TOCOPHEROL STABILITY IN CONTROLLED RELEASE PACKAGING FILMS

by

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ABSTRACT OF THE THESIS

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Increasing the shelf-life of packaged foods is of great interest to the food science community. Controlled release packaging (CRP) materials have been developed that are capable of releasing protective compounds (antioxidants, antimicrobials) into the food at rates suitable for long term protection. While the technical challenges of development have been met, FDA approval must be granted before these materials can be used commercially. One critical step in the FDA approval process for food contact materials is to ensure that no toxic products are formed during processing, and that sufficient levels of the added compound are retained to provide the stated functionality.

Tocopherol was chosen as an active compound for incorporation into CRP films as it is a natural, potent antioxidant with positive consumer recognition as Vitamin E. It has been used at low levels as a polymer stabilizer, with no problematic degradation reported. Tocopherol containing films were produced by cast film extrusion of LDPE, PP and a 50:50 blend of these polymers. Multiple extrusion conditions were chosen within standard processing parameters to apply varying degrees of thermal and mechanical

stress on the polymer melt. Resulting films were analyzed for their physical properties, recoverable tocopherol, volatile and non-volatile degradation products, as well as antioxidant activity assays.

This study showed that tocopherol recovery is high (92-100%) for all polymer compositions and processing methods, but greatest loss was seen in PP films subjected to high shear. GC-MS analysis shows only polymer additives, with no volatile degradation products formed from tocopherol. A reduction in the level of polymer volatiles was also noted. Analysis by LC-MS has provided tentative identification of two tocopherol dimers and tocoquinone, all previously documented degradation products of tocopherol. Little variation was observed between different processing methods. Antioxidant assays demonstrate that extracts from the tocopherol containing films exhibit as much, or greater antioxidant activity as unprocessed mixed tocopherols.

While further work remains to confirm the identities of the products identified and provide accurate quantification, this research supports the safety and antioxidant effectiveness of tocopherol containing extruded polymer films.

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DEDICATION

For M. W. Greene

Missed it by *that* much.

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Introduction

As the demand for foods with long-term stability increases, new technologies must be developed to counteract the degradation found during long term storage. One such technology is controlled release antioxidant packaging, which allow maintenance levels of antioxidant to be supplied to a food during its storage to retard lipid oxidation, the reaction most responsible for loss of food quality over time. While many obstacles still challenge development of functional controlled release packaging materials, one of the most important is the insurance of the stability of the active compound during production of the material.

The natural antioxidant tocopherol, also known as Vitamin E, was one of the first active compounds selected for inclusion in antioxidant packaging materials. As an antioxidant for food systems, tocopherol has several advantages including good heat stability, high effectiveness against lipid oxidation and being natural; perhaps most importantly, it provides a clean label preferred by consumers. Tocopherol has also been used in extruded polymers as a polymer antioxidant, indicating its suitability for such applications. Tocopherol occurs naturally as a mixture of four isomers, differing in the number and position of methyl groups on the ring. While previous groups chose to use only α-tocopherol, the most bioactive form, this study used mixed tocopherols rich in the γ isomer. While this reduces the biological Vitamin E activity, it increases the *in vitro* antioxidant activity, providing greater stabilization to the food product. With all isomers being present, it is possible that degradation of tocopherol during film production could

proceed differently than in polymers containing only α-tocopherol, leading to the formation of products not seen in α-tocopherol films.

Controlled release antioxidant films are produced by polymer extrusion, in which the active compound is first blended with the polymer resin, then extruded to produce the final film. This extrusion process utilizes both heat and shear to melt the polymer, which provide sufficient energy to break not only the intermolecular bonds and allow melting, but also the intramolecular bonds, leading to polymer degradation. The breakage of these intramolecular polymer bonds leads to the formation of polymer radicals, which left unchecked, can lead to decreased quality of the final product. . In normal film production, low levels of antioxidant, e.g. tocopherols or Irganox, are added to control radical reactions and stabilize the polymer; some antioxidant is degraded in the process. In antioxidant CRP production, much higher antioxidant levels are added to the polymer with the ultimate goal of releasing the excess antioxidant (not needed for polymer stabilization) into food. For this application, antioxidant reactions with the polymer must be minimized in order to preserve the required level in the final product.

In addition to simple loss of active material, the formation of degradation products must also be taken into consideration. Acceptance for use as a food contact material by the Food and Drug Administration (FDA) requires analysis of materials to demonstrate that no toxic products are released into the food to contaminate the food supply.

