STABILITY OF ASEPTICALLY PACKAGED FOOD AS A FUNCTION OF OXIDATION INITIATED BY A POLYMER CONTACT SURFACE

by

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(ABSTRACT)

In this study, low density polyethylene (LDPE) and polyethylene terephalate (PETE) resin beads were ground to a coarse powder and exposed to sterilization treatments applied to the food contact surface of packaging materials used in aseptically processed and packaged food. Electron paramagnetic resonance (EPR) analyzed free radical ($-CH_2CHCH=CHCH_2-$) production on the surface of LDPE exposed to heat (107°C) and treatments of heat (107°C) + 30% hydrogen peroxide solution (H_2O_2). As the temperature was raised from 100° to 200°C, peak intensity of carbon radicals produced gradually increased. The sensitivity of EPR prevented detection of free radicals on LDPE, exposed to H_2O_2 treatment, due to residual peroxide and H_2O condensation on the surface of LDPE.

D-limonene was placed in 12ml sealed glass vials containing a sodium citrate buffer solution (pH=3.7), under atmospheric O_2 (21%) conditions. Oxidation of d-limonene, placed in intimate contact with untreated, H_2O_2 treated, and ultraviolet (UV) light (650mW/cm^2) treated LDPE for 15 weeks, was measured to

determine the capacity of an oxidized polymer to initiate autoxidation. The oxidation of d-limonene in vials containing no polymer was also measured. Production of carvone and carveol were used as an index for oxidation. No polymer and UV treated samples showed significantly ($P<0.05$) higher levels of carvone and carveol than samples containing untreated and H_2O_2 treated LDPE. Samples containing no polymer oxidized d-limonene at the highest rate, but not significantly faster than solutions containing UV treated LDPE. Accumulation of carvone and carveol was zero order.

Linoleic acid, in a model food solution, was placed in intimate contact with untreated, H_2O_2 treated, and UV treated LDPE and PETE to determine the oxidation initiating effect of an oxidized food contact polymer. A model solution which contained linoleic acid with no polymer was also utilized. The accumulation of hexanal, evacuated from the headspace of glass test cells containing model food solution, was used as the index of oxidation. Test conditions of 5% and 21% O_2 were used to determine the effect of O_2 concentration. UV treatment of PETE showed the most significant ($P<0.05$) accumulation of hexanal over time. Untreated LDPE resulted in significantly ($P<0.05$) smaller accumulations of hexanal. Linoleic acid solution containing no polymer were shown to oxidize at a rate comparable to untreated and H_2O_2 treated PETE, and H_2O_2 and UV treated LDPE. Samples containing treated and untreated PETE oxidized significantly more rapidly than treated and untreated LDPE.

Dedicated to my parents, Dr. Ernest A. and Mary Jane Berends;
and my cousin, Joshua Sternke

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TABLE OF CONTENTS

ABSTRACT ii

DEDICATION iv

ACKNOWLEDGEMENTS v

TABLE OF CONTENTS vi

LIST OF TABLES AND FIGURES x

INTRODUCTION 1

SECTION I : LITERATURE REVIEW

POLYMER OXIDATION AND STABILIZATION 5

Free radical chain mechanism of autoxidation 5

Photo-oxidation 7

ESR/EPR measurement of free radical formation 8

Reactivity of free radicals with oxygen 11

Antioxidants 12

OFF-FLAVOR DEVELOPMENT IN CITRUS JUICES 13

Juice composition and loss mechanisms 13

Autoxidation of d-limonene 15

Antioxidants: commercial and naturally occurring 17

INTERACTION AT PRODUCT/PACKAGE INTERFACE 19

Absorption by the food contact polymer 19
1. Permeation 19
2. Absorption 21
Barrier properties of the package system 23
Effect of oxygen concentration on d-limonene 24
Effect of temperature and storage conditions on d-limonene . . . 25
Migration 26
PREVIOUS STABILITY STUDIES 26
OXIDATION OF LINOLEIC ACID 29
Mechanism of lipid oxidation 30
Singlet oxidation 32
Breakdown of linoleic acid 33
Kinetics of lipid oxidation 34
1. Monomolecular rate period 35
2. Bimolecular decomposition 35
Factors influencing the rate of oxidation 37
1. Oxygen concentration 37
2. Fatty acid composition 38
3. Pro-oxidant effects 38
4. Antioxidation 39
Analytical techniques for lipid oxidation analysis 40

1. Product model 40

2. Indices of oxidation 42

3. Hexanal 46

REFERENCES 48

SECTION II : LOW DENSITY POLYETHYLENE INITIATED OXIDATION OF D-LIMONENE

ABSTRACT 56

INTRODUCTION 57

MATERIALS AND METHODS 60

RESULTS AND DISCUSSION 65

CONCLUSIONS 72

REFERENCES 73

APPENDIX 75

SECTION III : POLYMER INITIATED OXIDATION OF LINOLEIC ACID

ABSTRACT 84

INTRODUCTION 85

MATERIALS AND METHODS 87

RESULTS AND DISCUSSION 93

CONCLUSIONS 100

REFERENCES 101

APPENDIX 105

VITA 133

LIST OF TABLES AND FIGURES

SECTION I

Figure 1. General autoxidation scheme for solid polymers 6

Figure 2. General autoxidation scheme of d-limonene via a free radical mechanism 16

Figure 3. Antioxidant termination of chain oxidation 18

Figure 4. Autoxidation scheme for lipid oxidation 32

SECTION II

Table 1. Carvone (μg/g of d-limonene) accumulation in samples containing no polymer, untreated, hydrogen peroxide treated, and ultraviolet light treated LDPE during 15 weeks of storage at an oxygen concentration of 21% 68

Table 2. Carveol (μg/g of d-limonene) accumulation in samples containing no polymer, untreated, hydrogen peroxide treated, and ultraviolet light treated LDPE during 15 weeks of storage at an oxygen concentration of 21% 69

Figure 1. Combined totals of carvone and carveol (μg/g of d-limonene) accumulation as a function of 4 different treatments (1-no polymer in contact with d-limonene; 2-untreated LDPE in contact with d-limonene; 3-hydrogen peroxide treated LDPE in contact with d-limonene; 4-ultraviolet light treated LDPE in contact with d-limonene) at an oxygen concentration of 21% 70

Table 3. Regression equations for the combined accumulations of carvone and carveol during 15 weeks of storage at an oxygen concentration of 21% 71

Appendix B. Percent recovery data of carveol and n-octanol (internal standard) from Prep-Sep extraction columns 76

Appendix C. Chromatogram of carveol and carvone peaks produced during the autoxidation of d-limonene 78

Appendix D. Carvone and carveol data collected from the gas chromatograph for vials containing no polymer at an oxygen concentration of 21% . 80

Appendix E. Carvone and carveol data collected from the gas chromatograph for vials containing untreated LDPE at an oxygen concentration of 21% 81

Appendix F. Carvone and carveol data collected from the gas chromatograph for vials containing hydrogen peroxide treated LDPE at an oxygen concentration of 21% 82

Appendix G. Carvone and carveol data collected from the gas chromatograph for vials containing ultraviolet light treated LDPE at an oxygen concentration of 21% 83

SECTION III

Figure 1. Hexanal accumulation as a function of polymer type (LDPE, PETE) and treatment of polymers exposed to model solution (1-no polymer; 2-untreated LDPE; 3-hydrogen peroxide treated LDPE; 4-ultraviolet light treated LDPE; 5-untreated PETE; 6-hydrogen peroxide treated PETE; 7-ultraviolet light treated PETE) at an oxygen concentration of 21% 94

Figure 2. Hexanal accumulation as a function of polymer type (LDPE, PETE) and treatment of polymers exposed to model solution (1-no polymer; 2-untreated LDPE; 3-hydrogen peroxide treated LDPE; 4-ultraviolet light treated LDPE; 5-untreated PETE; 6-hydrogen peroxide treated PETE; 7-ultraviolet light treated PETE) at an oxygen concentration of 5% 97

Appendix B. Gas chromatographic calibration data for hexanal 107

Appendix C. Gas chromatographic calibration curve for hexanal 108

Appendix D. Chromatogram of hexanal peak produced during the autoxidation of linoleic acid 109

Appendix F. Percent recovery data of hexanal from the Tenax TA 111

Appendix G. Schematic of gas flow apparatus and glass test cell allowing for headspace evacuation of hexanal into tenax trap 112

Appendix H. Hexanal data collected from the gas chromatograph for test cells containing no polymer at an oxygen concentration of 21% ... 114

Appendix I. Hexanal data collected from the gas chromatograph for test cells containing untreated LDPE at an oxygen concentration of 21% 115

Appendix J. Hexanal data collected from the gas chromatograph for test cells containing hydrogen peroxide treated LDPE at an oxygen concentration of 21% 116

Appendix K. Hexanal data collected from the gas chromatograph for test cells containing ultraviolet light treated LDPE at an oxygen concentration of 21% 117

Appendix L. Hexanal data collected from the gas chromatograph for test cells containing untreated PETE at an oxygen concentration of 21% 118

Appendix M. Hexanal data collected from the gas chromatograph for test cells containing hydrogen peroxide treated PETE at an oxygen concentration of 21% 119

Appendix N. Hexanal data collected from the gas chromatograph for test cells containing ultraviolet light treated PETE at an oxygen concentration of 21% 120

Appendix O. Hexanal levels (μg/g of linoleic acid) as a function of polymer type (LDPE, PETE) and treatment of polymers exposed to model solution (NP-no polymer; NT-untreated polymer; HP-hydrogen peroxide treated polymer; UV-ultraviolet light treated polymer) at an oxygen concentration of 21% 121

Appendix P. System flow rates and gas partitioner calibration for the 5% oxygen condition 122

Appendix Q. Headspace gas analysis during experiments conducted at an oxygen concentration of 5% 123

Appendix R. Hexanal data collected from the gas chromatograph for test cells containing no polymer at an oxygen concentration of 5% 125

Appendix S. Hexanal data collected from the gas chromatograph for test cells containing untreated LDPE at an oxygen concentration of 5% 126

Appendix T. Hexanal data collected from the gas chromatograph for test cells containing hydrogen peroxide treated LDPE at an oxygen concentration of 5% 127

Appendix U. Hexanal data collected from the gas chromatograph for test cells containing ultraviolet light treated LDPE at an oxygen concentration of 5% 128

Appendix V. Hexanal data collected from the gas chromatograph for test cells containing untreated PETE at an oxygen concentration of 21% . 129

Appendix W. Hexanal data collected from the gas chromatograph for test cells containing hydrogen peroxide treated PETE at an oxygen concentration of 5% 130

Appendix X. Hexanal data collected from the gas chromatograph for test cells containing ultraviolet light treated PETE at an oxygen concentration of 5% 131

Appendix Y. Hexanal levels (μg/g of linoleic acid) as a function of polymer type (LDPE, PETE) and treatment of polymers exposed to model solution (NP-no polymer; NT-untreated polymer; HP-hydrogen peroxide treated polymer; UV-ultraviolet light treated polymer) at an oxygen concentration of 5% 132

INTRODUCTION

Maintaining quality and shelf-stability of aseptically packaged products poses a significant problem due to the absorption and oxidation of select flavor and/or aroma compounds. Some variations in the shelf-life of aseptic foods are not explained by normal factors such as headspace oxygen, storage temperature, package defects or barrier properties of packaging materials. Foods and beverages sensitive to heat are processed and packaged aseptically for the purpose of providing a higher quality product with greater nutrient retention (David, 1992). Although a high quality product is produced compared to conventional canning, the product as received by the consumer is not always of superior quality. The problem is of particular importance in foods containing essential flavor and aroma compounds that are absorbed by the food contact surface. An oxidized polymer is thought to be a capable initiator of oxidative reactions in the food. Determination of aseptic stability/degradation must include the production of free-radicals by the food contact surface with implications for the production of off-flavors via autoxidation processes. Quantitative measurements of volatile absorption does not compensate for polymer initiated oxidation of these sensitive compounds.

The absorption of flavors by the food contact surface of aseptically packaged products, usually low density polyethylene (LDPE), has been the subject of

intensive research (Mannheim et al., 1987; Baner et al., 1991; Paik and Tigani, 1993; Sadler and Braddock, 1991; Sadler and Braddock, 1990; Mohney et al., 1988). The problem of sorptive loss has been compensated for by the addition of extra flavor compounds to a level significantly higher than required to maintain sufficient concentration for acceptable flavor and mouthfeel. Oxygen permeates the laminate structure, albeit slowly, via flex cracking of the aluminum foil barrier or by permeation through the barrier plastic layer. As the concentration of select flavor compounds increases a greater amount will be absorbed into the matrix of the polymer, through solvation processes, causing swelling and increased permeability to oxygen. As oxygen diffuses through the polymer, readily available flavor volatiles in the matrix and on the film surface will set the stage for the autoxidation process. Depending upon the concentration, flavor components could become layered (unequal dispersion in food with higher concentration at interface) at the food contact surface, increasing the rate of oxidation via chain reaction. This phenomena may have a greater impact on the quality and shelf-stability of aseptically packaged foods than simple loss of flavor through scalping.

The degradation of the food contact polymer, usually LDPE, via photoxidation, irradiation, thermal extrusion processes, or heat treatment with hydrogen peroxide could contribute to the reduction of shelf-stability of aseptically packaged foods, independent of all other factors. During the extrusion coating process of aseptic flexible laminate cartons, free radicals can be mechanically and

thermally produced. Most of these free radicals are quickly dissipated through recombination and quenching by atmospheric oxygen. These oxygenated compounds on the surface and in the bulk phase of the polymer are then available to absorb UV light during storage to continue this oxidation/degradation of the polymer. Package sterilization processes expose the food contact layer of aseptic packages to a 35% solution of hot H_2O_2 (85°C) followed by evaporation at temperatures of 100°C. Other manufacturers use UV light for film surface sterilization. These processes may be sufficient to create free radicals on the surface and throughout the matrix of the food contact polymer.

Mannheim et al. (1987) reported that the corona-treated and untreated contact surface of polyethylene accelerated the rate of ascorbic acid degradation immersed in solution, with the oxidized film having the greatest effect. Bojkow et al. (1976) and Mannheim et al. (1987) reported the use of elevated temperatures (320°C) by some manufacturers during the coating operation, may result in excessive surface oxidation and thus adversely affect the product (Leong et al., 1992). Although free radicals dissipate quickly, they may remain while product is introduced into the container. The formation of oxygenated compounds (C=O, C-OH, COOH, etc.) and possibly free radicals on the surface and/or the bulk phase of the polymer could initiate autoxidation of compounds essential to flavor quality. The affinity/absorption of d-limonene, fatty acids, and other select compounds to the food contact polymer could set the stage for polymer initiated

oxidation at the product/package interface, developing off-flavor and effectively reducing product storage life and quality.

The goal of this research is to determine if select flavor volatiles are degraded on the film surface causing an autoxidative chain reaction in the food. Assuming all independent variables involved in maintaining shelf-stability of aseptically packaged products can be controlled (oxygen content, temperature, absorption, migration, and light), we will test whether the oxidative state of the polymer could be involved in autoxidation of aseptically processed food components.

OBJECTIVES:

1. Determine if free radicals are produced on a polymer surface during the sterilization process of aseptic packaging.
2. Determine if the food contact layer acts as a free radical donor capable of initiating oxidation of flavor compounds, resulting in the development of off-flavors.
3. Determine the effect of flavor/polymer affinity and headspace oxygen concentration on the rate of oxidation of flavor compounds.

SECTION I: LITERATURE REVIEW

POLYMER OXIDATION AND STABILIZATION

Free Radical Chain Mechanism of Autoxidation

All polymers are oxidized and deteriorated to certain extents by molecular oxygen and other oxidizing species present in the atmosphere by a free radical chain mechanism (Kamiya, 1983). Numerous researchers have studied the degradation of polyethylene (PE) via oxidation since the 1940's when the British Rubber Producers Association developed the general fundamental mechanism for the uncatalyzed autoxidation of polyolefins (Jellinek, 1989; Gugumus, 1990; Cicchetti, 1970). Several oxidation schemes have been devised to represent the steps in which PE and other polymers may become oxidized and degraded (Kamiya and Niki, 1983; Cicchetti, 1970). The general autoxidation scheme for solid polymers can be described in three stages: initiation, propagation, and termination (Fig. 1).

Initiation:	Substrate ----> free radicals ($R\bullet$, $RO\bullet$, $RO_2\bullet$, $\bullet OH$, etc.)	(1)
Propagation:	$R\bullet + O_2$ ----> $ROO\bullet$	(2)
	$ROO\bullet + RH$ ----> $ROOH + R\bullet$	(3)
Termination:	$2R\bullet$ ----> R-R	(4)
	$RO_2\bullet$ ----> $ROOR + O_2$	(5)
	$R\bullet + RO_2\bullet$ ----> ROOR	(6)

Figure 1. General autoxidation scheme for solid polymers.

Initiation (1) begins with the abstraction of a hydrogen atom creating a free radical. There have been several possible mechanisms and/or reactions postulated for the generation of radicals. The triplet state oxygen molecule in the atmosphere is a radical and has been thought to abstract a hydrogen atom from organic compounds (Kamiya and Niki, 1983). Being endothermic this reaction is thermodynamically and kinetically unfavorable occurring slowly at ambient temperature. The bond dissociation energy of the C-H bond is >75 kcal/mole, while that of the H-O_2 bond is only 49 kcal/mole. At moderately elevated temperatures, however, this reaction becomes feasible (Kircher, 1987). Free radicals might also be initiated via mechanical fracture (mechano-radicals) of solid

polymers through scission of polymer chains (Sohma, 1979). Igarashi (1983) ground HDPE under liquid nitrogen using a hand grinder found free radical formation. He attributed the primary main chain radicals ($-CH_2-CH_2$) to mechanical fracturing of polymers, including chain scission, slippage and chain unfolding. During the formation of resin, polymer is heated to molten and mechanically sheared giving rise to free radicals and initiating autoxidation. As free radicals are formed they are immediately quenched by molecular oxygen and/or dissipated via recombination. The reaction of oxygen with the carbon radical gives a peroxy radical (2), which can then in turn abstract a hydrogen atom giving a hydroperoxide and creating another free radical (3), hence propagation. In the presence of sufficient oxygen, reaction (2) occurs extremely fast. In the absence of inhibitors, the rate of oxidation is dependent upon the competition between propagation (3) and the termination steps in autoxidation (4,5,6). The decay of hydroperoxides leads to generation of radicals and main chain cross-linking which accelerates the degradation of polymer chains (Jellinek, 1989).

Photo-oxidation

After resin manufacture and/or extrusion processes and subsequent storage, polymers are extremely sucseptible to photo-oxidation. The photo-oxidation of polymer films leading to physical and mechanical degradation has been extensively studied (Tsuji, 1973; Cicchetti, 1970; Tsuji, 1973; Shimada et al., 1970).

Hypothetically, most saturated polymers in pure form should not absorb UV sunlight reaching the earth (wavelengths above 295 nm). However, degradation of polymers does take place upon exposure to UV light of wavelengths above 300 nm (Osawa, 1983). Photo-oxidation must be attributable to chromophoric groups, such as hydroperoxides, carbonyl groups and oxygen-polymer charge transfer complexes, which become sensitized upon exposure to UV light. Photolysis of the carbonyl compounds occurs via Norrish type I and type II reactions, yielding chain scission and radical formation. Further oxidation of the carbonyl-bearing radical yields an accumulation of carboxylic acids, due to their insensitivity to UV light. Photochemical decomposition of hydroperoxides involves bimolecular reactions with neighboring chain segments yielding ketones and aldehydes.

The extended storage of a food contact polymer exposed to the atmosphere could continue the degradation process of radical formation, chain scission and production of oxygenated compounds, ultimately affecting the quality of a shelf-stable product.

ESR/EPR Measurement of Free Radical Formation

The phenomenon of free radical creation and subsequent reaction with atmospheric oxygen leading to the aging or degradation of polymers has been measured by numerous instruments over the past few decades applying a variety of methods. Calorimeters, monitoring heat generated or consumed by chemical

reactions or physical processes, have been used to monitor the oxidative reactions of polymers. Paulsson (1993), studying the oxidation of aged polyethylene using a microcalorimeter, reported normal heat flow values (μW/g) for samples exposed to nitrogen, air, and 100% oxygen at 70°C. He reported very little change in mechanical and other properties of aged, normal stabilized polyethylene until a sudden change leading to cracks was seen, which was attributed to a loss of oxidation stablizers. Microcalorimeters can be used to study the oxidative degradation of old or degraded polyethylene at moderate temperatures (70°C)(Paulsson, 1993).

Van der Mei et al. (1991) used attenuated total reflection infrared spectroscopy (ATR-IR) and x-ray photoelectron spectroscopy (XPS) to measure the aging effects of repeatedly glow-discharged (Gld) polyethylene. The absorption spectra of the IR revealed peaks attributable to carboxylic acid groups, carbonyl groups, and hydroxy groups after at least five Gld treatments. IR absorption was obviously not sensitive enough to reveal any surface modification for less than five Gld treatments. This is due to the depth of the surface layer probed (0.3-3 μm) and the insufficient concentration of oxygen-rich components in comparison with other components in the polyethylene film thickness analyzed (Van der Mei et al., 1991). XPS measured the intensity of C_{1s} and O_{1s} peaks, enabling calculation of the O/C concentration. XPS was successful in detection of hydrophilic groups, but could not detect the displacement of a hydrophilized

group away from the surface towards the bulk material due to the 2-5 nm probing depth. Garbassi et al. (1989) found that aging of oxygen glow-discharged polypropylene was undetectable by XPS.

Measurement and detection of free radical formation via gamma irradiation, ultraviolet irradiation, electron beam, plasma-irradiation, mechanical fracture and thermal exposure has been studied by a host of investigators using Electron Spin Resonance (ESR) or Electron Paramagnetic Resonance (EPR) (Shimada et al., 1970; Tsuji, 1973; Kuzuya et al., 1991; Sakaguchi et al., 1993). ESR allows for the sensitive detection of free radicals formed on the surface of polymers. The development of free radicals is dependent upon the method of initiation. Kuzuya et al. (1993) reported the midchain alkyl radical ($-CH_2CHCH_2-$) and allylic radical ($-CH_2CHCH=CHCH_2-$) upon exposure of polyethylene to plasma irradiation. Upon ultraviolet light irradiation of polyethylene the primary process of radical formation ($-CH_2-CH-CH_3$) is a Norrish type I reaction of carbonyl groups contained in the polymer (Anonymous, 1973). It was found that free radicals are present only in amorphous regions in which oxygen molecules can diffuse very easily, and that free radicals are produced from carbonyl groups in the amorphous regions only. Carbonyl groups in the crystalline regions do not contribute to radical formation probably due to light scattering (Anonymous, 1973). Sohma (1979) used ESR to study free radicals formed upon mechanical fracture of polypropylene under cryogenic conditions. Mechano-radicals are scission type (-

CH_2-CH-CH_3 and CH_2-CH-CH_2-), whereas irradiation produces the midchain radicals mentioned above.

Reactivity of Free Radicals with Oxygen

Most polymer radicals formed by high-energy irradiation are rapidly converted to peroxy radicals in the presence of oxygen at room temperature. Plasma-irradiated polyethylene, exposed to air, formed unstable peroxy radicals that undergo a rapid chain termination reaction through the hydroperoxide, consuming several moles of oxygen (Kuzuya et al., 1993). Sohma (1979) found differences in the reactivity of free-radicals formed by mechanical fracture and gamma-irradiation. At elevated temperatures (200 K), nearly 100% (complete conversion is never obtained due to the competition of recombination reactions) of the mechano-radicals had converted to peroxyradicals, while only 40% of the gamma-irradiated radicals had gone through conversion. This clearly shows the high reactivity of mechano-radicals with oxygen molecules. However, the accessibility of a reactant to a trapped polymer radical through diffusion of the reactant may be the most important consideration in determining the rate or occurrence of a reaction. If a reactive free-radical is trapped on the surface of a polymer, to which any reactive molecules are freely accessible, the reaction does occur rapidly (Sohma, 1979). Free-radicals in the matrix are less accessible to the diffusion of even small molecules like oxygen, but with time and/or elevated temperatures some fraction

of oxygen may penetrate to the trapped polymer radicals. The high reactivity of mechano-radicals can be attributable to the fact that they are trapped on fresh surfaces, in the amorphous region of polymers, produced by fracture, as compared with gamma-irradiated radicals which are produced in the crystalline region, less accessible to permeating reactant species (Sohma, 1979).

