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MASS TRANSFER OF α - TOCOPHEROL (VITAMIN E) FROM A MULTI-LAYER LAMINATE STRUCTURE AND ITS INFLUENCE ON PRODUCT STABILITY

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JU-FEN LIN

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Jack K. Giacin
Major professor

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MASS TRANSFER OF α-TOCOPHEROL (VITAMIN E) FROM A MULTI-LAYER LAMINATE STRUCTURE AND ITS INFLUENCE ON PRODUCT STABILITY

By

Ju-Fen Lin

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ABSTRACT

MASS TRANSFER OF α-TOCOPHEROL (VITAMIN E) FROM A MULTI-LAYER LAMINATE STRUCTURE AND ITS INFLUENCE ON PRODUCT STABILITY

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The antioxidant, α-tocopherol (ATP) was incorporated into a coextruded laminate film consisting of a heat seal layer, a core layer of high density polyethylene (HDPE) impregnated with ATP, and an outer HDPE layer. The rate of loss of the antioxidant from the laminate film was determined as a function of time and temperature and was found to follow a first order rate expression, with an activation energy of 28.87 kcal/mole.

The effectiveness of ATP and BHT impregnated laminate films to retard the oxidation of a packaged oat cereal product was also evaluated. The extent of oxidation was based on the level of hexanal in the product. Product packaged in pouches containing no antioxidant exhibited a significantly high level of lipid oxidation, as compared to the product packaged in the antioxidant impregnated laminate structures. Both antioxidants appeared to provide a similar level of effectiveness, in terms of retarding product oxidation.

DEDICATION

This thesis is dedicated to my parents and my sister, for their support, encouragement, patience, and love throughout this work.

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TABLE OF CONTENTS

LIST OF TABLES vii

LIST OF FIGURES ix

INTRODUCTION 1

LITERATURE REVIEW 4

- General Review of Oxidation 4
- Mechanism of Oxidation 5
- Methods of Measurement Oxidation 6
  - Peroxide Value (PV) 7
  - Thiobarbituric Acid Reactive Substances (TBARS) 8
  - Hexanal 9
- Composition of Oat Cereal 12
- General Review of Antioxidants. 14
- Mechanism of Antioxidant 18
- Characteristics of Hindered Phenolic Synthetic Antioxidants 19
- 2-(1,1-Dimethylethyl)-1,4-Benzenediol (TBHQ) 19
- 2-Tertiary-Butyl-4-Methoxy Phenol (BHA) 20
  - 3,5-Di-Tertiary-Butyl-4-Hydroxytoluene (BHT) .. 21
  - n-Propyl-3,4,5-Trihydroxybenzoate (PG) 23
- Characteristics of Natural Antioxidants 25
  - Ascorbic Acid 25
  - Rosmarinus Officinalis 26
  - α-Tocohperol 28
- Application of Antioxidant in Packaging Materials .. 31
- Theory of Migration 41
- Technology for Trapping Volatile 46
- Determination of Antioxidants 50

MATERIAL AND METHODS 52

- Cereal Product 52
- Packaging Material 52
- Film Storage Studies 55
- Antioxidant Loss Rate Studies 55
- High Pressure Liquid Chromatography (HPLC) Analysis
  - Extraction Procedure 56

Percent Recovery of Antioxidant for Extraction Procedure 56
HPLC Analysis 57
Product Stability Studies 59
Hexanal Quantification 63
Apparatus for Trapping of Volatiles 63
Hexanal Analysis Procedure 68
Hexanal Calibration Curve Procedure 70

Antioxidant Content of Cereal 71
Lipid Extraction 71
Antioxidant Content of Extracted Fat 72

RESULT AND DISCUSSION 74
Thickness of Packaging Materials 74
Loss of α-Tocohperol from HDPE Film 75
% Recovery for Antioxidant 79
Relative Rate Lossed of Antioxidants 87
Product Storage Studies 93
Retained Levels of Antioxidant in Packaging Sturctures 98
Antioxidant Content of Cereal Product 102

SUMMARY AND CONCLUSION 108

FUTURE STUDIES 110

APPENDICES 112

LIST OF REFERENCES 123

LIST OF TABLES

1. Antioxidants permitted in foods in the United States 16

2. Average thickness for lamination film 75

3. α-Tocopherol concentration (wt/wt%) from fresh roll at different time interval 76

4. Loss of α-tocopherol from coextruded laminate film at 23°C determined by HPLC analysis 80

5. Loss of α-tocopherol from coextruded laminate film at 30°C determined by HPLC analysis 81

6. Loss of α-tocopherol from coextruded laminate film at 40°C determined by HPLC analysis 82

7. Loss of α-tocopherol from coextruded laminate film at 50°C determined by HPLC analysis 83

8. Rate constants for the loss of α-tocopherol from the coextruded laminate film sturcture 84

9. The hexanal concentration of cereal product packaged in pouches fabricated from test coextruded laminate sturctures 96

10. α-Tocopherol content in packaging pouches as a function of storage time 101

11. BHT content in packaged cereal product following storage at 23°C 107

12. α-Tocopherol calibration curve 112

13. Hexanal data for the first calibration curve 114

14. Hexanal data for the second calibration curve 116

15. Thickness (μm) measurements performed by optical microscopy 118

16. Thickness (μm) measurements performed by micrometer 119

17. Percent recovery data and calculations 120

LIST OF FIGURES

1. The structure of TBHQ 20

2. The structure of BHA 21

3. The structure of BHT 22

4. The structure of PG 24

5. The structure of ascorbic acid 26

6. The structure of the rosemary antioxidant, rosmarinic acid? 27

7. Tocopherol structure 29

8. Cross section of three multi-layer structure 53

9. The storage arrangement for cereal package 61

10. Flow diagram of the test scheme 62

11. Schematic diagram of dynamic purge and trap system 64

12. The apparatus with the sparging tube attached 66

13. Photograph of dynamic purge and trap system 67

14. Loss of ATP from the coextruded laminate film as a function of time/temperature 85

15. Arrhenius plot of loss rate constant versus temperature 86

16. Relative percent loss of BHT and α-tocopherol from coextruded laminated film as a function of time (23°C) 90

17. Relative percent loss of BHT and α-tocopherol from coextruded laminated film as a function of time (30°C) 91

18. Relative percent loss of BHT and α-tocopherol from coextruded laminated film as a function of time (40°C) 92

19. Hexanal concentration in cereal product packaged in pouches fabricated from test coextruded laminated structures 97

20. Schematic of the mechanism of antioxidant activity of a polymeric packaging material 106

21. α-Tocopherol calibration curve 113

22. First hexanal calibration curve 115

23. Second hexanal calibration curve 117

INTRODUCTION

Antioxidants have been used as an additive in food production for a number of years to prevent lipid oxidation. Antioxidants function by delaying the onset of oxidation by acting as free radical scavengers or metal chelating agents, and may be classified as either synthetic or natural. A recent innovation has emerged in the use of antioxidants in the manufacture of polymeric packaging materials, such as high density polyethylene (HDPE), to minimize the effect of oxidative degradation (Food Engineering, 1979).

Antioxidants such as 3,5-di-tert-butyl-4-hydroxytoluene (BHT) have been incorporated within cereal packaging for a period of time (Charnas, 1992). As currently used, BHT not only stabilizes cereal liner films, but also functions as an antioxidant for the food product itself, inhibiting lipid oxidation and extending its shelf life.

Due to the high level of unsaturated fatty acids in cereal grains, such products are subject to attack by oxygen, resulting in lipid oxidation. Impregnating packaging materials with an antioxidant will act to inhibit product oxidation. For example, Hoojjat et al.(1987) reported that

BHT impregnated HDPE film retarded the rate of oxidation of an oatmeal cereal. The proposed mechanism of antioxidant activity involves the following three-step process:

(1) antioxidant diffusion through the polymer bulk phase;

(2) evaporation of antioxidant from the surface of the packaging materials; and (3)subsequent antioxidant sorption onto the surface of the packaged product.

BHT is widely used in food packaging. However, because of its low molecular weight, volatility and suspicion of being a cancer causing agent (Charnas, 1992), there has been considerable interest expressed in developing BHT alternatives (Monks, 1992).

Recently, α-tocopherol was evaluated for the rather unconventional use as a polymer stabilizer for packaging film, when used at low concentrations, such as 100 ppm(wt/wt) (Urata, 1988; Laermer, 1990). Researchers (Laermer et al., 1993; Zambetti, 1995) have described its utility in the processing of polyolefins such as polyethylene and polypropylene, where the α-tocopherol functions to inhibit polymer oxidation during thermal processing. Their studies showed polypropylene containing α-tocopherol had higher heat and color stability, when

compared to a polypropylene sample which had a commercial antioxidant added. α-Tocopherol also showed lower volatility and lower migration characteristics than other commercial antioxidants on the market.

The present study focuses on determining the effect of temperature on the mass transfer properties of BHT and α-tocopherol from a multi-layer laminate film. The laminates consisted of an inner heat seal layer, a core layer of high density polyethylene impregnated with the antioxidant, and an outer HDPE layer.

The specific objectives of this study included :

(1) To determine the rate of loss of α-tocopherol from the test laminate film as a function of time and temperature.

(2) To evaluate the effectiveness of α-tocopherol and BHT impregnated laminates in inhibiting lipid oxidation of a cereal product via an evaporation-sorption mechanism.

(3) Utilizing data obtained from the rate loss studies to develop a better understanding of the transfer mechanism of α-tocopherol from packaging film to product.

LITERATURE REVIEW

General review of oxidation

Lipids become rancid as a result of oxidation, and rancidity is a major cause of food deterioration. Oxidative reactions occur as a result of such factors as the presence of oxygen or heavy metals, exposure to light and heat, the degree of product lipid unsaturation, product enzymes etc.. Oxidation of unsaturated fatty acids has been reported to be the primary factor in product rancidity (Buck, 1984). Oxidation is the result of the loss of electrons from a molecule or atom, and the corresponding reduction of the recipient component.

Some fat- or oil- rich products, such as crackers and potato chips, are easily oxidized as a result of their large surface area. Product oxidation results in the development of rancid off-flavors, odors, discoloration of pigments, change in texture, loss of nutritional value, and even toxic oxidation products (Dziezak, 1986).

Mechanism of oxidation

Oxidation of fats and oils is believed to occur as an autocatalytic reaction, which involves the following three steps:

(1) initiation, when free radicals are produced. At this step, the flavor and odor of the substance are slightly affected; (2) propagation, when free radicals react with oxygen to produce hydroperoxide radicals (ROO•), which react further with other unsaturated hydrocarbons to regenerate the free radicals and yield hydroperoxides (ROOH); and (3) termination, which involves the combination of two radicals with the formation of stable products (Nawar, 1985; Dugan 1976; Paquette et al., 1985). The reaction sequence of the three-step oxidation process is as follows:

Initiation : $RH \xrightarrow{\text{activation}} R\bullet$ (free radicals)

Propagation : $R\bullet + O_2 \longrightarrow ROO\bullet$

$ROO\bullet + RH \longrightarrow ROOH + R\bullet$

Termination : $R\bullet + R\bullet \longrightarrow RR$

$R\bullet + ROO\bullet \longrightarrow ROOR$

$ROO\bullet + ROO\bullet \longrightarrow ROOR + O_2$

RH refers to any unsaturated fatty acid, which has an active C-H group adjacent to a double bound. In the initiation stage, this hydrogen atom is easily removed from a carbon next to a double bound, when it has been activated. With light, lipoxygenases or a metal catalyst present, the unsaturated fatty acid, RH is easily activated. In the propagation step, oxygen quickly adds to the free radicals, forming an unstable peroxy free radical (ROO•). This peroxy free radical abstracts a hydrogen atom producing a hydroperoxide (ROOH). Hydroperoxides (ROOH) are the major initial reaction products from the reaction of a fatty acid with oxygen. At the termination stage, hydroperoxides readily decompose into various secondary byproducts such as aldehydes, ketones and hydrocarbons. These byproducts are the major source of rancid odor and flavors (Pokorny, 1971; Nawar, 1985).

Method of Measurement Oxidation

Sensory analysis provides a direct method to evaluate product oxidation. However, it is not a quantitative technique to determine the extent of product oxidation, and it lacks reproducibility. The following three methods are

the most popular being used to determine the extent of product oxidation. (1) Peroxide value (PV); (2) Thiobarbituric acid reactive substance (TBARS); and (3) Hexanal analysis. These three methods provide reproducibility, sensitivity and quantitativeness (Gray, 1978; Melton, 1983).

Peroxide Value (PV)

Hydroperoxides, commonly called peroxides, are the primary products of lipid oxidation. Therefore, measuring the peroxide concentration will determine the level of oxidation. Numerous analytical procedures for the measurement of the peroxide value are described (Dahle, 1962; Agbo, 1992; Van de Voort et al., 1994; Lrevi, et al., 1991). The American Oil Chemists' Society (AOCS) official iodometric method Cd 8-53 is generally used. Peroxide values can be measured based on their ability to liberate iodine from potassium iodide, or to oxidize ferrous to ferric ions. However, the results of this method have been described as highly empirical and questionable. The results vary with details of the procedure used and the test is extremely sensitive to temperature changes (Gray, 1978).

The other errors associated with this method are: (1) iodide will be adsorbed at unsaturated bonds of the fatty material; and (2) the liberation of iodine from potassium iodide by oxygen present in the solution to be titrated (Mehlenbacher, 1960). Since the peroxides are likely to decompose to other secondary byproducts, it is not a good choice for measurement of oxidation over long periods of time. Therefore, the peroxide value is applicable only at the early stages of oxidation.

Thiobarbituric Acid Reactive Substances (TBARS)

Kohn and Liversadge (1944) observed that a pink color was found when animal tissue, which had been incubated aerobically, reacted with 2-thiobarbituric acid (TBA). This pink color was formed from the oxidation product and TBA. The oxidation product has been reported to be the dicarbonyl compound, malonaldehyde, which is generated from the degradation of unsaturated fatty acids (Dahle et al., 1962).

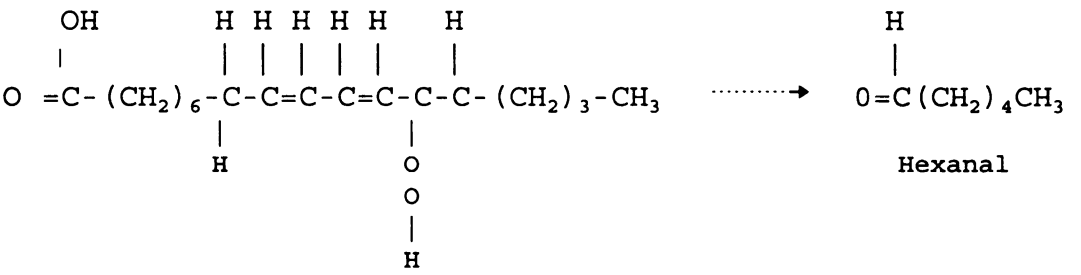
The TBA test originally had been used to measure the level of malonaldehyde in the product. When one mole of malonaldehyde reacts with two moles of TBA, this results in the formation of a pink chromagen. Absorbency of the pink

complex is monitored at 532 nm and the optical density recorded. This reaction has been used as a measure of the degree of lipid oxidation, particularly in meat systems (Tarladgis et al., 1960). An inherent problem associated with TBARS values is that other food components besides malonaldehyde, which are other oxidation products, can react with TBA to produce complexes that also absorb around 532 nm (Marcuse and Johansson, 1973). Therefore, TBARS values should be defined as resulting from compounds which react with TBA, producing a pink chromagan, rather than malonaldehyde exclusively. Also, the effect of other factors, such as acidity, heat and oxidizing agents on the TBA reagent will influence the accuracy of TBARS (Gray, 1978; Melton, 1983). However, this method is still applicable to estimate the rancidity development of lipid oxidation after proper modification (Melton, 1983).

Hexanal

Hexanal is the major secondary product of the oxidation of linoleic acid. Since linoleic acid has been found in cereal grain products, soybean oil and other vegetable oils, the accumulation of hexanal has been used as an excellent

indicator of the degree of oxidation in food products. Hexanal is derived from the 13-hydroperoxide of linoleic acid as follows (Dugan, 1976):



13-Hydroperoxide

Hexanal

Fritsch and Gale (1977) used hexanal as a measure of rancidity in low fat foods, such as ready-to eat breakfast cereal. They analyzed for hexanal in the headspace of oat, corn, wheat and mixed cereal to determine the degree of rancidity of the respective grain products. The data showed that when the hexanal concentration increased to between 5 and 10 ppm(wt/wt), a significant deterioration in product quality was observed, due to lipid oxidation. Heydanek and McGorrin (1981) analyzed the volatiles from rancid oat groats by using gas chromatography-mass spectrometry (GC-MS) and found that the data was in agreement with the results

reported by Fritsch and Gale (1977) and hexanal was the most abundant volatile product determined. Bruechert, et al.(1988) investigated the reaction of lipids and their breakdown products during extrusion. They found that in the lipid decomposition products of extruded samples, hexanal was the primary oxidation product of linoleic acid. Also, hexanal was a major peak in the chromatogram of each of the unextruded samples (Bruechert et al., 1988).