The focus of this research project was to investigate retention and degradation of mixed tocopherols in controlled release films using GC-MS and LC-MS techniques, volatile and non-volatile compounds found in the films are identified and quantified, allowing a determination of the extent of degradation. From this information it can be determined if degradation presents a significant problem, and if so, may suggest how it can be minimized for optimal film production.

Literature Review

CRP Rationale

Shelf Life

Current trends in consumer food preference are leading to the requirement of greater shelf life for both raw and ready to eat foods. With the decline in both food preparation time and the cooking skills of the population, sales of shelf-stable meal kits rose 48% between 1998 and 2003 [1]. There is also a trend amongst food retailers to shift from in-store packaging of meats to ‘case ready’ packages, which are packaged at the meat processing facility, in order to minimize labor costs and lost revenue due to product expiration [2].

Another factor creating demand for longer shelf life is the increasing transport distances travelled by food products between the farm and the final consumer [3]. As the global population increases, there will be a greater need for food trade between regions and nations. In the case of fresh foods, one of the greatest hurdles to this trade is the high cost of rapid transport required to delver foods before they become spoiled [4]. With increased shelf life, slower, more cost effective transportation methods can be employed while retaining food quality.

Lipid oxidation and food quality

While the prevention of microbial growth is the most important factor in the short-term storage of foods, long-term degradation proceeds by chemical, rather than biological pathways [5]. Lipid oxidation is the most significant chemical reaction governing the

shelf life of foods, leading to undesirable off-flavors and odors, and may also compromise the nutritional quality of foods [6]. Details of lipid oxidation are discussed later in this work.

Packaging

Traditionally, packaging served only to protect food products from contamination and degradation by external environmental factors such as dirt, microbes, light and vermin [7]. More recently, packaging materials that act to protect or preserve the food against internal factors have been developed. These products are known as Active Packaging materials, and include oxygen scavengers, carbon dioxide emitters, desiccants, ethylene removing materials, and antimicrobial packaging materials[8]. By using active packaging to maintain an ideal internal environment for fresh produce, shelf life can be extended significantly, enabling practical shipping and sales times [9].

Due to the oxygen dependence of lipid oxidation, packaging systems utilizing oxygen scavengers, vacuum packaging or flushing with an inert gas have been utilized to minimize oxidative degradation [1, 8, 10]. These methods do have limitations, especially in long term storage applications, as nearly all foods contain some level of oxygen, and to remove all traces of oxygen would be prohibitively expensive. It is also less suitable for liquid or semi-solid foods in which there is little or no package headspace [11].

Antioxidants

Another method by which foods are protected against oxidation is through the use of antioxidants [12]. Antioxidants are chemical compounds that are able to reduce the

overall oxidation of a substrate. The first class of antioxidants, divided into primary and secondary antioxidants, act upon radicals already present. In the case of primary antioxidants, this is accomplished by donation of a hydrogen atom to quench the radical, forming relatively unreactive antioxidant radical which is less likely to propagate the radical chain reaction.

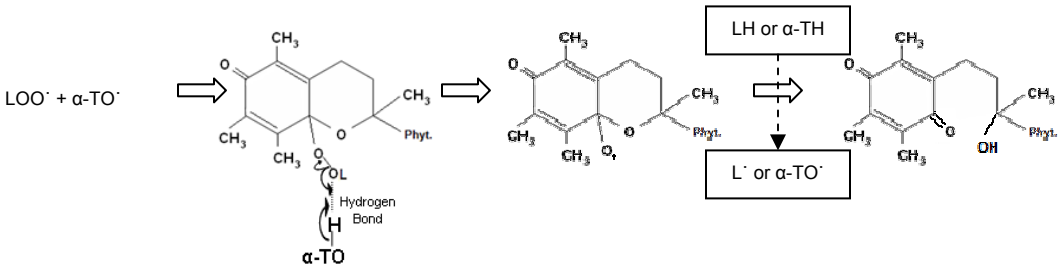
Primary antioxidants are typically phenolic compounds in which the radical can be stabilized by resonance on the aromatic ring. Secondary antioxidants act to enhance the activity of primary antioxidants by either regenerating active antioxidants from their radical form or by reducing the radical load of the system. Radical load reduction can be accomplished by non-radical decomposition of hydroperoxides, preventing their involvement in the branching chain reaction. Examples of such secondary antioxidants include reducing agents, organic acids and sulfur compounds. A second class of antioxidants acts to minimize the initial formation of radicals by eliminating the sources of oxidation. Such antioxidants include metal chelators, light absorbers and $^1O_2$ quenchers. Protective environments, including modified atmospheres and opaque packaging, may also be considered a class of environmental antioxidants.