Antioxidants

Practically all commercial polyolefins, the most widely used thermoplastic polymers, contain at least one antioxidant to protect them againts thermal and oxidative breakdown during processing and for end-use application (Munteanu, 1990). Polymers are attacked by oxygen during processing (only limited oxygen is involved in high temperature processes that generate C-centered radicals) and degraded by a number of physical and chemical causes during atmospheric aging (solar radiation, mechanical strain, oxygen and humidity) (Pospisil, 1983). Stabilizers protecting various organic subtrates against atmospheric oxygen and ultraviolet light have been used for years in an attempt to retard decomposition. The rate of oxidation can be limited (1) by using compounds that compete with the rate of ROOH homolysis in the propagation or chain branching step and (2) by scavenging radicals ($RO_2\cdot$, $R\cdot$) (Pospisil, 1983). Gugumus (1990) evaluated the efficacy of hindered-amine light stabilizers (HALS) alone or in combination with benzotirazole-type UV absorbers for the stabilization of polyethylene upon photo-

oxidation. He found the performance of HALS was outstanding in tapes and films, and synergistic effects between HALS and UV absorbers yielded the optimum stabilization. For example, HALS are not efficient in quenching ketones, but benzotriazole-type UV absorbers are very efficient quenchers of the Norrish type I reaction according to the long-range energy transfer mechanism.

OFF-FLAVOR DEVELOPMENT IN CITRUS JUICES

Juice Composition and Loss Mechanisms

The flavor of citrus juices combines taste, aroma, mouthfeel, and appearance by virtue of high concentrations of sugar and organic salts, plus a unique blend of more than 150 volatile flavor compounds at low concentrations that significantly contribute to the overall perceived aroma. The majority of volatile components that give citrus juices their characteristic flavor and aroma, making up only 0.2% by volume, come from the peel and essence oils added back to the concentrate before freezing. Citrus peel oils, due to their pleasant aromas, are extensively used as flavor enhancers in fruit drinks and carbonated beverages (Kealey and Kinsella, 1979). Peel oil is expressed from glands in the peel (carpels) during extraction of the juice through cold-pressing or distillation. During cold-pressing, oil sacs are ruptured mechanically and the oil is separated from the aqueous

emulsion through centrifugation. Essence oil is the oily layer separated by centrifugation during collection of aqueous essence from citrus waste streams, i.e., peel, pulp, and rag. Aqueous essence, predominately a water-ethanol mixture, also adds to citrus flavor (Moshonas and Shaw, 1986). The quantitative relationship of individual flavor components that make-up the essential oil of citrus is critical, however the mixture and proportion of these compounds during processing and storage have been difficult to measure and remain unidentified (Moshonas and Shaw, 1986).

The characteristic aroma of orange comes from constituents produced by the interconversion of acyclic alcohols and aldehydes in the peel (Kealey and Kinsella, 1979). Over 90% of the essential oil of orange is the non-polar, water-insoluble monoterpene d-limonene, however the major contribution of flavor is due to the minor oxygenated constituents, especially the aldehydes, esters, and alcohols. D-limonene is present at over 400 times its flavor threshold in water (Ahmed et al., 1978). The most easily oxidized components in citrus juices are terpenes, giving "terpeney" off-flavors, attributed to carvone and carveol formed by autoxidation of d-limonene (Ting and Newhall, 1978).

Acid-catalyzed reactions of terpenes are possible under dilute aqueous acid conditions (pH $\leq$6), causing a different flavor, but not necessarily an unpleasant taste or odor (Clark and Chamblee, 1992). These reactions involve hydrations of the terpenic double bonds, dehydrations, rearrangements, cyclizations, and

hydrolyses of esters, leading to profound flavor changes and increases or decreases in flavor intensity (Clark and Chamblee, 1992).

Autoxidation of d-Limonene

Numerous researchers have extensively studied the oxidation of d-limonene or monoterpenes via autoxidation (Bernhard and Marr, 1959), photo-oxidation (Stromvall and Petersson, 1992; Sato and Murayama, 1974), or via hydrogen peroxide (Wilson and Shaw, 1973), and singlet oxidation catalyzed autoxidation (Clark et al., 1981), producing various off-flavors and aromas. The general scheme for the autoxidation of d-limonene via a free radical mechanism, suggested by Flores and Morse (1952) is shown in Fig. 2.

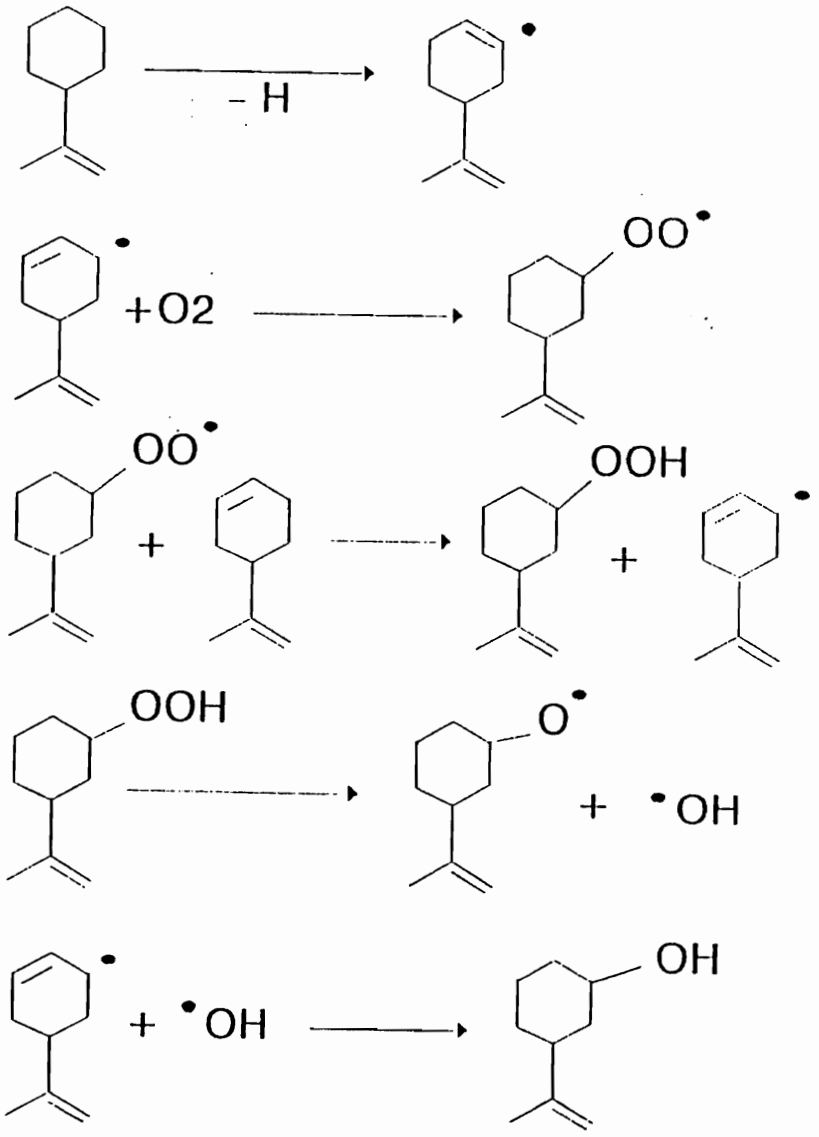


Figure 2. General autoxidation scheme of d-limonene via a free radical mechanism.

The previously mentioned methods vary only in their mode of initiation. The initiation stage of oxidation begins with the abstraction of a labile hydrogen atom

by any of the previously mentioned modes of initiation (i.e., thermal, light, singlet oxygen, etc.). Propagation begins with molecular oxygen readily binding to the unpaired electron forming a hydroperoxy radical. The hydroperoxy radical having a strong electron affinity abstracts a hydrogen from another molecule of d-limonene, giving a more stable hydroperoxide and a carbon radical. The carbon radical can then bind with another molecule of oxygen, continuing the chain reaction. Although, initially hydroperoxides are produced in high yields, the hydroperoxides can subsequently attach the double bond of another molecule by both polar and radical processes (Bernhard and Marr, 1959). Scission of the oxygen-oxygen bond of hydroperoxides takes place to yield a hydroxyl free radical. The oxygen radical bound to the d-limonene molecule can then react with a d-limonene free-radical to give carvone. The free hydroxyl group can then react with another d-limonene free-radical to yield carveol. Carveol can also be produced by the bound oxygen radical abstracting a hydrogen from a d-limonene molecule. Bernhard and Marr (1959) identified 2-limonyl hydroperoxide, carvone and carveol as the three main constituents produced from the oxidation of d-limonene.

Antioxidants: Commercial and Naturally Occurring

Antioxidants are substances that can inhibit or retard the onset of oxidation of autoxidizable substances. Hundreds of compounds, natural and synthesized have

been found to exert antioxidant activities (Nawar, 1985). Common commercial antioxidants found to extend the useful life of citrus oils are nordihydroguaiarctic acid (NDGA), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and alpha-tocopherol (Vitamin E). The mode of action for these antioxidants is via donation of a hydrogen atom or acceptance of free-radicals. The most probable number of oxidation chains terminated by one inhibitor molecule is two, with the reaction represented in two stages as illustrated in Fig. 3 (Nawar, 1985):

$$RO_2\cdot + AH_2 \text{ ------> } ROOH + AH\cdot$$

$$AH\cdot + AH\cdot \text{ ------> } A + AH_2$$

Figure 3. Antioxidant termination of chain oxidation.

Many citrus fruits, oranges in particular, have natural occurring antioxidant activity. Ting and Newhall (1978) found the flavedo (outer peel) extracts of oranges greatly extended the length of the oxidative induction period of d-limonene. The antioxidant seemed to be a tocopherol-like substance (i.e., non-polar in nature and soluble in petroleum ether), and not a flavonoid.

INTERACTION AT PRODUCT/PACKAGE INTERFACE

In flexible aseptic packaging systems there are a variety of factors that influence quality during extended storage life, one of which is the interaction of sensitive flavor compounds with packaging materials, i.e., absorption of volatiles, effect on barrier properties, migration of low molecular weight materials.

Absorption by the Food Contact Polymer

Flavor absorption of citrus juices in aseptically packed flexible cartons has been the subject of intensive research over the past few years. The problem is of particular importance in foods containing essential flavor and aroma compounds, such as orange juice and other citrus juices.

Permeation

The sorption of flavors into a polymer and subsequent diffusion through the polymer has the potential to remove flavor from a food product. This phenomema could occur broadly across all flavor components in a food product, or perhaps worse, the removal could occur selectively (DeLassus and Strandburg, 1991). The mechanism by which this occurs is known as permeation (P) that can be described by two components, the diffusion coefficient (D) and solubility coefficient (S), using the following equation: $P = D \times S$. Permeation is the mult-

step process of a small molecule species colliding with a polymer (food contact surface), adhering to the surface (adsorption), and with net motion from the inside surface of the package to the outside surface.

The diffusion coefficient is a kinetic term that describes how fast a permeant molecule can penetrate a polymer, and the time it takes to reach steady state. The measure of diffusion is determined by thermal and geometric effects (DeLassus and Strandburg, 1991). The solubility coefficient is a thermodynamic term that describes the number of permeant molecules that move into a polymer host. It is a function of temperature, chemical activities, intermolecular interactions, in addition to the state of the polymer in relation to its glass transition temperature (DeLassus and Strandburg, 1991).

The simple measure of permeability for modelling the interactions of flavor molecules with plastic packaging material is inadequate due to the normally large solubility coefficients and small diffusion coefficents. The time to reach steady state for packaging films can vary from a few minutes to many months for barrier films, which may extend past the storage time of the food. With large solubility coefficients, flavor depletition could occur before steady state is reached. Concentration as well as diffusion processes dependent upon time may take place, resulting in bulk phase swelling of the polymer and a non-ideal Henry's Law relationship (Mohney et al., 1988). Therefore, to estimate flavor loss, the solubility and diffusion coefficients must be calculated.

Absorption

Numerous researchers have shown the susceptibility of flavor volatiles to be absorbed by the food contact layer, usually low density polyethylene (LDPE), causing a reduction in the organoleptic quality of citrus juices (Durr et al., 1981; Marshall et al. 1985; Konczal et al., 1991; Saddler and Braddock, 1991). Mannheim et al. (1987) reported the d-limonene content of citrus juice was reduced 25% within 14 days of storage due to absorption by LDPE. Jeng et al. (1991) reported that absorption of d-limonene into the inner layer of an aseptic package was temperature dependent.

Halek and Luttmann (1991) found that flavor absorption into polypropylene (PP) and LDPE was dependent upon polarity of the compound. PP was found to absorb at a significantly higher rate than LDPE. Significant differences in sorption were found between hydrocarbon monoterpenes (i.e., d-limonene, myrcene, alpha-pinene) and the polar oxygenated monoterpenes (i.e., l-carvone, d,l-linalool, citral). The non-polar, hydrocarbon compounds were absorbed at a greater rate and quantity than the polar, oxygenated compounds for both polymers (i.e., d-limonene was absorbed more than carvone in every case). Using their sorption data, Halek and Luttmann (1991) estimated partition coefficients after 30 days for d-limonene and l-carvone in both polymers. They found limonene had significantly higher partition coefficient values than carvone for PP and LDPE, and concluded that structure and functional groups of the solutes

exert a strong influence on sorption behavior towards polymers of similar polarities. Fukamachi et al. (1993) found surface hydrolysis of ethylene-vinyl acetate copolymer (EVA) film resulted in a specific sorption depression behavior of volatile compounds (i.e., hydrocarbons, ethyl octanoate, decanal), without any mechanical property changes.

Dürr et al. (1981) studied the effects of pasteurization and filling, and storage time and temperature on the d-limonene concentration in orange juice. They discovered a decrease in d-limonene content prior to processing and after filling into Tetra Brik™ style packages, from 220 to 180 ppm, respectively. A decrease of 40% in d-limonene, due to absorption into the polyethylene lining of the flexible packages, was reported after 6 days of storage at 32°C. Baner et al. (1991) found temperature to have a significant effect on the sorption of aroma into LDPE and other materials. Polymers showed a significant decrease in partition coefficients as temperatures increased from 25° to 40°C.

The problem of sorptive loss has been compensated for by the addition of extra flavor to a level higher than required, to maintain sufficient concentration for acceptable flavor and mouthfeel. Although over 90% of the essential oil of orange is the monoterpene hydrocarbon, d-limonene, the major contributors to orange flavor comes from the minor oxygenated compounds, especially the aldehydes, esters, and alcohols (Wolford and Attaway, 1967). Halek and Luttmann (1991) have shown that the rates of sorption and ultimate equilibrium

partitioning of chemically different citrus-flavor compounds in food contact polymers were different. They suggest this could present a problem when compensating for flavor ingredient changes by adding extra amounts of those specific ingredients, since flavor profiles would still not remain constant over the storage period.

Barrier Properties of the Package System

Barrier properties of the carton must prevent or significantly retard the penetration of oxygen. Mannheim et al. (1987) reported that CO_2 permeated into flexible laminate cartons (generally composed of from inside to outside LDPE/PE ionomer/aluminum foil/PE ionomer or other laminating layer/paperboard/LDPE), suggesting that O_2 transmission would also be considerable and would affect oxidative reactions of product in these packages during storage. Sizer et al. (1988) reported that although made of barrier materials, most aseptic cartons have areas of vulnerability along folds, seals, lids, or creases which allow a very limited amount of oxygen permeation. This suggests that the amount of oxygen transmitted is insignificant compared to the amount usually found in the orange juice or in the headspace. Flavor absorption by the food contact layer PE has also been shown to increase permeability of O_2 (Mohney et al., 1988; Sadler and Braddock, 1990). Cold-pressed orange oil used extensively in the flavor industry is very prone to oxidation, causing the development of terpeney off notes

(Buckholz and Daun, 1978). In most cases the packaging of aseptically processed foods is the weakest and most fallible component of the aseptic process (Reuter, 1989). Conditions of storage and distribution may also contribute to deterioration and the loss of product quality.

Effect of Oxygen Concentration on d-Limonene

If a headspace in an aseptically processed and packaged product is required, the composition should be controlled to exclude oxygen. Oxygen dissolved in the product or contained in the headspace can be exasperated through product agitation and nitrogen purging while filling. Cold-pressed orange oil and citrus essential oils consist of a mixture of unsaturated mono- and sesquiterpenes which are highly susceptible to oxidative and photochemical degradations (Anandaraman and Reineccius, 1986). The leading causative effect for the change in flavor of orange juice during storage in aseptic cartons is not absorption of flavor compounds, but chemical degradation of flavoring components and the development of off-flavor components from resulting degradation products (Sizer et al., 1988).

Buckholz and Daun (1978) found orange oil and d-limonene to rapidly oxidize under ultravoilet light and in atmospheric conditions (21% O_2), yielding carvone and carveol as the main oxidation products. The concentrations of carvone and carveol increased significantly over time. Kutty et al. (1994) found d-limonene to

oxidize via first order reaction kinetics with the following end-products: d-carvone, carveol, limonene oxide, perrilaldehyde, linalool, and a hydrolysis product, alpha-terpineol.

Effect of Temperature and Storage Conditions on d-Limonene

The temperature of storage can have a significant effect on the shelf-stability of aseptic citrus juices. Sizer et al. (1988) found storage temperature to be the single most important factor in delaying flavor loss and achieving satisfactory shelf-life and quality. Graumlich et al. (1986) also found storage temperature to be the most important factor in determining shelf-life of aseptic orange juice and concentrate. Aseptic processing produces a higher quality orange juice than hot filling, however, storage at ambient temperature extinguishes any quality differences (Graumlich et al., 1986). Jeng et al. (1991) reported that only trace amounts of volatile components were absorbed by the inner packaging material when aseptic packages were stored at 4°C versus 37°C where large amounts were absorbed. They compared the rate of absorption and the rate of loss (volatile components remaining in the juice), and found that oxidation and thermal induced degradation may have a significant effect on the quality of aseptic orange juice. Absorption of volatile components may be important, but only to a certain extent (Jeng et al., 1991).

Migration

Migration of packaging components from LDPE have been shown to develop off-flavor in water and milk (Leong et al., 1992). Sizer et al. (1988) reported that additive-free polyethylene grades are typically used in aseptic packaging material, relieving the problem of migration of slip agents, plasticizers, or antioxidants.

PREVIOUS STABILITY STUDIES

Buckholz and Daun (1978) analyzed the thermal (38°C) oxidation of cold-pressed orange oil for 14 weeks by gas-liquid chromatography and sensory evaluation. California cold-pressed orange oil (25g) was placed in flint jars with a headspace of air, and placed in an oven. Samples were analyzed on a weekly basis by direct injection of oil onto the GLC column. Carvone and carveol, the two main autoxidative products of the orange oil, gave an initial lag phase when plotted versus time, followed by a sharp increase in concentration with time. Good correlation was obtained between sensory and instrumental analysis.

Moshonas and Shaw (1989) evaluated 250 mL cartons of commercial aseptically packaged orange juice stored at 0° (control), 21°, and 26°C with a sensory panel using hedonic ratings. Flavor scores of samples were significantly lower than the control juice and unacceptable after 1 week at 26°C and 2 weeks at

21°. A gas chromatographic profile also showed a 40% decrease in d-limonene content during storage.

Kutty et al. (1994) measured the headspace oxygen contents of sodium citrate-water buffers containing d-limonene with and without LDPE on a weekly basis for a 10-week period. Screw cap culture tubes containing sodium citrate buffer, 20-40 mesh LDPE, 10 μL of d-limonene, and headspace oxygen concentration of 21% were tumbled by rotation (12 rpm). The decrease in headspace oxygen was measured (oxygen probe), representing the oxidation of limonene in the sample, GC/MS analyses of the liquid portion of the sample yielded concentrations of carvone and carveol that were compared with time. They found limonene was readily sorbed into LDPE, and reported that a higher rate of oxidation was realized in the control (absence of LDPE powder) than in LDPE, suggesting limonene was protected from oxidation by being sorbed into the LDPE, and that absorption could be beneficial to flavor quality.

Shaw et al. (1993) analyzed the volatile constituents of orange juice by using a headspace GC technique. A 2 mL of juice in a glass vial sealed with a crimptop cap with TFE/silicone septum seal was heated to 80°C and analyzed in a Model HS-6 headspace sampler attached to a Perkin-Elmer Model 8500 GC with an FID detector.

Min et al. (1989) evaluated flavor stability of dry whole milk by measuring formation of volatile compounds in the headspace and oxygen disappearance in

the headspace. Volatile compounds (pentane and acetone) and oxygen content were analyzed by withdrawing headspace gas from test pouches with a gas-tight syringe, and injecting on the stainless steel column of a gas chromatograph.

Konczal et al. (1991) used a purge and trap procedure to determine the concentration of flavor compounds (ethyl-2-butyrate, hexanal, trans-2-hexanal, 1-hexanol) in apple juice. Nitrogen gas was bubbled through the sample in a modified gas washing bottle, and headspace volatiles trapped in a Tenax-GC (60/80 mesh) trap. Flavors were recovered from the trap with isopentane followed by centrifugation. On-line injected samples were analyzed using a gas chromatograph. Charara et al. (1992) also used a purge and trap procedure to determine the absorption of orange flavor by several different polymers.

Baner et al. (1991) developed a headspace gas analysis for evaluating aroma sorption by polymeric packaging materials. Headspace gas from a glass vial, containing aroma (ethyl acetate, n-hexanal, d-limonene, alpa-terpineol) injected from saturated headspaces and various film samples (i.e., LDPE), was withdrawn with a gas-tight syringe. Concentrations of aroma compounds over time were analyzed by gas chromatography.

OXIDATION OF LINOLEIC ACID

Lipid oxidation is one of the major causes of deterioration or spoilage in foods. Foods containing oils and fats can, in the presence of oxygen become unacceptable, effectively reducing product shelf-life (Nawar, 1985). Loss of acceptability and/or nutritional value often occurs because of the production of objectionable off-flavor and odor compounds, generally called rancid, from reactions involving oxygen absorption. The production of primary and secondary products (hydroperoxides, free radicals, endoperoxides, malonaldehyde, epoxides, alkanes, alkenes, hydrocarbons, alcohols, and acids) from lipid oxidation are possibly toxic to humans (Ajuyah et al., 1993). The factors which affect the rate of oxygen uptake by food products, such as oxygen concentration, light, temperature, and water activity (a_w) are important for process and product development, packaging and storage (Quast and Karel, 1972).

The effect of headspace oxygen concentration on the rate of lipid oxidation has been well documented (Koelsch et al. 1991; Labuza, 1971). Removal of oxygen from the headspace of a package to create a vacuum or replacing headspace air with nitrogen are verified approaches to extending the induction period of lipid oxidation and prolonging product shelf-life. However, eliminating oxygen is difficult due to air trapped in internal cavities of a product, package defects (seal integrity, pinholing), and the inability of modern machinery to

completely remove oxygen at reasonable rates of production. Even if the concentration of oxygen could be maintained at low levels of approximately 1% or lower, Tamsma et al. (1964) found that for certain products, oxygen concentrations lower than 1% can have a marked effect on the quality of the product (Quast and Karel, 1972).

Temperature and light will increase the rate at which fatty acids are oxidized. Light can be removed with packaging, and temperature is a function of the storage environment and/or processing conditions.

Mechanism of Lipid Oxidation

Karel (1981), Labuza (1971), and Nawar (1985) have discussed the mechanism of lipid oxidation in detail. A brief description of the general scheme follows. Lipid oxidation can occur by both enzymatic and non-enzymatic mechanisms, and is often autocatalytic, meaning the oxidation products themselves catalyze the reaction (Karel, 1981). Oxidation can be catalyzed by autocatalytic reaction products, metal ions, hydroperoxide decomposition or an enzyme, such as lipoxygenase. Metal ions decrease the induction period and reduce the activation energy of the initiation step. Several metal reactions are possible including catalyst decomposition, activation of molecular oxygen possibly to singlet oxygen, and direct radical initiation with substrate. Hydrolysis of the ester bonds in lipids by lipases (lipolysis), results in the liberation of free fatty acids. Free fatty acids

are more susceptible to oxidation than fatty acids esterified to glycerol (Nawar, 1985). Autoxidation, considered the main reaction involved in oxidative deterioration of lipids, focuses on a free-radical mechanism. The rate of reaction is affected by water activity (Labuza, 1971; Karel and Yong, 1981), oxygen concentration, temperature (Quast and Karel, 1972), and light.