Although hexanal is the oxidation product of a fatty acid, it was considered to undergo autoxidation under certain conditions. For example, Palamand and Dieckmann (1974) used a slow stream of air passing over hexanal and analyzed for the presence of compounds produced from autoxidation of hexanal. They found hexanal undergoes extensive breakdown and participates in a variety of reactions, resulting in the formation of a large member of compounds such as esters, lactones, cabonyl compounds, alcohols, hydrocarbons and acids. Furthermore, autoxidation of hexanal resulted in the formation of many compounds having significant odor and taste properties. Heydanek and McGorrin(1981) investigated the flavor chemistry of oat groats by using a Tenax headspace trapping system. The data

showed that by using either dry vacuum distillation or a vacuum steam distillation procedure to trap volatiles, hexanal is a major component in both methods. Jeon and Rassette (1984) monitored the shelf-life of potato chips by analyzing for hexanal as an indicator of potato chip quality or shelf-life. The results showed that hexanal concentrations in light exposed samples were moderately higher than those in the control (25°C at dark). The sensory results indicated that the amount of hexanal detected was related to off-flavor development in the potato chip.

Composition of Oat Cereal

Oat cereal contains the highest levels of lipid, as compared to other cereal grains. The composition of oat cereals have been reported by a number of authors (de la Roch et al., 1977; Price, 1975; Welch, 1975; Youngs, 1977). The major fatty acid composition of cereal grains report by de la Roche et al.(1977) is palmitic acid ($C_{16:0}$)(17.5-23.6%), oleic acid($C_{18:1}$)(26.5-46.5%), and linoleic acid ($C_{18:2}$)(33.2-46.2%).

Price (1975) analyzed the fatty acid composition of seven cereal grains. The fatty acid content was determined by saponification and extraction followed by gas-liquid chromatographic analysis. Linoleic, oleic, palmitic and linolenic were found to be the major fatty acids detected. Data showed that linoleic is the predominant unsaturated fatty acid in the seven cereal grains. The lipid formulation of oat cereal contains 36.34% of oleic acid and 42.02% of linoleic acid (Price, 1975).

Youngs et al.(1977) determined the relative fatty acid composition of free lipids and bound lipids from oat groat fractions by using a gas chromatographic procedure. They found that palmitic, oleic, and linoleic acid are the major fatty acids, both in free lipids and bound lipids.

Many authors have described the variation in fatty acid composition of oat groats from different cultivation and different growing environments. Hutchinson and Martin (1955) indicated that the environmental conditions affected the oil of oats. Beringer (1971) reported a higher ratio of unsaturated : saturated fatty acids in oat grains, when grain development took place at 12°C than at 28°C. In a study carried out with six varieties of oats, two of wheat

and two of barley, Welch (1975) found differences between the species in composition and in their response to sowing date. He also found that the content and degree of unsaturation of the oat total fatty acid was affected by the sowing season.

Although most cereals contain appreciable quantities of natural antioxidants, they may loss as high as 90% as a result of processing (Herting, 1969). Due to the high percentage of unsaturated fatty acids found in oat cereal, such oat grain products readily undergo autooxidation.

General Review of Antioxidants

The United States Food and Drug Administration (FDA) defines antioxidants as substances used to preserve food by retarding deterioration, rancidity or discoloration due to oxidation (21 Code of Federal Regulations (CFR) 170.3(o)(3)) (Dziezak, 1986). Antioxidants permitted for use as food additives are list in Table 1.

Antioxidants have been used in the U.S. since 1947 to stabilize fats (Stuckey, 1972). In general, the total concentration of antioxidant must not exceed 0.02% by weight, based on the fat content of the food (Nawar, 1985).

The main antioxidants used for foods are monohydric or polyhydric phenols, with various ring substitutions.

Antioxidants are generally classified as either synthetic or natural products. The four synthetic products, tertiary butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG), are the antioxidants most widely used for food antioxidants. In the United States, they can be legally used, under the food additive regulations, at concentrations of not over 200 ppm(wt/wt) of the fat content of food products (Dziezak, 1986). The natural antioxidants include tocopherols, ascorbic acid, lecithin, rosemary extract, gum guaiac, ascorbic acid, and others (Dougherty, 1988).

Table 1. Antioxidants permitted in foods in the United States (Source: Narwar, 1985)

Primary antioxidants	Synergists
Tocopherols	Citric acid and isopropyl citrate
Gum guaiac	Phosphoric acid
Propyl gallate	Thiodipropionic acid and its didodecyl, dilauryl, and dioctadecyl esters
Butylated hydroxyanisole (BHA)	
Butylated hydroxytoluene (BHT)	
2,4,5-Trihydroxybutyrophenone (THBP)	
4-Hydroxymethyl-2,6-di-*tert*-butylphenol	Ascorbic acid and ascorbyl palmitate
tert-Butylhydroquinone (TBHQ)	Tartaric acid
	Lecithin

Antioxidants can not reverse the oxidative process, or restore the food to its original quality, but they can interrupt the free-radical propagation step of oxidative reactions by contributing a hydrogen atom from the phenolic hydroxyl groups. They then become stable free radicals, which do not initiate nor propagate further oxidation of lipids (Sherwin, 1976). The effectiveness of an antioxidant is related to many factors, such as activation energy, rate constant, oxidation reaction potential and solubility properties. Its solubility and volatility affects accessibility to the peroxy radical sites and its permanence during storage or heating, respectively (Narwar, 1985).

For maximum efficiency, primary antioxidants are often used in combination with other phenolic antioxidants or with a metal sequestering agent. Synergism occurs when a mixture of antioxidants perform more activity than the individual antioxidants, if used separately (Weng, 1993). Two kinds of synergism are recognized. One is the action of mixed free radical acceptors (Uri, 1961). The other involves the combined action of a free radical acceptor and a metal chelating agent (Narwar, 1985).

No single antioxidant is suitable for all food products. Antioxidants must be added to freshly produced oil or fat before the autooxidation reaction has been initiated (Dougherty, 1988; Dziezak, 1986; Coulter, 1988). It is also necessary to select a proper antioxidant to meet the needs of a particular food item. The choice of antioxidant is dependent upon the nature of the food product, processing conditions and the method by which it is added. Various methods have been described for the incorporation of antioxidants into a snack food, these include : (1) directly sprayed onto food; (2) treated packaging materials; (3) treated animal or vegetable fats; and (4) treated essential oils and flavors (Buck, 1984). Correctly applied antioxidants will help to maintain the product's original freshness, flavor, odor and shelf-life.

Mechanism of antioxidant

Antioxidants have been used effectively and safely for many years to retard oxidative deterioration of foods. Antioxidants will inhibit or interfere with the formation of free radicals in food fats, terminating the oxidative reaction in its initiating step (Dougherty, 1988). The

mechanism by which the antioxidant (AH) acts to interfere with free radical formation is shown in the following sequence of reactions:

$$R\bullet + AH \longrightarrow RH + A\bullet$$
$$RO\bullet + AH \longrightarrow ROH + A\bullet$$
$$ROO\bullet + AH \longrightarrow ROOH + A\bullet$$
$$R\bullet + A\bullet \longrightarrow RA$$
$$RO\bullet + A\bullet \longrightarrow ROA$$

Antioxidants interrupt the autoxidation process by reacting with free fatty acid radicals or hydroperoxide radicals. The antioxidant free radicals are quite stable and will not further propagate the radical chain process (Everson et al., 1957).

Characteristics of Hindered Phenolic Synthetic Antioxidants

2-(1,1-Dimethylethyl)-1,4-Benzenediol (TBHQ)

2-(1,1-Dimethylethyl)-1,4-benzenediol, commonly known as TBHQ. The molecular weight of TBHQ is 166.22. Its melting range is 126.5°C-128.5°C. It is a white to tan crystallive solid. TBHQ is soluble in oil, propylene glycol, ethanol and slightly soluble in water. It is effective in providing oxidative stability to crude and

refined polyunsaturated oil, without encountering a problem of color. It is quite color stable even in the presence of metals. TBHQ exhibits good carry-through characteristics in the frying of potato chips. It was approved for use in the preservation of foodstuffs by FDA in 1972 (21 CFR 172.185) (Coulter, 1988). The structure of TBHQ is shown in Figure 1 (Coulter, 1988).

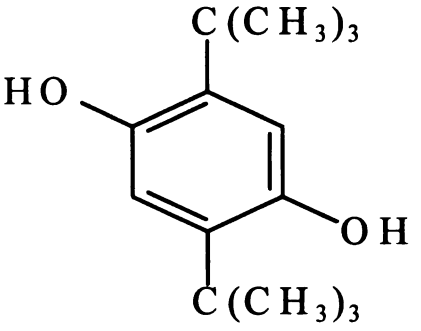
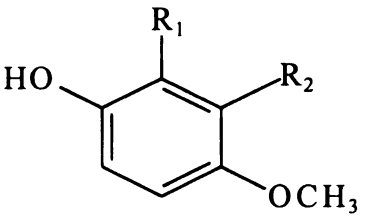


Figure 1. The structure of TBHQ

2-Tertiary-Butyl-4-Methoxy Phenol (BHA)

2-Tertiary-butyl-4-methoxy phenol, commonly known as BHA. The molecular weight of BHA is 180. It has a boiling point of 264°C-270°C and melting range at 48°C-55°C. It is a white solid which is soluble in fats, oils, propylene glycol, paraffin, and ethanol, but not soluble in water. BHA has a typical phenolic odor that may be noticeable if

the oil is subjected to high heat (Sims, 1972). The structure of BHA is shown in Figure 2 (Coulter, 1988).



10% R 1-H
R2-C(CH 3)3

90% R 1-C(CH 3)3
R2-H

Figure 2. The structure of BHA

BHA has excellent carry-through activity after hot processing, therefore, BHA is a popular antioxidant choice for dry breakfast cereal, animal fats and vegetable oils (Nawar, 1985). It is relatively effective when used in combination with other primary antioxidants. Because of its volatility, BHA has been incorporated as an additive to packaging material, from which it can migrate into food to prevent oxidation (Han et al., 1987).

3,5-Di-Tertiary-Butyl-4-Hydroxytoluene (BHT)

3,5-Di-tertiary-butyl-4-hydroxytoluene (BHT) is the most prevalently used synthetic antioxidant. It has been used as an antioxidant in the food industry since 1970 for animal fat, dry breakfast cereals, and emulsion stabilizers

(Nawar, 1985; Dugan, 1976). The molecular weight of BHT is 220. It has a boiling point of 265°C, and a melting point of 69.7°C. It is a white crystalline powdery solid, which is insoluble in water but is soluble in fats, oils and ethanol. The structure of BHT is as follows (Coulter, 1988):

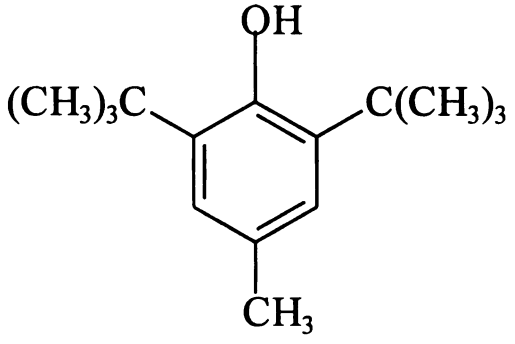


Figure 3. Structure of BHT

In accordance with good manufacturing practice (21 CFR 182.3173), BHT is listed as Generally Recognized As Safe (GRAS) for use in food, when the total content of antioxidants is not over 0.02% of the fat or oil content of the food (Coulter, 1988). In 1977, a proposal was made by the FDA to remove BHT from GRAS status and banning the substance from use in direct and indirect food packaging (Monks, 1992; Charnas, 1992). For the present, however, the official position of the FDA is that BHT still maintains

GRAS status (Monks, 1992). Because of the proposal, concerns had been raised regarding the safety and continued use of BHT in food packaging applications (Ho et al., 1994). Therefore, in order to meet consumer demands, it is necessary to find an antioxidant as a substitute for BHT.

n-Propyl-3,4,5-Trihydroxybenzoate (PG)

n-Propyl-3,4,5-trihydroxybenzoate, also known as PG, is the n-propyl ester of 3,4,5-trihydroxy-benzoic acid. The molecular weight of PG is 212. It begins to decompose when temperature is above 148°C. It has a melting range at 146°C-148°C. It is supplied as a solid white crystalline powder. It is soluble in propylene glycol and ethanol, slightly soluble in water, but not in fats or oils (Dziezak, 1986). PG is useful in inhibiting oxidation in oils and animal fats, meat products, including fresh and fresh frozen pork sausage, spices, and snacks. It was approved for food use by FDA in 1947. The FDA has also approved its use in chewing gum, based on levels not to exceed 0.01% total antioxidant weight. PG is the most effective of the synthetic antioxidants in preventing oxidative rancidity. However, it can also be the most problematic due to possible

formation of black or purple colored complexes, when it comes in contact with metallic ions such as iron or copper. To prevent this problem, liquid blends are offered in a propylene glycol solution combining propylgallate with a cheating agent such as citric acid. The structure of PG is shown in Figure 4 (Coulter, 1988).

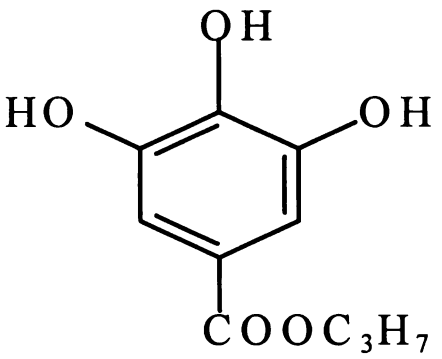


Figure 4. The structure of PG

Characteristics of Natural Antioxidants

Ascorbic Acid

Ascorbic acid, or vitamin C is a compound very widespread in natural. Ascorbic acid is constantly gaining importance as a natural food additive. It helps to improve the quality and increases the shelf-life of many food products (Dugan, 1976). In its removal of oxygen from air or food, ascorbic acid is oxidized to form dehydroascorbic acid, thereby asserting its antioxidant action (Narwar, 1985). Its ability to reduce components may be extended to antioxidants. For instance, ascorbic acid and its sodium salt, are hypothesized to regenerate phenolic antioxidants by contributing hydrogen atoms to phenoxyl radicals produced by lipid oxidation. By itself, ascorbic acid is freely water soluble and has hardly any antioxidant activity. It promotes the effectiveness of other antioxidants by combining as a synergist. Ascorbic acid has no restrictions on its usage levels and has GRAS status for use as a chemical preservative (21 CFR 182.3013) (Dziezak, 1986). Ascorbic acid is a highly soluble substance that has both acidic and strong reducing properties. These qualities are attributable to its enediol structure which is conjugated

with the carbonyl group in a latone ring. The structure of ascorbic acid is as follows:

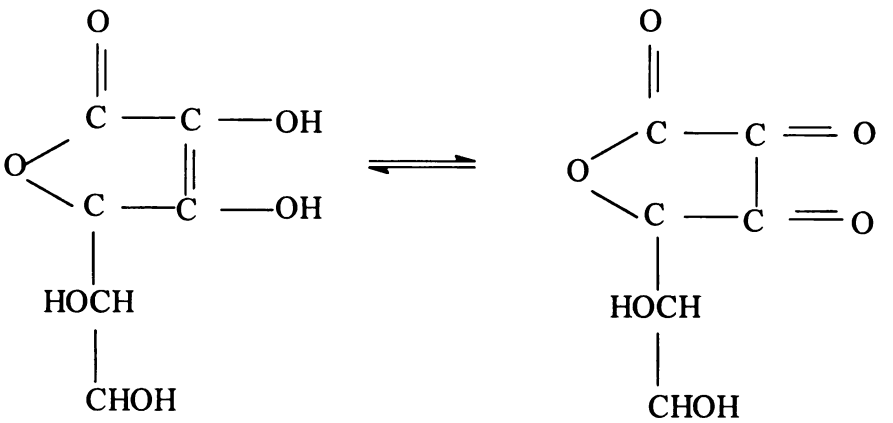


Figure 5. The structure of ascorbic acid

Rosmarinus Officinalis

Rosmarinus officinalis has been used for some time as an antioxidant in a number of foods (Liu et al., 1992; Pokrny, 1991). It is extracted from rosemary leaves by steam distillation. An alcoholic extraction of the leaves gives the crude rosemary extract, which can be directly used as a food grade antioxidant after evaporation of the alcohol (Yang, 1985). However, the extracts of rosemary have a strong odor and bitter taste and therefore, can not be used in most food products (Chang et al., 1977). To solve these problems, a molecular distillation followed by steam distillation was necessary. This gave an odorless and pale-

red/orange product of high activity (Loliger, 1983).

Loliger (1983) studied the effectiveness of rosemary extract compared with BHT/BHA and the results indicated that the antioxidant activity from rosemary is about the same as BHT/BHA, at corresponding concentrations. Loliger also analyzed the rosmarinus officinalis by a HPLC procedure and identified several of the compounds with very good antioxidant properties, such as rosmarinic acid, carnosol and carnosic acid. The structure of rosmarinic acid is shown in Figure 6.