Controlled release

One limitation of antioxidants is that they are consumed in the process of preventing oxidation [13], and this must be taken into consideration when designing strategies for long-term stabilization. Adding very high levels of antioxidants to the food during formulation prior to packaging to allow for long-term consumption is a common practice, but this often proves ineffective since at high concentration many antioxidants can exhibit

pro-oxidant effects [14]. In the case of tocopherol, this pro-oxidant activity is not caused as much by the abstraction of hydrogen from unoxidized lipids (LH) and from lipid hydroperoxides (LOOH) by the tocopherol radical itself, as by the tocopherol catalyzed breakdown of primary tocopherol oxidation product 8a-peroxy-α-tocopherone to form the tocopherone radical, which can abstract hydrogen from both lipids and tocopherol (Equation 1) [15].

Equation 1 - Proposed mechanism for tocopherol catalyzed decomposition of peroxyl radicals [16].



Tocopherol can also act to reduce metals present by donation of an electron,

$$\mathrm{TOH} + \mathrm{M}^{(n+1)+} \rightarrow TO^{\bullet} + H^{+} + Mn^{n+}$$

$$TO^{\bullet} + \mathrm{M}^{(n+1)+} \rightarrow TO^{+} + Mn^{n+}$$

and these reduced metals then act as oxidation initiators by Fenton type reactions[15]. This is due to the fact that the reaction rate for the abstraction of hydrogen by the tocopheroxyl radical from lipid hydroperoxide (5×10^{-1} $M^{-1}s^{-1}$) is far lower than that of both the rate of hydrogen abstraction from tocopherol by peroxyl radical (3.2×10^{6} $M^{-1}s^{-1}$) and the rate of tocopheroxyl radical bimolecular coupling (3×10^{3} - 5×10^{5} $M^{-1}s^{-1}$). There

are also federal regulations limiting the levels at which such materials may be safely added to foods.

The concept of controlled release packaging was developed to overcome this limitation. Controlled release packaging (CRP) is a food packaging material into which an active compound, such as an antioxidant or antimicrobial [16], has been introduced, and is then released at a specific rate suitable for the maintenance of food quality and safety throughout the desired shelf life. A schematic diagram of controlled release packaging is shown in Figure 1. Early CRP technology incorporated the synthetic antioxidants BHA [(1,1-dimethylethyl)-4-methoxy phenol] and BHT [2,6-di-tert-butyl-para-cresol] in a polymeric packaging material [17], but this has limitations due to the volatility and toxicity of these active compounds. Thus, current development in the food industry and in the military focuses on replacing BHA and BHT in foods and packaging with natural antioxidants. Early research by Wessling et al [18] found that a packaging material made of LDPE containing 360 and 2400 ppm of α-tocopherol slowed the oxidation of a linoleic acid emulsion proving the concept that a natural antioxidant could be used in such a manner.

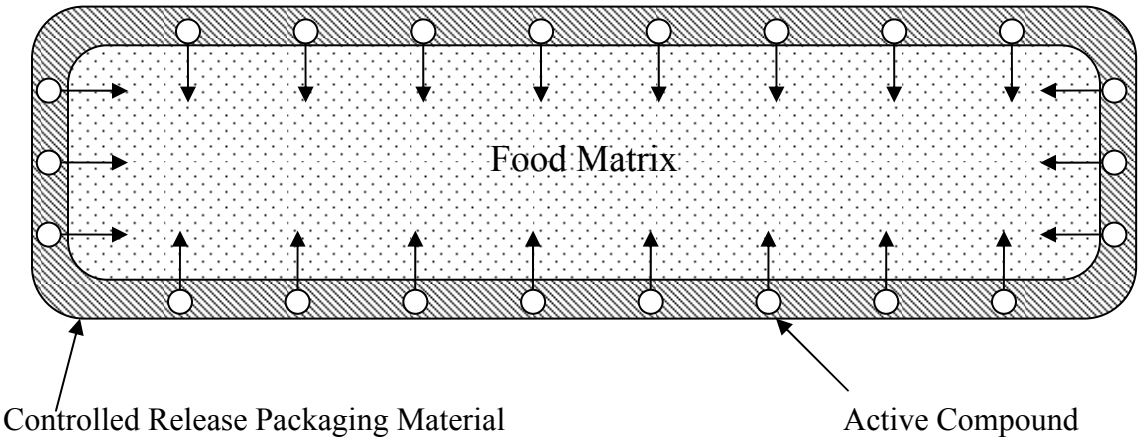


Figure 1 - Illustration of controlled release package

Conceptual framework

As the development of a workable CRP product is a highly complex and involved project and previous research in CRP had been largely empirical, a conceptual framework was developed for our group to use in coordinating and organizing the research into a systematic approach (Figure 2). This framework presents the four main classes of variables that must be taken into consideration: process variables, structure variables, property variables and food variables. It clearly shows the effects of those variables upon each other, with process variables influence structure variables, which in turn alter the property variables, which define the ultimate activity of the material. Food variables must also be considered, as the properties of the packaging material must be tailored to suit the composition, form and desired shelf life of the target food product.