Autoxidation, the reaction of unsaturated fatty acids with molecular oxygen, is commonly divided into three stages: initiation, propagation, and termination. Initiation begins with the abstraction of a hydrogen atom, requiring a high activation energy of 35 kal/mole (Nawar, 1985). Hydrogen atoms, alpha to a carbon-carbon double bond are labile and can easily be removed by a non-enzymatic catalyst. This forms an allylic free radical, forming a resonating structure. Monomolecular decomposition in the initiation stage occurs with the rate proportional to the square root of the extent of oxidation (Karel and Yong, 1972).

The propagation stage begins as molecular oxygen is consumed rapidly by reacting directly with the allylic free radical to form a peroxy radical. The peroxy free radical abstracts a hydrogen atom from another unsaturated fatty acid chain to form an unstable hydroperoxide. The abstraction of the hydrogen atom from the fatty acid creates another allylic free radical to react with oxygen, speeding up the rate of oxidation, and quickly increasing the hydroperoxide concentration. Hydroperoxides are then decomposed to give an aldehyde and an ester, among

other compounds. Bimolecular decomposition of hydroperoxides occurs with the rate proportional to peroxide concentration.

In the last stage of autoxidation, termination, allylic free radicals can react in many different ways forming non-radical products which can no longer react with molecular oxygen. The various breakdown products vary widely in their chemical and physical properties and how they impact flavor. The schematic of lipid oxidation is given in Figure 4.

initiation RH + oxygen + initiator ----> R'+ H

propagation R'+ oxygen ----> ROO'----> ROOH + R'

termination R'+ R'----> R-R, non-radical

ROO'+ ROO'----> ROOR + oxygen, non-radical

R'+ ROO'----> ROOR, non-radical

Figure 4. Autoxidation scheme for lipid oxidation

Singlet Oxidation

It is also possible for initiation to occur via a reaction of singlet oxygen with C=C bonds in RH and ROOH. Singlet oxygen can be formed through photo-chemical reactions in the presence of a sensitizer (Labuza, 1971). Plant and tissue pigments

such as chlorophyll, pheophytin, and myoglobin can act as sensitizers. Trace metals, high temperatures, and UV light have been postulated as possible initiators. Dulog (1964) found the initial rate of autoxidation initiated by singlet state oxygen was at least 10^5 to 10^6 times slower than for monomolecular decomposition at 25°C (Labuza, 1971). Therefore, initiation by singlet oxygen would show long induction periods, especially under conditions protected from UV light. The singlet state oxygen is more electrophilic than triplet state oxygen, reacting approximately 1500 times faster than triplet oxygen with moieties of high electron density, such as C=C bonds. Hydroperoxides will then cleave to initiate conventional free radical chain reactions (Nawar, 1985).

Formation of hydroperoxides by singlet oxygen proceeds via mechanisms that are different than for free radical autoxidation. Since oxygen is inserted at the ends of the double bond, linoleate produces 9-, 10-, 12-, and 13-hydroperoxides instead of 9-, and 13- from free radical autoxidation. There is general agreement that once the initial hydroperoxides are formed, the free radical chain reaction prevails as the main mechanism. Thus, giving formation of products based on free radical autoxidation (Nawar, 1985).

Breakdown of Linoleic Acid

In linoleic acid the double bonds are located at the 9,12-carbon positions. The most labile hydrogen atom on the 11-carbon is removed and a conjugated free

radical structure is formed at the 13- or 9-carbon position in its resonating form.

Hydroperoxides are decomposed to form a number of secondary products, one of which is the saturated aldehyde, hexanal. The 1,4-pentadiene structure of linoleic acid is more susceptible to oxidation (by a factor of 20) than the propene system of oleate. Other secondary products of linoleic acid include 2,4-decadienal, nonanal, and pentane. 2,4-Decadienal and nonanal can be further decomposed to form hexanal, pentane, and various other products (Fennema, 1985).

Kinetics of Lipid Oxidation

The rate of lipid oxidation is not a direct function of the degree of unsaturation, but increases rapidly as the number of double bonds increase. The increased rate is due to a reduction in activation energy required for removal of a labile methylene hydrogen atom situated between two double bonds. The activated bond once attacked forms a conjugated system which allows for resonance of the free radical and the myriad of products formed. In conditions where oxygen is not limiting, it is assumed that initially all the oxygen reacted is in the form of peroxides. Once the first hydroperoxides are produced, the chain reaction takes over. As each oxygen molecular reacts, one peroxide molecule, [ROOH], is formed (Koelsch et al., 1991).

Monomolecular Rate Period

The monomolecular decomposition of peroxides into free radicals occurs by the time many foods go into storage. A plot of the rate of oxidation vs. square root of the extent should give a straight line up to the point where either the substrate concentration decreases significantly or the peroxides decompose into secondary products (Labuza, 1971). The rate of oxidation for high levels of oxygen in the headspace, is dependent upon substrate concentration, and independent of oxygen concentration (Labuza, 1971).The breakdown of peroxides is monomolecular, therefore the rate of hexanal production is directly proportional to the peroxide level. This assumes the rate of formation of hexanal to be much faster than the rate of its disappearance, during lipid oxidation (Koelsch et al., 1991).

Bimolecular Decomposition

After monomolecular decomposition where an unsaturated lipid has been oxidized, hydroperoxide concentration builds up to a point at which a change in the initiation mechanism occurs, and peroxides begin to decompose faster than produced. In this period, the rate is directly proportional to the extent of peroxide concentration, assuming the rate of hexanal formation is equivalent to peroxide concentration (Koelsch et al., 1991).

Hall et al. (1985) described the formation of oxidative products as zero order, first order, and so forth. A mathematical model was developed for the overall

characterization of the formation of volatile fat oxidation products. It is generally accepted that the reaction has an initial linear phase (zero order), and gradually changes to an exponential phase. The point where the rate of oxidation changes from zero order to first order, the break point, describes the end of the initiation stage (induction period) and the beginning of the propagation stage. Hall et al. (1985) used the following linear models to describe zero and first order kinetics:

$$\text{zero order: } c = a_o + b_o t \qquad (1)$$

$$\text{first order: } c = a_1 e^{b1t} \; ; \; \log c = \log a_1 + b_1 t \qquad (2)$$

where: c =concentration of the volatile compound
t =storage time

A special nonlinear model was developed for applications that includes both zero and first order kinetics (Hall et al., 1985):

$$c = c_1 = a_1 e^{b(t-tb)} \; ; \; t > t_b \qquad (3)$$

where: t_b =break point

Hall et al. (1985) found the nonlinear mixed model offered a better description of the kinetics of fat oxidation than do models for first or zero order kinetics taken

separately.

Factors Influencing the Rate of Oxidation

Oxygen concentration, fatty acid composition, water activity, antioxidants, trace metals, temperature and light are all factors capable of modifying the rate of lipid oxidation.

Oxygen Concentration

The partial pressure of oxygen in the headspace of a product-package system has a direct effect on the rate of oxidation. At high oxygen concentrations, where the supply of oxygen is unlimited, the rate of oxidation is independent of oxygen pressure, but at very low oxygen pressure the rate is approximately proportional to oxygen pressure (Nawar, 1985). As seen by Koelsch (1989), at low oxygen partial pressures (approximately 1.2%) the rate of reaction was much less than at higher oxygen concentrations (approximately 15.4%). After a certain time period, the rate of lipid oxidation begins to escalate exponentially. Koelsch found the additional time needed for the rate to reach the exponential stage for oxygen concentrations of 1.2% versus 15.4% was 523 hours.

The propagation stage of autoxidation cannot occur in the absence of oxygen. A product-package system could be vacuum packed or nitrogen flushed to remove oxygen from the headspace surrounding the product, preventing autoxidation from

occurring. However, even with modern technology, lowering the oxygen concentration to 1% or less is rarely achieved. If oxygen could be completely eliminated from the environment surrounding a product, cavities within the food product may contain oxygen and this residual internal oxygen can initiate oxidation (Koelsch, 1989). Inadequate packaging, such as an inferior barrier, defective seals, damaged package, or pinholing, could allow oxygen to enter the package and initiate oxidation.

Fatty Acid Composition

Foods contain a mixture of fatty acids that significantly change the food's susceptibility to oxidation (Nawar, 1985). The number, position, and geometry of double bonds of the fatty acids present has a marked effect on the rate of oxidation. Cis or trans configurations, and conjugated double bonds affect the reactivity of the fatty acid. Free fatty acids oxidize at a greater rate than when esterified to glycerol (Nawar, 1985).

Pro-oxidant Effects

Transition metals, such as copper, cobalt, iron, manganese and nickel have major pro-oxidant effects on the rate of autoxidation. At very low levels of approximately 0.1 ppm, they work to decrease the induction period and increase the rate of oxidation. Trace metals are naturally present in all food tissues, and in

most edible oils which come from the soil (Nawar, 1985). Metal ions could also originate from the package (Strasburg, 1992).

Antioxidation

Antioxidants are added to many types of foods, as well as packaging materials to extend the shelf-life to at least equal the normal distribution and marketing time of foods. There are literally hundreds of compounds, both natural and synthetic, that possess antioxidant properties. However, their use in food is limited by health concerns and accompanying governmental regulations.

Antioxidants do not improve quality of the product or stop oxidation from occurring, but delay the onset or slow the rate of oxidation of autoxidizable materials. Antioxidants use various mechanisms to prolong the induction period of the autoxidation reaction, and generally function by interrupting the propagation of the free radical chain mechanism (Nawar, 1985). Phenols, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), act as free radical chain inhibitors by donating a hydrogen to a free radical (Labuza, 1971). BHA and BHT are typically used in conjunction with other primary antioxidants to achieve synergistic effects (Labuza, 1971). Tocopherols, naturally occurring antioxidants in vegetable oils, can survive oil processing in sufficient quantity to provide increased oxidative stability to the finished product (Nawar, 1985). Tocopherols, both natural and synthetic (alpha, gamma, delta, beta), act as free

radical chain stoppers by donation of a proton. Chelating agents such as ethylene diaminetetraacetic acid (EDTA), citric acid, and phosphates function as free radical production preventors by tying up metal catalysts.

Substantial differences in effectiveness of various antioxidants are noted when used with different types of oils or fat-containing foods. The different levels of effectiveness are due primarily to the differences in molecular structure between antioxidants. Therefore, selection of an antioxidant is product dependent. In addition to level of effectiveness, other factors such as ease of incorporation into the food, carry-through characteristics, sensitivity to pH, tendency to discolor or produce off-flavor, availability, and cost further complicate selection of an antioxidant (Nawar, 1985).

Analytical Techniques for Lipid Oxidation Analysis

Product Model

The matrix of a food product, product surface area, porosity, moisture content, and storage conditions could affect the ability of oxygen to reach oxidation-susceptible food components, influencing the rates of oxidation. Product models are often employed to reduce the variables involved in research. Flink (1971) as cited by Karel and Yong (1981) studied the influence of physical structure of freeze-dried emulsified systems on the oxidation behavior of the lipid component. He found that in freeze-dried emulsions where insoluble carbohydrates

(ungelatinized starch granules and microcrystalline cellulose) were the matrix-forming solute, dried powders were formed with all the lipid component present on the surface. In systems where proteins were used as the matrix-forming solute, the lipid was effectively encapsulated, with only small amounts of lipid present on the surface. The emulsions included a lipid (linoleic acid), water, nonvolatile solute-matrix former (microcrystalline cellulose), and an emulsifier. The insoluble carbohydrates were observed to have only surface lipid with no encapsulation and more uniform distribution than with soluble carbohydrates. This uniformity would allow for all lipid components to be exposed to headspace oxygen. When surface lipid is exposed to air, it is readily oxidized, whereas encapsulated lipid is well protected and unavailable as a site for oxygen to react (Karel and Yong, 1981). Koelsch (1989) adapted a product model consisting of microcrystalline carboxymethyl cellulose, soybean oil, tween 20, and distilled water. The product proved to be consistent and uniform throughout oxidation studies.

Product models are used in research to remove inconsistent variables which could influence the outcome of an investigation. Kogashiwa (1980) and numerous other researchers employed product models to eliminate inconsistent variables and maintain control over product characteristics in repetitive oxidation studies (Koelsch, 1989).

Indices of Oxidation

No single method for measuring the extent of lipid oxidation can possibly measure all oxidative events at once, be equally useful at all stages of the oxidative process, be applicable to all fats, all foods, or all conditions of processing (Nawar, 1985). Several methods, thiobarbituric acid test (TBA), peroxide value (PV), anisidine value (AV), the Kreis test, and more recently gas chromatography, have been used to quantify the extent of lipid oxidation.

In the TBA test, one mole of malonaldehyde, an oxidation product of unsaturated systems, reacts with two moles of thiobarbituric acid in solution to give a pink color. The greater the absorbance in the liquid state, the higher the TBA value, corresponding to a more rancid flavor (Nawar, 1985). The drawback to this method is that malonaldehyde is a very small component of oxidation, and is a secondary product of polyunsaturated systems of only 3 or more double bonds such as linolenic acid, not linoleic or oleic. Linolenic only comprises a small amount of the total fatty acid concentration in most vegetable oils. Thus, TBA tests can only be used in measuring oxidation of linolenic acid. Malonaldehyde has been known to react with proteins in an oxidizing system giving abnormally low TBA values (Nawar, 1985). TBA reagent can also react with other food components (sugars and carbohydrates) besides malonaldehyde (Nawar, 1985). This method may be more accurately defined as TBARS (thiobarbituric acid-reactive substances) to compensate for these compounds which produce the

characteristic pink chromagen (Kumor, 1986).

Peroxide Value (PV) is a measure of the ability of peroxide to liberate iodine from potassium iodide, or to oxidize ferrous to ferric ions (Nawar, 1985). Peroxides are the reaction intermediates of autoxidation, which react to form secondary reaction products. This test could be useful in the initial stages where peroxides are formed faster than they are decomposed, but as oxidation proceeds, peroxides decompose at a faster rate than they are formed. Therefore, as rancidity increases, the peroxide value may be decreasing, giving a poor correlation to sensory evaluation. Quast and Karel (1972) found that in oxidation of potato chips, in most cases peroxide value increased in storage initially, then decreased to a level lower than the starting value. Correlations between this test and development of rancid flavors have been attempted, but are inconsistent (Quast and Karel, 1972).

One of the first tests used to measure the extent of lipid oxidation was the Kreis test, which involved measurement of a red color believed to result from the reaction of epihydrin aldehyde (an isomer of malonaldehyde) or other oxidation products with phloroglucinol. The problem was the characteristic color sometimes developed in fresh non-oxidized foods, giving inconsistent results (Nawar, 1985). The Kreis test and the Oxirane, a colorimetric method based on the reaction of the oxirane group with picric acid, require direct product analysis. In direct product studies, the product must be removed from the test environment for

analysis. In a closed system, the test environment cannot be disturbed (Koelsch, 1989).

The above mentioned problems and inconsistencies with TBARs, PV, and direct product analysis led to the refining of chromatographic techniques to measure hexanal and other volatile derivatives. With the development of sensitive gas chromatograph (GC) instrumentation, the headspace technique as a measure of lipid oxidation has become widely accepted. Scholz and Ptak (1966) employed a direct injection technique in the analysis of cottonseed oil volatiles. They claimed peroxide values and rancid flavors and odors were not closely associated, and consequently chose gas chromatography for its correlation to the results obtained by flavor and odor testing panels.

Headspace isolation and concentration techniques have been developed to concentrate volatile vapors over an oxidizing product. Brinkman (1972) flushed simmering beef broth with purified nitrogen gas and collected flavor components in a porous polymer trap. Headspace trapping procedures feature a number of advantages such as small sample size (1-300g) needed, short preparation time, isolation and concentration of both low- and high boiling compounds, short sampling and analysis cycle, and reduced occurrence of artifacts (Sugisawa, 1981).

Adsorption polymers, which have a high affinity for, and reversible adsorption of, organic compounds are used for collection, concentration and subsequent GC analyses in a wide variety of applications. There are several polymer based traps

used in headspace analysis of volatiles, including Chromosorb, Porapak, and Tenax GC. Butler and Burke (1976) found Tenax GC to be good for high boiling components due to its high thermal stability and low retention volume. Good stability assures no volatiles will bleed from the trap onto the GC column during analysis, and complete regeneration of the porous polymer (Buckholz, 1980). Water vapor does not affect Tenax GC performance, which is an asset if quantifying lipid oxidation in a humidified system. Tenax GC can also be employed in the collection and desorption of volatiles of higher molecular weight. It is also excellent in adsorption of volatiles at room temperature and permits efficient desorption of the same volatiles at 200-300°C. Porous polymer traps can store collected volatiles for two weeks, and storage at 0 to 4°C or room temperature gave reproducible GC results (Sugisawa, 1981).

Analysis of hexanal via extraction from porous polymer traps has been adapted for use in products from fruit, vegetables, meats, and vegetable oils (Koelsch, 1989). When measuring the extent of lipid oxidation at constant oxygen concentrations, Koelsch used Tenax GC to isolate, concentrate, and quantify the amount of hexanal produced in the headspace of a test cell above oxidizing soybean oil. These 1/8 inch o.d. glass traps packed with the porous polymer can be inserted directly into the modified injection port of a gas chromatograph equipped with a flame ionization detector. If the GC injection port is incapable of receiving a glass trap, the hexanal can be extracted by washing the Tenax GC

with a solvent. Koelsch used HPLC grade 2-methylbutane, centrifuge, and then concentrated the solution into a septa seal vial. A syringe was then used for direct injection from the septa seal vial onto the GC column.

Hexanal

Hexanal is one of the major secondary oxidation products of linoleic acid (Frankel et al., 1981). Being a terminal product of oxidation, hexanal is less subject to further interaction with other food ingredients. Since linoleic acid comprises approximately 55% of the fatty acid content in soybean oil, and a large portion in many other vegetable oils, the accumulation of hexanal is an excellent indicator of the degree of rancidity in snack foods. Hallberg and Lingnert (1991), when boiling potato granules, claimed hexanal was the most abundant aldehyde formed. Fritsch and Gale (1977) used hexanal as a measure of rancidity in low fat foods, such as potatoes and soy products. When rancid odors were first noted, the hexanal concentration was found to be 5-10ppm. They found for these foods, a good prediction of the time required for rancidity at any temperature can be made from tests carried out at accelerated conditions. In foods whose fat contained an abundance of linoleic acid and less than 1ppm hexanal when fresh, an increase to 5ppm or more hexanal was found to indicate significant deterioration in quality due to lipid oxidation (Fritsch and Gale, 1977). Jeon et al (1984) tested the susceptibility of potato chips to oxidation under various

accelerated temperature conditions. Monitoring a sharp increase in n-hexanal was found to be a good index for accelerated stability testing.

In studying the correlation of flavor scores with instrumental measurement techniques, Goetz and Waltking (1990) found as the rate of flavor deterioration increased, the PV content decreased, and recommended that hexanal and pentane be used in predicting flavor scores. Being a stable end-product, the concentration of hexanal increases as a food product containing linoleic acid becomes increasingly rancid. Hexanal's relationship to flavor tests is not linear, but flavor tests are very subjective, and hexanal has proven to be a very effective indicator of lipid oxidation, allowing for good inferences for off-flavor development. With increased sensitivity of the gas chromatographic techniques, hexanal analysis is more reproducible, less time consuming, more simplistic, and provides a better relationship to sensory evaluation.

REFERENCES

Ahmed, E.M., Dennison, R.A., Dougherty, R.H., and Shaw, P.E. 1978. Flavor and Odor Thresholds in Water of Selected Orange Juice Components. J. Agric. Food Chem. 26: 187.

Ajuyah, A.O., Fenton, A.W., Hardin, R.T., and Sims, J.S. 1993. Measuring Lipid Oxidation Volatiles in Meats. J. Food Sci. 58: 270.

Anandaraman, S. and Reineccius, G.A. 1986. Stability of Encapsulated Orange Peel Oil. Food Technol. 11: 88.

Anonymous. 1973. Electron Spin Resonance Study of Polyethylene Irradiated with Ultraviolet Light: Effect of Crystallinity. J. Polym. Sci.: Polym. Chem. Ed. (Notes). 11: 897.

Baner, A.L., Kalyankar, V., and Shoun, L.H. 1991. Aroma Sorption Evaluation of Aseptic Packaging. J. Food Sci. 56: 1051.

Bernhard, R.A. and Marr, A.G. 1960. The Oxidation of Terpenes. I. Mechanism and Reaction Products of d-Limonene Autoxidation. Food Research 25: 517.

Buckholz Jr., L.L., Daun, H.K. 1978. Instrumental and Sensory Characteristics of Orange Oil Oxidation. J. Food Sci. 43: 535.

Buckholz Jr., L.L., Withcombe, D.A., and Henayk, D. 1980. Application and Characteristics of Polymer Adsorption Method Used to Analyze Flavor Volatiles from Peanuts. J. Agric. Food Chem. 28: 760.

Charara, Z.N., Williams, J.W., Schmidt, R.H., Marshall, M.R. 1992. Orange Flavor Absorption Into Various Polymeric Packaging Materials. J. Food Sci. 57: 963.

Cicchetti, O. 1970. Mechanisms of Oxidative Photodegradation and of UV Stabilization of Polyolefins. Adv. Polymer Sci. 7: 70.

Clark, Jr., B.C., and Chamblee, T.S. 1992. Acid-Catalyzed Reactions of Citrus Oils and Other Terpene-Containing Flavors. In *Off-Flavors in Foods and Beverages*, G. Charalambous (Ed.), 229. Elsevier Science Publishers B.V.

Clark, Jr., B.C., Jones, B.B., and Iacobucci, G.A. 1981. Characterization of the Hydroperoxides Derived from Singlet Oxygen Oxidation of (+)-Limonene. Tetrahedron, 37 (Supplement No. 1): 405.

David, J.R.D. 1992. Aseptic Processing of Foods: Market Advantages and Microbial Risks. In *Advances in Aseptic Processing Technologies*, R. Singh and P. Nelson (Ed.), 189. Elsevier Science Pub., England.

DeLassus, P.T. and Strandburg, G. 1991. Flavor and Aroma Permeability in Plastics. Food Packaging Technol., ASTM STP 1113, D. Henyon, Ed., American Society for Testing and Materials, Philidelphia, 64.

Dürr, P. and Schobinger, U. 1981. The contribution of some volatiles to the sensory quality of apple and orange juice odor. In *Flavour '81*, J. Schreier (Ed.), 179. Walter de Gruyter & Co., Berlin.

Dürr, P., Schobinger, U., and Waldvogel, R. 1981. Aroma Quality of Orange Juice after Filling and Storage in soft Packages and Glass Bottles. Lebensmittel-Verpackung 20: 91.

Fennema, O.R. 1985. Water and Ice. In *Food Chemistry*, O.R. Fennema (Ed.), 23. Marcell Dekker, New York.

Flores, H., and Morse, R.E. 1952. Deterioration of Orange Oil. Food Technol. 6: 6.

Frankel, E.N., Neff, W.E., and Selke, E. 1981. Analysis of autoxidized fats by gas chromatography-mass spectrometry: VII. Volatile thermal decomposition products of pure hydromperoxides from autoxidized and photosensitized oxidized methyl oleate, linoleate, and linolenate. Lipids. 16: 279.

Fritsch, C.W. and Gale, J.A. 1977. Hexanal as a Measure of Rancidity in Low-Fat Foods. J. Amer. Oil Chem. Soc. 54: 225.

Fukamachi, M., Matsui, T., Shimoda, M., Nakashima, M., and Osajima, Y. 1993. Sorption Depression of Flavors into Modified EVA Film by Lowering Film-Flavor Affinity. J. Agric. Food Chem. 41: 929.

Garbassi, F., Morra, M., Occhiello, E., Barino, L., Scordamaglia, R. 1989. Surf. Interface Anal. 14: 585.

Graumlich, T.R., Marcy, J.E., and Adams, J.P. 1986. Aseptically Packaged Orange Juice and Concentrate: A Review of the Influence of Processing and Packaging Conditions on Quality. J. Agric. Food Chem. 34: 402.

