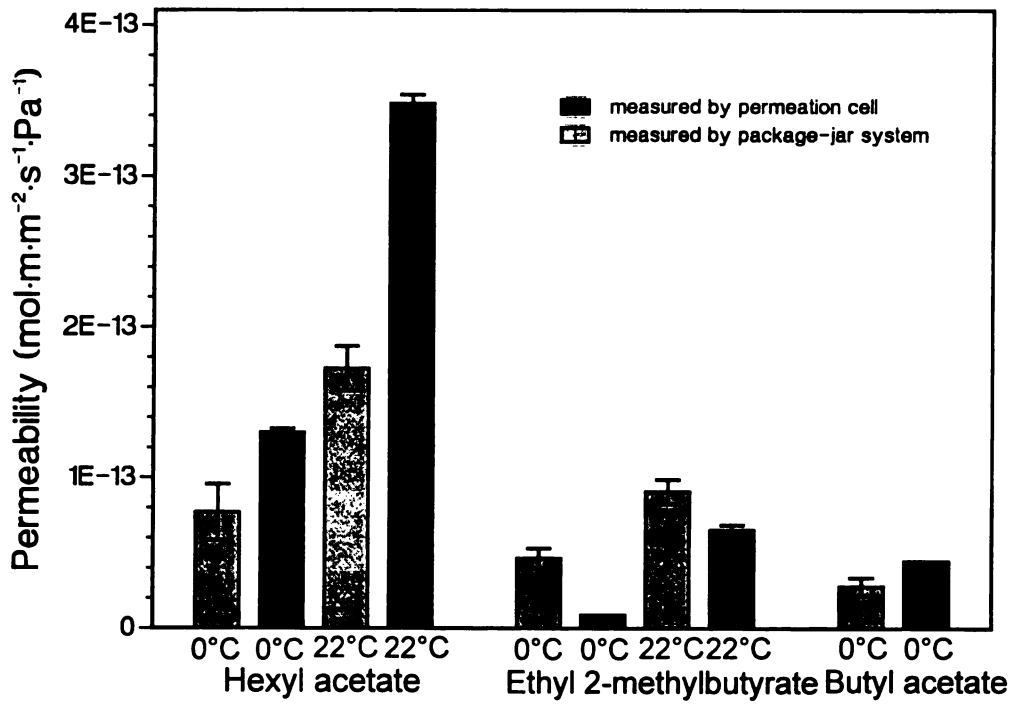

Permeability (mol·m·m⁻²·s⁻¹·Pa⁻¹)
1E-15
8E-16
6E-16
4E-16
2E-16
0
0
5
10
15
20
25
Storage duration (days)

Figure 7. Permeability of LDPE to selected volatiles as measured by the permeation cell and package-in-a-jar systems.


4E-13
3E-13
2E-13
1E-13
0
Permeability (mol·m·m⁻²·s⁻¹·Pa⁻¹)
measured by permeation cell
measured by package-jar system
0°C 0°C 22°C 22°C
Hexyl acetate
0°C 0°C 22°C 22°C
Ethyl 2-methylbutyrate
0°C 0°C
Butyl acetate