This thesis is focused on Pathway 5 (Figure 2), the stability of the active compound during processing. This is essential, since an unstable active compound will not survive

processing, and will not be present in high enough levels within the final packaging material to release at an appropriate rate for the food. To obtain FDA approval for food use of CRP, it must also be demonstrated that there are no undesirable degradation products formed during processing that may be released into the food, posing a potential health risk or reducing effectiveness.

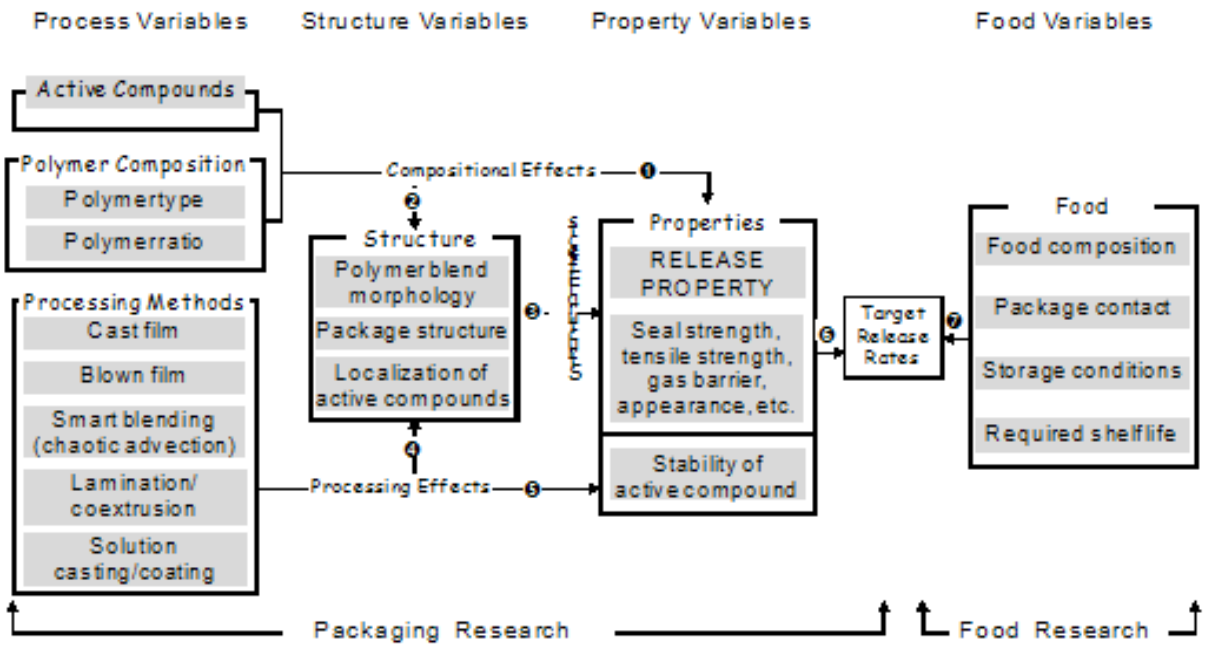


Figure 2 - Conceptual framework for CRP project (Balasubramanian, Schaich, and Yam, manuscript in preparation).

Legal and Regulatory Issues

In the United States, food regulation is managed by the Food and Drug Administration (FDA). FDA regulation also applies to food packaging materials, which are classified as food additives when they leach into the food. In order for a food packaging material to be approved for use by the FDA, it must be demonstrated to be safe and effective in the

application to which it is being applied. In addition, food packaging materials must be demonstrated not to release any contaminating materials into the food being packaged.

As defined in the Federal Food Drug and Cosmetic (FD&C) Act, Section 201(s), a food additive is:

"…any substance the intended use of which results or may reasonably be expected to result, directly or indirectly, in its becoming a component or otherwise affecting the characteristics of any food (including any substance intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food…), if such substance is not generally recognized… as having been adequately shown through scientific …to be safe under the conditions of its intended use …"[19]

Under this definition, unmodified tocopherol migrating from packaging material would not be considered a food additive, since tocopherol has been designated as generally regarded as safe (GRAS) when used as a chemical preservative according to good manufacturing practice[20].