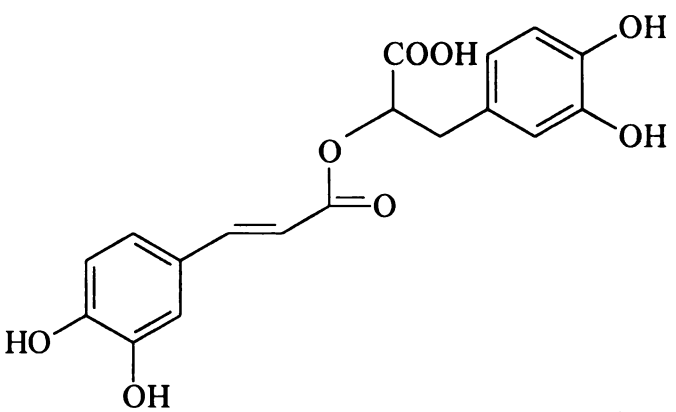
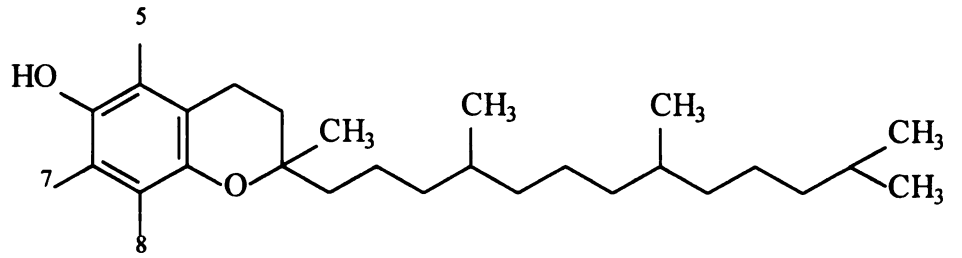


Figure 6. The structure of the rosemary antioxidant, rosmarinic acid

α-Tocohperol

α-Tocopherol(Vitamin E) has recently been shown to have application as an alternative antioxidant for polymer processing (Laermer et al., 1993). It occurs naturally in vegetable oils. α-Tocopherol is a natural antioxidant which belongs to a class of compounds exhibiting vitamin E activity. At least seven types of tocopherol methyl substituted forms of tocol have been isolated. Tocopherols exhibit antioxidative properties, and have been shown to be effective in delaying oxidation in a variety of foods (Dugan, 1976). The antioxidative nature of tocopherols is attributed to their phenolic structure. Tocopherols differ in their antioxidant activity. In general, antioxidant activity increases with decreasing vitamin E activity. The antioxidant activity of tocopherols increases in the following order: delta > gamma > beta > alpha. However, the relative activity of these compounds is significantly influenced by temperature and light conditions (Sherwin, 1976). The structure of the respective tocopherols is shown in Figure 7.



5,7,8 - Trimethyl = α-tocohperol

5,8 - Dimethyl = β-tocopherol

7,8 - Dimethyl = γ-tocopherol

8 - Methyl = δ-tocopherol

Figure 7. Tocopherol structures

The molecule weight of α-tocopherol is 430.72. It is a clear, viscous oily substances of pale yellow color, nearly odorless. It is insoluble in water, miscible at any ratio with vegetable oils, ethanol, ether, chloroform and acetone. It is heat stable and not volatile or steam distillable. Natural source tocopherols are regulated in the United States by the FDA in 21 CFR 182.3890 and by USDA at 9 CFR 318.7. In 1978 the FDA proposed to affirm GRAS status for mixed, concentrated tocopherols under 21 CFR 184.1894. Tocopherols have been approved for use as food additives in a number of countries which include: Canada,

Japan, Korea, Australia and all members of the European Economic Community (Dougherty, 1988).

The antioxidant effect of vitamin E in biological systems is well documented. It is believed that vitamin E functions in *vivo* as a free radical scavenger, by being oxidized to a tocopheroxyl radical. The tocopheroxyl radical is easily formed due to its stabilization by the pyran ring oxygen (Burton, 1983). In the human diet, vitamin E acts to prevent biological damage associated with the effects of aging, chronic disease, and exposure to atmospheric pollution, and has been considered as an anticarcinogen. Epidemiological studies indicated that vitamin E, alone or in combination with other antioxidants can decrease the incidence of certain forms of cancer by quenching free radicals, or reacting with their products (Packer, 1992).

α-Tocopherol has been shown to be effective in improving the oxidative stability of fats and is used at relatively low concentrations (100 to 300 ppm of fat weight) in a wide variety of food products to retard oxidative deterioration. (Dougherty, 1988; Coulter, 1988)

Application of Antioxidants in Packaging Materials

Additives have been incorporated into food products and packaging materials for a number of years (Smith, 1993). Recently, consumer demands have lead to more interest towards reducing the levels of food additives in processed foods. However, elimination of additives such as antioxidants may reduce the shelf-life of the product. Therefore, manufactures have attempted to reduce the total amount of antioxidant consumed with their products by incorporating less into the product, and more into the packaging material (Food Engineering, 1979).

Since oxygen first attacks food at the surface, impregnating packaging materials with an antioxidant has long been used to protect the product from oxidative rancidity and thus prolong shelf life. The proposed mechanism of antioxidant activity involves the following 3 step process: (1) antioxidant diffusion through the polymer bulk phase; (2) evaporation of antioxidant from the surface of the packaging material; and (3) subsequent antioxidant sorption onto the surface of the packaged product.

Antioxidant stabilizers have been incorporated into polyolefin resin to prevent degradation, improve heat

resistance and control color change. The basic steps associated with thermal degradation are similar to an oxidation reaction. When the polymer undergoes thermal degradation, the breaking of chemical bonds results in formation of reactive products such as free radicals and unstable hydroperoxides. Antioxidants, therefore, not only stabilize food packaging liner films during fabrication but as indicated above, due to their volatility and tendency to migrate, also become an antioxidant for the food product itself. Various studies have been described dealing with methods of adding antioxidants indirectly, showing the positive utilization of the absorption phenomenon, in which the vapor from the antioxidants moves into the food. (Coulter, 1989). For example, Hoojjat et al. (1987) demonstrated the effectiveness of a BHT impregnated film to retard lipid oxidation of a packaged oatmeal cereal, through the migration of antioxidant from the package to the product via an evaporation/sorption mechanism.

There are a number of studies reported dealing with the migration characteristics and the stability of BHT in polyolefin films. Urata (1988) observed that when compared to vitamin E and Inganox®-1010 (Trade name of CGP-CIBA-

GEIGY Corporation) in polypropylene resin, BHT showed the greatest loss by heat deterioration at 100°C. Urata also reported that polypropylene (PP) resin, containing various concentrations of BHT as a stabilizer, was found to decrease in weight by heat deterioration and to be become brittle after 2 days heating at 130°C. When compared with vitamin E as a stabilizer, the higher the quantity of vitamin E added, the greater the number of days before the PP resin became brittle.

Since BHT is somewhat volatile, even at ambient temperature, it readily migrates into food (Dziezak, 1986; Ho, 1994). Currently, BHT is added to plastic resins in concentrations of 3000 ppm(wt/wt) for breakfast cereal liner film (Monks, 1992). The BHT migrates to the cereal, acting as a preservative. Migration of BHT from polymer films has been widely studied. Terada and Naito (1989) reported that the higher the concentration of BHT in polyethylene (PE) film, the higher the BHT migration levels into a food model. Their studies showed that migration occurs when the product/package system are in contact with each other, or through the gaseous phase alone. They also found the levels of BHT migrating increased with time, but the level of

migration as a function of time varys significantly with the food.

Till et al. (1988) studied the migration of BHT from high density polyethylene (HDPE) to food and food simulates. They found that BHT migrated to fatty foods at a faster rate than to aqueous foods. The data also showed that temperature is a factor affecting the diffusion coefficient of BHT through HDPE. The higher the temperature, the higher the diffusion coefficient value. Therefore, the packaging material which has been impregnated with antioxidant must be stored properly before use. Figge et al. (1976) studied the migration of BHT by a radio-tracer technique. They used several ^{14}C-labeled plastic additives incorporated into resin samples which were compounded and molded into test sheets. The test sheets were then stored for various periods of time in contact with selected foods, at several different temperatures. They found that the amount of labeled additives migrating from HDPE is higher than from polyvinyl chloride (PVC). Bailey (1995) studied the mass transfer of BHT from a multi-layer lamination film and concluded that the rate of loss of BHT from the respective surface layers is controlled by surface evaporation, the

mass transfer coefficients and diffusion coefficients. Bailey also noted that the rate of loss of BHT from the heat seal layer was greater than that from the HDPE surface layer. The differences in antioxidant loss rates could affect the quality of a packaged product, due to the transfer rate of additive to the product.

For many years, film manufactures used BHT as a stabilizer to prevent degradation. Recently, however, BHT has been suspected to be a cancer-causing agent (Charnas, 1992; Ho, 1994). As a result, many companies, such as Quantum Chemical Co., Mobil Chemical Co., and Exxon Chemical Co., have eliminated BHT from their resin and while the FDA has not taken any action with regard to the continued use of BHT, public concern has lead polyolefin producers and food manufactures to actively seek alternate antioxidants (Monk, 1992). Some costly, high molecular weight hindered phenols are being considered as alternative antioxidants by film manufactures (Monks, 1992). These antioxidants provide more permanent protection than BHT, while using the same additive level. They also diminished the color problem during processing. Therefore, most resin and additive suppliers

recommend high molecular weight hindered phenols as the best alternatives to BHT (Monk, 1992).

In contrast, a study recently reported by the America Health Foundation (Monk, 1992) showed that BHT acts as an anticarcinogen, when ingested in small doses. In this study, rats were fed a potent liver carcinogen with 0 to 6000 ppm BHT. Data showed that rats eating all but the highest dosages of BHT developed less cancer than those who were denied the antioxidant (Monks, 1992).

α-Tocopherol (ATP) or vitamin E is said to be unquestionably one of the safest, most environmentally acceptable additives that can be used for polymer stabilization. The use of vitamin E in the plastics area is relatively new and has been used as an alternative for BHT in food packaging since the mid-1980s (Urata, 1988; Ho, 1994). α-Tocopherol consists of an aromatic ring, which gives the molecule an enhanced redox potential and allows it to act as a extremely effective scavenger of free radicals. Also, the long side chain provides good solubility in the polymer (McMurrer, 1991). Based on its chemical structure, it is thought that α-tocopherol is 250 times more reactive toward peroxy radicals in styrene than BHT (Burton, 1986).

The properties of ATP make it effective in preventing polymer damage associated with the effect of long-term storage, processing temperature, and exposure to atmospheric pollutants. Laermer and Schuster (1990) observed the melt stability of polypropylene resin containing either ATP, BHT or Irganox®-1010 and found ATP to be superior to the conventional antioxidants at very low concentrations, or in synergistic mixtures with other additives. When ATP was combined with synergistic additives, it also showed better color stability for PP. To provide the best processing and color stability, a secondary antioxidant, typically a phosphorous-based compound, is often incorporated to work synergistically with the primary phenolic antioxidants (McMurrer, 1991).

Secondary antioxidants such as phosphites and phosphonites provided very important synergism for primary phenolic antioxidants. They must be hydrolytically stable to be blended and mixed thoroughly and homogeneously with the primary antioxidants. However, the commonly used phosphites are usually hydrolytically unstable and contribute to mixing/handing problems during transfer. They also occasionally form black specks during extrusion at high

temperature and for long residence time. A series of studies have been described which evaluated a "phosphite free" system to stabilize polyolefins. For example, Young et al. (1995) examined a "phosphite free" system based on a patented formulation of α-tocopherol, Irganox®-1010, and other combinations of antioxidants, such as Irganox®-1010/Ultranox®-626, and Irganox®-1010/α-tocopherol. The data showed that using α-tocopherol alone was the most effective for HDPE and was found to improve film color and to reduce the formation of gels in films. For PP resin, the combination of α-tocopherol and Irganox®-1010 offered excellent color and melt flow control. The results demonstrated that α-tocopherol can replace a phenolic antioxidant/phosphite combination as the primary antioxidant system, or replace the phosphite portion to achieve optimum performance. Therefore, the problems associated with phosphites can be eliminated or minimized. For overall performance, a "phosphite free" system offers balanced properties, as well as excellent economics and it is to be considered as the most cost effective system (Young et al., 1995).

Due to its excellent thermal stability, ATP will not begin to volatilize until 300°C. Its limited volatility reduces migration from the polymer matrix into food products (Smith, 1993). However, although ATP is very stable, it still has the potential to migrate to food products (Packaging, July 1992). The migration of ATP from a film to a series of food simulate systems was studies by Laermer et al. (1993). The authors reported that 8.4% of the initial ATP additive present in HDPE film will migrate into heptane, 3.8% of the initial ATP will migrate to 50% ethanol, and no detectable level of migration was indicated with 8% ethanol and 4% acetic acid as the food simulant. Another study on the migration of ATP from drug packaging was carried out by Hoffmann-La Roche's research group (Laermer et al., 1993). In these studies, strips of HDPE containing various additives were cut from the side wall of HDPE bottles and immersed in 100% ethanol in a sealed container. Data showed that only 5% of the initial ATP present in the HDPE bottle wall was extracted, as compared with 25% of BHT and 13% of Irganox®-1076 (Trade name of CGP-CIBA-GEIGY Corporation). It is beneficial to both the drug manufacturer and consumers to have less antioxidants extracted into a drug product.

"Odor and plastic taste" problems have challenged resin producers for years. During polymer processing, the heat and shear energy often causes bond-scission of polymer chains, leading to the formation of various low molecular weight compounds. Those compounds which are entrapped in the polymer matrix can evaporate in air as off-odor volatiles or migrate to food as off-taste compounds. The off-odors and off-taste can arise from a variety of materials such as monomers, plasticizers, oligomers, solvents, etc.. Antioxidants have been used in plastic bottles to reduce their so called plastic taste. Laermer et al. (1993) and Ho (1994) have shown that HDPE incorporated with 100 ppm(wt/wt) ATP exhibited less taste and odor responses, when compared to 500 ppm(wt/wt) of Irganox®-1076 or 250 ppm of BHT. This data also agreed with the results of Zambetti (1995), that ATP provided a 73%-83% reduction in aldehyde levels which are responsible for the unacceptable taste and odor of HDPE containers. ATP has been used as a flavor retainer by resin manufactures and food companies as well. A taste test carried out by Sensory Spectrum of Chatham (Laermer et al., 1993) demonstrated that any level of ATP incorporated into the HDPE liner structure retarded

the loss of flavor from a cereal product over the test period and temperature (37°C and 3 months). The development of gels in both blown film and cast film has been a problem to converters. Studies showed that the addition of ATP reduced the gel counts for both LLDPE and HDPE by 80%, when compared to the unstabilized resin. Thereby, reducing waste and allowing film to be processed at higher temperatures, with faster throughput. In marketing, the cost of ATP is three times higher than other standard commercial antioxidants. However, it can be used at significantly lower dosages - 100 ppm for PE application and 250 ppm (or less) for PP. Because it is typically used at 1/4 to 1/5 the effect dosage of other antioxidants, ATP is more cost-effective than conventional antioxidants (Laermer et al., 1993).

Theory of Migration

Migration is described as the transfer of material from plastic to food, under specified conditions. The migration of a plastic ingredient to food may take place through the headspace between the package and food. There are two types of migration processes which have been defined in food

packaging, namely: global migration and specific migration. Global migration refers to the total transfer of all migrating species from plastic into packaged food. Specific migration refers to one or more identifiable species that is a constituent of the packaging material (Giacin, 1980). The rate of migration is affected by the following factors: (1) the solubility of additives in the polymer system; (2) the diffusion coefficient within the bulk of the polymer structure; and (3) the rate at which it volatilizes from the polymer surface (Calvert, 1979).

Solubility of the additives has been recognized as an important factor in additive compatibility. Hawkins et al.(1969) demonstrated that the rates of loss of typical stabilizing additives are significant, relative to the lifetime of the polymer. If migration from the packaging material to a contained product is to occur, the migrant has to diffuse to the polymer surface, followed by dissolution of the migrant accumulated at the surface to the contact phase. Therefore, the loss rate of additives is determined by the rate of volatilization from the polymer surface and the diffusion coefficient within the bulk of the polymer.

A mathematical expression has been described by Crank (1975) for the additive loss from a film by surface evaporation with finite boundary conditions. According to this model, the total amount of additive leaving the polymer in time (t), is expressed as a fraction of the corresponding amount leaving at infinite time.

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=1}^{\infty} \frac{2L^2 \exp(-\beta_n^2 T)}{\beta_n^2(\beta_n^2 + L^2 + L)} \qquad (1)$$

where M_t = amount of additive leaving the polymer in time t
M_∞ = amount of additive leaving the polymer at infinite time
$T = Dt/l^2$
$L = l\alpha/D$
l = half of film thickness, cm
t = time, second
D = diffusion coefficient of additive in polymer, cm^2/sec
α = mass transfer constant of additive from polymer, cm/sec
β_n values are the positive roots of $\beta_n \tan\beta_n = L$

Calvert and Billingham (1979) used Eq.(1) to describe the loss of additives from polymer film and sheet. In order to apply Eq.(1), they assumed that polymer degradation will proceed rapidly to sample failure, when the average concentration of additive falls to 10 percent of its initial

value, i.e., when M_t/M_∞ = 0.9. Eq.(2) is obtained when M_t/M_∞ = 0.9, and n=1.

$$\frac{2L^2 - \exp(-\beta^2 T)}{\beta^2(\beta^2 + L^2 + L)} = 0.1 \qquad (2)$$

They plotted Eq.(2) for a variation of L value as a function of T and concluded that with high L values (thick film, rapid evaporation and low diffusion rate), the failure time is diffusion dominated and independent of α. The failure time is then given by Eq.(3)

$$t = 0.87\ l^2/D \qquad L > 10 \qquad (3)$$

At the lower L value (thin film, slow evaporation and fast diffusion rate), Eq.(2) becomes a line of unit slope obeying Eq.(4)

$$\log L + \log T = 0.383 \qquad (4)$$

leading to the failure time given by Eq.(5)

$$t = 2.42\ l/\alpha \qquad L < 0.6 \qquad (5)$$

According to Eq.(3) and Eq.(5), the additives loss from a thick film is dominated by bulk phase diffusion and loss from a thin film is determined by surface evaporation.