Gugumus, F. 1990. Photo-oxidation and Stabilization of Polyethylene. In

Mechanisms of Polymer Stabilization, G. Scott (Ed.), 169. Elsevier Science Pubs. Co., New York.

Halek, G.W. and Luttmann, J.P. 1991. Sorption Behavior of Citrus-Flavor Compounds in Polyethylenes and Polypropylenes. In *Food and Packaging Interactions II*, American Chemical Society Symposium Series 473: 212.

Hall, Anderson, Lingnert, and Olofsson 1985. Flavor Changes During Storage: Kinetics of the Formation of Volatile Fat Oxidation Products and Other Volatile Compounds. J. Food Quality 7: 153.

Hallberg, M.L. and Lingnert, H. 1991. Lipid Oxidation in Potato Slices Under Conditions Simulating to Production of Potato Granules. J. Amer. Oil Chem. Soc. 68: 167.

Hughes, D.E. 1985. Irreversible Reaction Kinetics of the Aerobic Oxidation of Ascorbic Acid. Anal. Chem. 57: 555.

Igarashi, M. 1983. Free-Radical Identification by ESR in Polyethylene and Nylon. J. Poly. Sci: Poly. Chem. Ed. 21: 2405.

Jellinek, H.H.G. 1989. The Cu-Catalyzed Oxidation of Isotactic Polypropylene and Low Density (Branched) Polyethylene Including a Brief Discussion of the Basic Autoxidation Scheme (BAS). In *Degradation and Stabilization of Polymers 2*, H.H.G. Jellinek (Ed.), 295. Elsevier Science Pubs. Co., New York.

Jeng, J., Chen, C., Lin, S., Chen, C. 1991. Interaction of Orange Juice and Inner Packaging Material of Aseptic Product. In *Food and Packaging Interactions II*, American Chemical Society Symposium Series 473: 187.

Jeon, I.S. and Bassette, R. 1984. Analysis of n-Pentanal and Hexanal as Indices of Potato Chip Shelf-Life. J. Food Quality 7: 97.

Kalus, W.H. and Filby, W.G. 1986. Loss of Ascorbic Acid During Simulated Industrial Blanching Experiments. Lebensm.-Wiss. u.-Technol. 19: 7.

Kamiya, Y. and Niki, E. 1983. The Role of Hydroperoxides in the Oxidative Degradation of Polymers. In *Degradation and Stabilization of Polymers 1*, H.H.G. Jellinek (Ed.), 337. Elsevier Science Pubs. Co., New York.

Kanner, J., Fishbein, J., Shalom, P., Harel, S., and Ben-Gera, I. 1982. Storage Stability of Orange Juice Concentrate Packaged Aseptically. J. Food Sci. 47: 429.

Karel, M. and Yong, S. 1981. Autoxidation-Initiated Reactions in Foods, in *Water Activity: Influences on Food Quality*, L.B. Rockland and G.F. Stewart (Eds.), 511. Academic Press, New York.

Kealey, K.S. and Kinsella, J.E. 1979. Orange Juice Quality with an Emphasis on Flavor Components. CRC Critical Reviews in Food Science and Nutrition 11: 1.

Kircher, K. 1987. Degradation of Polymers During Processing, in *Chemical Reactions in Plastics Processing*, 192. Hanser Publishers, New York.

Koelsch, C.M. 1989. A System for the Measurement of the Rate of Lipid Oxidation at Constant Oxygen Concentrations and Relative Humidity. Michigan State University, School of Packaging, East Lansing, MI. M.S. Thesis.

Koelsch, C.M., Labuza, T.P. and Downes T.W. 1991. Hexanal Formation via Lipid Oxidation as a Function of Oxygen Concentration: Measurement and Kinetics. J. Food Sci. 56: 816.

Konczal, J.B., Harte, B.R., Hoojjat, P., Giacin, J.R. 1991. Apple Juice Flavor Compound Sorption by Sealant Films. J. Food Sci. 56: 1024.

Kumor, E.A. 1986. The Characterization and Stability of the Lipids in Oat Cereal. Michigan State University, Dept. of Food Science and Human Nutrition, East Lansing, MI. M.S. Thesis.

Kutty, V., Braddock, R.J., Sadler, G.D. 1994. Oxidation of d-Limonene in Presence of Low Density Polyethylene. J. Food Sci. 59: 402.

Kuzuya, M., Noguchi, A., Ito, H., Kondo, S.-I., Noda, N. 1991. Electron Spin Resonance Studies of Plasma-Induced Polystyrene Radicals. J. Polym. Sci.: Part A: Polym. Chem. Ed. 29: 1.

Kuzuya, M., Niwa, J., Ito, H. 1993. Nature of Plasma-Induced Surface Radicals of Powdered Polyethylene Studied by Electron Spin Resonance. Macromolecules 26: 1990.

Labuza, T.P. 1971. Kinetics of Lipid Oxidation. CRC Crit. Reviews in Food Tech. 2: 355.

Labuza, T.P., Tannenbaum, S.R., and Karel, M. 1970. Water Content and Stability of Low-Moisture and Intermediate-Moisture Foods. Food Tech. 24: 543.

Leong, C.M.O., Harte, B.R., Partridge, J.A., Ott, D.B., Downes, T.W. 1992. Off-Flavor Development In Milk Packaged In Polyethylene-Coated Paperboard Cartons. J. Dairy Sci. 75: 2105.

Mannheim, C.H., Miltz, J., and Letzter, A. 1987. Interaction Between Polyethylene Laminated Cartons and Aseptically Packed Citrus Juices. J. Food Sci. 52: 737.

Marshall, M.R., Adams, J.P., and Williams, J.W. 1985. Flavor absorption by aseptic packaging materials. Proceedings ASEPTIPAK '85. Third International Conference and Exhibition on Aseptic Packaging, 299, Princeton, NJ.

Min, D.B., Lee, S.H., Lindamood, J.B., Chang, K.S., and Reineccius, G.A. 1989. Effects of Packaging Conditions on the Flavor Stability of Dry Whole Milk. J. Food Sci. 54: 1222.

Mohney, S.M., Hernandez, R.J., Giacin, J.R., Harte, B.R., Miltz, J. 1988. Permeability and Solubility of d-Limonene Vapor in Cereal Package Liners. J. Food Sci. 53: 253.

Moshonas, M.G. and Shaw, P.E. 1989. Flavor Evaluation and Volatile Flavor Constituents of Stored Aseptically Packaged Orange Juice. J. Food Sci. 54: 82.

Moshonas, M.G. and Shaw, P.E. 1986. Quantities of Volatile Flavor Components in Aqueous Orange Essence and in Fresh Orange Juice. Food Technol. 11 :100.

Munteanu, D. 1990. Analysis of Antioxidants and Light Stabilizers in Polymers by Modern Liquid Chromatography. In *Mechanisms of Polymer Stabilization*, G. Scott (Ed.), 211. Elsevier Science Pubs. Co., New York.

Nagy, S. 1980. Vitamin C Contents of Citrus Fruit and Their Products: A Review. J. Agric. Food Chem. 28: 8.

Nawar, W.W. 1985. Lipids. In *Food Chemistry*, O.R. Fennema (Ed.), 139. Marcell Dekker, New York.

Paik, J.S. and Tigani, M.A. 1993. Application of Regular Solution Theory in Predicting Equilibrium Sorption of Flavor Compounds by Packaging Polymers. J. Agric. Food Chem. 41: 806.

Paulsson, L.-E. 1993. Oxidation of Polyethylene Monitored by Microcalorimetry at 70°C. Thermochimica Acta 214: 141.

Pospisil, J. 1983. Antioxidants. In *Degradation and Stabilization of Polymers 1*, H.H.G. Jellinek (Ed.), 193. Elsevier Science Pubs. Co., New York.

Quast, D. and Karel, M. 1972. Effects of Environmental Factors on the Oxidation of Potato Chips. J. Food Sci. 58: 220.

Rouseff, R.L., Nagy, S., Naim, M., and Zahavi, U. 1992. Off-Flavor Development in Citrus Juice Products. In *Off-Flavors in Foods and Beverages*, G. Charalambous (Ed.), 211. Elsevier Science Publishers B.V.

Sadler, G.D. and Braddock, R.J. 1991. Absorption of Citrus Flavor Volatiles by Low Density Polyethylene. J. Food Sci. 56: 35.

Sadler, G.D. and Braddock, R.J. 1990. Oxygen Permeability of Low Density Polyethylene as a Function of Limonene Absorption: An Approach to Modeling Flavor "Scalping". J. Food Sci. 55: 587.

Sakaguchi, M., Yamaguchi, T., Shimada, S., Hori, Y. 1993. ESR Study on Molecular Motion of Chain End Radicals of Polyethylene Molecules Anchored on Fresh Surfaces of Polyethylene and Polytetrafluoroethylene. Macromolecules 26: 2612.

Sakai, Y., Watanabe, H., Takai, R., Hasegawa, T. 1987. A Kinetic Model for Oxidation of Ascorbic Acid and Beta-Carotene. J. of Food Processing and Preservation. 11: 197.

Sato, T. and Murayama, E. 1974. The Unsensitized Photooxidation of (+)-Limonene, 1,2-Dimethylcyclohexene, and *endo*-Dicyclopentadiene. Bulletin of the Chemical Society of Japan 47: 715.

Scholz, R.G. and Ptak, L.R. 1966. A Gas Chromatographic Method for Measuring Rancidity in Vegetable Oils. J. Amer. Chem. Soc. 43: 596.

Shaw, P.E., Buslig, B.S., and Moshonas, M.G. 1993. Classification of Commercial Orange Juice Types by Pattern Recognition Involving Volatile Constituents Quantified by Gas Chromatography. J. Agric. Chem. 41: 809.

Shimada, S., Kashiwabara, H., Sohma, J. 1970. ESR Studies of the Photodegradation of Polyethylene Containing Trapped Allylic Free Radicals. J. Polym. Sci.: Part A-2. 8: 1291.

Sizer, C.E., Waugh, P.L., Edstam, S., Ackerman, P. 1988. Maintaining Flavor and

Nutrient Quality of Aseptic Orange Juice. Food Technol. 43: 152.

Sohma, J. 1979. Electron Spin Resonance Studies of the Mechanical Degradation of Polymers. In *Developments in Polymer Degradation-2*, N. Grassie (Ed.), 99. Applied Science Pubs., England.

Strasburg, G. 1992. Personal Communication. Food Science and Human Nutrition, Michigan State University, East Lansing, MI.

Strömvall, A.M., and Petersson, G. 1992. Protection of terpenes against oxidative and acid decomposition on adsorbent cartridges. J. of Chromatography, 589: 385.

Sugisawa, H. 1981. Sample Preparation: Isolation and Concentration. In *Flavor Research*, K. Teranishi, R.A. Flath, and H. Sugisawa (Ed.), p.11. Marcell Dekker, New York.

Tamsma, A., Pallansch, M.J., Mucha , T.F., and Patterson, W.I. 1964. Factors related to the flavor stability of foam-dried whole milk: Effects of oxygen level. J. Dairy Sci. 44: 1644.

Tannenbaum, S.R., Archer, M.C. and Young, V.R. 1985. Vitamins and Minerals. In *Food Chemistry*, O.R. Fennema (Ed.), 488. Marcel Dekker Inc., NY.

Ting, S.V. and Newhall, W.F. 1978. Occurrence of a Natural Antioxidant in Citrus Fruit. Florida Agricultural Experiment Stations Journal, Series No. 1885: 57.

Toledo, R.T. 1986. Postprocessing Changes in Aseptically Packed Beverages. J. Agric. Food Chem. 34: 405.

Tsuji, K. 1973. Comparison of Photo- and Radiation Induced Radical Formation in Polyethylene by ESR Spectroscopy. J. Polym. Sci.: Polymer Chem. Ed. 11: 1407.

Tsuji, K. 1973. ESR Study on Radical Conversions in Irradiated Low-Density Polyethylene. J. Polym. Sci.: Polym. Chem. Ed. 11: 467.

Van der Mei, H.C., Stokroos, I., Schakenraad, J.M., and Busscher, H.J. 1991. Aging effects of repeatedly glow-discharged polyethylene: influence on contact angle, infrared absorption, elemental surface composition, and surface topography. J. Adhesion Sci. Technol. 5: 757.

Waltking, A.E. and Goetz, A.G. 1990. Instrumental Determination of Flavor

Stability of Fats and Fatty Foods and Its Correlation with Sensory Flavor Responses. CRC Critical Reviews in Food Sci. and Nutrition 19: 99.

Wilson, C.W., and Shaw, P.E. 1973. (+)-Limonene Oxidation with Selenium Dioxide-Hydrogen Peroxide. J. Org. Chem 38: 1684.

Wong, M., Stanton, D.W., and Burns, D.J.W. 1992. Effect of Initial Oxidation on Ascorbic Acid Browning in Stored Kiwifruit Juice and Model Concentrates. Lebensm.-Wiss. u.-Technol., 25: 574.

SECTION II: LOW DENSITY POLYETHYLENE INITIATED OXIDATION OF D-LIMONENE

(Paper formatted for submission to the Journal of Food Science)

ABSTRACT

Free radical ($-CH_2CHCH=CHCH_2-$) production on the surface of low density polyethylene (LDPE), exposed to heat (107°C) and treatments of heat (107°C) + 30% hydrogen peroxide (H_20_2) solution, was analyzed using electron paramagnetic resonance (EPR). As the temperature was raised from 100° to 200°C, the peak intensity of the carbon radicals gradually increased. The sensitivity of EPR, prevented detection of free radicals exposed to H_2O_2 due to residual peroxide and H_2O condensation on the surface of LDPE. D-limonene was placed in 12ml sealed glass vials containing sodium citrate buffer solution under atmospheric oxygen conditions (21% O_2). Oxidation of d-limonene, placed in intimate contact with untreated, H_20_2, and ultraviolet (UV) light (650mW/cm^2) treated LDPE. The oxidation of d-limonene in vials containing no polymer was also measured. The accumulation of carvone and carveol, oxidation products of d-limonene, were used as an index for oxidation. No polymer and UV treated samples showed significantly ($P<0.05$) higher levels of carvone and carveol than samples

containing untreated LDPE and H_2O_2 treated LDPE. Samples containing no polymer oxidized d-limonene at the highest rate (zero order), but the rate was not significantly ($P>0.05$) higher than the UV treated samples.

Key Words: free radicals, d-limonene, oxidation, sterilization

INTRODUCTION

Maintaining quality and shelf-stability of aseptically packaged citrus juices poses a significant problem due to the absorption and oxidation of select flavor and/or aroma compounds. Some variations in the shelf-life of aseptic foods are not explained by normal factors such as headspace oxygen, storage temperature, package defects or barrier properties of packaging materials. Foods and beverages sensitive to heat are processed and packaged aseptically for the purpose of providing a higher quality product with greater nutrient retention (David, 1992). Although a high quality product is produced compared to conventional canning, the product as received by the consumer is not always of superior quality.

The absorption of flavors by the food contact surface of aseptically packaged citrus juices, usually low density polyethylene (LDPE), has been the subject of intensive research (Mannheim et al., 1987; Baner et al., 1991; Paik and Tigani,

1993; Sadler and Braddock, 1991; Sadler and Braddock, 1990; Mohney et al., 1988). The volatile flavor component most often studied in oxidation and absorption research is d-limonene, the chief constituent in cold-pressed citrus oils. The problem of sorptive loss has been compensated for by the addition of extra flavor compounds to a level significantly higher than required to maintain sufficient concentration for acceptable flavor and mouthfeel. Depending upon the concentration, volatiles could become layered (unequal dispersion in food with higher concentration at interface) at the food contact surface, increasing the rate of oxidation via chain reaction. This phenomena may have a greater impact on the quality and shelf-stability of aseptically packaged foods than simple loss of flavor through scalping.

The degradation of the food contact polymer, usually LDPE, via photoxidation, irradiation, thermal extrusion processes, or heat treatment with hydrogen peroxide could contribute to the reduction of shelf-stability of aseptically packaged citrus products. During the extrusion coating process of aseptic flexible laminate cartons, free radicals can be mechanically and thermally produced. Most of these free radicals are quickly dissipated through recombination and quenching by atmospheric oxygen. These oxygenated compounds on the surface and in the bulk phase of the polymer are then available to absorb UV light during storage to continue this oxidation/degradation of the polymer.

Package sterilization processes expose the food contact layer of aseptic

packages to a 35% solution of hot H_2O_2 (85°C) followed by evaporation at temperatures of 100°C. Other manufacturers use UV light for film surface sterilization. These processes may be sufficient to create free radicals on the surface and throughout the matrix of the food contact polymer. Mannheim et al. (1987) reported that the corona-treated and untreated contact surface of polyethylene accelerated the rate of ascorbic acid degradation immersed in solution, with the oxidized film having the greatest effect. Bojkow et al. (1976) and Mannheim et al. (1987) reported the use of elevated temperatures (320°C) by some manufacturers during the coating operation may result in excessive surface oxidation and thus adversely affect the product (Leong et al., 1992). Kutty et al. (1994) found that d-limonene, absorbed in LDPE, oxidized less rapidly than d-limonene in solution, indicating that LDPE had a protective effect on d-limonene oxidation. Although free radicals dissipate quickly, they may remain while product is introduced into the container. The formation of oxygenated compounds (C=O, C-OH, COOH, etc.) and possibly free radicals on the surface and/or the bulk phase of the polymer could initiate autoxidation of compounds essential to flavor quality. The affinity/absorption of d-limonene and other select compounds to the food contact polymer could set the stage for polymer initiated oxidation at the interface, developing off-flavor and effectively reducing product storage life and quality.

The goal of this research was (1) to determine if free radicals are produced on

LDPE during the sterilization process of aseptic packaging, (2) and to determine if the food contact layer acts as a free radical donor initiating oxidation of flavor compounds in citrus juices, resulting in the development of off-flavors.

MATERIALS AND METHODS

Free radical determination in LDPE as a function of mechanical fracture and temperature

Commercially available LDPE resin beads (Tetra Pak Research Center, Buffalo Grove, IL) were frozen (-86°C) for 24 hours, then shredded to an average diameter of 0.05mm in a laboratory size Wiley mill (Arthur H. Thomas Co., Philadelphia, PA). LDPE powder was filled into Nuclear Magnetic Resonance (NMR) tubes, approximately 1.5cm from the base of the tube. Ground LDPE packed tubes were analyzed by EPR (f=9.63hz, At=10db, Mod.=2.5, SW=500g, CF=2965G, swp time=100s, time const.=100) to determine the presence of carbon or organic radicals ($-CH_2CHCH=CHCH_2-$). EPR allows for the sensitive detection of free radicals formed on the surface of polymers. Carbon radicals present in sufficient concentration are represented by a broad peak on the EPR spectra. Free radical production was analyzed at three different temperatures (100, 150, 200°C), using room temperature (21°C) as the control. A sweep was

made at room temperature to assure absence of free radicals. The heating block temperature was then raised, and a sweep made at two specific time intervals (3 and 10 min.) at each temperature platform. Experiments were completed in duplicate.

Free radical determination in LDPE as a function of hydrogen peroxide treatment

LDPE resin (Dow 724; Tetra Pak Research Center, Buffalo Grove, IL) was prepared as in the previous experiment. Approximately 1.0mg of the shredded LDPE powder was placed in the base of an NMR tube. The tube was spun horizontally over a flame, bringing the LDPE to melt temperature, and coating the base and inside walls of the tube with a uniform layer of LDPE to a height of 1.5cm from the base. The process was repeated until a coating thickness of 0.5 to 1.0mm was achieved. The tubes were then cooled in atmospheric conditions (21°C, 21% O_2) for 4 hours prior to testing.

The LDPE coated tube was placed in the heating block, equilibrated (10 min.) to a temperature of 85°C, and analyzed via EPR to obtain a baseline. A long-nosed glass eye-dropper was then forced down the length of the tube and 1 drop of H_2O_2 (30%; Fisher Scientific, Pittsburgh, PA) was deposited into the base. A second glass eye-dropper, attached to an air or nitrogen tank was placed 3/4 of the way down the tube, aiding in dissipation of residual H_2O_2. Nitrogen was utilized to prevent the interaction of oxygen with carbon radicals, which could not

be detected on EPR spectra due to the broadness of the peak formed by oxygen radicals. EPR analyses of samples began immediately after H_2O_2 was expelled from the base of the tube. Free radical formation was analyzed at temperatures of 85 and 100°C, under nitrogen and air environments.

LDPE initiated d-limonene oxidation

A 15-week experiment was performed to determine the rate of formation of carvone and carveol, the two primary oxidation products of d-limonene. Amber colored, 12ml septa-seal auto sampler vials (19x65mm; National Scientific Company, Lawrenceville, GA) were utilized as test cells for all experiments. A 0.2M citrate buffer model solution (pH 3.7) with 0.1% (w/w) sodium azide was pipetted (6ml) into each vial. The model solution was adapted from an earlier study by Kutty et al. (1994) in which d-limonene oxidation experiments were performed. To the sodium citrate buffer, 100μl of 99.9% pure d-limonene (Tastemaker, Lakeland, FL) was added.

Added to 3 separate vials, containing solutions of d-limonene, were polymer which had been treated with H_2O_2, ultraviolet light, and not treated (unoxidized). A fourth vial contained only the d-limonene solution. Triplicate vials were created for each of the 4 treatments, allowing for 12 samples to be analyzed at each sampling time.

Polymer resin was frozen and ground using a laboratory Wiley mill as in

the previous experiments. For treatment with H_2O_2 (30%), the coarse polymer powder was weighed into a micropore filter cup and placed in a preheated convection oven (107°C) for 1.5 min. Immediately upon removal, polymer powder was exposed to 10ml of hydrogen peroxide for 20s and evacuated with vacuum. While still under vacuum pressure, powder was rinsed with 20ml distilled/deionized water, sufficient to reduce the residual peroxide concentration to <0.5ppm. Residual concentrations were checked using Peroxide Test strips (EM Science, Gibbstown, NJ). Ultraviolet light treatment was accomplished by exposing a single layer of polymer powder to 650mW/cm^2 of UV light (254nm) from a distance of 15.2cm for 30s in a Spectroline Ultraviolet Cabinet (Spectronics Corp., Westbury, NY). H_2O_2 or UV light treated polymer was weighed (0.3g) and placed into the appropriate 12ml sample vials, containing d-limonene solutions, immediately post-treatment, which represented time zero. Storage, sterilization, and experimental procedures were under atmospheric oxygen (21%) condition. Once filled, vials were placed in a plexi-glass™ apparatus and rotated end-over-end at 5.5rpm in a laboratory size tumbler, assuring constant contact between d-limonene and polymer.

Headspace gas analyses

At specified time intervals, vials were removed from the tumbler and headspace gas samples withdrawn using a gas-tight syringe. Gas samples were analyzed using

a Fisher-Hamilton Gas Partitioner (Fisher Scientific Co., Pittsburgh, PA) to monitor any decrease in oxygen concentration, representing oxidation of d-limonene. Gas samples were taken prior to volatile extraction.

Volatile extraction procedure and gas chromatographic analysis

Septa-seal caps were removed from sample vials and n-octanol was added as an internal standard. D-limonene oxidation products, carvone and carveol, were removed from the model solutions using Pre-Sep Extraction Columns. Analytes were washed from extraction column using methanol, and analyzed by a 5890 Hewlett-Packard gas chromatograph (Fisher Scientific, Pittsburgh, PA) equipped with dual ionization detection (F.I.D.). A Hewlett-Packard 3392A integrator (Fisher Scientific, Pittsburgh, PA), interfaced to the gas chromatograph, was utilized to quantify carvone and carveol (μg/g). Aliquots of extractant were injected with a 5μl syringe (Hamilton Co., Reno, NV). Volatile separation took place in a SPB-5 column (Supelco Inc., Bellefonte, PA). The gas chromatograph was programmed at an initial temperature of 60°C for 2min. with a 25°C/min. increase to 145°C for 2 min. The temperature was then increased at a rate of 25°C/min. to a final temperature of 220°C.

Recovery studies were performed by preparing three standard concentrations of carvone and carveol in water. Standards were then poured into extraction cartridges and removed through the extraction process. Average area

response of three injections determined percent recovery of carvone and carveol from solution using extraction cartridges was 88%.

Statistical Analysis System (SAS Institute Inc., Cary, NC) was utilized to perform regression analysis of carvone and carveol accumulation over time.