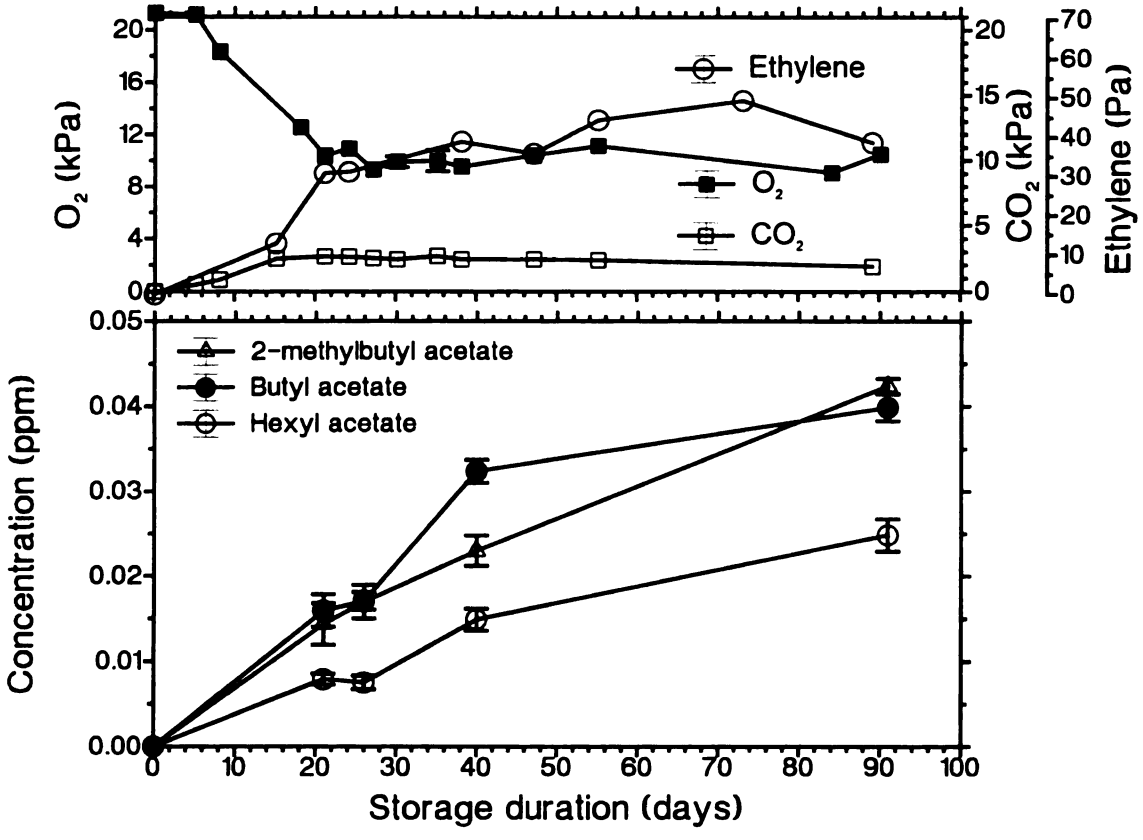

Ethylene
O2
CO2
O2 (kPa)
CO2 (kPa)
Ethylene (Pa)
2-methylbutyl acetate
Butyl acetate
Hexyl acetate
Concentration (ppm)
Storage duration (days)

Figure 8. Effect of storage duration on O_2, CO_2, ethylene and volatile partial pressures in packages that maintained approximately 10 kPa O_2 during MAP storage of apple at 0°C. Each symbol represents 5 replications ± SE.

Figure 9. Effect of storage duration on O_2, CO_2, ethylene and volatile partial pressures in packages that maintained approximately 8 kPa O_2 during MAP storage of apple at 0°C. Each symbol represents 5 replications ± SE

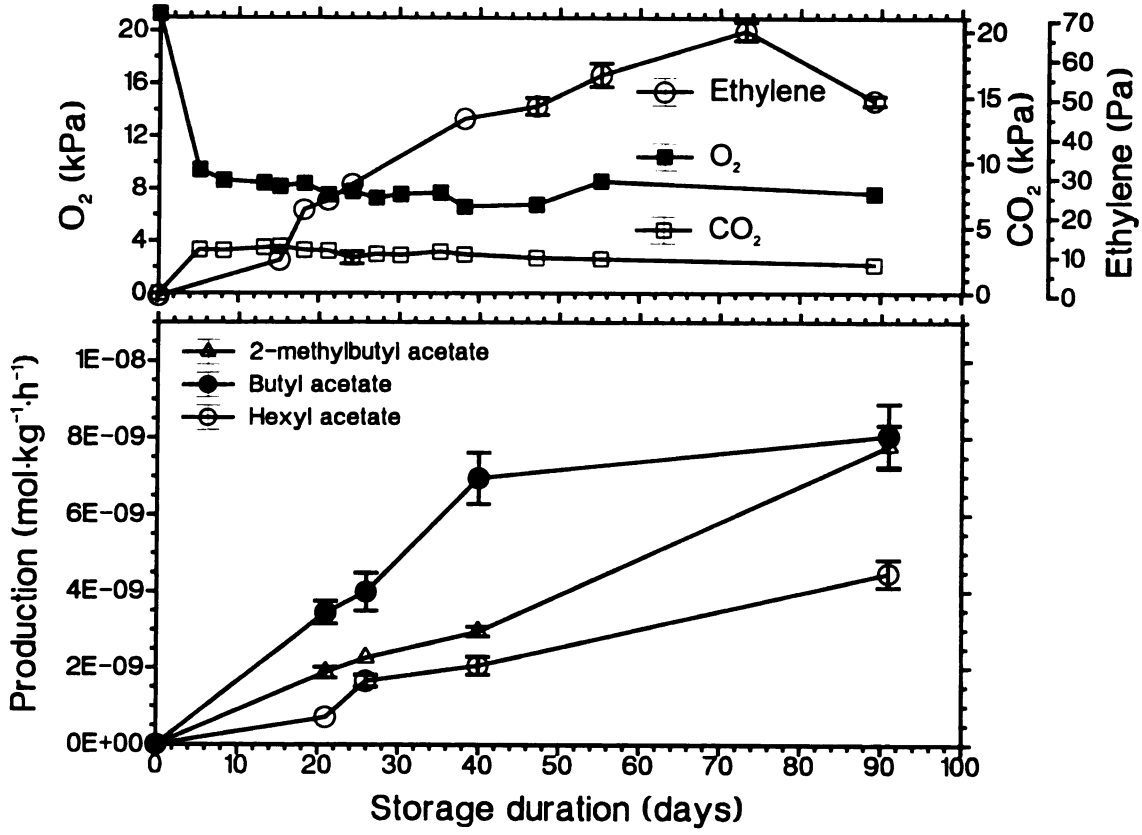

O2 (kPa)
CO2 (kPa)
Ethylene (Pa)
Ethylene
O2
CO2
2-methylbutyl acetate
Butyl acetate
Hexyl acetate
Production (mol·kg-1·h-1)
Storage duration (days)

Figure 10. Effect of storage duration on O_2, CO_2, ethylene and volatile partial pressures in packages that maintained approximately 4 kPa O_2 during MAP storage of apple at 0°C. Each symbol represents 5 replications ± SE.