Diffusivity, or diffusion coefficient (D), is defined as the tendency of a substance to permeate through the polymer bulk phase. The driving force is dependent on a

concentration gradient, where the dissolved species diffuses from a high concentration to a low concentration area (Giacin, 1980). The rate of diffusion is defined by Fick's first (6) and second (7) laws(Crosby, 1981):

$$\frac{1}{A}\frac{dm}{dt} = -D\frac{dc}{dx} \qquad (6)$$

where m = mass of the component transferred
t = time
c = migrant concentration
x = path of diffusion
D = diffusion constant
A = area of plane cross which diffusion occur

Fick's second law is applied when the diffusion process is over an infinite surface (i.e., a sheet).

$$\frac{dc}{dt} = D\frac{d^2c}{dx^2} \qquad (7)$$

In this expression, the diffusion coefficient is assumed to be constant and independent of the concentration. Therefore, the concentration of migration in the contact phase is a function of time, and product affinity.

There are three different types of packaging materials/contact phase systems which can be defined in terms of migrate diffusivity. System I : non-migrating; system II : independently migration; and system III : leaching. In system I, migration occurs only from the

packaging surface with near zero diffusion, therefore little if any migration takes place. In system II, migration take place and the diffusion coefficient is measurable under the test conditions and the specified contact time. In leaching, components of the contacting phase interact with the polymer and increase the diffusion coefficient of the migrating species through the polymer bulk phase (Crosby, 1981).

Technique for Trapping Volatiles

Techniques that have been used for trapping volatiles include solvent extraction, steam distillation, liquid-liquid extraction, simultaneous steam distillation-solvent extraction, and dynamic headspace or purge and trap procedures (Risch, 1989). Analytical procedures for the detection and identification of volatiles present in food products have been employed for many years. Dynamic headspace/gas chromatography has been recognized as one of the most effective and sensitive techniques for the analysis of volatile organic compounds (Vallejo-Cordoba, 1993). Headspace volatiles sampling technique can be achieved by:

(1) direct injection of volatiles into a gas chromatograph (GC) ,and (2) indirectly sorbing volatiles by sorption trap.

Direct headspace sampling has been widely used. In 1971, Dupuy et al. described a direct GC procedure for determining volatiles in vegetable oils. Two years later, the technique was further applied to the direct GC examination of volatiles in salad oils and shortenings (Dupuy et al., 1973). Other researchers have confirmed the validity of instrumental analysis as a reliable means of quantifying components contributing to food flavor, and their relationship to product quality, and shelf life. However, while measurement of volatiles by direct injection GC offers the advantage of no loss of volatiles due to handling, the sample size is limited and no appreciable concentration of headspace volatiles can be achieved. In many instances, the concentration of volatiles in the headspace is too low for direct analysis (Clark, 1975; Murray, 1977). Therefore, collection and concentration of these substances may be necessary to achieve the needed analytical sensitivity. Recently, porous sorbant polymers have been used for the collection, concentration, and subsequent GC analysis in a wide variety of applications

(Macku and Shibamoto, 1991). The most commonly used concentration method for headspace samples is the application of adsorbents such as Tenax (Buchkholz et al., 1980), Carbotrap, and Porapak Q (Jennings, 1972; Clark, 1975). Adsorption on a solid sorbent is an extensively utilized preconcentration technique. Once the components have been adsorbed, sample recovery can be achieved either by solvent desorption or thermal desorption. The advantages of the thermal desorption technique are the absence of a solvent peak and higher sensitivity, as the whole sample can be analyzed in an undiluted form (McCaffrey, 1994).

P-2,6-Diphenylene oxide (Tenax GC) has been widely used as an adsorbent in purge-and-trap/gas chromatography-mass spectrometry procedures (Buckholz et al., 1980; Galt, 1984). The reason why Tenax GC is a good candidate for an adsorbent trap is the fact that Tenax GC has a very high affinity for organic compounds. Also, Tenax GC is relatively hydrophobic, which is important in view of the large volume of water vapor produced upon heating food products for volatiles analysis by the dynamic purge and trap procedure. A further advantage of Tenax GC is that it has a high temperature limit of 375°C (Olafsdottir, 1985). This high

thermal stability assures no volatiles will bleed from the trap onto the GC column during analysis, and complete regeneration of the porous polymer is achieved (Buckholz et al., 1980). One of the most critical aspects of dynamic headspace sampling is the type of the adsorbent used in the trap. Ideally, the adsorbent should retain volatiles during purge and release them during the thermal desorption step. In addition, an adsorbent should be hydrophobic so that it will not adsorb water vapor during the purging step and thus interfere with the gas chromatographic analysis following desorption. Withycombe et al. (1978) used several polymers to trap the headspace volatiles from hydrolyzed vegetable protein and found that sampling capacities depended on the nature of both the adsorbants and sorbates. Adsorption tube evaluation has been discussed by McCaffrey and MacLachlan (1994). Adsorbents with the highest surface area will tend to have the highest sampling capacities. However, no single adsorbent polymer will be best for every sampling application. The adsorbent must be chosen to fit a particular problem (Withycombe, 1978).

Determination of Antioxidants

Analytical methods used to determine antioxidant levels in both foods and polymers have been widely reported. A UV spectrophotometic procedure has been described by Terada and Naito (1989) to measure the concentration of BHT in different types of food systems. Bailey (1995) also described a UV spectrophotometic technique to determine the BHT level in a multi-layer lamination. The limit of this method is that those compounds which absorb light energy at wavelength ranges behind or above the ultraviolet and visible region can not be detected. Wyatt and Sherwin (1979) used direct-sampling gas chromatography to measure the loss of BHA in polyethylene. Thin-layer chromatography is another useful method to analyze for antioxidants. Till et al. (1982) used this technique to determine the level of radio-labeled BHT in HDPE plaques. Numerous researchers have used high pressure liquid chromatography procedures (HPLC) to analyze for the levels of antioxidant in both polymer and food systems (Till, 1982; Han, 1987; Schwope, 1987; Hoojjat, 1987; Chase , 1994). Hoojjat et al. (1987) concluded that the results from HPLC analysis agreed well

with results obtained by a UV spectrophotometer method. However, while the above methods are useful for the analysis of antioxidants, there are various problems associated with these analyses. Such problems arise from three factors: (1) the low chemical of thermal stability of antioxidants; (2) the low concentration of antioxidants present in food and polymer samples; and (3) the solubility of antioxidants in a polymer matrix (Wheeler, 1968).

MATERIAL AND METHODS

Cereal Product

The cereal product used in this study was a commercial type oat cereal, and was formulated to contain no incorporated additives (e.g. antioxidant). The product was obtained from General Mills. Inc. (Minneapolis, MN)

Packaging Material

Three multi-layer laminate films were used to fabricate the flexible pouches evaluated in this study. All of the films contained an inner heat seal layer (Surlyn-EVA), a core high density polyethylene (HDPE) layer and an outer HDPE layer. The primary difference between the three test films was in the nature and level of antioxidant incorporated within the core layer. Film I contained α-tocopherol in the core layer, Film II contained 3,5-di-tertiary-butyl-4-hydroxytoluene (BHT) in the core layer, and Film III had no antioxidant incorporated into the core layer. Figure 8 shows a cross sectional view of the respective multi-layer structures.

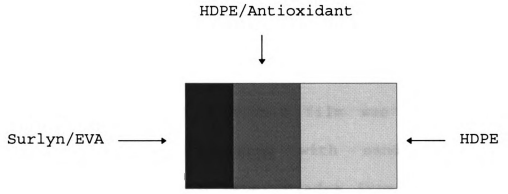


Figure 8. Cross section of three multi-layer structure

The film containing BHT in the core layer (Film II), and Film III, which contained no antioxidant in the core layer, were provided by the United Film Corporation (Odon, IN). Film I, which contained α-tocopherol in the core layer was obtained from the Bemis Company, Inc. (Terre Haute, IN).

The thickness of each layer was analyzed by optical microscopy at the Composite Materials and Structures Center laboratory (MSU). The polymer film was mounted in an acrylic matrix and polished with sand paper using consecutively finer sand paper grades from 120-4000 grit. When a mirror finish was achieved, the samples were mounted in the Olympus Model BHS Optical Microscope, and examined under 50x and 2.5x lenses. Precise measurement of each layer in the polymer required a calibration photo be made to use as a grid. Each division of the grid was equal to 10μ.

The total concentration of BHT incorporated into the laminate film structure was 0.157 percent (wt/wt). The total concentration of α-tocopherol incorporated into the laminate film structure was 0.0238 percent (wt/wt).

Both concentration levels were determined by high pressure liquid chromatography (HPLC) analysis.

Film Storage Studies

The three test films were stored at room temperature (23°C ± 2°C) and 50%RH ± 2% until samples were required for testing. Approximately six to eight feet of film was removed from the roll stock prior to sampling to insure evaporated losses of antioxidant were minimized.

Antioxidant Loss Rate Studies

Studies of antioxidant loss depletion from the test films, as a function of time and temperature, were carried out on the α-tocopherol impregnated film sample (Film I). Film samples measuring 12 inch by 12 inch were mounted with magnets to a metal frame, and the test film samples stored in a temperature controlled oven which circulated air through it (Blue M, Electric stabil-Therm, Electric Oven). The storage temperatures were 23, 30, 40, and 50°C, respectively. The level of retained antioxidant was determined as a function of storage time at each test temperature. Antioxidant levels in the film samples were determined by a high pressure liquid chromatography (HPLC) procedure.

High Pressure Liquid Chromatography (HPLC) Analysis

Extraction Procedure

Film samples were removed from the constant temperature chambers after predetermined storage time intervals, and cut into pieces 0.5 inch by 0.5 inch. Approximately 2.0 grams of film were placed in an extraction thimble, and extracted with 110 ml of acetonitrile (EM Science, 99.9+% HPLC grade) in a soxhlet extraction apparatus for 24 hours. A second extraction was performed to insure complete removal of the antioxidant from the film sample. The extracts were brought up to a 100 ml volume using acetonitrile. The samples were then filtered through a 0.45μm (HV4-0.45μm,4mm, Millipore) size filter, and transferred to 4 ml vials for analysis by HPLC.

Percent Recovery of Antioxidant for Extraction Procedure

A recovery study was carried out to determine the percentage loss of antioxidant from the extraction procedure. A standard solution of antioxidant was prepared by weighing approximately 0.02 gram α-tocopherol, transferring the weighed sample to a 200 ml volumetric flask

and diluting with acetonitrile to volume. The specific concentration of the α-tocopherol standard solution was determined by an HPLC procedure in triplicate. An average area response was used as a basis for determining percent recovery.

110 ml of the standard α-tocopherol solution was transferred to the soxhlet extraction apparatus. No film was placed in the extraction thimble. After 24 hours of continuous operation, the extractant solution was recovered and brought up to a volume of 100 ml using acetonitrile. The sample was filtered through 0.45μm size filter (HV4-0.45μm,4mm, Millipore), then transferred to a 4 ml vial for HPLC analysis. The area responses of three replicate injections of the extractant solution were averaged. The averages was divided by the area response set as a basis for 100% recovery, as described previously.

HPLC Analysis

Analysis of α-tocopherol was carried out on a Waters Model 150-C ALC/GPC, with a Waters Data Module (Model 730) and a Waters 486 Tunable absorbance detector. The chromatographic conditions were as follows:

1) Column : Delta-Pak HPI C18 300 A (3.9mm × 150mm), Waters

2) Solvent : Pure Methanol (J.T.Baker Inc,99.9+% HPLC grade)

3) Flow rate : 1.0 ml/minute

4) Detection wavelength : 280nm

5) Amount injected : 10μl

6) Elution time : 3.65 minute

Analysis for BHT also used the Waters HPLC system. The chromatographic conditions were as follows:

1) Column : Delta-Pak HPI C18 300A (3.9mm × 150mm)

2) Solvent : Methanol : Water = 85 : 15

3) Flow rate : 1.0 ml/minute

4) Detection wavelength : 280nm

5) Amount injected : 10 μl

6) Elution time : 3.7 minute

The concentration of antioxidant in the test film was determined by comparison of detector response to a standard calibration curve which was constructed from standard solutions of known concentration. A standard solution of antioxidant was prepared by accurately weighing 0.01 gram of antioxidant, transferring the weighted sample to a 100 ml volumetric flask and diluting with acetonitrile to volume.

The primary standard solution was then used to prepare a series of standard solutions of known concentration by a serial dilution procedure. For construction of the calibration curve a 10 µl sample was withdrawn from the respective standard solutions and injected directly into the HPLC. The calibration data are shown in Appendix A.

The concentration of antioxidant in the film was determined by substitution into the expression.

$$\text{antioxidant} = \frac{AU \times CF \times V_{total} \times 100}{V_{injection} \times Wt._{polymer}} \qquad (8)$$

CF = calibration factor (g/AU)

AU = average area units from integrator

V_{total} = total extractant phase volume (100ml)

$V_{injection}$ = sample volume injected (10µl)

$Wt._{polymer}$ = weight of polymer sample (g)

Product Stability Studies

Approximately 25 grams of oat cereal product were packaged in 4.5 inch by 5 inch test pouches. The pouches were fabricated from the three test films. The pouches were

sealed with an impulse sealer (Sencorp System Inc. Model No-16TP). The heating time was 0.8 second, the cooling time was 1 second and the pressure was 35 psi. All test pouches were mounted on a wooden sampling rack in an environmentally controlled room. The storage environment was monitored using a hygrometer. Temperature and relative humidity were maintained at 23°C ± 2°C and 50%RH ± 2%. The storage arrangement is shown in Figure 9.

Test pouches made from the respective film structures and filled with product were removed from storage every two weeks for the first two months of storage. After two months, filled pouches were removed monthly. Packaged product was sampled on a monthly basis for a period of eleven months. After sampling the pouches, the cereal product was removed from the package and the following analysis performed on the product: (1)Hexanal analysis to determine the extent of lipid oxidation; and (2) the level of sorbed antioxidant present in the cereal product. The pouches were also assayed to determinate the relative concentration of antioxidant retained as a function of the storage time. A flow diagram of the test scheme is shown in Figure 10.

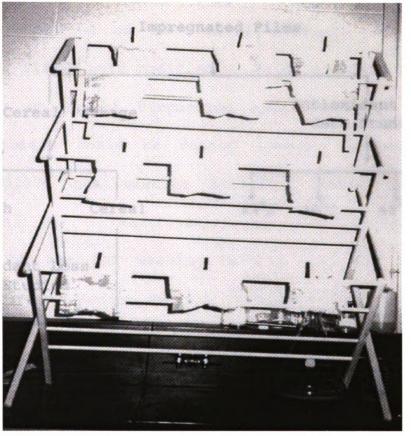


Figure 9. The storage arrangement for cereal package

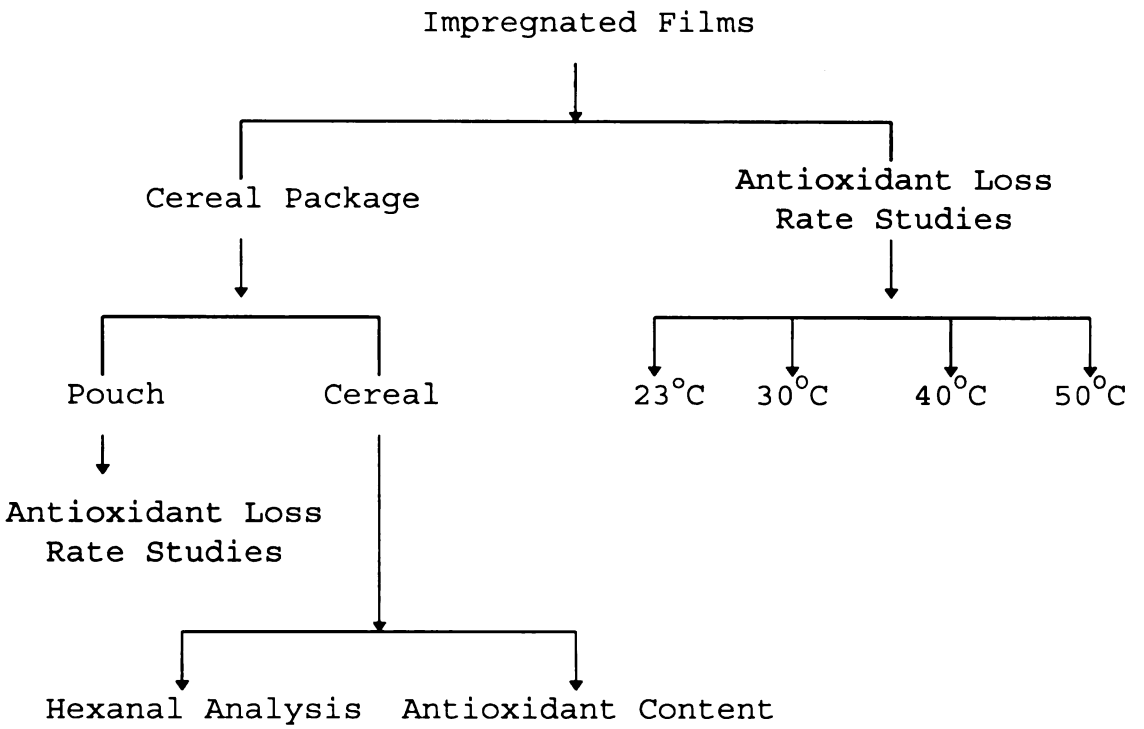


Figure 10. Flow diagram of the test scheme

Hexanal Quantification

Apparatus for Trapping of Volatiles

A dynamic gas purge and trap system was designed for headspace sampling of the cereal product. Six 250 ml Erlenmeyer flasks were modified to 29/42 standard taper male joints to which the dispersion tube assembly of a gas washing bottle could be fitted (Stopper assemblies for Corning 31770 gas washing bottles, Fisher Scientific, Pittsburgh, PA). Modification of the dispersion tube assembly of the gas washing bottles and the Erlenmeyer flasks were performed by the Chemistry Department Glass Blowing Shop at MSU. A schematic diagram of the dynamic purge and trap system is shown in Figure 11. As shown, a cylinder of compressed nitrogen was interfaced to a dispersing manifold which consisted of three flow meters and needle valves, all connections were through 1/8" O.D. copper tubing and swagelok fitting. Flow meters were used to provide a continuous indication that a constant rate of flow of nitrogen was maintained to the individual purge and trap cells. Gas flow was regulated with NU PRO needle valves, type B-25G.