The mixture doctrine states that it is permissible for packaging materials to incorporate a mixture of polymers and additives without the need for specific approval if all of the materials making up the package have been individually approved for use in such applications, and are being used according to any restrictions they may have. This eliminates the need to evaluate and grant approval for every conceivable combination of materials that are deemed appropriate. The exception to this is the case in which components of the combination react to form a new compound. In this case, the resulting compound must be evaluated and approved before the mixture can be approved [21].

Taking both of these regulations into consideration, FDA approval of a tocopherol releasing antioxidant packaging material is dependent upon the stability of the embedded tocopherol during the production and storage of the material. If it could be demonstrated that there is no reaction or degradation of the tocopherol, or that any degradation products are also GRAS, such packaging materials would be acceptable for food contact according to existing regulation. Were there to be non-GRAS products resulting from film processing, further analysis of the safety and migration of such products would be required before FDA approval could be granted.

Lipid Oxidation

Lipid oxidation is an autocatalytic, free-radical mediated reaction that degrades polyunsaturated fatty acids [22]. As shown in the classical presentation (Figure 3), lipid oxidation proceeds as a sequence of three stages: initiation, propagation and termination.

Initiation of lipid oxidation involves the formation of an initial lipid free radical by the presence of metals, ionizing radiation, singlet oxygen, heat or other free radicals in the system [23] (Reaction 1, Figure 3). This first radical, a highly reactive alkyl radical (L_1•), is formed by the abstraction of a hydrogen atom from the fatty acid (LH), removal of an electron from the double bond, or addition of thermal energy to break covalent bonds anywhere in the acyl chain.

CLASSICAL FREE RADICAL CHAIN REACTIONS OF LIPID OXIDATION

Initiation ***(formation of ab initio lipid free radical)***

$$L_1H \xrightarrow{k_i} L_1^{\bullet} \quad (1)$$

Propagation

Free radical chain reaction established

$$L_1^{\bullet} + O_2 \underset{k_\beta}{\overset{k_o}{\rightleftharpoons}} L_1OO^{\bullet} \quad (2)$$

$$L_1OO^{\bullet} + L_2H \xrightarrow{k_{p1}} L_1OOH + L_2^{\bullet} \quad (3)$$

$$L_2OO^{\bullet} + L_3H \xrightarrow{k_{p1}} L_2OOH + L_3^{\bullet} \text{ etc.} \dashrightarrow L_nOOH \quad (4)$$

Free radical chain branching (initiation of new chains)

$$L_nOOH \xrightarrow{k_{d1}} L_nO^{\bullet} + OH^{-} \text{ (reducing metals)} \quad (5)$$

$$L_nOOH \xrightarrow{k_{d2}} L_nOO^{\bullet} + H^{+} \text{ (oxidizing metals)} \quad (6)$$

$$L_nOOH \xrightarrow{k_{d3}} L_nO^{\bullet} + {}^{\bullet}OH \text{ (heat and uv)} \quad (7)$$

$$\left.\begin{matrix} L_nO^{\bullet} \\ L_nOO^{\bullet} \\ HO^{\bullet} \end{matrix}\right\} + L_4H \xrightarrow[k_{p1}]{k_{p2}} \left.\begin{matrix} L_nOH \\ L_nOOH \\ HOH \end{matrix}\right\} + L_4^{\bullet} \quad \begin{matrix} (8a) \\ (8b) \\ (8c) \end{matrix}$$

$$L_1OO^{\bullet} + L_nOOH \xrightarrow{k_{p4}} L_1OOH + L_nOO^{\bullet} \quad (9)$$

$$L_1O^{\bullet} + L_nOOH \xrightarrow{k_{p5}} L_1OH + L_nOO^{\bullet} \quad (10)$$

Termination ***(formation of non-radical products)***

$$\left.\begin{matrix} L_n^{\bullet} \\ L_nO^{\bullet} \\ L_nOO^{\bullet} \end{matrix}\right\} + \left.\begin{matrix} L_n^{\bullet} \\ L_nO^{\bullet} \\ L_nOO^{\bullet} \end{matrix}\right\} \xrightarrow[k_{t3}]{k_{t1}\ k_{t2}} \text{Radical recombinations} \quad (11a)$$

polymers, non-radical monomer products (11b)

(ketones, ethers, alkanes, aldehydes, etc.) (11c)

$$\left.\begin{matrix} LOO^{\bullet} \\ LO^{\bullet} \end{matrix}\right\} \xrightarrow[k_{ts2}]{k_{ts1}} \text{Radical scissions}$$

non-radical products (12a)

(aldehydes, ketones, alcohols, alkanes, etc.) (12b)

i - initiation; o-oxygenation; β- O_2 scission; p-propagation; d-dissociation; t-termination; ts–termination/scission

Figure 3 - Classical free radical reactions proposed for lipid oxidation [24].