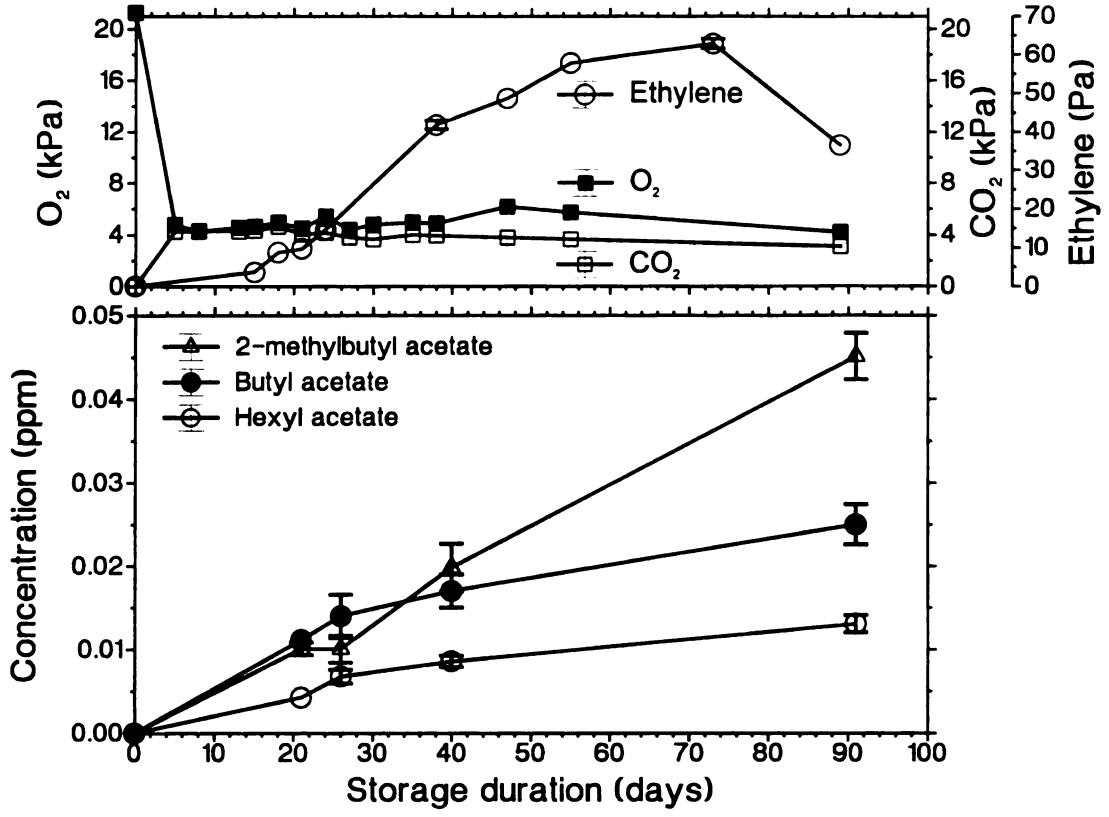

O2 (kPa)
CO2 (kPa)
Ethylene (Pa)
Ethylene
O2
CO2
Concentration (ppm)
2-methylbutyl acetate
Butyl acetate
Hexyl acetate
Storage duration (days)

Figure 11. The effect of O_2 partial pressure and storage duration on ethylene production rate during MAP storage of apple fruit at 0°C. Each symbol represents 5 replications ± SE

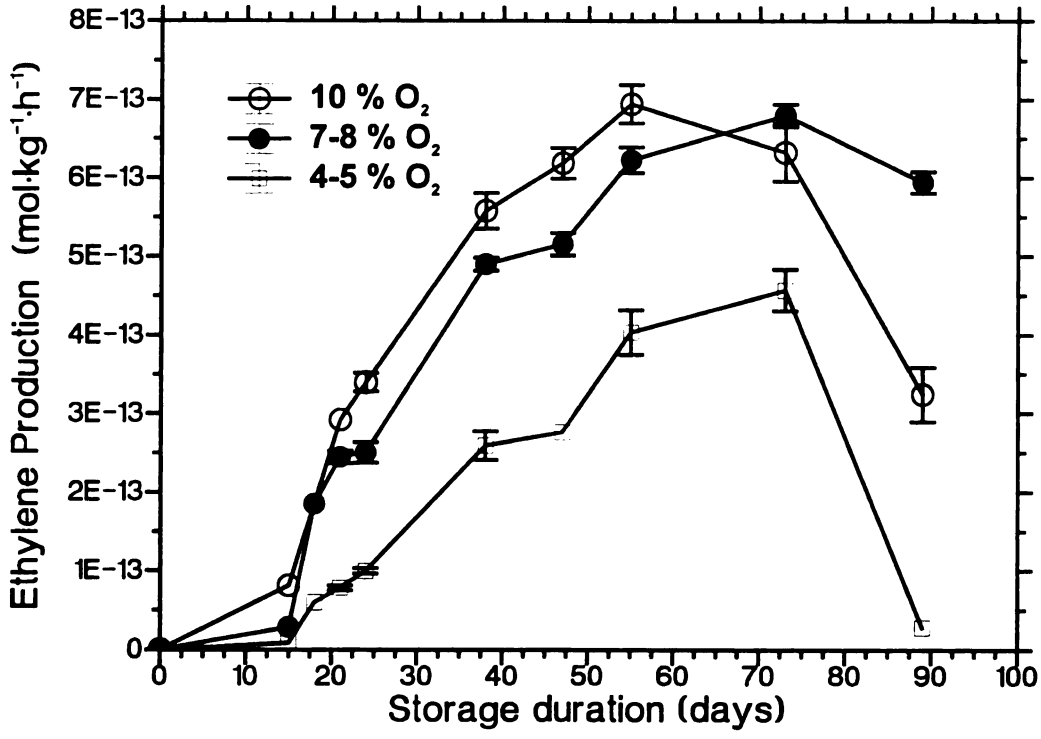

8E-13
7E-13
6E-13
5E-13
4E-13
3E-13
2E-13
1E-13
0
Ethylene Production (mol·kg⁻¹·h⁻¹)
10 % O2
7-8 % O2
4-5 % O2
0
10
20
30
40
50
60
70
80
90
100
Storage duration (days)