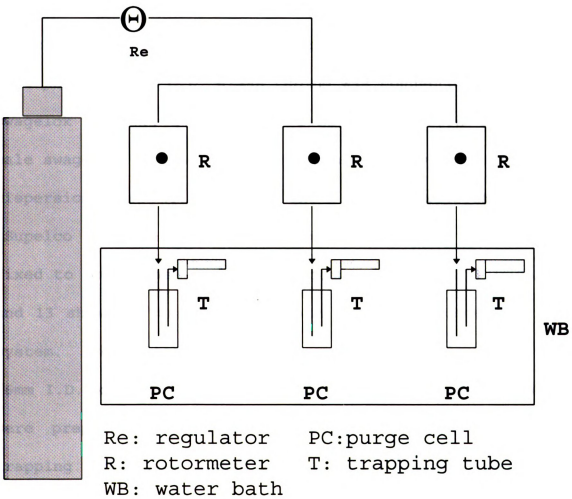


Figure 11. Schematic diagram of dynamic purge and trap system

The trapping system was designed to ensure that the sample was continuously flushed with nitrogen gas and the desorbed volatiles conveyed to the trapping tube attached. The sorption trap was connected to the exit port of the dispersion head via swagelok adapters. The dispersion head exit port of 8 mm O.D. glass tubing was connected by a 5/16" swagelok nut and a series of reducing adapters to a 1/4" male swagelok fitting. The sorption trap was mounted to the dispersion head with a 1/4" thumb wheel swagelok fitting (Supelco Inc., Bellefonte, PA) for easy removal and could be fixed to both glass and metal desorption traps. Figures 12 and 13 show the trapping cell and the whole purge and trap system. The glass thermal desorption tubes-Carbotrap 300 (6mm I.D. x 4 mm I.D. x 11.5 cm) used in the present study were prepacked by Supelco Inc. (Bellefonte, PA). The trapping tubes were packed with 300 micrograms of Carbotrap C absorbent, 200 micrograms of Carbotrap B absorbent and 125 micrograms of Carbosieve S-III absorbent.

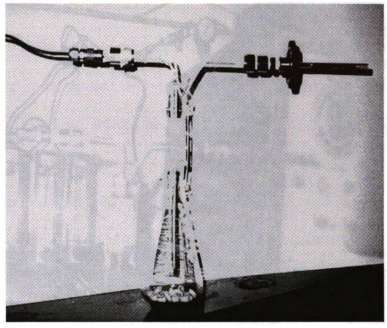


Figure 12. The apparatus with the sparging tube attached

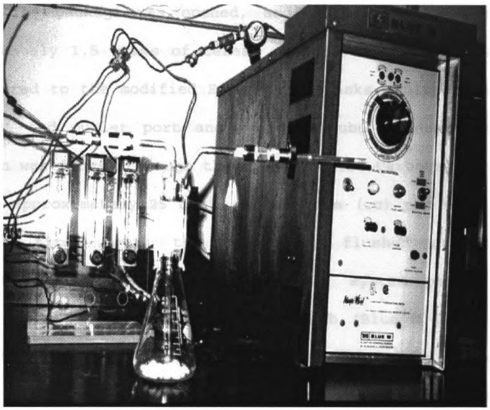


Figure 13. Photograph of Dynamic Purge and Trap System

Hexanal Analysis Procedure

Individual packaged cereal product samples were removed from the environmental chamber as a function of storage time. The package was opened, and the product removed. Approximately 1.5 grams of cereal product were weighed and transferred to the modified Erlenmeyer flasks equipped with an inlet and outlet port and sparging tube as described. Nitrogen was flowed through the flask and the Carbotrap at a rate of approximately 25 cubic centimeters (cc) per minute. For the first hour, the flask was flushed at room temperature to remove oxygen from the system prior to heating. After one hour, the water bath (Blue M Constant Temperature Bath, Blue Island, IL) was turned on and allowed one hour to reach 55°C. The modified Erlenmeyer flasks were placed in the water bath and the system purged for 24 hours. Repetitive analyses of the cereal sample showed no additional hexanal detected. Each sample was analyzed in triplicate.

After 24 hours trapping, the Carbotrap tubes containing trapped volatiles were transferred to the thermal desorption unit (Model 890, Dynatherm Analytical Instruments, Inc.) which was interfaced to a gas chromatograph for

quantification. The sorbed volatiles were desorbed by heating for 6 minutes at 340°C, with the valve and transfer line held at 230°C to maintain the desorbed compounds in the vapor phase, while being transferred to the gas chromatograph. Helium was used as a carrier gas through the thermal desorption unit at a flow rate of 7.5 ml./minute at 40 psi. After sample desorption, the sorbant tubes were conditioned at 340°C for 40 minute prior to re-use.

Gas chromatographic (GC) analyses were carried out with a Hewlett-Packard 5890A gas chromatograph, equipped with dual flame ionization detectors (Avondale, PA, USA). The GC conditions were as follows:

Column : Supelco, SPB5 (30m × 0.32nm, 10um)

Helium carrier : 7.5 ml/minute

H2 : 30 ml/minute

Nitrogen : 30 ml/minute

Air : 400 ml/minute

Initial temperature : 40°C

Initial time : 6 minute

Rate : 5 degree/minute

Final temperature : 200°C

Final time : 10 minute

Hexanal was eluted at a retention time of 8.5 minutes. The concentration of hexanal in the cereal product was determined by substitution into the following equation :

$$\text{Conc. (Wt/Wt)} = \frac{AU \times CF}{Wt._{total}} \qquad (9)$$

AU = average area units response from integrator

CF = Calibration factor (gm/Au)

$Wt._{total}$ = Weight (gm) of cereal sample

Hexanal Calibration Curve Procedure

A standard solution of hexanal was prepared by accurately weighing 0.04 gram hexanal and transferring the weighed sample to a 100 ml volumetric flask and diluting with methanol to volume. The primary standard solution was then used to prepare a series of standard solutions of known concentration by a serial dilution procedure. To prepare a standard calibration curve to establish the linearity and sensitivity of the thermal desorption procedure, a 1.2µl sample was withdrawn from the respective standard solutions and the sample injected directly onto the Carbotrap tube. The Carbotrap tube was then transferred to the thermal

desorption unit. The GC conditions were as follows: programmed at an initial temperature of 40°C for six minutes, followed by heating at a rate of 5°C per minute to a final temperature of 200°C which was held for ten minutes. The calibration data are shown in Appendix B.

Antioxidant content of Cereal

Lipid extraction

The cereal was extracted for total lipid using the method of Hoojjat (1987). A 40 gram sample of cereal was removed from the test pouches. A weighed quantity of the product was mixed in a blender (WARD Montgomery, 12 speed) at low speed with 50 ml chloroform and 100 ml methanol for 2 minutes. An additional 50 ml of chloroform was added and blended for 30 seconds. After 30 seconds, 50 ml of HPLC grade water was added and the solution blended another 30 seconds. The proportion of chloroform, methanol and water is 2:2:1, respectively.

The mixture was filtered by vacuum filtration. After removing the cereal residue, the filtrate was transferred to a separatory funnel. The chloroform layer (bottom layer) was collected and concentrated using a Buchi Rotavapor Model

RE III series with a 50°C water bath and water aspirator pressure. After removing the chloroform, a yellow oily layer remained. This layer was then transferred to a screw capped vial and stored at -18°C for later analysis.

Antioxidant Content of Extracted Fat

Antioxidant content of the cereal product following storage was determined using the method of Hoojjat (1987). A 0.5 gram sample of extracted oil was weighted into a 4 ml vial and 3 ml of acetonitrile was added. The mixture was shaken by hand for 30 second and allowed to stand for approximately 15 minutes. After two phase separation, the top layer (acetonitrile) was collected, filtered and analyzed by HPLC for its antioxidant content (Analytical conditions were as previously described).

The antioxidant content of the cereal product is determined by substitution into the following equation:

Antioxidant content (g/g cereal)

$$= \frac{AU \times CF \times V_{total} \times Wt._{total}}{V_{injection} \times Wt._{sample} \times Wt._{cereal}} \qquad (10)$$

CF = calibration factor (g/AU)

AU = average area units from integrator

V_{total} = total solution phase volume (3ml)

$V_{injection}$ = sample volume injected (10µl)

$Wt._{total}$ = total weight of extracted oil (g)

$Wt._{sample}$ = weight of oil sample assayed (~0.5g)

$Wt._{cereal}$ = weight of cereal sample extracted (~40g)

RESULTS AND DISCUSSION

Thickness of Packaging Materials

The optical microscopy procedure used to determine the thickness of the respective layers of the α-tocopherol impregnated laminate was found to give good resolution of the heat seal layer, but could not resolve the middle and outer HDPE layer. However, values of the total laminate thickness and the heat seal layer were obtained. Thickness measurements, were repeated five times, and the data obtained is presented in Appendix C. The averaged values obtained are summarized in Table 2. The total film thickness was also measured by a micrometer (Model 549 Micrometer, Testing machines, Inc. Amityville, NY) and the results are summarized in Table 2 for comparison. Ten measurements were recorded and are tabulated in Appendix D. The thickness values determined by Bailey (1995) for Film II are also summarized in Table 2.

Table 2. Average thickness for lamination film

	heat seal layer	HDPE layer	Total [b] thickness	Total [c] thickness
Film I	14.7 μm	44.3 μm	59.0 μm	59.1 μm
Film II [a]	10.16 μm	45.72 μm	61.9 μm	62 μm

[a] Source: Bailey (1995)
[b] Measured by microscopy
[c] Measured by micrometer

As shown in Table 2, the total thickness of Film I measured by optical microscopy and by micrometer is 59μm and 59.1μm, respectively, providing good agreement between the two procedures.

Loss of α-Tocopherol from Coextruded Laminate Film

The relative loss of α-tocopherol from the test film, as a function of time and temperature, was determined by the HPLC procedure previously described. The initial concentration of α-tocopherol was determined at different time intervals, between receipt of the roll stock and termination of the studies, to insure a constant concentration. The results are summarized in Table 3. The test film was reported to contain 275 ppm(wt/wt) gm α-

tocopherol per gm total film weight, according to the supplier (Bemis Company, Terre Haute, Indiana). The concentration level of α-tocopherol reported by the supplier is assumed to be the level added to the resin. Losses during processing could account for the somewhat lower α-tocopherol level determined by HPLC analysis. As shown, the level of α-tocopherol is reasonably constant over the course of the study.

Table 3. α-Tocopherol concentration (wt/wt%) of laminate roll stock at different time intervals

Storage Day[a]	0	100	120	170	200	380	Avg.
wt/wt%[b]	0.0238	0.022	0.023	0.0218	0.02	0.0185	0.0215

[a] Time of storage from date of receipt

[b] Average of three replicate sample analysis, with an average standard deviation of ± 5%

The results of monitoring the loss of α-tocopherol from the test film, at 23, 30, 40, and 50°C, by HPLC analysis, are summarized in Tables 4 through Table 7, respectively.

The results are also presented graphically in Figure 14, where the relative percent α-tocopherol remaining in

the film is plotted as a function of time for the studies carried out at 23, 30, 40, and 50°C, respectively. According to Han et al. (1987), the rate of loss of an additive from the test film follows a first-order or pseudo first-order rate expression:

$$\ln(C_t/C_o) = -kt \qquad (11)$$

where C_o is the initial concentration of α-tocopherol in the film and C_t is the concentration (wt/wt%) at any time (t); k is the loss rate constant, and t is the time interval. Graphical analysis (see Figure 14) indicated that a first order expression provided a good description of the rate loss of α-tocopherol from the lamination, up through greater than 85% loss. The rate constants determined from Equation (11) for the different temperatures are summarized in Table 8.

As shown in Figure 14, a linear relationship was found for the plot of $(C_t/Co)\times100$ versus time at the four different temperatures of test evaluated in the rate loss studies. The following expressions were derived from a least squares fit of the rate loss data.

$$23^\circ C \quad (C_t/Co)\times100 = 92.954 * \exp(-0.0004t) \qquad (12)$$

$$30^\circ C \quad (C_t/Co)\times100 = 82.159 * \exp(-0.0021t) \qquad (13)$$

$40^{\circ}C$ $(C_t/Co)\times100 = 129.46 * \exp(-0.0068t)$ (14)

$50^{\circ}C$ $(C_t/Co)\times100 = 114.67 *\exp(-0.0284t)$ (15)

The R^2 values were 0.9914, 0.9069, 0.9177, and 0.9461 for 23, 30C, 40C, and $50^{\circ}C$, respectively. At $30^{\circ}C$, the relative percent loss of α-tocopherol from the test film was 83% after 720 hours, while a sample stored at $50^{\circ}C$ showed loss of the same amount after 64 hours. The relationship between the rate loss constant and temperature is illustrated in Figure 15, where K is plotted as a function of temperature $[1/T(^{\circ}k)]$.

The activation energy for the loss of α-tocopherol from the laminate structure was determined by solution of the Van't Hoff-Arrhenius equation:

$$k = k_o \exp(-E/RT) \quad (15)$$

where E is activation energy (kcal/mole), R is the gas constant (1.98 cal/degree•mole), k_o is the pre-experential constant, and T is temperature ($^{\circ}K$). As shown in Figure 15, the loss of α-tocopherol was found to follow the Arrhenius relationship. From the slope of the Arrhenius plot, the activation energy for the loss of ATP from the HDPE was determined to be 28.87 kcal/mole. Bailey (1995) determined

the activation energy for the loss of BHT from Film II, which is a similar multi-layer laminate film, to be 26 kcal/mole.

Calvert and Billingham (1979) proposed that the loss of BHT and other simple low molecular weight additives from thin films is controlled by evaporation, while diffusion controls the loss of additive from thick films and bulk solids. The multi-layer lamination used in this study has a thickness of 59μm. It was therefore assumed that the rate of α-tocopherol loss is controlled by surface evaporation, since the sample was in film form. In analysis of the rate loss data, it was further assumed that the additive (i.e. α-tocopherol) had attained an equilibrium partition distribution between the respective layers of the laminate.

% Recovery for Antioxidant

The % recovery of α-tocopherol carried through the soxhlet extraction/HPLC analysis procedure was determined to be 97%. The data and calculation are given in Appendix E.