During the propagation stage, this initial alkyl radical sets off an autocatalytic chain reaction, generating many more free radicals. The alkyl radical reacts almost immediately with any available O_2 in the system to form a lipid peroxyl radical ($L_1OO\bullet$) (Reaction 2, Figure 3). This peroxyl radical retains sufficient oxidation potential to abstract a hydrogen atom from another lipid chain (Reactions 3-4, Figure 3), forming a lipid hydroperoxide (L_1OOH) and a new lipid alkyl radical ($L_2\bullet$) which will undergo the same reaction with oxygen, propagating the chain. Additionally, the hydroperoxide can be degraded by metals, heat, and light to form lipid alkoxyl radicals ($LO\bullet$) and either hydroxide anions (^{-}OH) in the presence of metals (Reaction 5, Figure 3), or hydroxyl radicals ($\bullet OH$) in the presence of heat and light (Reaction 7, Figure 3). $LO^{\bullet}$ and $\bullet OH$ then start new oxidation chains (called branching) by abstracting hydrogens from other lipid acyl chains (Reaction 8, Figure 3) or from already-formed lipid hydroperoxides (Reaction 10, Figure 3). Rate constants for these abstractions, as well as those for the degradative reactions discussed in the following paragraphs, are presented in Table 1.

The termination step occurs by two mechanisms: radical recombination, in which two radicals react to form a non-radical product, and radical scission, in which alkoxyl radicals undergo scission and form end products by reacting with water. These non-radical molecules do not propagate the chain reaction, but can generate undesirable end products, such as polymers, ketones, and aldehydes are responsible for the characteristic off-odors and flavors in oxidized food products [25]. Because the sensory detection threshold for many of these volatiles is very low, often at part per billion levels, only a

Table 1 - Comparison of rate constants for competing reactions in lipid oxidation [24].

		ROO•	LO•
H abstraction, LH	nonpolar organic	<1-400 $M^{-1}s^{-1}$	$10^4 – 10^7$ M^{-1} s^{-1}
	polar, aqueous		10^6 - 10^8 L $M^{-1}s^{-1}$
H abstraction, LOOH	nonpolar organic	600 $M^{-1}s^{-1}$	2.5 x 10^8 M^{-1} s^{-1}
	polar, aqueous	NA	NA
Cyclization	nonpolar organic	10^1-10^3 s^{-1}	10^4 - 10^5 s^{-1}
	polar, aqueous	NA	NA
Addition	nonpolar organic	NA	$10^4 – 10^8$ M^{-1} s^{-1}
	polar, aqueous	NA	NA
β-scission	oleate	1-8 s-1	$10^3 – 10^5$ s^{-1} org
	linoleate	27-430 s^{-1}	$10^4 – 10^5$ s^{-1} polar org $10^6 – 10^7$ s^{-1} aq
Dismutation[b]	nonpolar organic	10^6-10^9 L $M^{-1}s^{-1}$	10^9 - 10^{10} $M^{-1}s^{-1}$
	polar, aqueous	10^7-10^8 L $M^{-1}s^{-1}$	NA
	oleate-OO•	10^6 $M^{-1}s^{-1}$	

[a] Data included authentic fatty acids whenever possible, plus primarily cumyl, tetralinyl, and *t*-butyl peroxyl and alkoxyl radicals.

[b] Dismutation is self-reaction of LOO• . It can be either a propagation reaction in oils,

$R_1OO^{\bullet} + R_2OO^{\bullet} \longrightarrow [R_1OOOOR_2] \longrightarrow {}_1O^{\bullet} + {}^{\bullet}OOOR_2 \longrightarrow R_1O^{\bullet} + O_2 + {}^{\bullet}OR_2$

or termination reaction in systems where water is present, e.g. in emulsions,

$R_1OO^{\bullet} + R_2OO^{\bullet} \longrightarrow R_1OOR_2 + O_2$

Dismutation contributions are strongly system dependent and important only at high oxidation levels

NA: data not available

small amount of lipid must oxidize before a food becomes unacceptable to the consumer [26]. Indeed, most foods are considered fully rancid at 1% oxidation.