RESULTS AND DISCUSSION

Free radical determination in LDPE

EPR analysis of LDPE exposed to mechanical fracture and temperature showed free radical production to increase as temperature increased. As the heating block temperature was raised from 100°C to a final temperature of 200°C, the peak intensity of the carbon radicals gradually increased.

Exposing LDPE to H_2O_2 at elevated temperatures proved to be difficult for EPR analysis. Upon exposure to hot LDPE, the hydrogen peroxide degraded to its constituents (H_2O and O_2). Due to the sensitivity of the EPR, the residual hydrogen peroxide and H_2O condensation build-up prevented analysis for approximately 7 min. after exposure. Once analysis proceeded, EPR spectra revealed similar results to those obtained in the previous experiment. This suggests that hydrophobic recovery (recombination) could have occurred, and/or formation of oxygen radicals, or that no radicals were formed within the 7 min.

where analysis was prevented. These results, although inconclusive, do allow for the possibility of free radical formation within the time frame that a H_2O_2 sterilized food contact surface would be exposed to a sensitive flavor ingredient. Oxygen radicals, undetected by EPR, may also contribute to initiation of autoxidation of food components.

LDPE initiated d-limonene oxidation

Headspace analysis of sample vials to monitor reduction in available oxygen, corresponding to a reduction in d-limonene due to oxidation, proved to be inadequate. The error associated with the gas partitioner was larger than the extremely small $\%O_2$ reduction realized within the headspace. No significant differences between treatments were found.

The oxidation of d-limonene produced carvone and carveol as stable end-products of the reaction, which proved to be a good index for oxidation. The Prep-Sep Extraction Columns provided an excellent method for removal of analytes from solution. The concentration of carvone and carveol increased over time, indicating a reduction in d-limonene due to autoxidation. The accumulation of carvone and carveol followed that of a zero order rate reaction (Fig 1). During the length of the experiment, 10 samples were taken over a 15 week (105 day) period.

Carvone and carveol (μg/g of d-limonene) levels at each sampling time for

vials containing untreated, hydrogen peroxide, and ultraviolet light treated LDPE, and vials without polymer, oxidized in a 21% O_2 environment are given in Tables 1 and 2, respectively. Over the 15 weeks, significantly more carvone was produced than carveol, suggesting that carvone was a more stable oxidation product of d-limonene and/or that carveol was further oxidized over time in favor of carvone.

The concentrations of carvone and carveol at each sampling time for all 4 treatments, combined to give the total oxidation of d-limonene (Fig. 1). A regression analysis (Table 3) of the combined carvone and carveol effect was performed, providing zero order rate constants for each regression line shown in Fig. 1. The samples containing no polymer showed the greatest amount of d-limonene breakdown during the 15 week storage time compared to the solutions containing treated and untreated polymer. These results are similar to those reported by Kutty et al. (1994), in which they contributed the increased rate of d-limonene oxidation to the absorption and protection of d-limonene in LDPE. Although the no polymer samples showed greater accumulation of carvone and carveol combined over time, there was no significant ($P>0.05$) difference between the no polymer and sample vials containing UV light treated polymer. Both the samples containing no polymer and UV light treated polymer were significantly ($P<0.05$) different from the untreated and H_2O_2 treated samples. These results show that d-limonene in continued contact with a polymer treated with UV light degraded more readily than when in contact with untreated or H_2O_2 treated

Table 1 - Carvone (μg/g d-limonene) accumulation in samples containing no polymer, untreated, H_2O_2, and UV light treated polymer during 15wks of storage at 21%O_2

Time (days)	No Polymer[a]	Untreated[a]	H_2O_2[a]	UV light[a]
0	359.4	359.4	359.4	359.4
7	720.3	479.9	468.4	636.0
20	998.9	297.8	342.5	740.6
28	1158.0	309.3	372.2	575.2
40	1692.0	326.0	511.7	604.9
52	1501.5	294.5	404.4	826.3
64	1784.5	332.2	437.3	999.3
75	1633.5	477.2	413.8	1431.2
85	2044.8	471.1	418.4	1566.5
95	2318.0	546.9	549.5	1682.9
105	2441.2	555.9	692.1	2609.7

[a]average of triplicate samples

Table 2 - Carveol (μg/g d-limonene) accumulation in samples containing no polymer, untreated, H_2O_2, and UV light treated polymer during 15wks of storage at 21%O_2

Time (days)	No Polymer[a]	Untreated[a]	H_2O_2[a]	UV light[a]
0	343.2	343.2	343.2	343.2
7	528.2	438.9	443.0	516.9
20	828.4	358.0	391.6	620.1
28	552.9	359.5	385.6	484.6
40	642.3	406.8	544.5	492.6
52	612.3	374.8	399.4	596.6
64	841.6	411.9	376.6	613.0
75	525.1	446.3	400.9	703.8
85	746.0	460.8	462.0	825.4
95	906.0	542.9	569.0	919.8
105	1353.5	433.9	548.2	1403.3

[a]average of triplicate samples

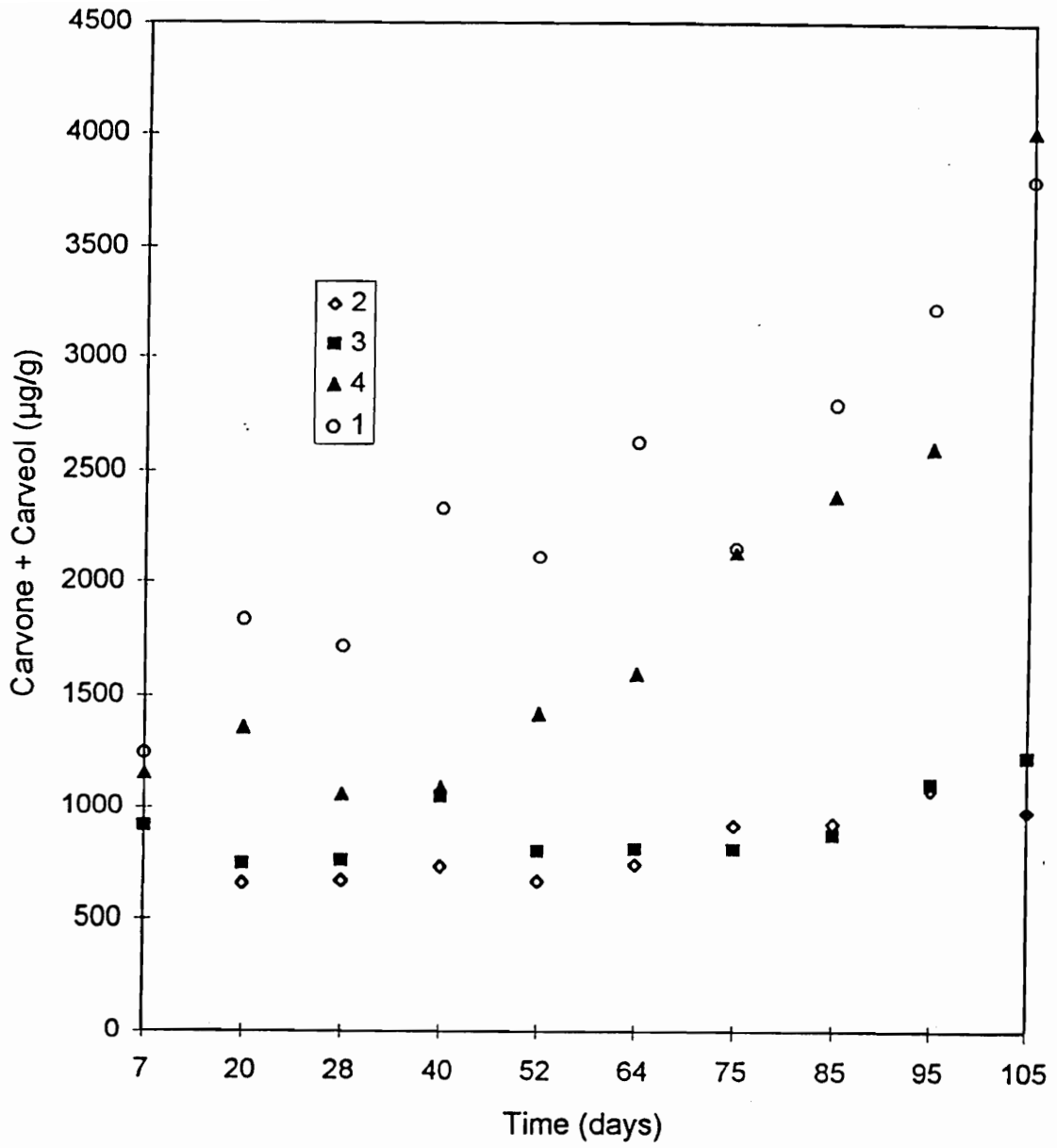


FIGURE 1. Mean (n=3) combined totals of carvone and carveol (μg/g of d-limonene) accumulation as a function of 4 different treatments (1-no polymer in contact with d-limonene; 2-untreated LDPE in contact d-limonene; 3-H_2O_2 treated LDPE in contact with d-limonene; 4-UV light treated LDPE in contact with d-limonene) at 21% O_2

Table 3 - Regression equations for the combined accumulation of carvone and carveol during 15wks of storage at 21%O_2

Treatment	Rate Equation	r^2
no polymer	c = 22.8(t) + 1039	0.85
untreated LDPE	c = 2.9(t) + 671	0.42
H_2O_2 treated LDPE	c = 3.1(t) + 732	0.30
UV light treated LDPE	c = 23.5(t) + 557	0.73

polymer, suggesting that UV light had a greater oxidizing effect on the surface of the LDPE than H_2O_2 under the experimental conditions. The untreated and H_2O_2 treated samples showed no significant ($P>0.05$) difference upon comparison.

There are numerous factors that could account for the above experimental results, including LDPE absorption of d-limonene and/or breakdown products (carvone and carveol), and a change in surface polarity of the polymer upon treatment which may have retarded absorption. However, we can state that an oxidized polymer has the ability to contribute to the development of off-flavors in aseptically packaged products, and could result in the reduction in shelf-life.

CONCLUSION

Ultraviolet light treated LDPE, in intimate contact with d-limonene, increased the rate of oxidation of d-limonene compared to untreated LDPE. An oxidized polymer in contact with food, can increase the rate at which sensitive food components are oxidized, increasing off-flavors and odors, and could contribute to a reduction in quality and/or shelf-stability. In determining the shelf-life of an aseptically processed food, an assesment of the food contact polymer must be made in regards to not only migration, scalping, barrier properties, and integrity, but also its contribution to initiating oxidation.

REFERENCES

Baner, A.L., Kalyankar, V., and Shoun, L.H. 1991. Aroma Sorption Evaluation of Aseptic Packaging. J. Food Sci. 56: 1051.

Bojkow, E., Washuttl, J., Foissy, E., Prey, T., and Steiner, I. 1976. Zum Problem der geschmacklichen Beeinflussung von Molkereiprodukten durch Packmittel. 1. Mitteilung: Sensorische und analytische Untersuchungen an PE-folien und PE-beschichteten Verbundstoffen fur die Verpackung pasteurisierter Trinkmilch. Osterr. Milchwirtsch. 31 Wiss. Beil 4: 15. Cited in Leong, C.M.O., Harte, B.R., Partridge, J.A., Ott, D.B., and Downes, T.W. 1992. Off-Flavor Development In Milk Packaged In Polyethylene-Coated Paperboard Cartons. J. Dairy Sci. 75: 2105.

David, J.R.D. 1992. Aseptic Processing of Foods: Market Advantages and Microbial Risks. In *Advances in Aseptic Processing Technologies,* R. Singh and P. Nelson (Ed.), 189. Elsevier Science Publishers, London, England.

Kutty, V., Braddock, R.J., and Sadler, G.D. 1994. Oxidation of d-Limonene in the Presence of Low Density Polyethylene. J. Food Sci. 59: 402.

Leong, C.M.O., Harte, B.R., Partridge, J.A., Ott, D.B., and Downes, T.W. 1992.

Off-Flavor Development In Milk Packaged In Polyethylene-Coated Paperboard Cartons. J. Dairy Sci. 75: 2105.

Mannheim, C.H., Miltz, J., and Letzter, A. 1987. Interaction Between Polyethylene Laminated Cartons and Aseptically Packed Citrus Juices. J. Food Sci. 52: 737.

Mohney, S.M., Hernandez, R.J., Giacin, J.R., Harte, B.R., and Miltz, J. 1988. Permeability and Solubility of d-Limonene Vapor in Cereal Package Liners. J. Food Sci. 53: 253.

Paik, J.S. and Tigani, M.A. 1993. Application of Regular Solution Theory in Predicting Equilibrium Sorption of Flavor Compounds by Packaging Polymers. J. Agric. Food Chem. 41: 806.

Sadler, G.D. and Braddock, R.J. 1991. Absorption of Citrus Flavor Volatiles by Low Density Polyethylene. J. Food Sci. 56: 35.

Sadler, G.D. and Braddock, R.J. 1990. Oxygen Permeability of Low Density Polyethylene as a Function of Limonene Absorption: An Approach to Modeling Flavor "Scalping". J. Food Sci. 55: 587.

APPENDIX A

Quantification of carveol and carvone

Using gas chromatography, area units (AU) of carvone and carveol were obtained at each sampling period for each treatment in triplicate. The averaged area units were then used to quantify concentration (micrograms per gram of d-limonene) as a function of d-limonene. Total micrograms of both carveol and carvone were quantified separately with the following equation:

$$M_g = AU\ (CF)\ (V_s)/V_i$$

where: M_g =micrograms (μg) of carveol or carvone
AU =area units from gas chromatograph
CF =calibration factor (1.35E-06μg of carveol/AU; 1.79E-06μg of carvone/AU)
V_s =sample volume
V_i =volume injected into gas chromatograph

The concentrations of carveol and carvone per grams of d-limonene (μg/g) are determined by the following equation:

$$C_c = M_g/D_w$$

where: C_c =micrograms of carvone and carveol per gram of d-limonene (μg/g)
D_w =grams of linoleic acid

APPENDIX B

A percent recovery study of carveol and n-octanol (internal standard) was performed by injecting 1μl aliquots of both compounds into 6ml of distilled and deionized water contained in five separate 12ml glass vials used for experimentation. Carveol and n-octanol were extracted from the distilled water solutions with the experimental procedure using Prep-Sep Extraction Columns and flushing with methanol to a volume of 6ml. Another five 12ml sample vials containing methanol were injected with 1μl aliquots of both compounds to provide standards. Duplicate injections of the five extracts and standards were then analyzed by gas chromatograhy to determine percent recovery.

		Standard solutions	
Sample #	Injection Vol. (μl)	n-Octanal (AU)*	Carveol (AU)*
1	0.85	264380	157450
2	0.85	266146	158021
3	0.85	268121	158363
4	0.85	264002	155416
5	0.85	269648	157984
	Average =	266639	157484
		Extract solutions	
1	0.85	249000	140780
2	0.85	251328	141519
3	0.85	244119	138544
4	0.85	252682	143728
5	0.85	249303	140113
	Average =	249286	140936

*average of duplicate injections

Percent Recovery Calculations for Prep-Sep Extraction Cartridges

%Recovery= Ea/Sa * 100

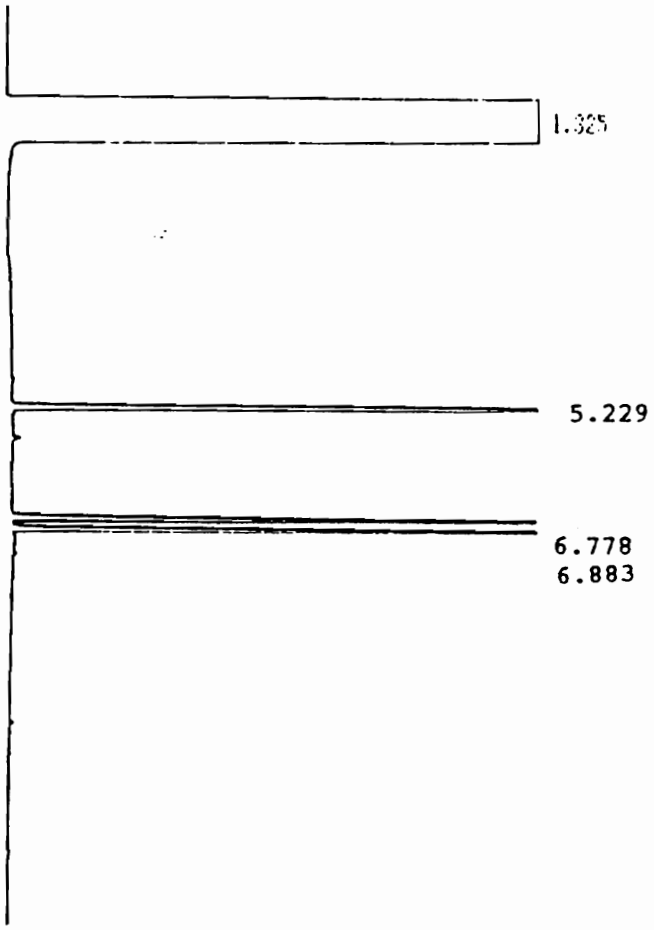
where: Ea= average area response (AU) for extraction samples
Sa= average area response (AU) for standard samples

n-Octanol:

%Recovery= {249286 / 266639} * 100 = 93.5%

Carveol:

%Recovery= {140936 / 157484} * 100 = 89.5%



APPENDIX C. Chromatogram of carveol and carvone peaks produced during the autoxidation of d-limonene. N-Octanol, carveol and carvone are the peaks corresponding to the retention times of 5.229, 6.778, and 6.883 minutes, respectively.

APPENDICES D through G show the carveol and carvone data collected from the gas chromatograph for test vials containing no polymer, and test vials containing untreated, HP treated, and UV treated LDPE at an oxygen concentration of 21%.

APPENDIX D

Model solution containing no polymer

Sample #	Time (day)	Carveol* (μg/g)	Carvone* (μg/g)		Sample #	Time (day)	Carveol* (μg/g)	Carvone* (μg/g)
1	0	351.8	365.6		1	64	760.8	1644.3
2		330.4	345.5		2		863.2	1678.9
3		347.5	366.7		3		900.8	2030.2
Average =		343.2	359.3		Average =		841.6	1784.5
1	7	528.9	682.0		1	75	589.4	1866.5
2		558.4	714.4		2		450.6	1481.0
3		497.3	764.6		3		535.3	1552.9
Average =		528.2	720.3		Average =		525.1	1633.5
1	20	767.7	958.3		1	85	763.2	2228.4
2		913.6	1074.9		2		838.4	2216.0
3		804.0	963.6		3		636.3	1690.1
Average =		828.4	998.9		Average =		746.0	2044.8
1	28	561.0	1285.9		1	95	939.5	2400.8
2		485.2	1141.7		2		976.4	2441.8
3		612.5	1046.5		3		810.8	2111.3
Average =		552.9	1158.0		Average =		906.0	2318.0
1	40	664.7	1723.2		1	105	1244.6	2317.6
2		631.4	1700.0		2		1284.4	2512.3
3		650.6	1652.8		3		1531.5	2493.7
Average =		642.3	1692.0		Average =		1353.5	2441.2
1	52	618.8	1666.9					
2		614.8	1247.4					
3		603.2	1590.1					
Average =		612.3	1501.5					

*All concentrations are averages of duplicate injections

APPENDIX E

Model solution containing untreated polymer

Sample #	Time (day)	Carveol* (μg/g)	Carvone* (μg/g)		Sample #	Time (day)	Carveol* (μg/g)	Carvone* (μg/g)
1	0	351.8	365.6		1	64	408.6	324.8
2		330.4	345.5		2		412.0	381.4
3		347.5	366.7		3		415.1	285.1
Average =		343.2	359.3		Average =		411.9	332.2
1	7	439.6	451.7		1	75	447.3	395.8
2		426.3	462.4		2		469.0	556.0
3		450.8	525.7		3		422.5	479.9
Average =		438.9	479.9		Average =		446.2	477.2
1	20	376.4	312.4		1	85	431.1	405.8
2		350.1	293.0		2		458.1	494.5
3		347.4	288.0		3		493.2	513.1
Average =		358.0	297.8		Average =		460.8	471.1
1	28	321.4	266.9		1	95	516.2	541.3
2		383.3	354.6		2		544.3	539.9
3		373.7	306.6		3		568.2	559.5
Average =		359.5	309.3		Average =		542.9	546.9
1	40	388.8	295.3		1	105	423.2	563.2
2		415.7	317.7		2		443.5	593.4
3		416.0	364.9		3		435.1	511.0
Average =		406.8	326.0		Average =		433.9	555.9
1	52	358.0	304.2					
2		387.3	291.0					
3		379.0	288.5					
Average =		374.8	294.5					

*All concentrations are averages of duplicate injections

APPENDIX F

Model solution containing hydrogen peroxide treated polymer

Sample #	Time (day)	Carveol* (μg/g)	Carvone* (μg/g)		Sample #	Time (day)	Carveol* (μg/g)	Carvone* (μg/g)
1	0	351.8	365.6		1	64	417.9	411.2
2		330.4	345.5		2		370.0	356.3
3		347.5	366.7		3		341.9	544.4
Average =		343.2	359.3		Average =		376.6	437.3
1	7	441.3	483.1		1	75	371.3	273.9
2		434.3	452.3		2		384.7	389.8
3		453.6	469.8		3		446.7	577.6
Average =		443.0	468.4		Average =		400.9	413.8
1	20	384.8	338.4		1	85	419.1	301.7
2		359.8	308.6		2		462.6	382.5
3		3430.3	380.5		3		504.1	570.8
Average =		391.6	342.5		Average =		462.0	418.4
1	28	353.0	293.5		1	95	487.8	457.7
2		421.6	325.8		2		595.3	597.9
3		382.1	497.2		3		623.8	592.9
Average =		385.6	372.2		Average =		569.0	549.5
1	40	440.3	371.4		1	105	568.2	708.2
2		627.9	593.1		2		565.6	777.2
3		565.4	562.6		3		510.8	591.0
Average =		544.5	511.7		Average =		548.2	692.1
1	52	426.0	493.3					
2		378.1	349.0					
3		394.1	371.0					
Average =		399.4	404.4					

*All concentrations are averages of duplicate injections

APPENDIX G

Model solution containing ultraviolet light treated polymer

Sample #	Time (day)	Carveol* (μg/g)	Carvone* (μg/g)		Sample #	Time (day)	Carveol* (μg/g)	Carvone* (μg/g)
1	0	351.8	365.6		1	64	607.2	1198.8
2		330.4	345.5		2		546.6	858.9
3		347.5	366.7		3		685.3	834.2
Average =		343.2	359.3		Average =		613.0	896.2
1	7	509.6	646.1		1	75	1003.1	1821.8
2		534.1	646.8		2		569.5	1255.5
3		506.9	615.2		3		538.7	1216.3
Average =		516.9	636.0		Average =		703.8	1431.2
1	20	846.8	1058.0		1	85	882.8	1729.0
2		495.4	546.8		2		802.4	1541.9
3		518.2	616.9		3		791.1	1428.5
Average		621.1	740.6		Average =		825.4	1566.5
1	28	459.8	532.2		1	95	975.8	1801.5
2		481.1	581.9		2		951.1	1716.5
3		512.7	611.4		3		832.6	1530.8
Average =		484.6	575.2		Average =		919.8	1682.9
1	40	479.3	501.5		1	105	1297.2	2308.1
2		526.5	661.8		2		1277.7	2396.8
3		472.0	651.5		3		1634.9	3124.2
Average =		492.6	604.9		Average =		1403.3	2609.7
1	52	617.5	870.0					
2		553.6	709.6					
3		618.9	899.9					
Average =		596.6	826.3					

*All concentrations are averages of duplicate injections

SECTION III: POLYMER INITIATED OXIDATION OF LINOLEIC ACID

(Paper formatted for submission to the Journal of Food Science)

ABSTRACT

Low density polyethylene (LDPE) and polyethylene terephalate (PETE) resin beads were ground and exposed to treatments of heat (107°C) + 30% hydrogen peroxide solution (H_2O_2) or ultraviolet (UV) light (650mW/cm^2), similar to aseptic sterilization techniques of food contact polymers. A model food solution containing linoleic acid was exposed to treated and untreated polymers to determine the oxidation initiating effect of oxidized food contact polymers. The accumulation of hexanal, evacuated from the headspace of sealed glass test cells containing model food solution, was used as the index of oxidation. Test conditions of 5% and 21% O_2 were maintained within test cells to determine the effect of oxygen concentration.