Table 4. Loss of α-tocopherol (ATP) from coextruded laminate film at 23°C, determined by HPLC analysis

Time (hours)	ATP Concentration[a] (wt/wt%)	Relative % ATP $(C_t/Co)\times100$
0	0.022	100
672	0.014	62.4
1344	0.012	53.5
4368	0.003	14.3
5040	N/A	N/A

[a] Average of three replicate sample analyses, with an average standard deviation of ± 5%

Table 5. Loss of α-tocopherol (ATP) from coextruded laminate film at 30°C, determined by HPLC analysis

Time (hours)	ATP Concentration[a] (wt/wt%)	Relative % ATP $(C_t/Co)\times100$
0	0.0230	100
48	0.0180	77.7
96	0.0140	59.0
144	0.0150	65.70
192	0.0120	52.1
240	0.0130	56.7
288	0.0086	36.7
336	0.0090	38.6
384	0.0080	34.8
480	0.0065	28.1
528	0.0060	26.0
576	0.0050	21.0
624	0.0050	22.5
672	0.0075	30.8
720	0.0040	17.4

[a] Average of three replicate sample analyses, with an average standard deviation of ± 5%

Table 6. Loss of α-tocopherol (ATP) from coextruded laminate film at 40°C, determined by HPLC analysis

Time (hours)	ATP concentration[a] (wt/wt%)	Relative % ATP $(C_t/Co)\times100$
0	0.0180	100.00
25	0.0177	97.8
49	0.0160	90.9
72	0.0158	87.2
96	0.0120	64.5
149	0.0100	57.4
198	0.0070	37.7
240	0.0062	34.4
312	0.0040	20.8
384	0.001	5.5

[a] Average of three replicate sample analyses, with an average standard deviation of ± 5%

Table 7. Loss of α-tocopherol (ATP) from coextruded laminate film at 50°C, determined by HPLC analysis

Time (hours)	ATP Concentration[a] (wt/wt%)	Relative % ATP $(C_t/Co)\times100$
0	0.0283	100
8	0.0215	75.8
16	0.0212	74.7
24	0.0189	66.6
32	0.0142	50.1
40	0.0123	43.5
48	0.0108	38.1
56	0.0065	22.9
64	0.0049	17.4
72	0.0033	11.6

[a] Average of three replicate sample analyses, with an average standard deviation of ± 5%

Table 8. Rate constants for the loss of ATP from the coextruded laminate film structure

Temperature (°C)	Loss Rate Constant k (hr^{-1})
23	0.0004
30	0.0021
40	0.0068
50	0.0284

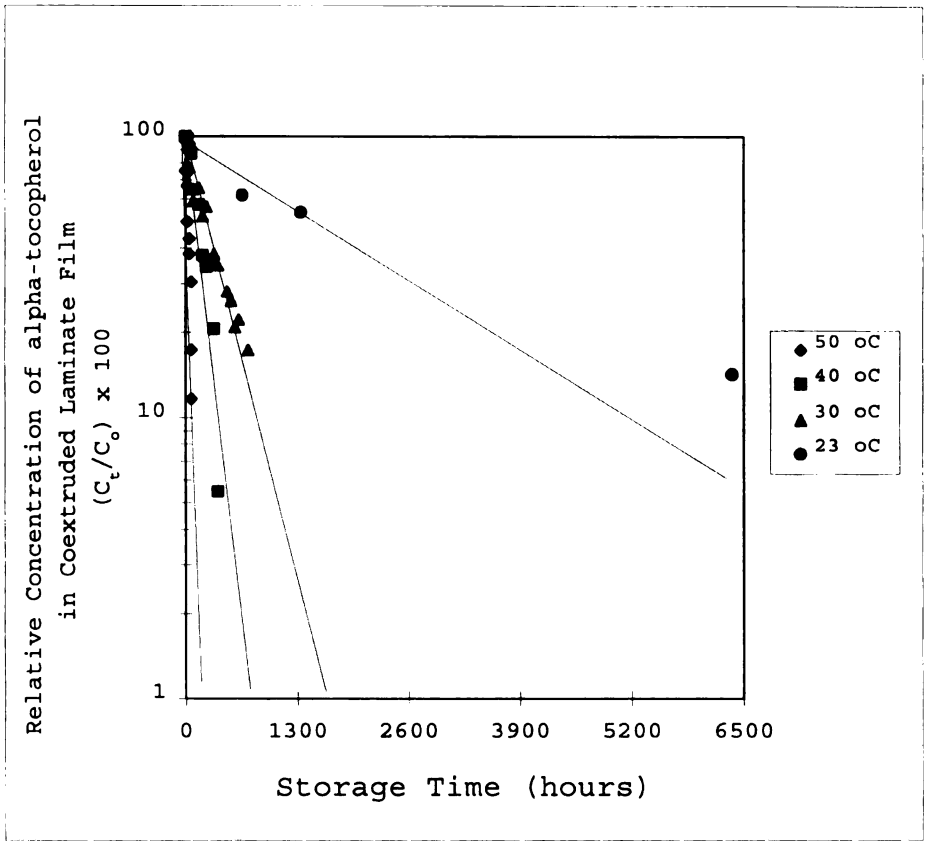


Figure 14. Loss of ATP from the coextruded laminate film as a function of time/temperature

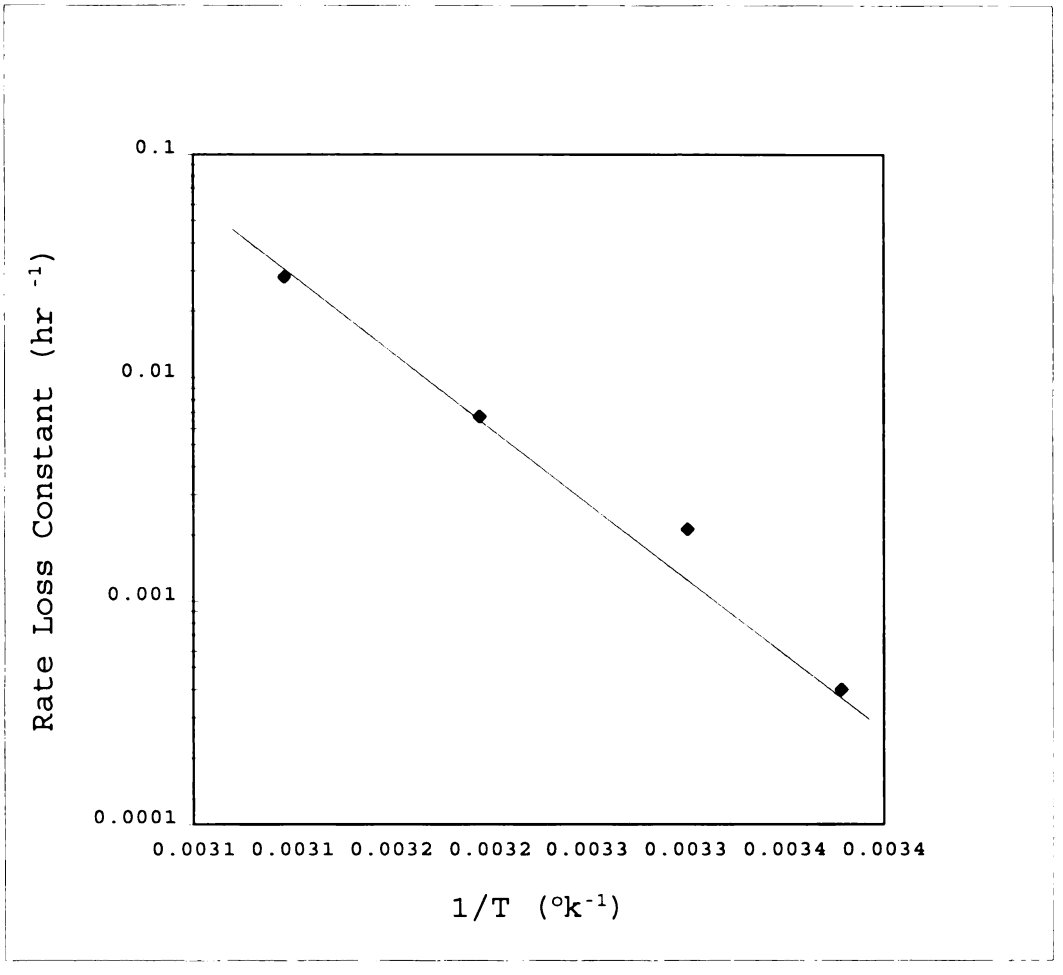


Figure 15. Arrhenius plot of loss rate constant versus temperature

Relative Rate Losses of Antioxidants

The relationship between the rate of loss of BHT and α-tocopherol from the laminate structures evaluated in the present study is shown graphically in Figures 16, 17 and 18, where the relative percent antioxidant remaining in the film is plotted as a function of time for rate loss studies carried out at 23, 30 and 40°C, respectively. The rate loss data for BHT from Film II was reported by Bailey (1995). As can be seen, the α-tocopherol exhibits a significantly lower rate of loss, as compared to the loss rate of BHT from the lamination. The linear expression derived for the α-tocopherol rate loss at ambient temperature (23°C) gave a rate constant of 0.0004 (hr^{-1}). When compared to the loss rate constant of BHT from the laminate at 23°C (k=0.0043 hr^{-1}), the rate of loss of BHT from the laminate was approximate 11 times greater than the rate of loss of α-tocopherol. At 30°C, the rate of loss of BHT from the laminate was approximately 10 times greater than the rate of loss of α-tocopherol, while the rate of loss of BHT at 40°C was also an order of magnitude greater than the rate of loss of α-tocopherol.

The lower rate of loss of α-tocopherol from the laminate may be attributed to a higher rate of diffusion of BHT through the laminate surface layers, or a difference in the rate of evaporation of the BHT from the respective surface layers, as well as to the equilibrium partition distribution of the antioxidants between the respective layers of the lamination. Antioxidant transport is related to the solubility of the additive within the respective layers of the lamination and the diffusion of the additive through it. Differences in either the solubility or diffusivity can affect the transmission characteristics of the respective antioxidants. The solubility difference depends primarily on the difference in the physico-chemical nature of the migrating species and the respective laminate layers and will be reflected in the partition distribution of the antioxidant between the laminate layers. On the other hand, differences in antioxidant diffusivity is determined largely by the size and shape of the molecule, and by the degree of aggregation among the diffusing species within the polymer layers.

The fact that the initial concentration of the antioxidant BHT was significantly higher than the initial

level of α-tocopherol incorporated into the laminate may also be a contributing factor to the observed lower rate loss for α-tocopherol.

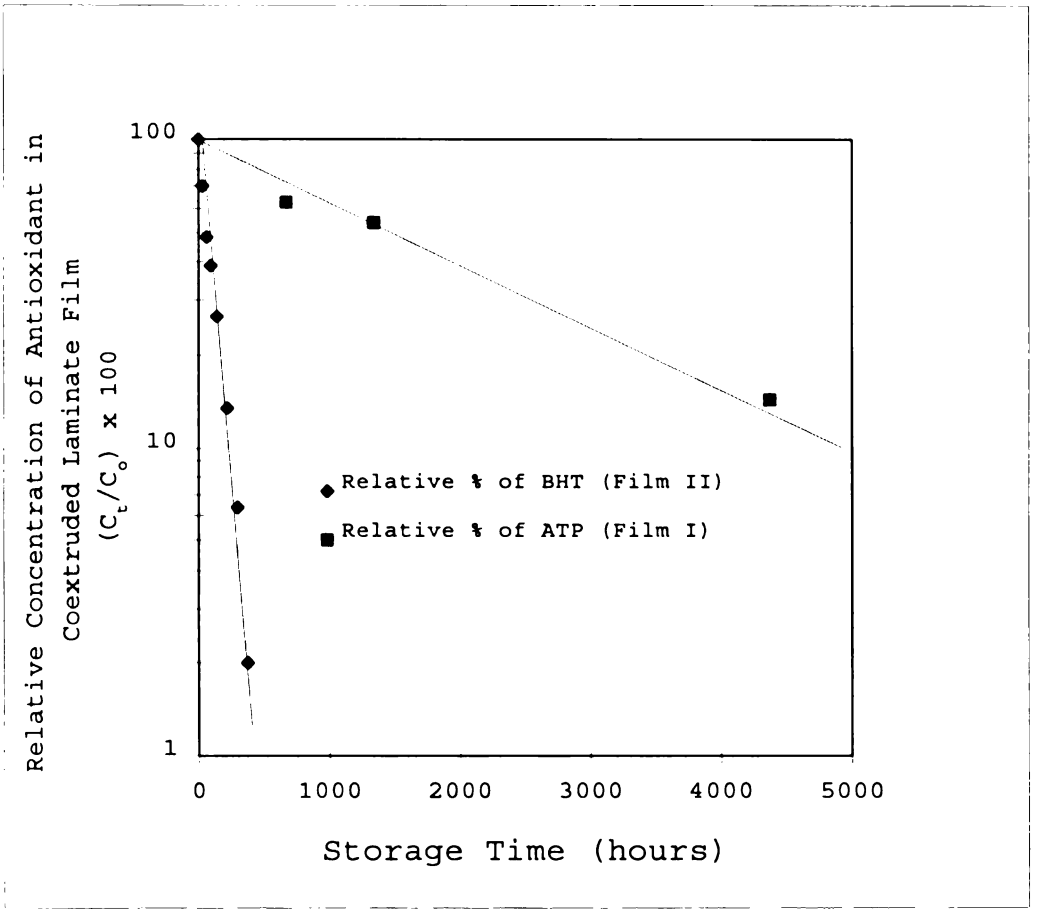


Figure 16. Relative percent loss of BHT and α-tocopherol from coextruded laminated films as a function of time (23°C)

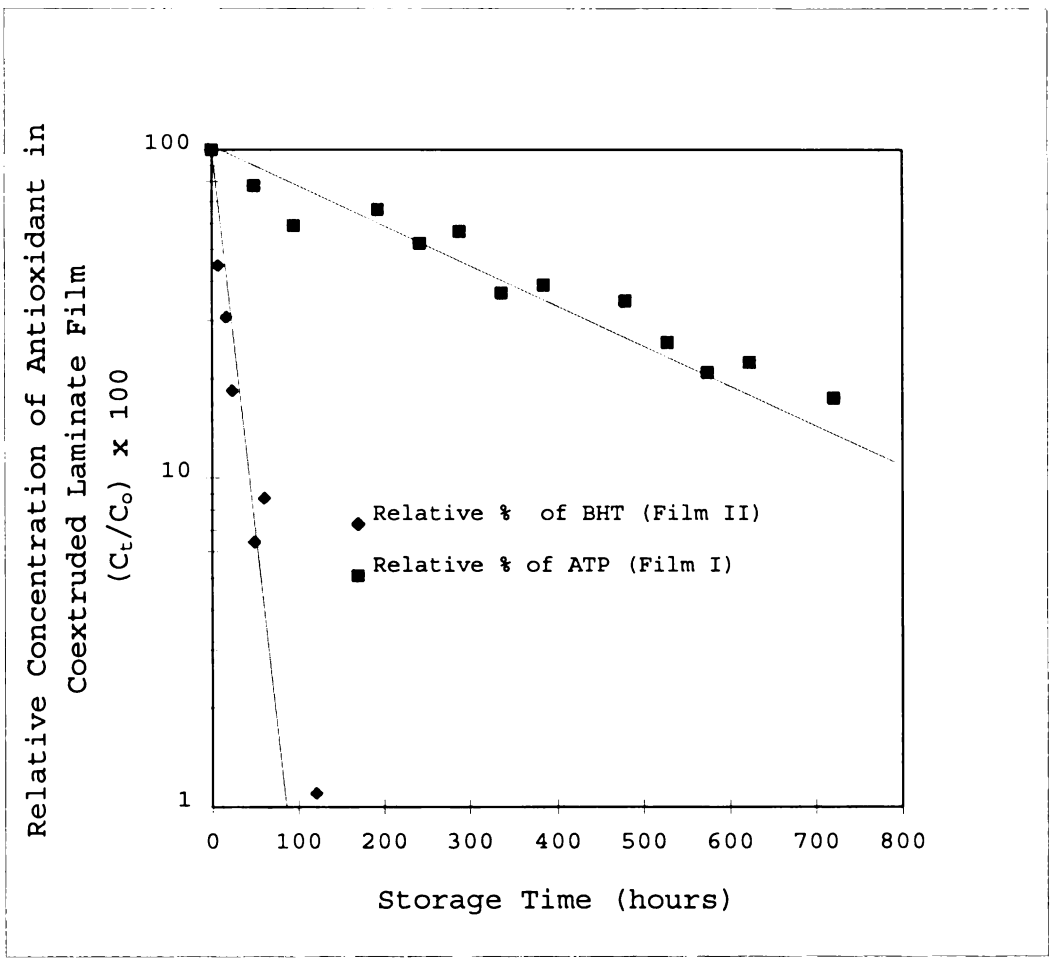


Figure 17. Relative percent loss of BHT and α-tocopherol from coextruded laminated films as a function of time (30°C)

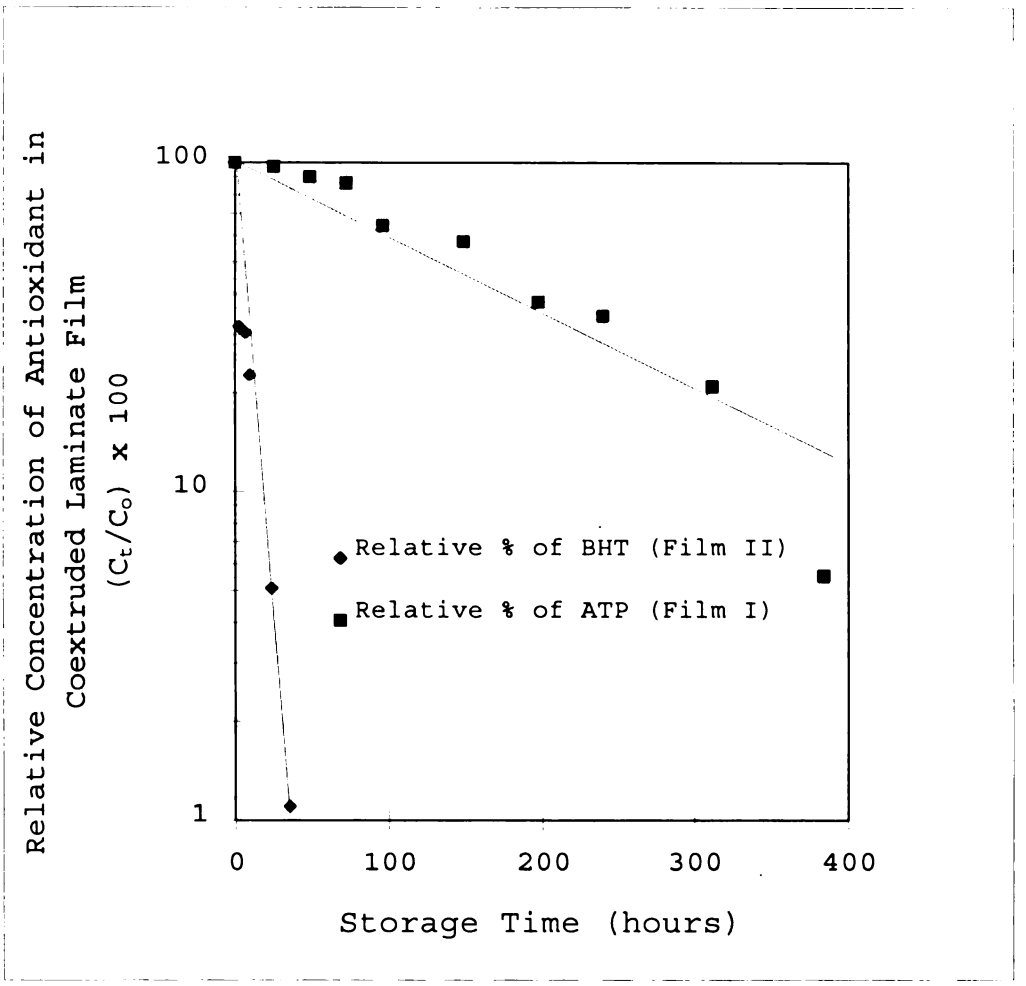


Figure 18. Relative percent loss of BHT and α-tocopherol from coextruded laminated films as a function of time (40°C)

Product Storage Studies

The results of hexanal analysis carried out on cereal product packaged in pouch I (α-tocopherol impregnated pouch structure), pouch II (BHT impregnated pouch structure), and pouch III (control pouch structure) are present in Table 9. The calibration factor applied for analyses carried out from week 0 through week 20 was based on the initial hexanal calibration curve constructed. The remaining calculations were all based on a calibration factor derived from a second hexanal calibration curve (see Appendix B). The relationship between the extent of lipid oxidation and the respective laminate package structures evaluated in the present study is shown graphically in Figure 19, where the hexanal concentration in the cereal product is plotted as a function of storage time. As shown, up through 20 weeks of storage the levels of hexanal detected in the cereal product packaged in the three test structures were very similar. Based on a one-way analysis of variance, by Tukey's analysis from 0 to 20 weeks, there was no statistically significant difference ($p>0.05$) between the levels of hexanal in the cereal product packaged in the three test pouches. After the 20th week of storage, however, product packaged in

pouches without antioxidant exhibited a significantly higher level of hexanal ($p<0.05$), as compared to the product packaged in the antioxidant impregnated laminate structures. Over a 44 week storage period, there appeared to be no significant difference ($p>0.05$) between the α-tocopherol impregnated pouches and BHT impregnated pouches in terms of hexanal levels detected. The statistical analysis carried out is presented in detail in Appendix F. Although the BHT and α-tocopherol have different characteristics such as volatility and stability, both appeared to provide similar effectiveness in terms of retarding lipid oxidation of the cereal product.