It must be pointed out, however, that this traditional description of lipid oxidation is incomplete and not fully accurate because peroxyl radicals and alkoxyl radicals both undergo several alternate reactions in competition with hydrogen abstraction[24]. These are shown in Figure 4. The rates of these alternate reactions are comparable (Table 1), so they clearly have the potential to compete with hydrogen abstraction in oxidizing lipids. Rearrangement reactions to form epoxides and epidioxides are perhaps the most important competitors. Scission and addition reactions become more active in secondary stages of lipid oxidation. The balance between the various pathways changes with the food system and environmental conditions, and thus is totally system-dependent:

a) H abstraction from other fatty acid chains is favored in neat oils and in the lipid interior of membranes when there is close contact between lipid chains and no other H sources are present. In organic solvents, H abstraction dominates at moderate lipid concentrations (enough substrate is present to supply hydrogens) and increases with solvent polarity and temperature (H abstraction is an energy-intensive process). However, in solvents, with antioxidants, or in the presence of other food molecules, particularly proteins, H abstraction from lipids (i.e. propagation) must compete with H abstraction from non-lipid sources. This terminates lipid chains but transfers oxidation to other components of the system.

b) Cyclization is most important when oxygen abstractable hydrogens are limited, e.g. in neat lipids, aprotic solvents, and low lipid concentrations. Cyclization does not

occur with monounsaturated fatty acids but increases dramatically with the degree of polyunsaturation and under conditions where radicals form at internal positions (e.g. photosensitization). Cyclization is unaffected by temperature so becomes relatively less important as temperature increases.

c) Scission is favored when water is present and in polar protic organic solvents that provide protons necessary to stabilize scission products. Scission can propagate secondary reactions in low water systems, but in high water systems scission becomes a termination reaction as all scission radicals formed are rapidly quenched by water and increased hydrolysis yields tertiary lipid oxidation products. Since scission involves bond rupture, the reaction increases markedly with temperature.

e) Addition reactions are usually slower than hydrogen abstraction and are most active in neat lipids with conjugated double bonds. Hence, addition reactions contribute little in early stages but become more competitive as oxidation chains are established, and they are often the dominant reaction in extensively oxidized oils with very high radical concentrations, leading to characteristic polymerization of oils. Addition is not competitive when H atom sources are readily available.

The critical contribution of antioxidants can be deduced from this simple summary. Antioxidants prevent transfer of radicals to other lipid molecules, thereby stopping propagation and terminating individual lipid radical chains. Stopping radical transfers early in the oxidation sequence prevents accumulation of reactive intermediates, co-oxidation of other important molecules, and formation of secondary products responsible for toxicity, odors and flavors, polymerization, and texture changes.

At the same time, $LOO^{\bullet}$ quenching by antioxidants increase the rate of lipid hydroperoxide production. This increases the supply of LOOH protons available for faster reaction with radicals (pro-oxidant). It also increases the levels of precursor for $LO^{\bullet}$, which react faster and more specifically than $LOO^{\bullet}$. Whether the latter is pro-or anti-oxidant in effect depends on the presence of LOOH decomposers (e.g. metals, light, heat) in the specific system.

Internal cyclization is the dominant reaction at low oxygen pressures, which is the condition for storage of most food products, as well as in dilute lipid systems. Epidioxides and epoxides are very reactive with lipids, are extremely reactive with proteins and nucleic acids (source of food quality deterioration and in vivo toxicity), and generate breakdown products (hence flavors and odors) different from the aldehydes normally expected. Internal rearrangements can be circumvented by provision of reactive hydrogens from antioxidants.

Scission reactions require the presence of active proton sources to drive the scission reactions forward and to immediately stabilize the scission products. The proton source is normally water. Whether antioxidants can replace water in facilitating scission reactions is not known, but could contribute to pro-oxidant effects at high levels. Hence, antioxidants may exert two opposing effects: hastening $LO^{\bullet}$ scission to various aldehydes, etc. that produce rancid odors and flavors versus quenching the scission radicals and preventing initiation of secondary oxidation chains. This argues against high levels of

antioxidants in food formulations and for careful control of antioxidant levels during storage.

Addition reactions leading to polymerization and crosslinking, both with lipids and with other molecules such as proteins, are most active at high radical concentrations and when lipid conjugation has increased due to oxidation. Control of early stages of lipid oxidation by delivery of antioxidants at appropriate levels should thus prevent most addition reactions that contribute to quality degradation in foods where lipids are actively oxidizing.