Figure 12. Effect of storage temperature and storage duration on ethylene production rate of apple fruit during MAP storage (10% O_2). Each symbol represents 5 replications ± SE

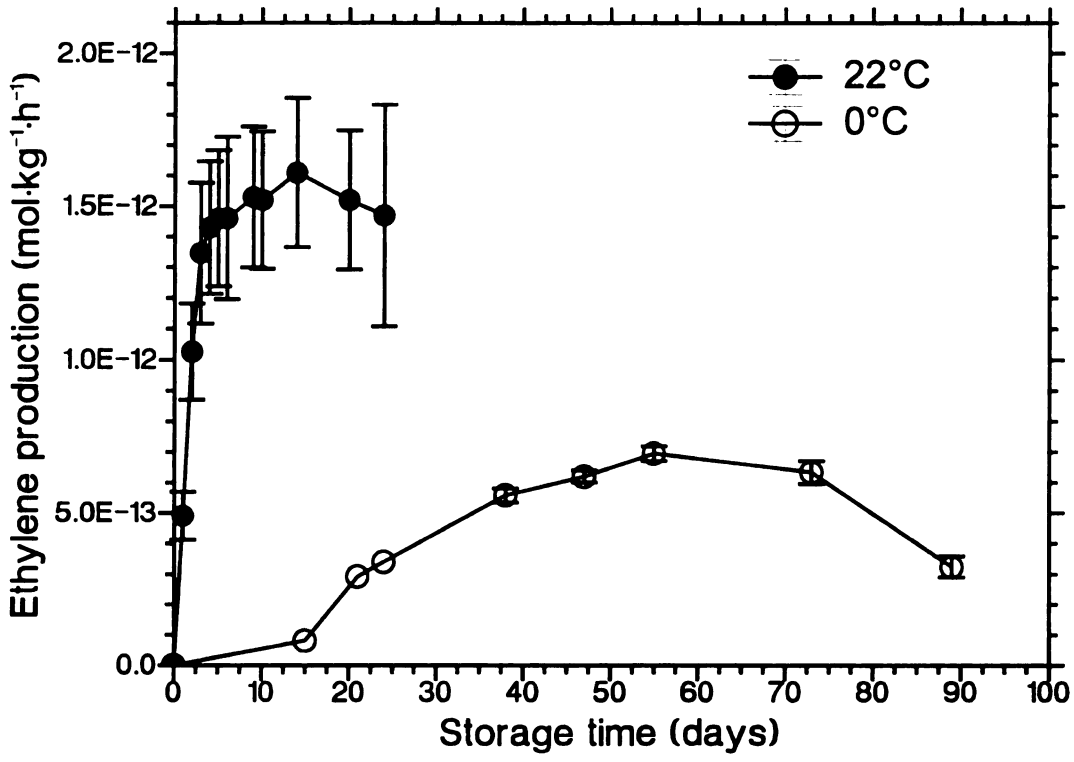

2.0E-12
1.5E-12
1.0E-12
5.0E-13
0.0
Ethylene production (mol·kg⁻¹·h⁻¹)
0
10
20
30
40
50
60
70
80
90
100
Storage time (days)
22°C
0°C

Figure 13. Changes in hexyl acetate, butyl acetate, and 2-methylbutyl acetate production rate during MAP storage of apple under different package atmosphere and storage duration. Storage temperature was 0°C. Each symbol represents 5 replications ± SE