Fritsch and Gale (1971) monitored the concentration of hexanal in oat, corn and wheat cereals stored at 37°C for 12 weeks. They determined a level of 5mg/kg (5ppm, wt/wt) to be indicative of significant deterioration in quality. As shown in Table 9, after 44 weeks, the cereal samples had not yet reached this level of hexanal concentration when held at 23°C and 50%RH. McWeeny (1968) studied a series of reactions associated with food systems, which included: (1) the oxidation of unsaturated fats and oil; (2) oxidized flavor in milk fat; and (3) vitamin lose in fried foods. He

stated that the rate of a chemical reaction is affected by temperature and sometimes a negative rate of change of the reaction can occur when the substrate is involved in an alternate pathway. In the current study, the low concentrations of hexanal determined may be attributed to the low relative humidity and temperature conditions of the storage environment.

In normal distribution, according to packaging scientists at General Mills Inc. (Culter, 1995), a cereal product has a shelf life of approximately nine months. In this study, the cereal product packaged in the antioxidant impregnated packaging structures showed decreasing levels of hexanal produced, as well as a reduction in the rate of oxidation, when compared to the product stored in laminate pouches to which no antioxidant was incorporated. These results provide supportive evidence for the effectiveness of the evaporation/sorption mechanism of antioxidant activity.

Table 9. The hexanal concentration of cereal product packaged in pouches fabricated from test coextruded laminate structures

Hexanal Concentration (μg/g cereal product)

Storage Time (weeks)	Pouch I [a] [d]	Pouch II [b] [d]	Pouch III [c] [d]
0	0.141	0.141	0.141
2	0.163	0.171	0.164
4	0.147	0.182	0.168
6	0.165	0.187	0.190
8	0.172	0.162	0.180
12	0.180	0.201	0.162
16	0.202	0.164	0.191
20	0.206	0.182	0.234
28	0.249	0.188	0.382
36	0.265	0.296	0.460
44	0.289	0.324	0.552

[a] pouch I - α-tocopherol impregnated pouch structure

[b] pouch II - BHT impregnated pouch structure

[c] pouch III - control pouch structure

[d] Average of three replicate sample analyses, with an average standard deviation of ± 5%

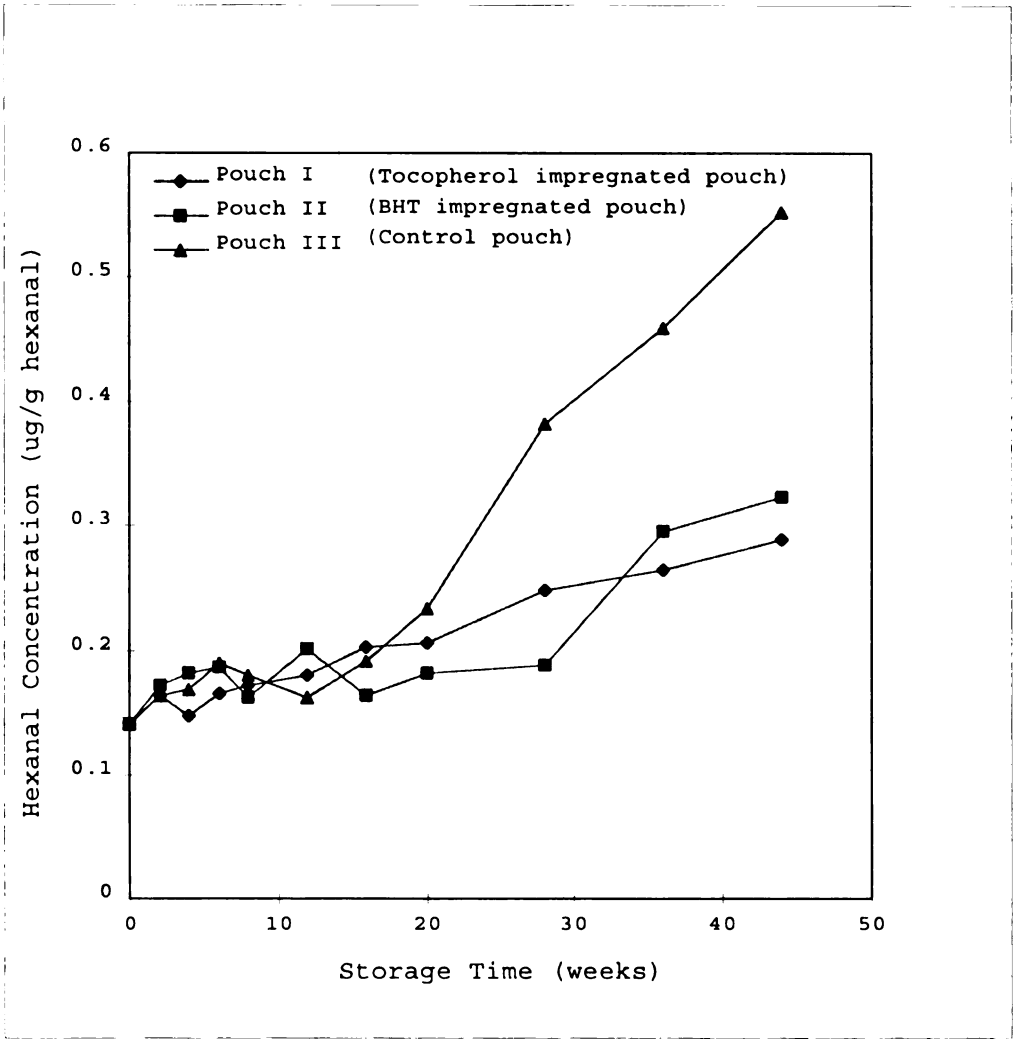


Figure 19. Hexanal concentration in cereal product packaged in pouches fabricated from test coextruded laminated structures

Retained Levels of Antioxidant in Package Structures

After removing the cereal product, the BHT and α-tocopherol content of the pouch material was determined as a function of storage time. No BHT was found in the BHT impregnated laminate structure after 4 weeks storage at 23°C and 50%RH. Bailey (1995) carried out a series of studies monitoring the loss of BHT from the test Film II and found no detectable level of BHT after storage for 3.2 weeks at 23°C. These result were in agreement with the present finding. In earlier studies, Hoojjat et al. (1987) determined the rate of loss of BHT from HDPE at 21.5°C, and found approximately 80% of the initial quantity of BHT lost after 1 weeks storage. The results of the studies involving determination of the α-tocopherol levels lost from the package structure with time are summarized in Table 10. Comparing these results with those obtained from the laminate film (see Table 4) showed the rate of loss of α-tocopherol from the package to be somewhat less than that from the laminate film directly. In determining the rate of loss of antioxidant from the laminate film, the outer and inner surface layers are both exposed to the external environment, which acts as a infinite sink. However, when

the laminate film is fabricated to a package, the outer film surface is exposed to the external environment, while the heat seal layer is in contact with a finite environment. This may account for the observed differences in the losses of α-tocopherol.

Loss of an additive, such as α-tocopherol or BHT, from the surface of a polymeric structure, like the laminate film studied, is determined by its solubility within the respective layers of the lamination and the diffusion coefficient of the additives in the polymer. Calvert and Billingham (1979) pointed out that BHT loss from thick films and bulk solids is determined by the rate of diffusion through the film, while antioxidant losses from thin films and fibers are controlled by their volatility from the surface. A number of studies have shown that BHT has high steam volatility (Dziezakm 1986; Figge, 1976; Till, 1982). Therefore, BHT is easily volatilized from the surface of the polymer into either the package headspace or to the external environment. Compared to BHT, α-tocopherol is much more stable while present in a polymer film. Its long side chain provides good solubility in the polymer and it does not begin to volatilize until temperatures reach 300°C

(McMurrer, 1991). Studies show that only 100 ppm(wt/wt) of α-tocopherol in HDPE film are needed to enhance flavor retention and shelf life (Ho et al., 1994; Laermer, 1993).

Table 10. α-Tocopherol content in packaging pouches as a function of storage time

Storage time (weeks)	α-tocopherol concentration[a] (%wt/wt)	Relative % remaining in packaging pouches
0	0.0238	100
4	0.0193	81.0
6	0.0192	80.7
8	0.0185	77.8
12	0.0124	51.9
16	0.0116	48.6
20	0.0115	48.6
28	0.0071	29.3
36	N/A[b]	N/A[b]

[a] Average of three replicate sample analysis, with an average standard deviation of ± 5%

[b] N/A - Not available

Antioxidant Content of Cereal Product

A schematic diagram of the mechanism by which antioxidant impregnated materials may control lipid oxidation is presented in Figure 20. As shown, the mechanism requires diffusion of antioxidant through the polymer bulk phase, evaporation of the antioxidant from the surface of the packaging material, diffusion of the antioxidant in air and, lastly, sorption of antioxidant onto the product surface.

To establish the validity of this mechanism of antioxidant activity, storage studies were carried out as described and the product analyzed for the extent of lipid oxidation and for antioxidant content (i.e., extent of antioxidant sorption) as a function of time. The results of analysis for BHT levels in the cereal product packaged in the BHT impregnated laminate pouch are summarized in Table 11. As shown, after 4 week of storage, approximately 15% of the BHT initially present in the packaging film was transferred or sorbed by the product, while no detectable level of BHT was found to remain in the packaging structure.

The results further show that the BHT concentration in the product decreased with increased storage time. It can

be assumed that the sorbed BHT is undergoing oxidative reactions as part of its antioxidant function, thus accounting for its decrease in concentration with time.

The level of α-tocopherol present in the cereal product could not be determined, due to difficulties experienced with achieving chromatographic separation of α-tocopherol from the other compounds extracted from the cereal product. However, from the data shown in Table 10, it was assumed that some level of α-tocopherol migrated to the food product to provide antioxidant activity. Alternatively, the α-tocopherol incorporated in the laminate structure may act to retard lipid oxidation of the oat cereal product by inhibiting polymer surface catalyzed oxidative reactions. This assumes rapid diffusion of the α-tocopherol from the core layer, to the heat seal polymer surface. Precedent is found in the literature for surface catalyzed oxidation reactions involving polyolefins which are thermally processed at elevated temperatures resulting in their oxidation and the formation of hydroperoxide functionality on the polymer surface (Mannheim et al., 1987; Mannheim et al., 1988; Culter, 1992).

The migration of BHT has been widely studied by a number of investigators. Schmope et al. (1986) studied the migration of the antioxidants, BHT and Irganox®-1010 from LDPE to food and food-simulating liquids. They found BHT transferred into the food phase more rapidly than Irganox®-1010. The rate of migration will also depend on the nature of the contact media and the polymeric contact phase. Figge et al. (1976) reported that BHT migration from PVC into various food products was much lower than from HDPE. Till et al. (1982) stated that BHT migration from HDPE to fatty foods occurs at a faster rate than to aqueous foods.

There is little information available concerning the migration of α-tocopherol. Laermer et al. (1993) studied the migration of α-tocopherol from HDPE film to both heptane and a 50% aqueous ethanol solution by an immersion procedure and found that 8.4% of the initial α-tocopherol additive present in the HDPE film migrated to the heptane while 3.8% transferred to the ethanol solution.

Most cereal grains contain appreciable quantities of α-tocopherol (Herting, 1969; Slover, 1983; Slover, 1969). Manufacture of oatmeals resulted in relatively little or no decrease of vitamin E, but more extensive processing

increased losses to about 90% (Herting, 1969). In this study, the level of α-tocopherol initially present in the product was not determined for reasons stated above.

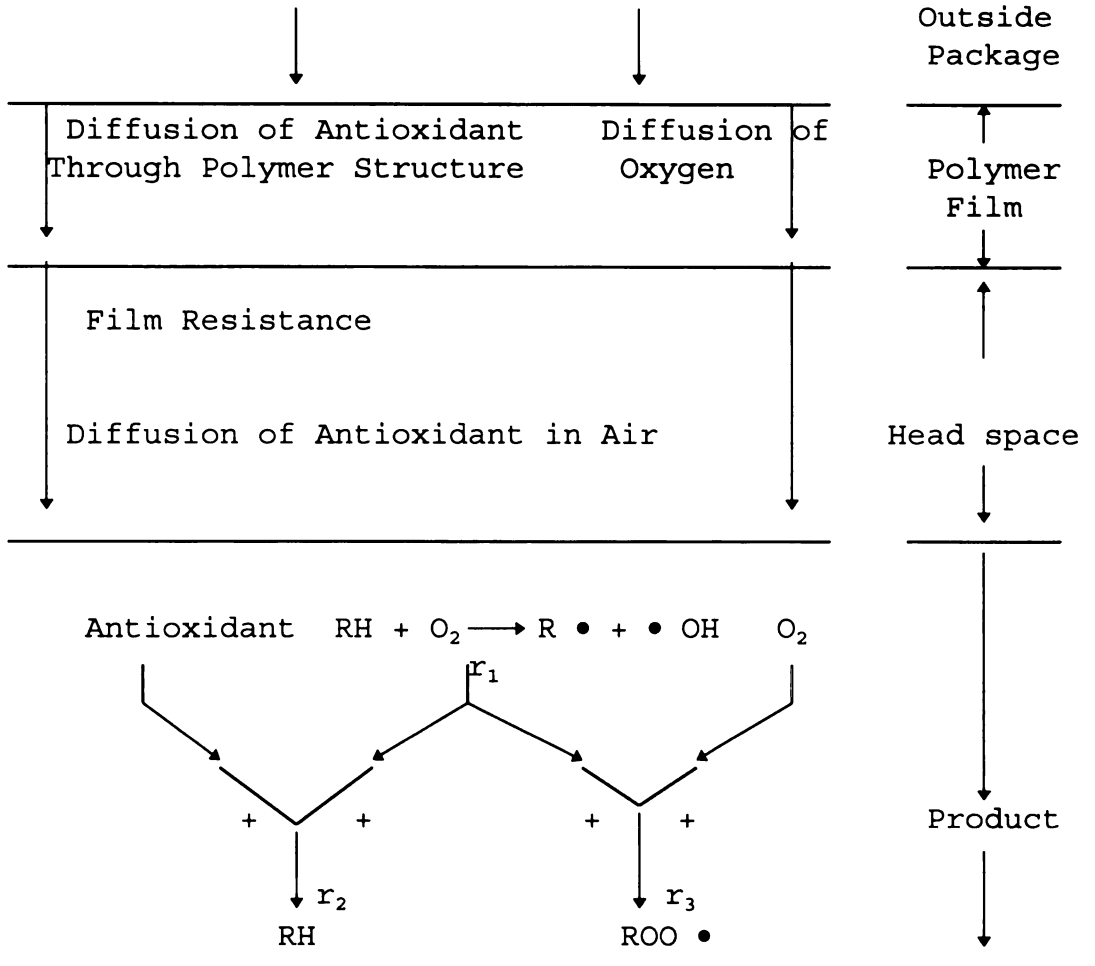


Figure 20. Schematic of the mechanism of antioxidant activity of a polymeric packaging material

Table 11. BHT content in packaged cereal product following storage at 23°C

Storage time (Weeks)	Quantity (μg)	Relative percent (wt/wt) distribution of BHT in cereal product
0	4024.97[b]	100
4	597.36[a]	14.84
6	408.81[a]	10.16
8	362.41[b]	9.00
12	357.44[b]	8.88
16	223.00[a]	5.54
28	119.96[b]	2.98

[a] Average of two replicate sample analysis

[b] Average of three replicate sample analysis

SUMMARY AND CONCLUSIONS

α-Tocopherol loss from the multi-layer laminate structure was determined as a function of time and temperature. Rate loss studies were carried out at 23, 30, 40, 50°C, respectively. Graphical analysis indicated that a first order rate expression provided a good description of the rate of loss of the antioxidant from the laminate structure. The temperature dependency of the transport process associated with the loss of antioxidant from the laminate film, over the temperature range studied, was found to follow the Arrhenius relationship. The determined activation energy value was 28.87 kcal/mole. The calculated loss rates for α-tocopherol from the laminate film were compared with those reported for BHT from a similar multi-layer laminate structure and were found to be significantly lower.