UV light treated PETE showed the most significant ($P<0.05$) accumulation of hexanal over time or oxidative effect. Untreated LDPE provided the least significant ($P<0.05$) oxidative effect. Linoleic acid solutions containing no polymer were shown to oxidize at a rate comparable to untreated and HP treated

PETE, and H_2O_2 and UV treated LDPE. Samples containing treated and untreated PETE oxidized significantly more rapidly than treated and untreated LDPE, indicating that the chemical structure of the polymer had a significant effect. The 21% O_2 concentration showed significantly greater production of hexanal over time, however, significant differences between treatments were independent of O_2 concentration.

Key Words: polymer oxidation, free radicals, hexanal

INTRODUCTION

Maintaining the quality of aseptically processed and packaged foods for extended periods is challenging. The shelf-stability of an aseptically processed and packaged food is affected by a number of factors, including product characteristics, the environment the product is exposed to during distribution, and the properties of the package (Harte, 1987). Some variations in the shelf-life of aseptic foods cannot be explained by factors such as headspace oxygen, distribution and/or storage temperature, or barrier properties of packaging materials. Assuming a high quality product is packaged in a hermetic fashion with an acceptable level of headspace oxygen, interactions at the product/package

interface become the most important factors affecting shelf-life. Significant research has been accomplished in the areas of flavor scalping or absorption of sensitive flavor components by the food contact polymer (Charara et al., 1992; Konczal et al., 1991; Moshonas and Shaw, 1989; Sadler and Braddock, 1991), increased oxygen permeation through food contact polymers due to swelling upon absorption (Johansson and Leufven, 1995; Piergiovanni et al., 1995; Lindberg, 1995), and migration of polymer material into the foodstuff (Culter, 1992; Feigenbaum, 1995; Leong et al., 1992). The degradation of the food contact polymer, usually low density polyethylene (LDPE), via photoxidation, irradiation, thermal extrusion processes, or heat treatment with hydrogen peroxide, individually or in combination, could be a factor in determination of aseptic product stability/degradation.

Aseptic package sterilization processes expose plastic packaging materials to extreme conditions, sufficient to create free radicals in the process of oxidizing the food contact surface. Mannheim et al. (1987) reported that corona-treated and untreated contact surface of polyethylene accelerated the rate of ascorbic acid degradation immersed in solution, with the oxidized film having the greatest effect. Bojkow et al. (1976) and Mannheim et al. (1987) reported the use of elevated temperatures by some polymer film manufacturers during paperboard coating operations, may result in excessive surface oxidation and thus adversely affect the product (Leong et al., 1992). Although free radicals dissipate quickly,

they may remain while the product is introduced into the container. The formation of oxygenated compounds and possibly free radicals on the surface and/or bulk phase of the food contact polymer could initiate autoxidation of compounds essential to flavor quality. The affinity of fatty acids and other sensitive flavor compounds to the polymer/product interface could set the stage for polymer initiated oxidation, developing off-flavor and odor, effectively reducing product storage life and quality.

Our objectives were to: (1) determine if the food contact layer acts as a free radical donor initiating oxidation of flavor compounds, (2) and to determine the effect of flavor/polymer affinity and headspace oxygen concentration, upon the development of off-flavors.

MATERIALS AND METHODS

Model Solution

A model product was developed to simulate a fluid-like, oxygen sensitive product, using linoleic acid as the oxidizable substrate and hexanal as the index for oxidation. Hexanal, one of the major secondary oxidation products of linoleic acid, is a terminal product less subject to further interaction with other food ingredients. The collection of volatiles, especially hexanal, have been extensively

used as a measure of rancidity (Fritsch and Gale, 1977; Hallberg and Lingnert, 1991). The model consisted of linoleic acid, 24.5%v/v (Fisher Scientific, Pittsburgh, PA), distilled water (75%v/v), and tween 20, 0.5%v/v (Fisher Scientific, Pittsburgh, PA). Ingredients were added into a 100ml blender cup and mixed at high speed with a laboratory blender for 3 minutes to reach desired consistency. Immediately, a 2.5ml aliquot of the model solution was pipetted into each glass test cell.

Polymer Preparation

To determine the effect of polymer polarity on initiating autoxidation of linoleic acid, two structurally different polymers were utilized. LDPE (Tetra Pak Research Center, Buffalo Grove, IL) and polyethylene terephalate (PETE) resin beads (Eastman Chemical Co., Kingsport, TN) were frozen (-86°C) for 24 hours. Frozen, brittle beads were then shredded to an average diameter of 0.5mm in a laboratory size Wiley mill (Arthur H. Thomas Co., Philadelphia, PA). For treatment with hydrogen peroxide (30%), coarse polymer powder was placed into a micropore filter cup and placed in a preheated convection oven (107°C) for 1.5 min. Immediately upon removal, polymer powder was exposed to 10ml of hydrogen peroxide (H_2O_2) for 20s and evacuated under vacuum. While still under vacuum pressure, powder was rinsed with 20ml distilled/deionized water, sufficient to reduce the residual peroxide concentration to <0.5ppm. Residual

concentrations were checked using Peroxide Test strips (EM Science, Gibbstown, NJ).

Ultraviolet light treatment was accomplished by exposing a single layer of polymer powder to 650mW/cm^2 of UV (254nm) light from a distance of 15.2cm for 30s in a Spectroline Ultraviolet Cabinet (Spectronics Corp., Westbury, NY). Hydrogen peroxide or UV light treated polymer (0.3g) was weighed and placed in the appropriate test cells immediately post-treatment. Storage and sterilization procedures were conducted in atmospheric oxygen (21% O_2) conditions.

System Design for Headspace Evacuation

A system, utilizing headspace evacuation of volatiles, was designed to quantify the rate of lipid oxidation. The system was developed to allow for the accumulation of hexanal as a result of the oxidation of linoleic acid. The production of hexanal was a function of oxygen concentration and polymer treatment. The study was conducted at ambient temperature (23±2°C) in an temperature controlled environmental chamber, in the absence of light.

A system of glass rotameters (0 to 60 scale, Cole Parmer, Chicago, IL), ball valves, and glass test cells were connected by one-eighth inch copper tubing using Swagelok fittings (Crawford Fitting Co., Solon, OH). A purified air stream was utilized for the ambient oxygen conditions (21% O_2), and a purified nitrogen stream (<0.5% O_2) purged the air stream to produce the lower oxygen

concentration (5% O_2). The gas stream of specified mixture was split into eight streams to expedite sampling. The purity of air and nitrogen gases were analyzed by withdrawing several samples and injecting them into a Fisher-Hamilton Gas Partitioner (Fisher Scientific Co., Pittsburgh, PA).

Test Cell Design and Headspace Trapping Procedure

Glass test cells (5ml) were developed at Virginia Tech Department of Chemistry to allow for oxidation of substrate under static conditions, and dynamic headspace evacuation of volatiles. The test cells consist of a 15cm long glass tube (1.5cm i.d.) designed with a threaded inlet and outlet port at opposite ends, at a 90° angle to the tube. Septa-seal, teflon coated caps (National Scientific Company, Lawrenceville, GA) were attached to each port. Once model solution and treated polymer were introduced into the test cell, a 23ga. hypodermic needle connected to the appropriate gas mixture (21%, 5% O_2) pierced the septa-seal inlet port to initiate the headspace equilibration process (15 min.). A second needle pierced the outlet port to allow for dynamic flow. During the equilibration process, headspace samples were withdrawn with a syringe and analyzed via gas partitioner to monitor test cell oxygen concentration. Needles were removed and test cells rotated end-over-end (5.5rpm) in a laboratory size tumbler. This was noted as time zero. The constant rotation of each test cell on its lateral axis promoted constant contact between polymer and lipid phase, and aided in forcing volatiles

into the gas phase. At specified time intervals, dependent upon oxygen concentration, test cells were removed from tumbler and the appropriate gas mixture was introduced through the inlet port, evacuating headspace volatiles through the outlet port. A glass capillary tube (6.5cm x 0.7cm i.d.) containing Tenax TA, 0.3g of 35/65mesh (Tekmar, Cinncinati, OH), attached to the outlet port syringe, absorbed flushed volatiles. Test cell headspace containing volatiles including n-hexanal was flushed for 30 min. prior to Tenax traps being removed. Headspace gas mixtures were static between sampling times.

Extraction Procedure and Gas Chromatographic Analysis

Hexanal was extracted from the Tenax TA by pipetting 2ml of HPLC grade 2-methylbutane (Fisher Scientific Co., Pittsburgh, PA) into the capillary tube and centrifuging (International Centrifuge, International Equipment Co., Boston, MA) for 2min. at 490g. Extractant was concentrated to 0.5ml under nitrogen prior to analysis.

A gas chromatograph (5890 Hewlett Packard, Fisher Scientific, Pittsburgh, PA) equipped with dual ionization detection (F.I.D.), interfaced with a integrator (Hewlett Packard 3392A, Fisher Scientific, Pittsburgh, PA), was used to quantify hexanal (μg/g). Aliquots of extractant were injected with a cooled 5μl syringe (Hamilton Co., Reno, NV). Volatile separation took place in a SPB-5 column (Supelco Inc., Bellefonte, PA). The gas chromatograph was programmed at an

initial temperature of 35°C for 2 min. with a 35°C/min. temperature increase to 165°C for 2 min. The temperature was then increased at a rate of 25°C/min. to a final temperature of 220°C.

Recovery studies were performed by preparing three standard concentrations of hexanal in 2-methylbutane. Standards were injected directly into Tenax TA, where hexanal was removed through the extraction process. Average area response of three injections determined percent recovery of hexanal from Tenax TA was 82.3%.

Experimental Design and Test Conditions

A three variable factorial design was utilized. The variables were polymer type (LDPE; PETE), polymer treatment (hydrogen peroxide treated resin, HP; ultraviolet light treated resin, UV; no treatment to resin, NT; no resin, NR), and headspace oxygen concentration (5%, 21%). Fourteen total treatments were split into two separate 7 treatment experiments, with respect to oxygen concentration. Experiments were conducted in triplicate under an ambient temperature (23±2°C), constant oxygen concentration, and in the absence of light. Statistical Analysis System (SAS Institute Inc., Cary, NC) was utilized to determine the significance between and within the two polymers and seven treatments at each sampling interval. Tukey's Studentized Range Test (HSD) was used for further comparisons.

RESULTS AND DISCUSSION

The oxidation of linoleic acid produced hexanal as a stable secondary reaction product, which proved to be an excellent index for oxidation. At time zero, the level of hexanal in the headspace of the test cells was undetectable by gas chromatograph. The accumulation of hexanal followed that of a first order rate reaction. There was an initial lag period of constant hexanal formation with time, before a period of exponential increase. All experiments were continued past the stage of exponential hexanal increase. The length of each experiment was dependent upon oxygen concentration, approximately 10.5 days at 21% O_2 and 20.5 days at 5% O_2.

Effect of LDPE and PETE Treated Resin on the Oxidation of Linoleic Acid at an Oxygen Concentration of 21%

Hexanal (μg/g of linoleic acid) levels at each samping interval for LDPE, PETE, and no polymer, represented as first order rate accumulations, at 21% O_2 concentration are given in Fig. 1. Treated LDPE and PETE proved to significantly accelerate the formation of hexanal compared to untreated polymer and no polymer samples. At each sampling interval there was a significant ($P<0.05$) difference in the amount of hexanal produced between polymer treatments with the treated (H_2O_2, UV) polymers showing the highest hexanal

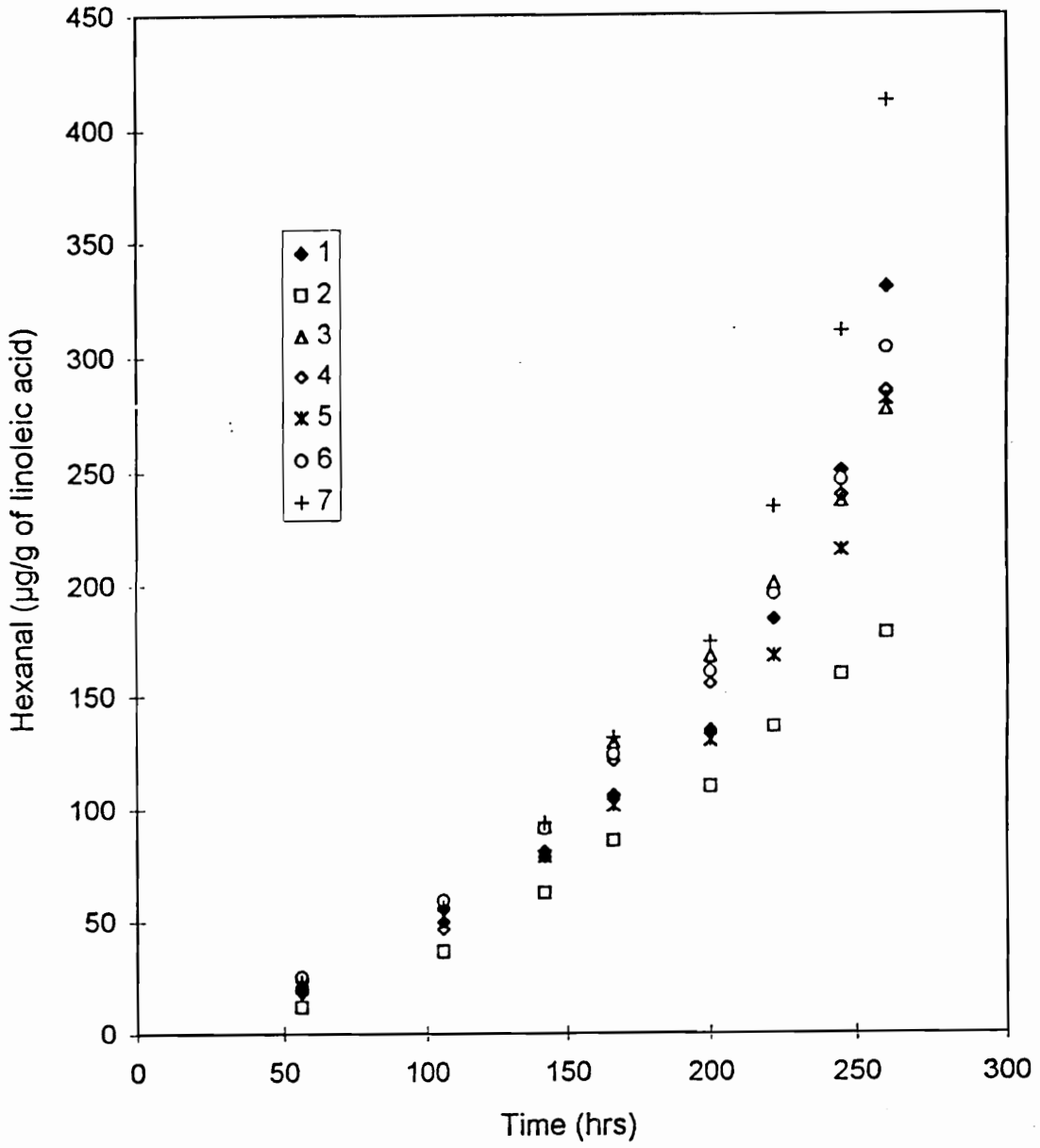


FIGURE 1. Mean (n=3) hexanal accumulation as a function of polymer type (LDPE, PETE) and treatment of polymers exposed to model solution (1-no polymers; 2-untreated LDPE; 3-hydrogen peroxide treated LDPE; 4-ultraviolet light treated LDPE; 5-untreated PETE; 6-hydrogen peroxide treated PETE; 7-ultraviolet light treated PETE) at at oxygen concentration of 21%.

levels.

After 56 hours linoleic acid in contact with treated (H_2O_2, UV) LDPE and PETE showed significantly greater oxidation as compared to untreated LDPE polymer. Glass test cells containing no polymer also showed significantly greater oxidation than untreated LDPE, but were not significantly different than treated polymer or untreated PETE. This initial sampling suggests the possibility of linoleic acid being absorbed into the untreated LDPE, retarding the effects of autoxidation. It also indicates that sensitive volatile compounds in foodstuffs may react differently towards an oxidized food contact layer.

After 200 hours, the formation of hexanal was significantly ($P<0.05$) greater for the H_2O_2 and UV treated polymers compared to the untreated polymers. The LDPE and PETE treated samples were significantly more oxidized, at this time interval, than the sample without polymer. Samples containing PETE showed larger amounts of hexanal being formed for all treatments as compared to LDPE. The samples containing untreated LDPE oxidized the slowest by a significant margin compared to all other treatments.

For PETE UV treated samples the production of hexanal began to increase exponentially around 222 hours, making it significantly different from all LDPE samples, H_2O_2 and untreated PETE samples, and the samples with no polymer added. Linoleic acid in contact with UV light treated PETE resin in solution, autoxidized at a faster rate than linoleic acid in solution without polymer. This

suggests that PETE exposed to UV light under specific conditions, becomes oxidized via free radical formation, and has the ability to contribute to the formation of off-flavor and odor in aseptically packaged products.

By the end of the experiment (260 hours), PETE UV treated samples had again produced significantly ($P<0.05$) greater oxidation of linoleic acid compared to the six other treatments. The UV light created an oxidative effect on the surface of PETE which allowed for the production of more than twice the amount of hexanal over the last sampling interval of 15 hours than all other treatments, except for the samples with no polymer. There was no significant ($P>0.05$) difference between untreated PETE, H_2O_2 treated PETE, UV treated LDPE, or H_2O_2 treated LDPE. Untreated LDPE, however, showed significantly ($P<0.05$) smaller hexanal production over most sampling intervals. This trend became increasingly evident as experimental time increased.

Effect of LDPE and PET treated resin on the oxidation of Linoleic Acid at an oxygen concentration of 5%

The experiments conducted at a headspace oxygen concentration of 5% showed similar results to those at 21% O_2. Hexanal levels at each sampling interval for treated and untreated LDPE, PETE, and no polymer in a 5% O_2 environment, represented as first order, are given in Fig. 2. At every sampling interval there was a significant ($P<0.05$) difference in the oxidation of linoleic acid between

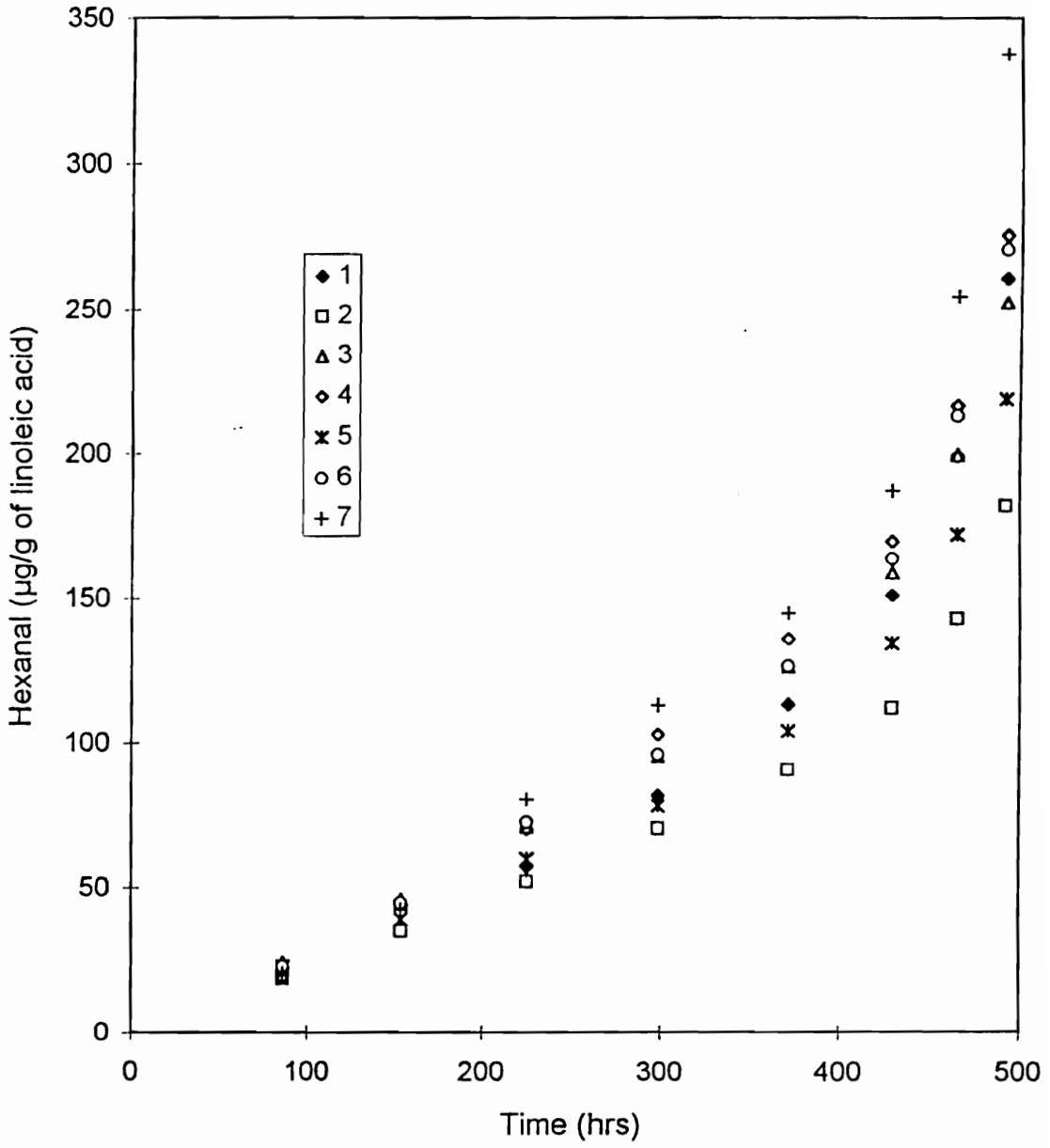


FIGURE 2. Mean (n=3) hexanal accumulation as a function of polymer type (LDPE, PETE) and treatment of polymers exposed to model solution (1-no polymers; 2-untreated LDPE; 3-hydrogen peroxide treated LDPE; 4-ultraviolet light treated LDPE; 5-untreated PETE; 6-hydrogen peroxide treated PETE; 7-ultraviolet light treated PETE) at at oxygen concentration of 5%.

treatments, with the H_2O_2 and UV treated polymers showing the greater effect. Linoleic acid in contact with a polymer oxidized by exposure to heat + hydrogen peroxide and/or ultraviolet light formed hexanal more rapidly than linoleic acid in contact with untreated polymer or no polymer.

After only 154 hours, the samples containing treated polymer showed a significantly ($P<0.05$) greater level of hexanal formation compared to samples containing untreated polymer and samples without polymer. This suggests the possible increased degradative effect of an oxidized polymer in contact with a highly oxidizable food component. The difference between H_2O_2 and UV, or PETE and LDPE was not significant ($P>0.05$).

By the third sampling (225 hours), the UV treated PETE showed the most rapid breakdown of linoleic acid to hexanal, as was the case with the experiment conducted at an oxygen concentration of 21%. UV treatment of PETE and LDPE provided a significantly more rapid oxidation of linoleic acid than H_2O_2 treatment of the polymers. These trends continued through the completion of the experiments. Samples containing PETE showed a greater oxidative effect than LDPE over the final three sampling intervals.

PETE samples treated with ultra-violet light accumulated the most hexanal over time compared to all other treatments. This suggests that the oxidation of PETE could contribute to the off-flavors and odors produced via autoxidation of lipid and/or other oxygen sensitive ingredients that migrate to the product/package

interface. Untreated LDPE samples showed the lowest accumulation of hexanal, which could indicate several lipid/polymer interactions. Linoleic acid has an affinity for non-polar, hydrocarbon polymers, and could have been absorbed by the LDPE, retarding the effects of oxidation by slowing the progress of oxygen. In contrast, untreated PETE samples had significantly greater hexanal production than untreated LDPE, possibly due to less affinity towards linoleic acid. The fact that treated LDPE samples showed significant oxidation compared to untreated samples may have been a result of free radicals in the treated LDPE that initiated linoleic acid oxidation. The oxidized polymer surface having less affinity for linoleic acid may account for minimal increases in hexanal formation. A significant amount of hexanal produced could have been absorbed by untreated LDPE while in the test cell. There was significantly more hexanal produced in test cells containing PETE compared to LDPE, indicating that PETE either was more readily oxidized and to a greater extent than LDPE, and/or absorption of linoleic acid, and possibly hexanal, was greater in LDPE.