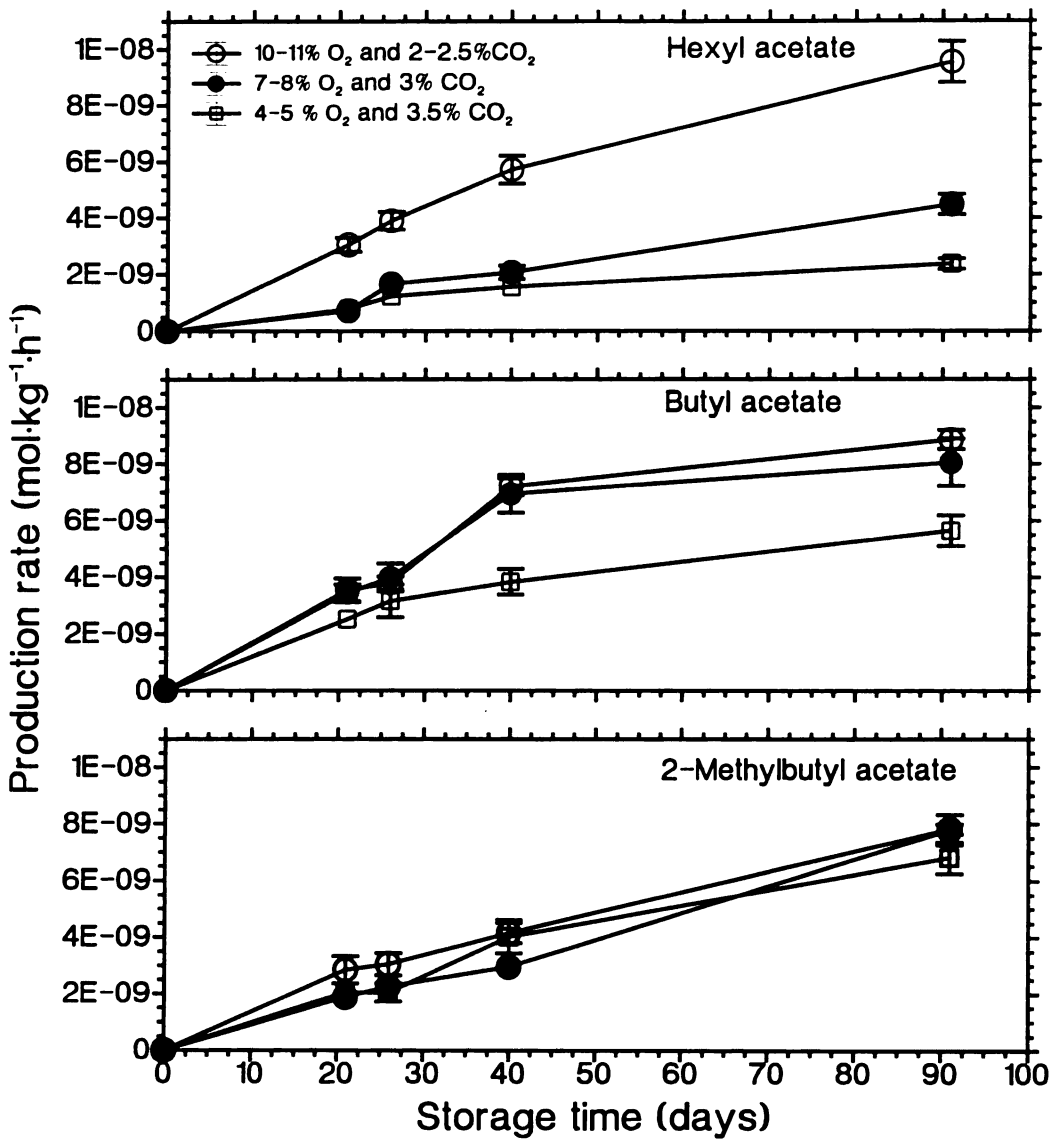

10-11% O2 and 2-2.5%CO2
7-8% O2 and 3% CO2
4-5 % O2 and 3.5% CO2
Hexyl acetate
Butyl acetate
2-Methylbutyl acetate
Production rate (mol·kg-1·h-1)
1E-08
8E-09
6E-09
4E-09
2E-09
0
0
10
20
30
40
50
60
70
80
90
100
Storage time (days)

Figure 14. Concentration of 2-methylbutyl acetate in the headspace of packages containing apple fruit, which were further enclosed in jars, in the headspace of those same jars and in the headspace of jars containing apple fruit not enclosed in packages. The storage temperature was 22°C. Each symbol represents 5 replications ± SE