Based on the results of product storage studies, oat cereal product packaged in pouches containing no added antioxidant exhibited a significantly high level of lipid oxidation, as compared to product packaged in the antioxidant impregnated laminate structures. Both antioxidants (BHT and α-tocopherol) were found to provide a

similar level of effectiveness, in terms of retarding product oxidation. The results of product analysis for the extent of oxidation and levels of transferred antioxidant provided supportive evidence for the evaporation/sorption mechanism of antioxidant activity.

Knowledge of the rate of additive loss from a packaging structure can have significant practical applications in the packaging field, where the transfer of additives, such as antioxidants from the package to the product, plays a critical role in maintenance of product quality. The evaporation/sorption process provides a means of decreasing the levels of antioxidant present in the food stuff, and can avoid the direct incorporation of antioxidants into the food system. Antioxidant transfer via the evaporation/sorption mechanism offers economics advantages as well, since the antioxidant incorporated into the packaging material can both stabilize the packaging material during fabrication and retard product oxidation during distribution and storage.

FUTURE STUDIES

Future studies in this area may include determining the rate of loss of the antioxidant from the heat seal layer and outer HDPE layer, as well as determination of the diffusion coefficient of α-tocopherol through the respective laminate layers. Studies may also include determining the solubility of α-tocopherol in the respective polymer layers of the lamination. Such data will provide a better understanding of solubility and mobility characteristic of α-tocopherol in both the heat seal layer and HDPE layer and can provide a means of optimizing antioxidant transfer to a contained product, based on judicious selection of the package laminate structure and laminate layer in which the antioxidant is initially incorporated.

Other areas of study may involve developing an appropriate analytical procedure to allow monitoring the extent of transfer of antioxidant (i.e. α-tocopherol) from the package to the contained product, and evaluating the effect of relative humidity and temperature on the oxidation of a food system stored in antioxidant impregnated laminate film structure.

The mass transfer characteristics of other commercial antioxidants, such as the Irganox series and their capacity to retard lipid oxidation also warrants investigation.

APPENDICES

APPENDIX A

Table 12. α-Tocopherol calibration curve

Sample	Grams of α-Tocopherol Injected	Area Response
1a	1.0E-08	9251
1b	1.0E-08	9405
1c	1.0E-08	8673
Average	1.0E-08	9110
2a	2.0E-08	20654
2b	2.0E-08	20531
2c	2.0E-08	20930
Average	2.0E-08	20705
3a	3.0E-08	34364
3b	3.0E-08	33064
3c	3.0E-08	33870
Average	3.0E-08	33756
4a	4.0E-08	45620
4b	4.0E-08	44715
4c	4.0E-08	46183
Average	4.0E-08	45506
5a	5.0E-08	57035
5b	5.0E-08	56727
5c	5.0E-08	57877
Average	5.0E-08	57369

CF = 8.24E-13 gm/AU

APPENDIX A

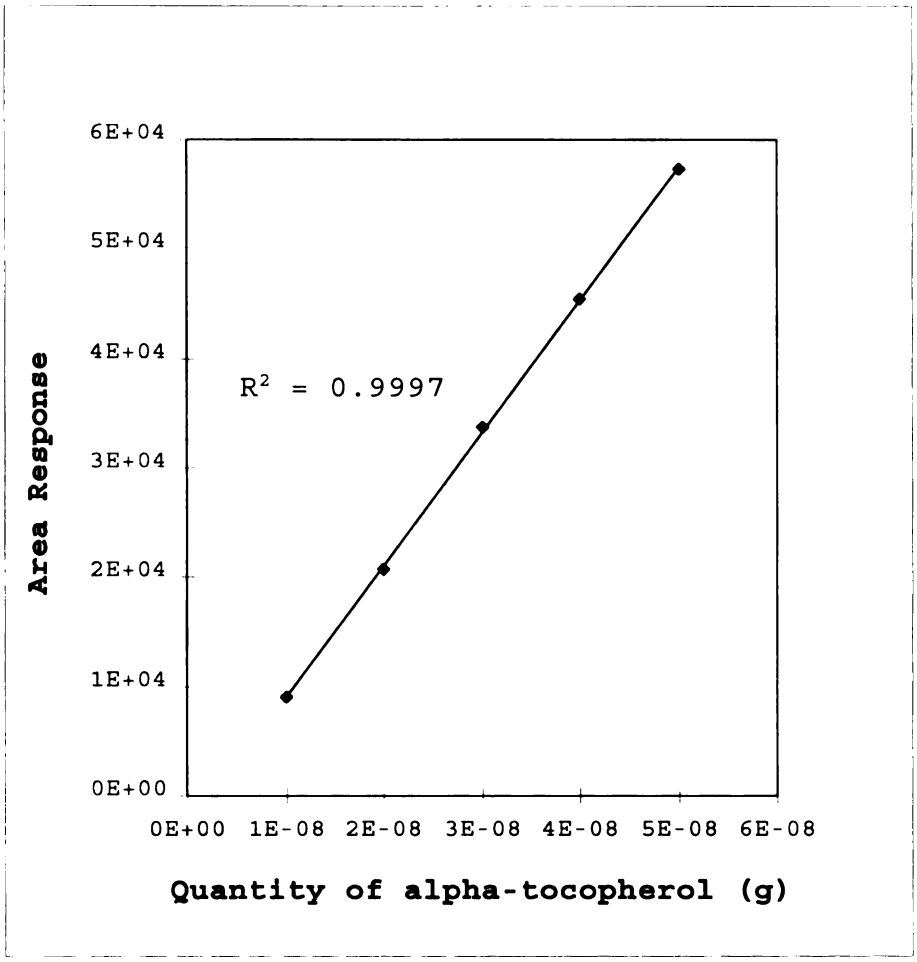


Figure 21. α-Tocopherol calibration curve

APPENDIX B

Gas Chromatography Hexanal Calibration Data

Table 13. Hexanal data for the first calibration curve

Grams of Hexanal Injected	Area Response[a]
1.20E-07	2101586
1.44E-07	2627925
1.68E-07	3235168
1.92E-07	3615613
2.16E-07	3832848
2.40E-07	4833968
2.64E-07	5260066
2.88E-07	5609180
3.12E-07	6538632
3.36E-07	6922237

[a] Average of three replicate sample analysis, with an average standard deviation of ± 5%

CF = 4.47E-14 gm/AU

APPENDIX B

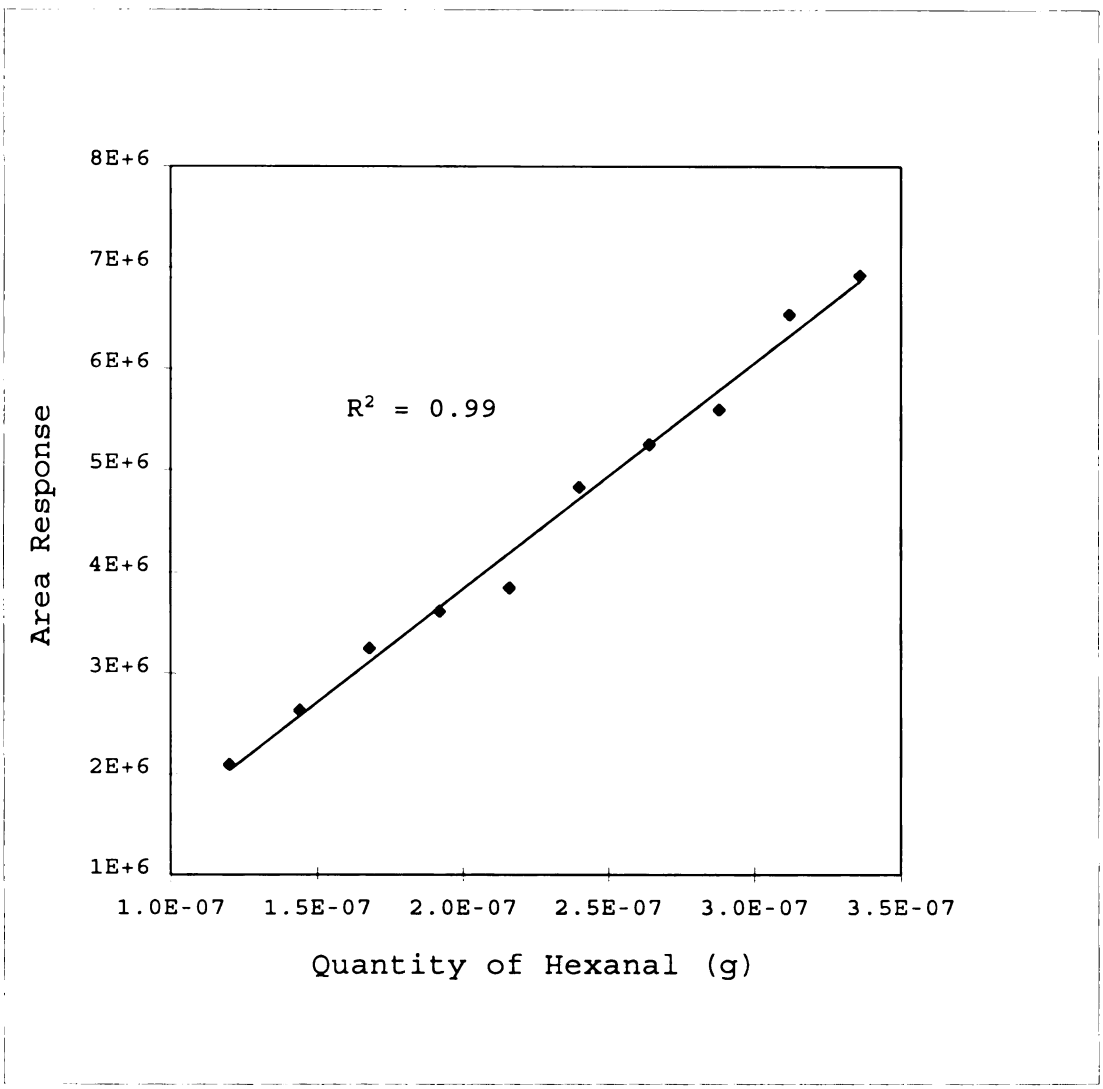


Figure 22. First hexanal calibration curve

APPENDIX B

Gas Chromatography Hexanal Calibration Data

Table 14. Hexanal data for the second calibration curve

Grams of Hexanal Injected	Area Response[a]
2.4E-08	476738
1.15E-07	2225511
1.88E-07	3571791
2.26E-07	4488571
3.07E-07	5652685
3.73E-07	6914579

[a] Average of three replicate sample analysis, with an average standard deviation of ± 5%

C.F = 4.34E-14 gm/AU

APPENDIX B

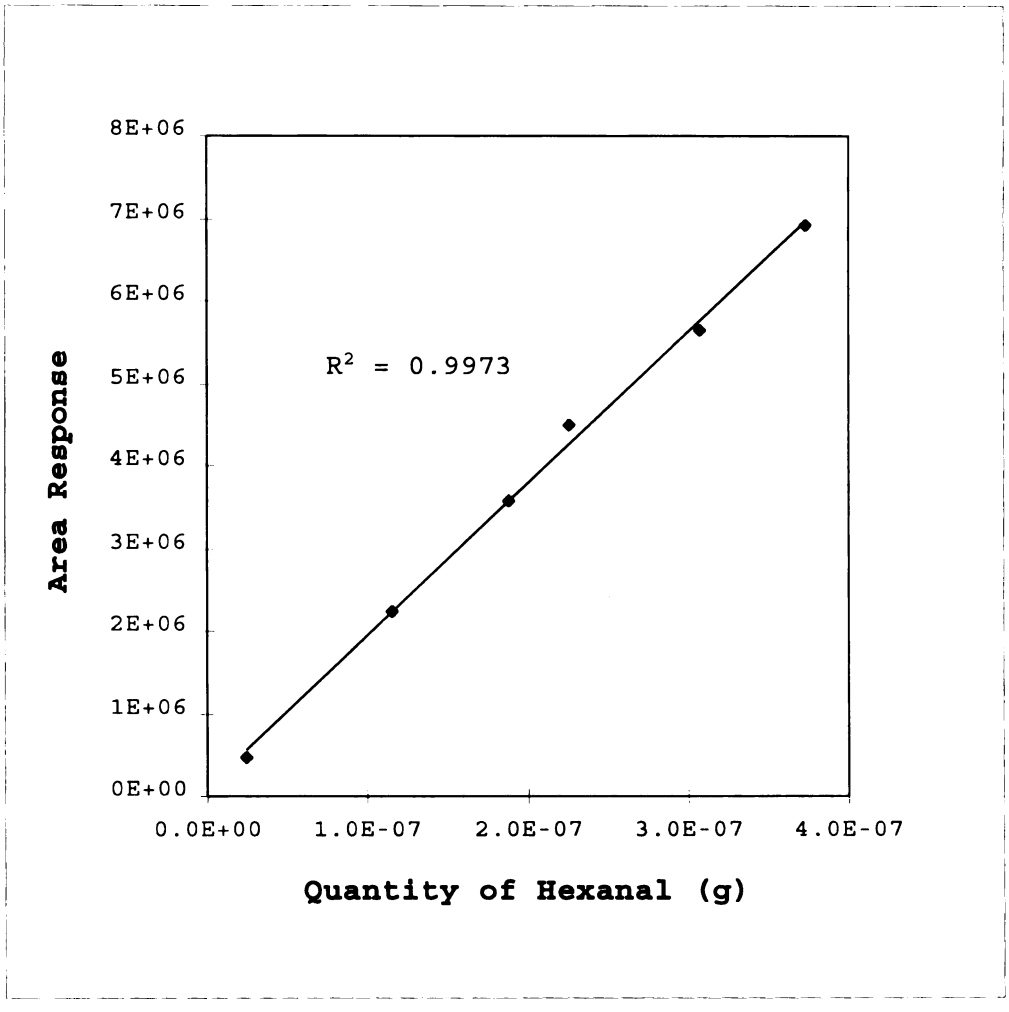


Figure 23. Second hexanal calibration curve

APPENDIX C

Table 15 . Thickness(μm) measurements performed by optical microscopy

Sample	Surlyn/EVA	Total HDPE layer	Total Film Thickness
1	15.9	44.0	59.9
2	14.5	43.2	57.7
3	12.6	47.2	59.8
4	17.2	42.1	59.3
5	13.4	45.1	58.5
Average	14.7	44.3	59.0
Standard Deviation	± 1.86	± 1.95	± 0.93

APPENDIX D

Table 16. Thickness(μm) measurements performed by micrometer

Sample	Total Thickness
1	53.3
2	58.4
3	68.6
4	68.6
5	58.4
6	58.4
7	55.8
8	55.8
9	58.4
10	55.8
Average	59.1
Standard Deviation	± 5.25

APPENDIX E

Table 17. Percent recovery data and calculations

Injection	Initial Concentration (gm/ml)	Concentration of extract solution (g/ml)
1	0.000144	0.000133
2	0.000131	0.000133
3	0.000136	0.000134
Average	0.000137	0.000133
Recovery		97.08%

APPENDIX F

One-Way Analysis of Variance (0-20 Weeks)

Analysis of Variance on quantity

Source	DF	SS	MS	F	P
Sample	2	0.00022	0.00011	0.10	0.905
Error	69	0.07738	0.00112		
Total	71	0.07761			

Level	N	Mean	StDev
1	24	0.16670	0.04347
2	24	0.17079	0.02236
3	24	0.16754	0.03122

Pooled StDev = 0.03349

Individual 95% Cis For Mean Based on Pooled StDev

```
-----+-------+------+----
     (----------*---------)
        (----------*----------)
      (----------*---------)
-----+-------+-------+---
   0.160   0.170   0.180
```

Tukey's Pairwise comparisons

Family error rate = 0.0500
Individual error rate = 0.0193
Critical value = 3.39

APPENDIX F

One-Way Analysis of Variance (28-44 Weeks)

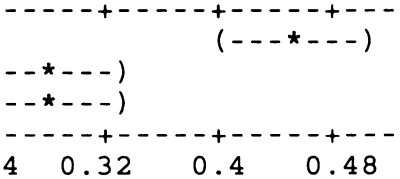
Analysis of Variance on quantity

Source	DF	SS	MS	F	P
Sample	2	0.23090	0.11545	30.39	0.000
Error	24	0.09118	0.00380		
Total	26	0.32208			

Level	N	Mean	StDev
1	9	0.46467	0.07634
2	9	0.26933	0.06496
3	9	0.26767	0.03674

Pooled StDev = 0.06164

Individual 95% Cis For Mean Based on Pooled StDev



Tukey's Pairwise comparisons

Family error rate = 0.0500
Individual error rate = 0.0198
Critical value = 3.53

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