As was expected, the accumulation of hexanal in the 5% O_2 environment was significantly less over the same time period as the 21% O_2 condition. Twice as much time was needed to reach the exponential phase of hexanal production for the 5% O_2 condition. The oxygen concentration showed no significant interaction between treatments.

CONCLUSION

Polymer sterilization procedures of aseptic packaging material could significantly impact quality and shelf-stability of the aseptically processed food. An oxidized food contact polymer could autoxidize labile food components via free radical initiation, contributing to an increase in off-flavor and reduction in shelf-life. The chemical structure or polarity of the polymer chosen for the food contact layer in an aseptic package could have a significant effect on the rate at which off-flavor and odor compounds are produced. A polar polymer, such as PETE, contains oxygenated compounds which may accelerate oxidation of the film surface. A non-polar, hydrocarbon polymer initially lacks excess oxygenated compounds, reducing its sensitivity to sterilization procedures, providing less surface oxidation which could initiate oxidation of sensitive food constituents in contact with such polymers.

REFERENCES

Bojkow, E. , Washuttl, J., Foissy, E., Prey, T., and Steiner, I. 1976. Zum Problem der geschmacklichen Beeinflussung von Molkereiprodukten durch Packmittel. 1. Mitteilung: Sensorische und analytische Untersuchungen and PE-folien und PE-beschichteten Verbundstoffen fur di Verpackung pasteurisierter Trinkmilch. Osterr. Milchwirtsch. 31 Wiss. Beil. 4: 15-23. Cited in Leong, C.M.O., Harte, B.R., Partridge, J.A., Ott, D.B., and Downes, T.W. 1992. Off-Flavor Development in Milk Packaged in Polyethylene-Coated Paperboard Cartons. J. Dairy Sci. 75: 2105.

Charara, Z.N., Williams, J.W., Schmidt, R.H., and Marshall, M.R. 1992. Orange Flavor Absorption Into Various Polymeric Packaging Materials. J. Food Sci. 57: 963.

Culter, J. 1992. Minimizing Plastic Package/Product Interactions-An Unfilled Need. J. of Plastic Film and Sheeting. 8: 28.

Feigenbaum, A., Marqu'e, D., and Riquet, A-M. 1995. Compatibility of Plastic Materials with Foodstuffs: Mechanistic and Safety Aspects of Ionized Polypropylene. P. Ackerman, M. Jagerstad, and T. Ohlsson (Ed.), 87. The Royal

Society of Chemistry, Science Park, Cambridge.

Fritsch, C.W. and Gale, J.A. 1977. Hexanal as a Measure of Rancidity in Low-Fat Foods. J. Amer. Oil Chem. Soc. 54: 225.

Hallberg, M.L. and Lingnert, H. 1991. Lipid Oxidation in Potato Slices Under Conditions Simulating Production of Potato Granules. J. Amer. Oil Chem. Soc. 68: 167.

Harte, B. 1987. Packaging of Aseptic Products. Ch. 6, In *Principles of Aseptic Processing and Packaging*, P.E. Nelson, J.V. Chambers, and J.H. Rodriguez (Ed.), 63. The Food Processors Institute, Washington, D.C.

Johansson, F. and Leufv'en, A. 1995. Food Packaging Polymers as Barrier Against Aroma Vapours and Oxygen in Fat or Humid Environments. In *Foods and Packaging Materials-Chemical Interactions,* P. Ackerman, M. Jagerstad, and T. Ohlsson (Ed.), 114. The Royal Society of Chemistry, Science Park, Cambridge.

Konczal, J.B., Harte, B.R., Hoojjat, P., Giacin, J.R. 1991. Apple Juice Flavor Compound Sorption by Sealant Films. J. Food Sci. 56: 1024.

Leong, C.M.O., Harte, B.R., Partridge, J.A., Ott, D.B., and Downes, T.W. 1992. Off-Flavor Development in Milk Packaged in Polyethylene-Coated Paperboard Cartons. J. Dairy Sci. 75: 2105.

Lindberg, B. 1995. Interaction Phenomena and Barrier Properties of Plastic Packaging Material in Relation to Foodstuff. In *Foods and Packaging Materials-Chemical Interactions,* P. Ackerman, M. Jagerstad, and T. Ohlsson (Ed.), 133. The Royal Society of Chemistry, Science Park, Cambridge.

Mannheim, C.H., Miltz, J., and Letzter, A. 1987. Interaction Between Polyethylene Laminated Cartons and Aseptically Packed Citrus Juices. J. Food Sci. 52: 737.

Moshonas, M.G. and Shaw, P.E. 1989. Flavor Evaluation and Volatile Flavor Constituents of Stored Aseptically Packaged Orange Juice. J. Food Sci. 54: 82.

Piergiovanni, L., Nicoli, P., Tinelli, L., and Vestrucci, G. 1995. Oxygen Permeability of an OPP Bottle Filled with Different Food Stimulants. In *Foods and Packaging Materials-Chemical Interactions,* P. Ackerman, M. Jagerstad, and T. Ohlsson (Ed.), 124. The Royal Society of Chemistry, Science Park, Cambridge.

Sadler, G.D. and Braddock, R.J. 1991. Absorption of Citrus Flavor Volatiles by Low Density Polyethylene. J. Food Sci. 56: 35.

APPENDIX A

Gas Chromatograph Calibration Procedure

The solutions used in the calibration procedure were hexanal (d= 0.834g/ml) and dichloromethane (used due to the low boiling point of 2-methylbutane). Volumetric flasks used for the procedure were washed, rinsed with dichloromethane and dried in a conditioned 100°C air convection oven. While flasks were dried, the purity of the dichloromethane solvent was evaluated using the GC. The GC conditions were specified in the manuscript.

Three 0.8 microliter injections, with a 5μl syringe, of dichloromethane were made into the GC. No peaks near the retention time of hexanal were evident. After one hour in the oven, flasks were taken out an cooled to room temperature, at which time the flasks could be labeled with appropriate concentrations.

1. 5μls of hexanal were added to 50mls of dicholormethane in a 50ml volumetric flask providing an initial solution with a hexanal concentration of 83.4ppm.

(0.834g/ml)(0.005ml/50ml)(1E+06)= 83.4ppm

2. 1ml of the 83.4ppm solution was added to 25ml of dichloromethane in a 25ml volumetric flask.

(83.4ppm)(1ml/25ml)= 3.34ppm

3. 1ml of the 83.4ppm solution was added to 10ml of dichloromethane in a 10ml volumetric flask.

(83.4ppm)(1ml/10ml)= 8.34ppm

4. 2ml of the 83.4ppm solution was added to 10ml of dichloromethane in a 25ml volumetric flask.

(83.4ppm)(2ml/10ml)= 16.68ppm

5. 10ml of the 83.4ppm solution was added to 25ml of dicholormethane in a 50ml volumetric flask.

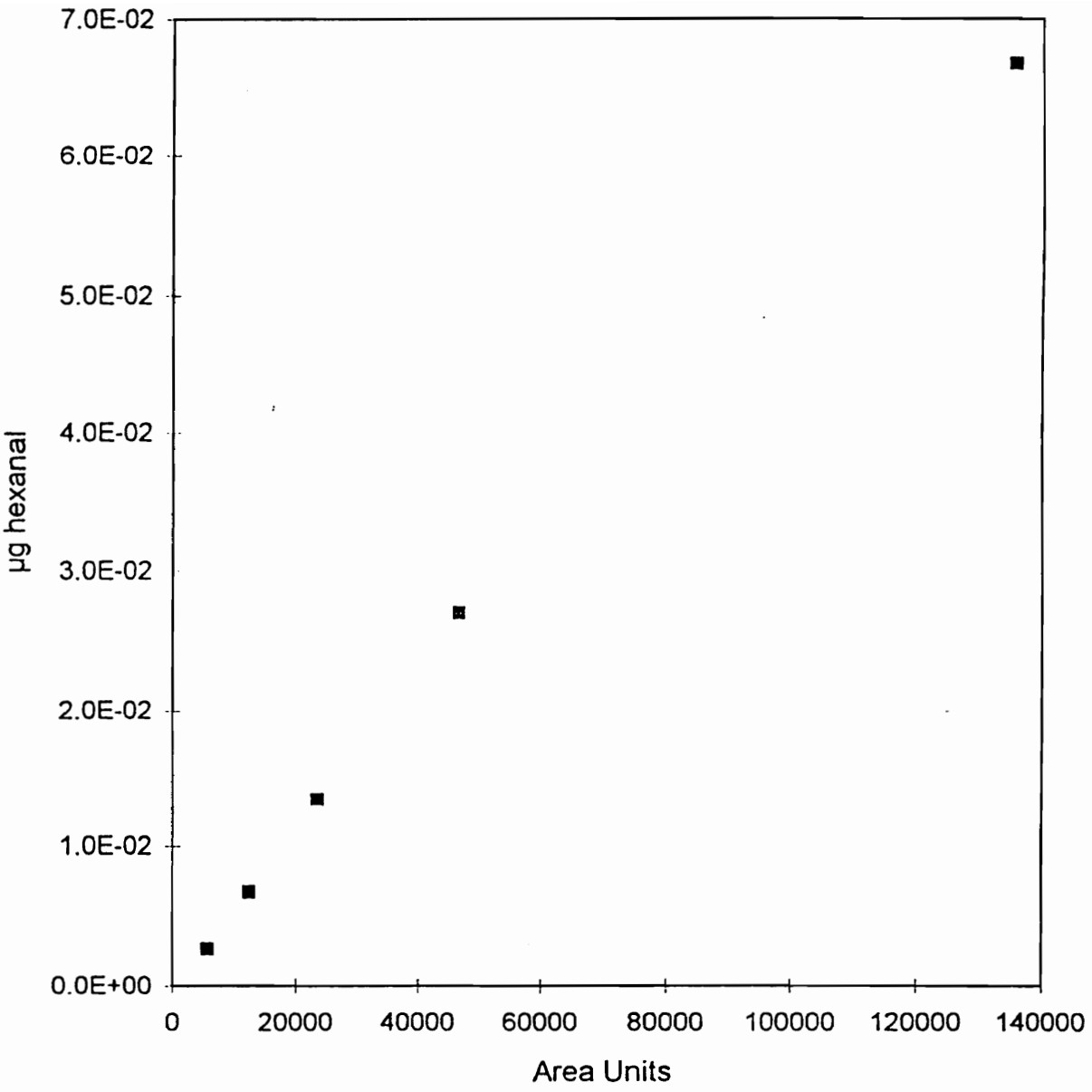
(83.4ppm)(10ml/25ml)= 33.36ppm

Three 0.8μl injections from each flask were made into the gas chromatograph, using the same 5μl syringe for all injections. After each the syringe was washed with dichloromethane and acetone, and heated in a 100°C oven for 15min. to remove all traces of solvent. The results from the three injections at each concentration were averaged and values plotted.

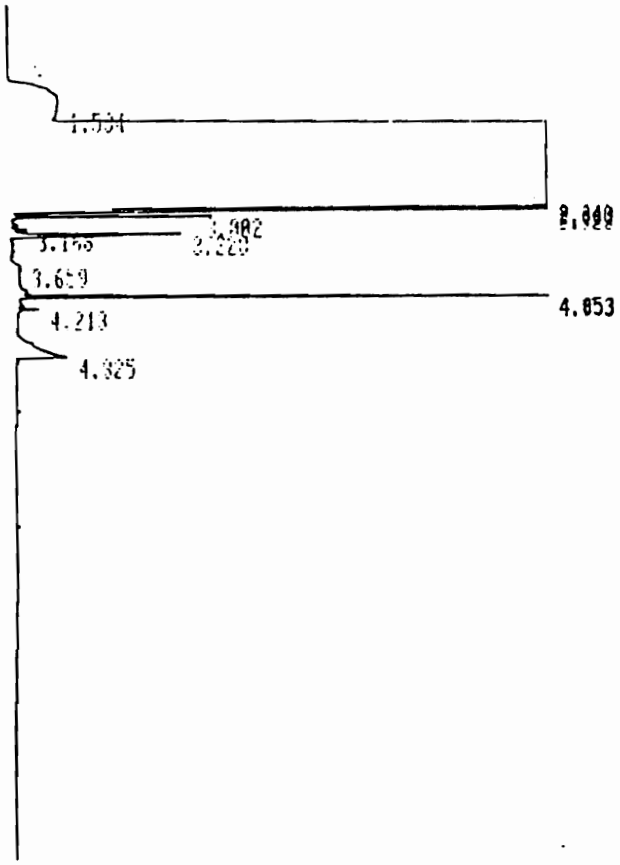
APPENDIX B

Gas Chromatographic Calibration Data for Hexanal

Sample	Volume Inj. (μl)	Hexanal Inj. (g) (E-09)	Area Response (AU)
1a	0.8	2.67	5833
1b	0.8	2.67	5484
1c	0.8	2.67	5510
		Average =	5609
2a	0.8	6.67	12094
2b	0.8	6.67	13037
2c	0.8	6.67	12168
		Average =	12433
3a	0.8	13.34	23859
3b	0.8	13.34	23611
3c	0.8	13.34	23082
		Average =	23517
4a	0.8	26.88	45898
4b	0.8	26.88	46691
4c	0.8	26.88	47335
		Average =	46641
5a	0.8	66.72	133416
5b	0.8	66.72	137036
5c	0.8	66.72	136185
		Average =	135546



APPENDIX C. Gas chromatographic calibration curve for hexanal. Each point represents the geometric mean of 3 responses.



APPENDIX D. Chromatogram of hexanal peak produced during the autoxidation of linoleic acid. Hexanal is the peak corresponding to a retention time of 4.053 minutes.

APPENDIX E

Quantification of hexanal

Using gas chromatography, area units (AU) of hexanal were obtained at each time interval for each test cell in triplicate. The averaged area units were then used to quantify hexanal concentration (micrograms per gram of linoleic acid) as a function of linoleic acid. Total grams of hexanal were quantified with the following equation:

$$H_g = AU\ (CF)\ (V_s)/V_i$$

where: H_g =micrograms (μg) of hexanal
AU =area units from gas chromatograph
CF =calibration factor (5.9E-07μg of hexanal/AU) determined from slope of calibration curve
V_s =sample volume
V_i =volume injected into gas chromatograph

The concentration of hexanal per grams of linoleic acid (μg/g) is determined by the following equation:

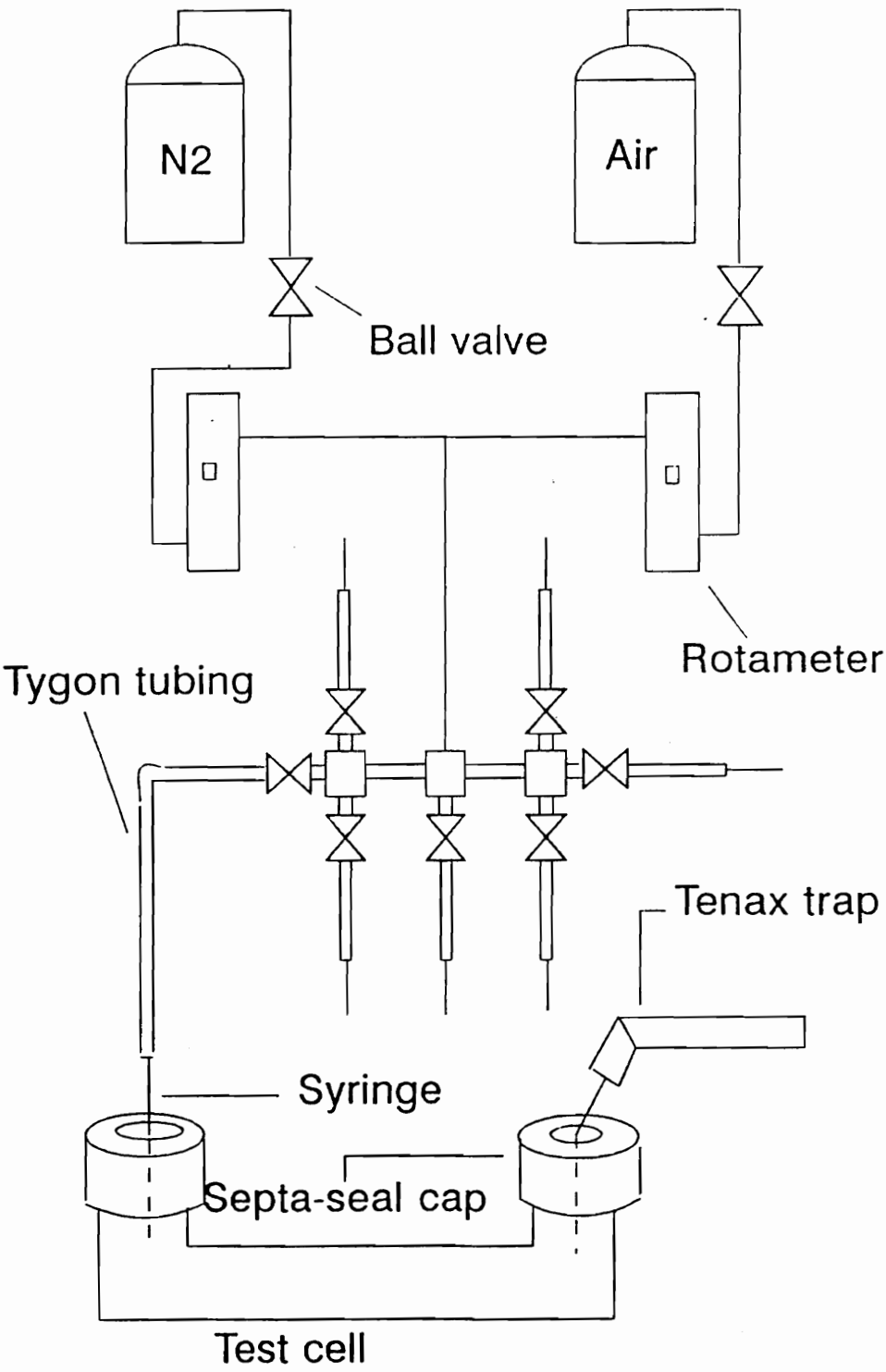
$$H_c = H_g/L_w$$

where: H_c =micrograms of hexanal per gram of linoleic acid (μg/g)
L_w =grams of linoleic acid

APPENDIX F

Tenax TA traps were conditioned overnight at 235°F with a gas flow of 20ml/min. A percent recovery study of hexanal from the Tenax TA was performed by injecting hexanal into the top of the glass tube containing tenax. The hexanal was extracted from the tenax using 2ml of 2-methylbutane. Recovery studies, using two different amounts of hexanal, were run in triplicate.

Sample #	Grams Injected	Area Response (AU)
1a	8.34E-04	18211
1b	8.34E-04	19381
1c	8.34E-04	18654
Average Area Response= 18749		
Grams of Hexanal Recovered= 6.91E-04		
Percent Recovery= 82.8%		
2a	16.68E-04	37654
2b	16.68E-04	36997
2c	16.68E-04	36215
Average Area Response= 36955		
Grams of Hexanal Recovered= 13.63E-04		
Percent Recovery= 81.7%		
Average Percent Recovery= 82.3%		



APPENDIX G. Schematic of gas flow apparatus and glass test cell allowing for headspace evacuation of hexanal into tenax trap.

APPENDICES H through N show the hexanal data collected from the gas chromatograph for test cells containing no polymer, and all test cells containing untreated, H_2O_2 treated, and UV treated PETE and LDPE at an oxygen concentration of 21%.

APPENDIX H

No polymer at an oxygen concentration of 21%

Sample	Time	Vs (ml)	Vi (μl)	AU	μg of hexanal	hexanal (μg/g)	Total hexanal
1	56	0.5	0.9	27428	8.99	18.0	18.0
2			0.65	24481	11.11	22.3	22.3
3			0.85	27055	9.39	18.8	18.8
						Average=	19.7
1	106	0.5	0.8	38693	14.3	28.6	46.6
2			0.75	34563	13.6	27.2	49.5
3			0.85	49866	17.3	34.7	53.5
						Average=	49.8
1	142	0.5	0.7	35530	15.0	30.0	76.6
2			0.85	37630	13.1	26.2	75.6
3			0.7	34624	14.6	29.2	82.7
						Average=	78.3
1	166	0.5	0.75	35938	14.1	28.3	104.9
2			0.65	31675	14.4	28.8	104.4
3			0.75	33085	13.0	26.1	108.8
						Average=	106.0
1	200	0.5	0.90	45189	14.8	29.7	134.6
2			0.95	44034	13.7	27.4	131.8
3			0.55	27006	14.5	29.0	137.8
						Average=	134.7
1	222	0.5	0.70	56914	24.0	48.0	182.6
2			0.85	65473	22.7	45.5	177.3
3			0.75	67840	26.7	53.4	191.2
						Average=	183.7
1	245	0.5	0.75	87844	34.6	69.2	251.8
2			0.65	69854	31.7	63.5	240.8
3			0.55	61698	33.1	66.3	257.5
						Average=	250.1
1	260	0.5	0.85	110095	38.2	76.5	328.3
2			0.8	114724	42.3	84.7	325.6
3			0.7	93312	39.3	78.8	336.3
						Average=	330.1

APPENDIX I

Untreated LDPE at an oxygen concentration of 21%

Sample	Time	Vs (ml)	Vi (μl)	AU	μg of hexanal	hexanal (μg/g)	Total hexanal
1	56	0.5	0.55	8183	4.4	8.8	8.8
2			0.65	12798	5.8	11.6	11.6
3			0.55	13896	7.5	14.9	14.9
						Average= 11.8	
1	106	0.5	0.85	35367	12.3	24.6	33.4
2			0.75	33467	13.1	26.4	38.0
3			0.85	34239	11.9	24.5	38.7
						Average= 36.7	
1	142	0.5	0.75	37596	14.8	29.6	63.0
2			0.90	35619	11.7	23.4	61.4
3			0.55	22779	12.1	24.5	63.2
						Average= 62.5	
1	166	0.5	0.95	33450	9.9	19.8	82.8
2			0.75	32753	12.9	25.8	87.2
3			0.70	28469	12.0	24.0	87.2
						Average= 85.7	
1	200	0.5	0.65	26224	11.9	23.8	106.6
2			0.95	43159	12.7	25.5	112.7
3			0.90	33814	11.1	22.2	109.4
						Average= 109.6	
1	222	0.5	0.60	30932	15.2	30.5	137.1
2			0.85	35420	12.3	24.6	137.3
3			0.70	29546	12.5	24.9	134.4
						Average= 136.3	
1	245	0.5	0.60	25294	12.4	24.9	162.0
2			0.70	25034	10.6	21.1	158.4
3			0.70	27279	11.5	23.0	157.4
						Average= 159.3	
1	260	0.5	0.85	28934	10.0	20.1	182.1
2			0.95	25380	7.9	15.8	174.2
3			0.95	30550	9.5	19.0	176.4
						Average= 177.6	

APPENDIX J

Hydrogen peroxide treated LDPE at an oxygen concentration of 21%

Sample	Time	Vs (ml)	Vi (μl)	AU	μg of hexanal	hexanal (μg/g)	Total hexanal
1	56	0.5	0.55	19885	10.7	21.4	21.4
2			0.60	26145	12.9	25.7	25.7
3			0.85	32272	11.2	22.4	22.4
						Average=	11.8
1	106	0.5	0.65	39643	18.0	36.0	57.4
2			0.55	31795	17.1	34.2	59.9
3			0.90	51446	16.9	33.8	56.2
						Average=	57.8
1	142	0.5	0.60	37709	18.5	37.1	94.5
2			0.80	42849	15.8	31.6	91.6
3			0.70	39154	16.5	33.1	89.3
						Average=	91.8
1	166	0.5	0.65	44888	20.4	40.8	135.3
2			0.90	54192	17.8	35.6	127.1
3			0.90	56054	18.4	36.8	126.1
						Average=	129.5
1	200	0.5	0.80	54260	20.0	40.1	175.4
2			0.80	47330	17.5	35.0	162.1
3			0.65	42211	19.2	38.4	164.4
						Average=	167.3
1	222	0.5	0.75	45635	17.9	35.9	211.4
2			0.80	36983	13.6	27.3	189.4
3			0.75	45943	18.1	36.2	200.6
						Average=	200.5
1	245	0.5	0.70	46735	19.7	39.5	250.8
2			0.70	36947	15.6	31.2	220.6
3			0.70	46280	19.5	39.1	239.7
						Average=	237.0
1	260	0.5	0.75	53918	21.2	42.5	293.3
2			0.70	40356	17.0	34.1	254.7
3			0.75	52427	20.6	41.3	281.0
						Average=	276.3