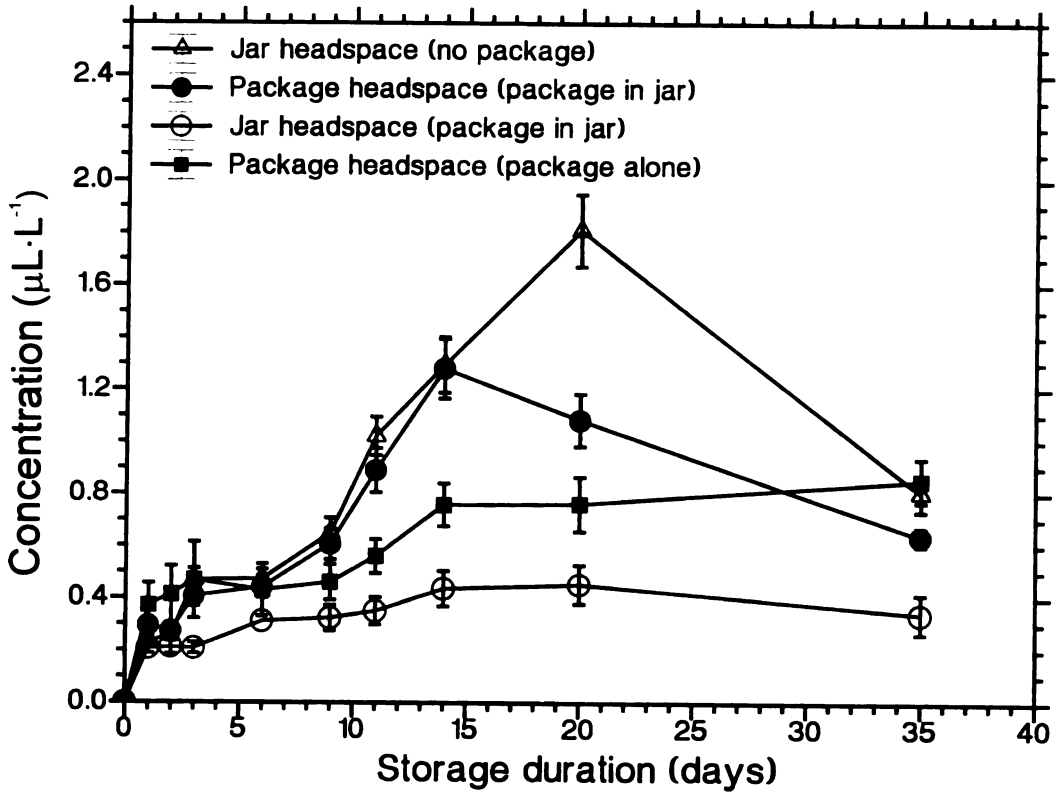

Jar headspace (no package)
Package headspace (package in jar)
Jar headspace (package in jar)
Package headspace (package alone)
Concentration (μL·L⁻¹)
Storage duration (days)
0.0
0.4
0.8
1.2
1.6
2.0
2.4
0
5
10
15
20
25
30
35
40

Figure 15. Concentration of hexyl acetate in the headspace of packages containing apple fruit, which were further enclosed in jars, in the headspace of those same jars and in the headspace of jars containing apple fruit not enclosed in packages. The storage temperature was 22°C. Each symbol represents 5 replications ± SE

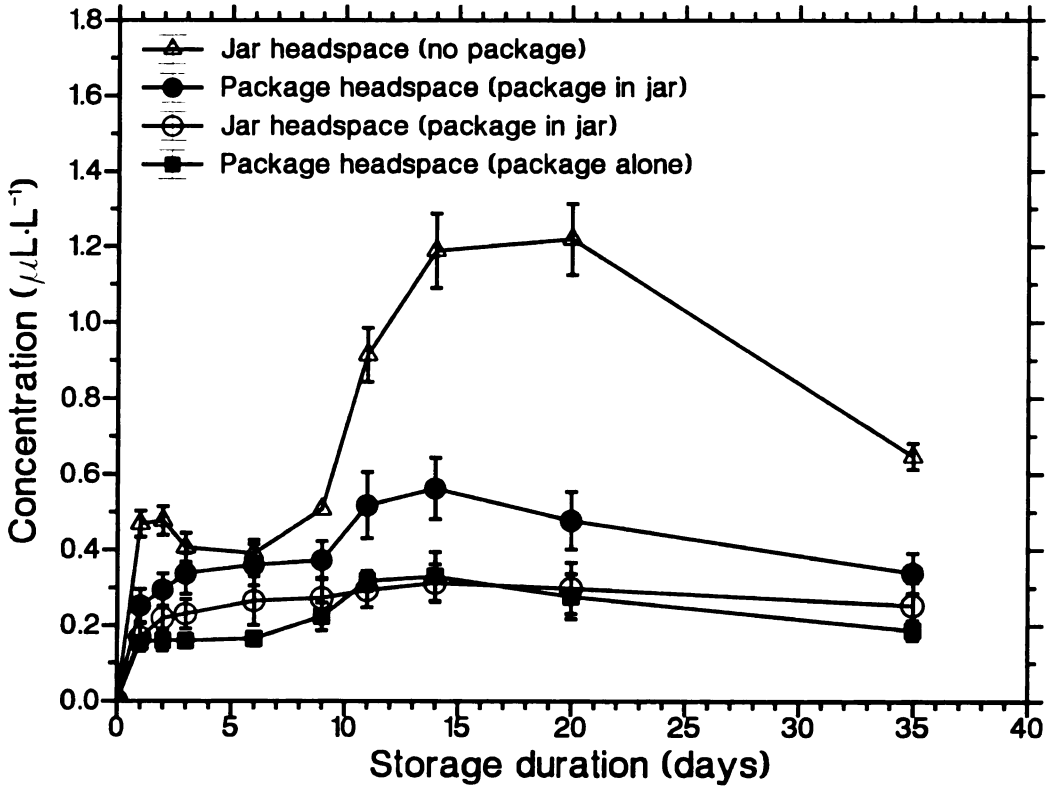

Jar headspace (no package)
Package headspace (package in jar)
Jar headspace (package in jar)
Package headspace (package alone)
Concentration (μL·L⁻¹)
Storage duration (days)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
0
5
10
15
20
25
30
35
40

Figure 16. Concentration of α-farnesene in the headspace of packages containing apple fruit, which were further enclosed in jars, in the headspace of those same jars and in the headspace of jars containing apple fruit not enclosed in packages. The storage temperature was 22°C. Each symbol represents 5 replications ± SE