APPENDIX K

Ultraviolet light treated LDPE at an oxygen concentration of 21%

Sample	Time	Vs (ml)	Vi (μl)	AU	μg of hexanal	hexanal (μg/g)	Total hexanal
1	56	0.5	0.70	21432	9.0	18.1	18.1
2			0.45	15704	10.3	20.6	20.6
3			0.60	17199	8.5	16.9	16.9
						Average=	18.5
1	106	0.5	0.90	47694	15.6	31.3	49.4
2			0.65	29594	13.4	26.9	47.5
3			0.70	31220	13.2	26.4	43.3
						Average=	46.7
1	142	0.5	0.55	33933	18.2	36.5	85.9
2			0.65	37708	17.1	34.3	81.8
3			0.85	45631	15.8	31.7	75.0
						Average=	80.9
1	166	0.5	0.60	41791	20.5	41.2	127.0
2			0.60	44446	21.9	43.8	125.6
3			0.75	45888	18.0	36.2	111.2
						Average=	121.3
1	200	0.5	0.75	41335	16.3	32.6	159.6
2			0.70	39170	16.5	33.1	158.6
3			0.70	42865	18.1	36.2	147.3
						Average=	155.2
1	222	0.5	0.75	50522	19.9	39.8	199.4
2			0.70	41211	17.4	34.8	193.4
3			0.85	65144	22.6	45.3	192.6
						Average=	195.1
1	245	0.5	0.75	58375	23.0	46.0	245.4
2			0.75	47299	18.6	37.3	230.7
3			0.75	63181	24.9	49.8	242.4
						Average=	239.5
1	260	0.5	0.70	52543	22.1	44.4	289.7
2			0.65	40959	18.6	37.2	267.9
3			0.75	67620	26.6	53.3	295.7
						Average=	284.4

APPENDIX L

Untreated PETE at an oxygen concentration of 21%

Sample	Time	Vs (ml)	Vi (μl)	AU	μg of hexanal	hexanal (μg/g)	Total hexanal
1	56	0.5	0.75	26527	10.4	20.9	20.9
2			0.70	28765	12.1	24.3	24.3
3			0.70	25426	10.7	21.5	21.5
						Average=	22.2
1	106	0.5	0.90	45156	14.8	29.6	50.5
2			0.80	40740	15.0	30.1	54.4
3			0.80	42470	15.7	31.4	52.8
						Average=	52.6
1	142	0.5	0.90	44480	14.6	29.2	79.7
2			0.65	25771	11.7	23.4	77.8
3			0.95	43521	12.8	25.7	78.5
						Average=	78.7
1	166	0.5	0.80	30239	11.2	22.3	102.1
2			0.65	25924	11.8	23.6	101.4
3			0.80	30239	11.2	22.3	100.9
						Average=	101.4
1	200	0.5	0.80	41738	15.4	30.8	132.9
2			0.85	39670	13.8	27.6	128.9
3			0.85	40801	14.2	28.4	129.2
						Average=	130.4
1	222	0.5	0.75	49749	19.6	39.2	172.1
2			0.75	45646	17.9	36.0	164.9
3			0.80	48102	17.7	35.5	164.8
						Average=	167.3
1	245	0.5	0.70	56828	23.9	47.9	220.1
2			0.85	58659	20.4	40.8	205.7
3			0.70	67084	28.3	56.6	221.4
						Average=	215.7
1	260	0.5	0.80	82195	30.3	60.7	280.8
2			0.85	81491	28.3	56.6	262.3
3			0.65	85686	38.9	77.9	299.3
						Average=	280.8

APPENDIX M

Hydrogen peroxide treated PETE at an oxygen concentration of 21%

Sample	Time	Vs (ml)	Vi (μl)	AU	μg of hexanal	hexanal (μg/g)	Total hexanal
1	56	0.5	0.45	17098	11.2	22.5	22.5
2			0.65	29190	13.2	26.5	26.5
3			0.70	31681	13.4	26.7	26.7
						Average=	25.2
1	106	0.5	0.80	39829	14.7	29.4	51.9
2			0.75	46189	18.2	36.4	62.9
3			0.60	37371	18.4	36.8	63.5
						Average=	59.4
1	142	0.5	0.80	40565	15.0	30.0	81.8
2			0.85	45147	15.7	31.4	94.3
3			0.80	45594	16.8	33.7	97.2
						Average=	91.1
1	166	0.5	0.90	45026	14.8	29.6	111.4
2			0.70	41194	17.4	34.8	129.1
3			0.55	32825	17.6	35.3	132.5
						Average=	124.3
1	200	0.5	0.75	40719	16.0	32.1	143.5
2			0.55	37097	19.9	39.9	168.9
3			0.65	40771	18.5	37.1	169.6
						Average=	160.7
1	222	0.5	0.65	38778	17.6	35.3	178.7
2			0.70	42889	18.0	36.2	205.1
3			0.65	35736	16.2	32.5	202.0
						Average=	195.3
1	245	0.5	0.70	63973	27.0	54.0	232.7
2			0.75	59428	23.4	46.8	251.9
3			0.70	60458	25.4	51.0	253.1
						Average=	245.9
1	260	0.5	0.80	82802	30.5	61.2	293.9
2			0.75	70339	27.7	55.4	307.4
3			0.85	80400	27.9	55.9	308.9
						Average=	303.4

APPENDIX N

Ultraviolet light treated PETE at an oxygen concentration of 21%

Sample	Time	Vs (ml)	Vi (μl)	AU	μg of hexanal	hexanal (μg/g)	Total hexanal
1	56	0.5	0.80	30526	11.3	22.5	22.5
2			0.65	25368	11.5	23.1	23.1
3			0.80	32820	12.1	24.2	24.2
						Average=	23.3
1	106	0.5	0.65	36838	16.7	33.5	56.0
2			0.45	22286	14.6	29.3	52.3
3			0.80	48561	17.9	35.9	60.1
						Average=	56.2
1	142	0.5	0.65	42243	19.2	38.4	94.4
2			0.85	52072	18.1	36.2	88.5
3			0.60	38047	18.7	37.5	97.6
						Average=	93.5
1	166	0.5	0.80	47589	17.5	35.1	129.6
2			0.65	44538	20.2	40.5	129.0
3			0.60	38774	19.1	38.2	135.8
						Average=	131.5
1	200	0.5	0.80	52344	19.3	38.7	168.2
2			0.85	64173	22.3	44.6	173.6
3			0.70	50311	21.2	42.5	178.2
						Average=	173.4
1	222	0.5	0.70	71726	30.2	60.5	228.8
2			0.60	66277	32.6	65.3	238.9
3			0.60	59321	29.2	58.4	236.7
						Average=	234.8
1	245	0.5	0.75	104180	41.0	82.1	310.9
2			0.65	80426	36.5	73.1	312.0
3			0.75	93661	36.8	73.8	310.4
						Average=	311.1
1	260	0.5	0.60	112729	55.4	111.0	421.9
2			0.60	100550	49.4	99.0	411.0
3			0.65	102168	46.4	92.9	403.3
						Average=	412.1

APPENDIX O. Hexanal levels ($\mu g/g$ of linoleic acid) as a function of polymer type (LDPE, PETE) and treatment of polymers exposed to model solution (NP-no polymers; NT-untreated polymer; HP-hydrogen peroxide treated polymer; UV-ultraviolet light treated polymer) at oxygen concentration of 21%.

		LDPE			PETE		
Time (hrs.)	NP	NT[a]	HP[a]	UV[a]	NT[a]	HP[a]	UV[a]
0	0	0	0	0	0	0	0
56	19.6	11.8	23.2	18.5	22.2	25.2	23.3
106	49.8	36.7	57.8	46.7	52.6	59.4	56.2
142	78.3	62.5	91.8	80.9	78.7	91.1	93.5
166	106.0	85.7	129.5	121.3	101.4	124.3	131.5
200	134.7	109.6	167.3	155.2	130.4	160.7	173.4
222	183.7	136.3	200.5	195.1	167.3	195.3	234.8
245	250.1	159.3	237.0	239.5	215.7	245.9	311.1
260	330.1	177.6	276.3	284.4	280.8	303.4	412.1

[a]average of triplicate samples

APPENDIX P

System Flow Rates and Gas Partitioner Calibration for the 5% Oxygen Condition

The tank pressures and flow rates needed to establish the desired O_2 concentration (5±0.5%) were determined. An equilibration time of 15min. was given prior to testing for O_2 concentration. Gas was analyzed by removing samples from the syringe end of the tygon tubing, used for flushing the test cells during experimentation. 10cc of gas was removed and analyzed using a Hewlett-Packard Gas Partitioner.

Trial #	Tank*	Flow Rate (ml/min.)	O_2 Conc. (%)
1	Air	30	12.4
	N_2	31	
2	Air	30	9.7
	N_2	32	
3	Air	30	5.3
	N_2	33	
4	Air	30	3.1
	N_2	34	
5**	Air	30	4.9
	N_2	32.5	

*Tank pressure was held constant at 40psi for both air and nitrogen tanks throughout calibration

**Rotameter settings for trial #5 were used throughout all experiments

APPENDIX Q

Headspace Gas Analysis during experiments conducted at an Oxygen Concentration of 5%

Two glass sample vials (5ml), with teflon septa-seal closures, were used for gas analysis throughout experiments to monitor headspace gas composition. The two vials were filled with 2ml of the model solution and flushed with the same gas mixture as glass test cells at time zero. At each sampling interval the headspace gas composition of the vials was analyzed using a gas partitioner, then flushed with a fresh gas mixture at the same time as test cells. The following table gives gas composition at the time of flushing and sampling.

Sampling Time (hours)	$\%O_2$ Concentration Flushing Gas Comp.	$\%O_2$ Concentration Sampling Gas Comp.
0	5.2	5.3
86	5.4	5.5
154	5.0	5.2
225.5	4.9	5.0
298.5	5.1	5.1
371.5	5.4	5.4
429	4.8	4.9
465	4.7	4.9
492	4.7	5.0

APPENDICES R through X show the hexanal data collected from the gas chromatograph for test cells containing no polymer, and all test cells containing untreated, H_2O_2 treated, and UV treated PETE and LDPE at an oxygen concentration of 5%.

APPENDIX R

No polymer at an oxygen concentration of 5%

Sample	Time	Vs (ml)	Vi (μl)	AU	μg of hexanal	hexanal (μg/g)	Total hexanal
1	86	0.5	0.65	21739	9.9	19.8	19.8
2			0.70	27293	11.5	23.0	23.0
3			0.70	26484	11.2	22.4	22.4
						Average=	21.7
1	154	0.5	0.70	18448	7.8	15.6	35.3
2			0.70	18454	7.8	15.6	38.6
3			0.75	20058	7.9	15.8	38.1
						Average=	37.4
1	225.5	0.5	0.65	22649	10.3	20.6	55.9
2			0.80	25994	9.6	19.2	57.8
3			0.75	24156	9.5	19.0	57.2
						Average=	57.0
1	298.5	0.5	0.70	26066	11.0	22.0	77.9
2			0.60	23503	11.6	23.1	81.0
3			0.75	36457	14.3	28.7	85.9
						Average=	81.6
1	371.5	0.5	0.65	33455	15.2	30.4	108.3
2			0.60	31235	15.4	30.8	111.7
3			0.65	33724	16.6	33.2	119.1
						Average=	113.1
1	429	0.5	0.60	38497	18.9	37.9	146.3
2			0.70	44881	18.9	37.9	149.6
3			0.65	41374	18.8	37.6	156.7
						Average=	150.9
1	465	0.5	0.60	53978	26.5	53.2	199.4
2			0.65	53384	24.2	48.5	198.1
3			0.70	51658	21.8	43.6	200.3
						Average=	199.3
1	492	0.5	0.70	69727	29.4	58.9	258.3
2			0.75	78641	30.9	61.9	260.1
3			0.65	70311	31.9	63.9	264.3
						Average=	260.9

APPENDIX S

Untreated LDPE at an oxygen concentration of 5%

Sample	Time	Vs (ml)	Vi (μl)	AU	μg of hexanal	hexanal (μg/g)	Total hexanal
1	86	0.5	0.85	31710	11.0	22.0	22.0
2			0.75	23733	9.3	18.7	18.7
3			0.85	22406	7.8	15.6	15.6
						Average=	18.8
1	154	0.5	0.85	27482	9.5	19.1	41.1
2			0.80	21951	8.1	16.2	34.9
3			0.65	16328	7.4	14.8	30.4
						Average=	35.5
1	225.5	0.5	0.70	20155	8.5	17.0	58.2
2			0.85	24788	8.6	17.2	52.1
3			0.90	23497	7.7	15.4	45.8
						Average=	52.0
1	298.5	0.5	0.90	24511	8.0	16.1	74.3
2			0.70	18851	7.9	15.9	68.1
3			0.70	25764	10.9	21.7	67.6
						Average=	70.0
1	371.5	0.5	0.70	22800	9.6	19.2	93.5
2			0.60	23353	11.5	23.0	91.1
3			0.65	21693	9.8	19.7	87.3
						Average=	90.6
1	429	0.5	0.60	20164	9.9	19.9	113.4
2			0.75	28096	11.1	22.1	113.2
3			0.65	24487	11.1	22.3	109.6
						Average=	112.0
1	465	0.5	0.65	34047	15.5	31.0	144.3
2			0.60	32111	15.8	31.6	144.8
3			0.70	35089	14.8	29.6	139.2
						Average=	142.8
1	492	0.5	0.75	47919	18.8	37.8	182.1
2			0.65	43611	19.8	39.6	184.5
3			0.70	48102	20.3	40.6	179.8
						Average=	182.1

APPENDIX T

Hydrogen peroxide treated LDPE at an oxygen concentration of 5%

Sample	Time	Vs (ml)	Vi (μl)	AU	μg of hexanal	hexanal (μg/g)	Total hexanal
1	86	0.5	0.65	25577	11.6	23.3	23.3
2			0.80	37530	13.8	27.7	27.7
3			0.65	25543	11.6	23.2	23.2
						Average=	24.7
1	154	0.5	0.65	26595	12.1	24.2	47.4
2			0.55	18513	9.9	19.9	47.6
3			0.55	17781	9.5	19.1	42.3
						Average=	45.8
1	225.5	0.5	0.65	28812	13.1	26.2	73.6
2			0.80	33168	12.2	24.5	72.1
3			0.75	30251	11.9	23.8	66.2
						Average=	70.6
1	298.5	0.5	0.85	43424	15.1	30.2	103.8
2			0.55	19661	10.5	21.1	93.2
3			0.60	22479	11.1	22.1	88.3
						Average=	95.1
1	371.5	0.5	0.65	34741	15.8	31.6	135.4
2			0.65	36229	16.4	32.9	126.2
3			0.70	34235	14.4	28.9	117.2
						Average=	126.2
1	429	0.5	0.70	36759	15.5	31.0	166.4
2			0.65	38748	17.6	35.2	161.4
3			0.70	37318	15.7	31.5	148.7
						Average=	158.8
1	465	0.5	0.60	41727	15.5	41.1	207.5
2			0.70	49855	21.0	42.1	203.5
3			0.70	46717	19.7	39.4	188.1
						Average=	199.7
1	492	0.5	0.75	69622	27.4	54.9	262.4
2			0.70	59234	25.0	50.0	253.5
3			0.70	63587	26.8	53.7	241.8
						Average=	252.5

APPENDIX U

Ultraviolet light treated LDPE at an oxygen concentration of 5%

Sample	Time	Vs (ml)	Vi (μl)	AU	μg of hexanal	hexanal (μg/g)	Total hexanal
1	86	0.5	0.70	25689	10.8	21.7	21.7
2			0.60	17646	8.7	17.4	17.4
3			0.70	23190	9.8	19.6	19.6
						Average=	19.5
1	154	0.5	0.65	26664	12.1	24.2	45.9
2			0.45	15568	10.2	20.4	37.8
3			0.70	35771	15.1	30.2	49.8
						Average=	44.5
1	225.5	0.5	0.70	29415	12.4	24.8	70.8
2			0.70	38841	12.2	24.5	62.3
3			0.80	36292	13.4	26.8	76.6
						Average=	69.9
1	298.5	0.5	0.65	34028	15.4	30.9	101.7
2			0.60	30997	19.1	38.3	100.5
3			0.65	33037	15.0	30.0	106.6
						Average=	102.9
1	371.5	0.5	0.70	42585	17.9	35.9	137.6
2			0.60	30997	15.2	30.5	131.1
3			0.70	32348	15.9	31.9	138.5
						Average=	135.7
1	429	0.5	0.75	40568	16.0	32.0	169.6
2			0.70	41428	17.5	35.0	166.0
3			0.70	41940	17.7	35.4	173.9
						Average=	169.8
1	465	0.5	0.70	54272	22.9	45.8	215.4
2			0.75	60308	23.7	47.5	213.6
3			0.70	55421	23.4	46.8	220.6
						Average=	216.5
1	492	0.5	0.70	74598	31.4	63.0	278.4
2			0.70	69940	29.5	59.0	272.6
3			0.75	71353	28.1	56.2	276.9
						Average=	275.9

APPENDIX V

Untreated PETE at an oxygen concentration of 5%

Sample	Time	Vs (ml)	Vi (μl)	AU	μg of hexanal	hexanal (μg/g)	Total hexanal
1	86	0.5	0.60	24200	11.9	23.8	23.8
2			0.65	25523	11.6	23.2	23.2
3			0.60	23488	11.5	23.1	23.1
						Average=	23.4
1	154	0.5	0.75	19637	7.7	15.5	39.3
2			0.75	20352	8.0	16.0	39.2
3			0.80	22611	8.3	16.7	39.8
						Average=	39.5
1	225.5	0.5	0.80	25616	9.4	18.9	58.2
2			0.80	25995	9.6	19.2	58.4
3			0.65	24100	10.9	21.9	61.7
						Average=	59.5
1	298.5	0.5	0.65	17465	7.9	15.9	74.1
2			0.70	22068	9.3	18.6	77.1
3			0.65	22704	10.3	20.6	82.4
						Average=	77.8
1	371.5	0.5	0.65	31947	14.5	29.0	103.1
2			0.65	26028	11.8	23.7	100.7
3			0.70	30967	13.1	26.1	108.5
						Average=	104.1
1	429	0.5	0.60	30984	15.2	30.5	133.7
2			0.65	31597	14.3	28.7	129.4
3			0.60	31901	15.7	31.4	139.9
						Average=	134.3
1	465	0.5	0.60	38684	19.0	38.1	171.8
2			0.70	43081	18.2	36.4	165.8
3			0.65	42204	19.2	38.4	178.3
						Average=	172.0
1	492	0.5	0.75	58911	23.2	46.4	218.2
2			0.60	50436	24.8	49.7	215.5
3			0.65	48770	22.1	44.3	222.6
						Average=	218.8

APPENDIX W

Hydrogen peroxide treated PETE at an oxygen concentration of 5%

Sample	Time	Vs (ml)	Vi (μl)	AU	μg of hexanal	hexanal (μg/g)	Total hexanal
1	86	0.5	0.95	34454	10.7	21.4	21.4
2			0.70	30615	12.9	25.8	25.8
3			0.60	23723	11.7	23.4	23.4
						Average=	23.4
1	154	0.5	0.70	28267	11.9	23.9	45.3
2			0.70	25335	10.7	21.4	47.2
3			0.65	20411	9.3	18.6	41.9
						Average=	44.8
1	225.5	0.5	0.80	36915	13.6	27.3	72.6
2			0.80	35737	13.2	26.4	73.6
3			0.90	42818	14.0	28.1	70.0
						Average=	72.1
1	298.5	0.5	0.60	22546	11.1	22.2	94.8
2			0.60	24573	12.1	24.2	97.8
3			0.60	25397	12.5	25.0	95.0
						Average=	95.9
1	371.5	0.5	0.65	38741	14.5	35.2	130.0
2			0.65	32372	14.7	29.4	127.3
3			0.55	24932	13.4	26.8	121.8
						Average=	126.4
1	429	0.5	0.65	43651	19.8	39.7	169.7
2			0.65	40766	18.5	37.1	164.3
3			0.75	44697	17.6	35.2	157.0
						Average=	163.7
1	465	0.5	0.55	47850	25.7	51.4	221.1
2			0.70	58667	24.7	49.5	213.8
3			0.70	55966	23.6	47.2	204.3
						Average=	213.1
1	492	0.5	0.75	69515	27.3	54.8	275.8
2			0.75	76251	30.0	60.1	273.9
3			0.70	70356	29.7	59.4	263.7
						Average=	271.1

APPENDIX X

Ultraviolet light treated PETE at an oxygen concentration of 5%

Sample	Time	Vs (ml)	Vi (μl)	AU	μg of hexanal	hexanal (μg/g)	Total hexanal
1	86	0.5	0.65	25045	11.4	22.8	22.8
2			0.65	22385	10.2	20.3	20.3
3			0.80	27211	10.0	20.1	20.1
						Average=	21.1
1	154	0.5	0.90	35816	11.7	23.5	46.3
2			0.55	19274	10.3	20.7	41.1
3			0.70	24621	10.4	20.7	40.9
						Average=	42.7
1	225.5	0.5	0.65	42243	19.2	38.4	84.7
2			0.85	52072	18.1	36.2	77.3
3			0.60	38047	18.7	37.5	78.4
						Average=	80.1
1	298.5	0.5	0.60	34866	17.1	34.3	119.0
2			0.60	31160	15.3	30.7	107.9
3			0.75	42469	16.7	33.5	111.8
						Average=	112.9
1	371.5	0.5	0.60	31823	15.6	31.3	150.4
2			0.65	37596	17.1	34.2	142.1
3			0.60	30043	14.8	29.6	141.4
						Average=	144.6
1	429	0.5	0.65	47816	21.7	43.5	193.8
2			0.65	43056	19.5	39.1	181.3
3			0.60	45908	22.6	45.2	186.6
						Average=	187.2
1	465	0.5	0.65	71611	32.5	65.1	258.9
2			0.70	79242	33.4	66.9	248.1
3			0.65	77390	35.1	70.4	257.0
						Average=	254.7
1	492	0.5	0.65	97489	44.2	88.6	347.5
2			0.60	86664	42.6	85.3	333.5
3			0.70	89633	37.8	75.7	332.6
						Average=	337.9

APPENDIX Y. Hexanal levels (μg/g of linoleic) as a function of polymer type (LDPE, PETE) and treatment of polymers exposed to model solution (NP-no polymers; NT-untreated polymer; HP-hydrogen peroxide treated polymer; UV-ultraviolet light treated polymer) at oxygen concentration of 5%.

		LDPE			PETE		
Time (hrs.)	NP	NT[a]	HP[a]	UV[a]	NT[a]	HP[a]	UV[a]
0	0	0	0	0	0	0	0
86	21.7	18.8	24.7	19.5	23.4	23.5	21.1
154	37.4	35.5	45.8	44.5	39.5	44.8	42.7
225	57.0	52.0	70.6	69.9	59.5	72.1	80.1
298	81.6	70.0	95.1	102.9	77.8	95.9	112.9
371	113.1	90.6	126.2	135.7	104.1	126.4	144.6
429	150.9	112.0	158.8	169.8	134.3	163.7	187.2
465	199.3	142.8	199.7	216.5	172.0	213.1	254.7
492	260.9	182.1	252.5	275.9	218.8	271.1	337.9

[a]average of triplicate samples

VITA

Corey Lee Berends was born on April 27, 1969 in Grand Forks, North Dakota. He graduated from Grosse Ile High School, on Grosse Ile, MI, in June of 1987. He spent six years at Michigan State University in East Lansing, MI, studying in the School of Packaging. During his six years at MSU he coached, participated in, and was president of the school's rowing team. He received his B.S. in 1991 and M.S. in 1993 in Packaging Engineering. In the fall of 1993 he began his Ph.D. program at Virginia Polytechnic Institute and State University in the Food Science and Technology Department.