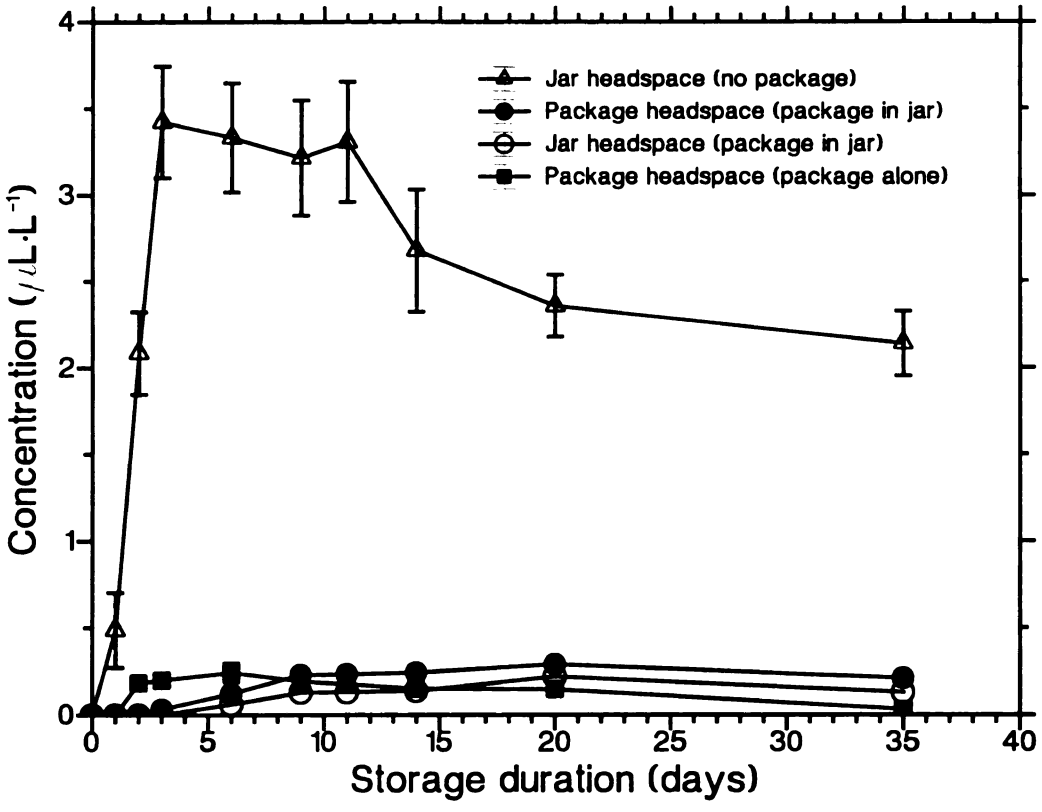
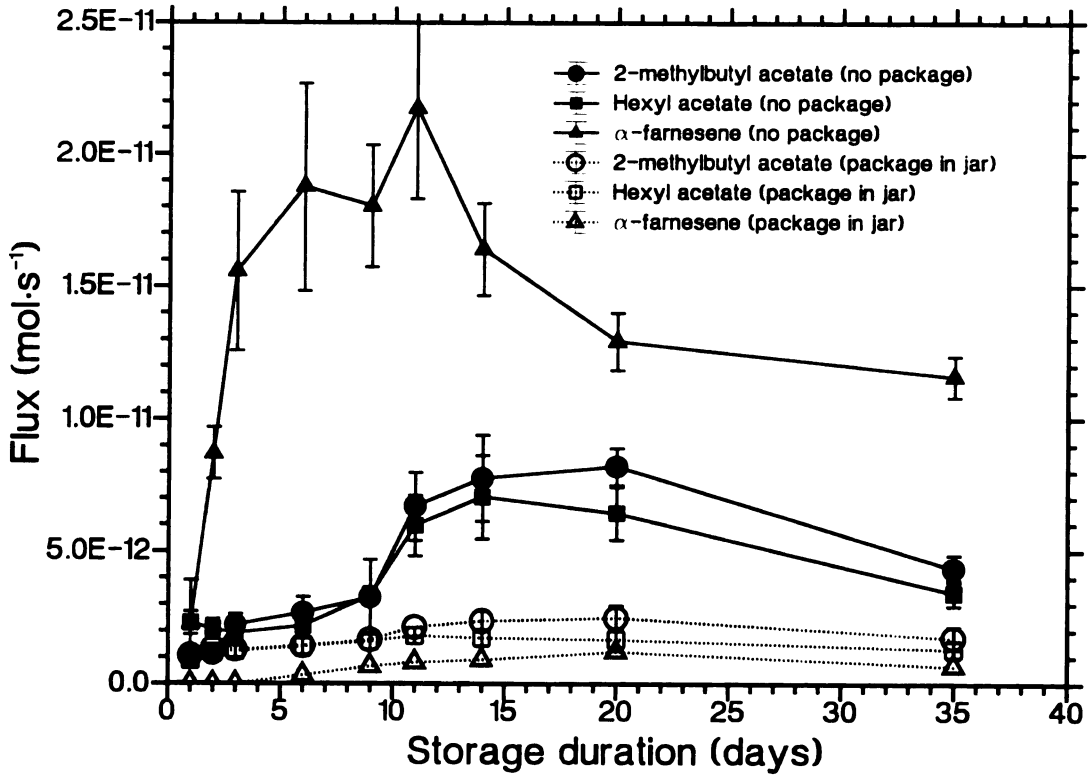

Jar headspace (no package)
Package headspace (package in jar)
Jar headspace (package in jar)
Package headspace (package alone)
Concentration (μL·L⁻¹)
Storage duration (days)
0
1
2
3
4
0
5
10
15
20
25
30
35
40

Figure 17. The flux of volatiles released from the package-in-a-jar and jar only system during MAP storage of apple. The O_2 level in the package was similar to that in glass jars with no package. Storage temperature was 22°C. Each symbol represents 5 replications ± SE.


2.5E-11
2.0E-11
1.5E-11
1.0E-11
5.0E-12
0.0
Flux (mol·s⁻¹)
0
5
10
15
20
25
30
35
40
Storage duration (days)
2-methylbutyl acetate (no package)
Hexyl acetate (no package)
α-farnesene (no package)
2-methylbutyl acetate (package in jar)
Hexyl acetate (package in jar)
α-farnesene (package in jar)

CHAPTER V:

SUMMARY AND CONCLUSIONS

The first purpose of these studies was to develop a simple and fast volatile sampling (collection and concentration) method for the analysis of major apple volatiles. The new volatile collection methodology and a specially designed package-in-a-jar system were used to simultaneously measure volatile production, concentration, and the permeability of package to those volatiles. The effects of packaging film to volatile profile during storage were also investigated.

Capillary GC column segments were installed in gas tight syringes and used as needles for volatile analysis. Using capillary column needles resulted in an increase in GC response in the range of 2- to 15-fold relative to a standard stainless steel needle having no internal coating. The number of times the syringe plunger was pumped (pump number) was the factor that most influenced GC response. To achieve response in certain range, the saturation for a particular needle/volatile combination, a pump number of 40 was required. Large non-polar molecules such as hexyl acetate required higher pump number to reach equilibrium than smaller, more polar molecules like butanol. Like syringe pumping number, injection volume and needle length affected GC response in a needle and volatile dependent manner. As injection volume was increased from 10 to 80μL, GC response to butanol and ethyl-2-methylbutyrate increased linearly. GC response to hexyl acetate experienced little increase in response to increasing injection volume in the range of 10 to 80μL. The results indicated that the minimal volume of 50 μL of sample or other gas (such as N_2 or air) was needed for pushing the adsorbed and/or absorbed volatile from needle

to the column when a capillary needle was used. When the length of capillary glass, and capillary steel needles was increased from 2.5 to 6.5 cm, GC response increased 225% and 214%, respectively. When the length of capillary glass, and capillary steel needles was increased from 2.5 to 6.5 cm, GC response increased 225% and 214%, respectively. The GC response for capillary column needles decreased when the sampling temperature was increased.

Since capillary GC column needle will work with both flow through and low volume sealed system such as modified atmosphere packaging (MAP), it permits volatile production to be measured for products in the package under its storage environment. Therefore, capillary GC column needle may be a useful tool for determining the dynamic relationship between flavor volatile synthesis and package atmosphere for packaged produce. In addition, by changing the column needle coating, one may create needles that are more specifically matched to the volatiles of interest, leading to enhanced analytical sensitivity.

The effect of polymers used in packaging on the aroma of the packaged product has been little explored. Using a package-in-a-jar system we are able to simultaneously measure volatile production by plant organ (*Malus domestica* Borkh. cv. Golden Delicious) and the permeability of the packaging film to those volatiles. In this system, apple fruit were placed into a glass container or sealed in a low density polyethylene (LDPE) package and subsequently placed into a glass container or were placed into a package alone. Air or a modified atmosphere was slowly passed through the glass containers such that the O_2

level was similar to that in the packages in containers. The package and jar headspaces were sampled using direct gas sampling and capillary column needles. Odor-active volatiles were analyzed by gas chromatography. The effect of temperature, atmosphere and film presence to some major volatile compounds was determined. The package permeability to hexyl acetate, 2-methylbutyl acetate, and -farnesene was, respectively, 287-, 130-, and 172- fold higher than those to ethylene.

The O_2, CO_2, and ethylene profile changes during MAP storage were easily measured in a package-in-jar system with capillary needles. When atmospheric composition changes from 10% O_2 + 2% CO_2 to 5% O_2 + 5% CO_2, ethylene production was reduced significantly. However, when O_2 concentration decrease from 10% to 8% and CO_2 concentration increase from 3% to 5%, the rise in ethylene production rate was delayed slightly.

The package-in-jar system can be used to estimate production rate under actual storage environment. At 0°C, as O_2 decreased in concentration from 10% to 5% (v/v), hexyl acetate and butyl acetate production declined significantly; 2-methylbutyl acetate production however, was not affected by this range of atmospheric composition. This can be taken to indicate the production of 2-methyl butanol (a branched alcohol derived from amino acids) for 2-methylbutyl acetate formation is not as O_2 concentration dependent as straight-chain alcohol.

Compared with 0°C, there was a higher volatile concentration and production rate for fruit held at room temperature. Under the same atmospheric composition, when storage temperature increased from 0°C to 22°C the

production rate of hexyl acetate and 2-methylbutyl acetate increased 11.27- and 17.15-fold, respectively.

At the same O_2 partial pressure, non-packaged fruit evolved greater amounts of all volatiles than packaged fruit. The flux of -farnesene, hexyl acetate and 2-methylbutyl acetate was 26.6-, 1.7-, and 1.4-fold higher, respectively, for fruit in a glass container. The reduced production found for packaged apples was probably due to loss of volatiles to the packaging material. The sorption of -farnesene and some other volatiles into LDPE film apparently alters the aroma profile of packaged produce relative to a flow-through system.

The package-in-a-jar measurement system and capillary GC column needle may be a useful methodology for determining the dynamic relationship between flavor volatile synthesis and package atmosphere for packaged produce.