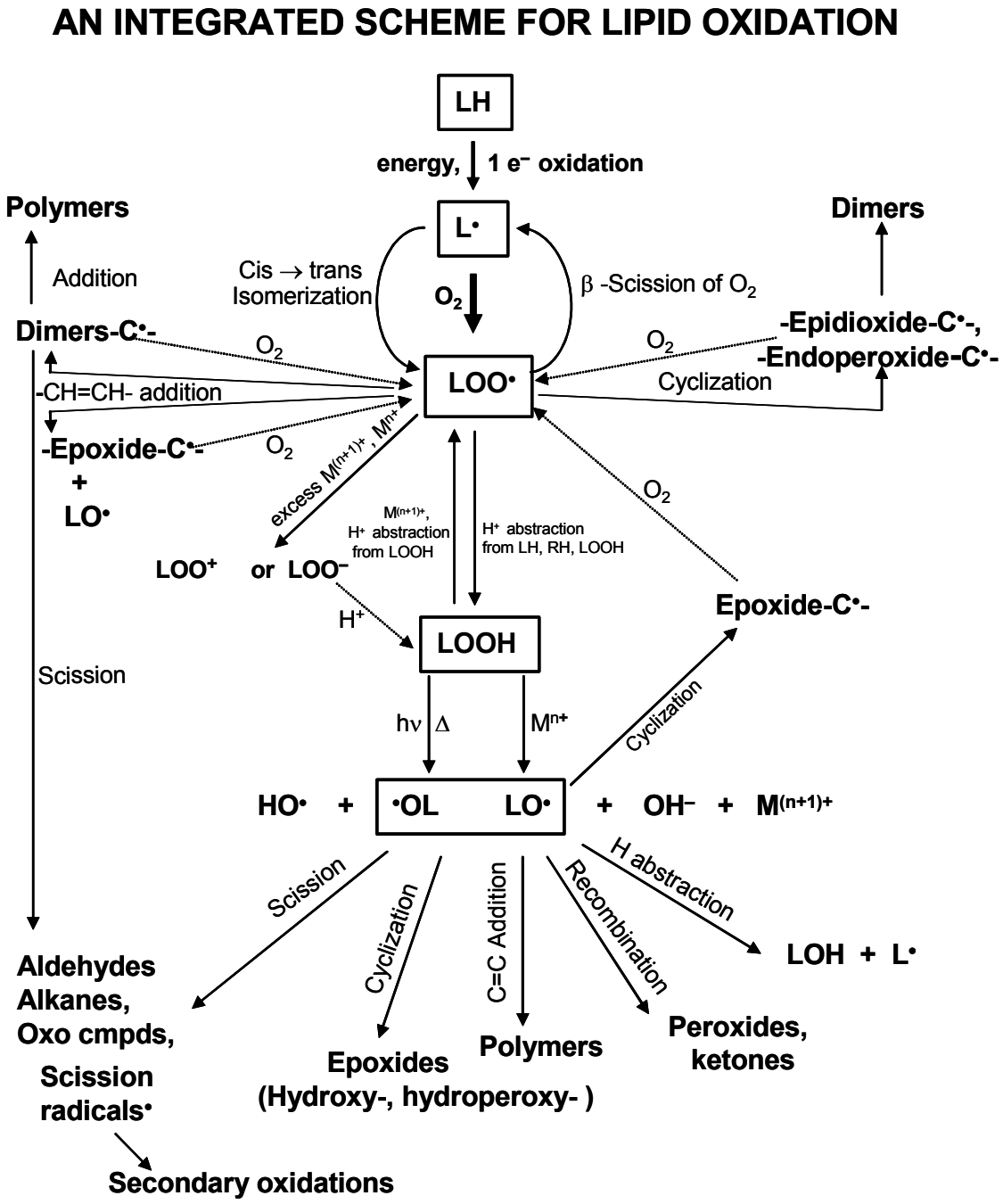


Figure 4 - An integrated scheme for lipid oxidation [24].

Tocopherol

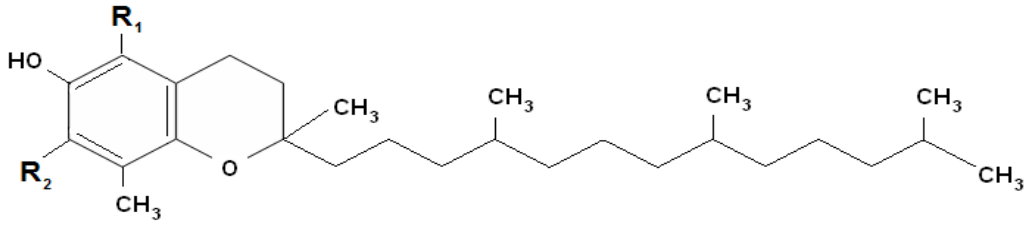
Tocopherol discovery

First described in 1925 as a factor critical for reproduction called Vitamin E [27], this compound was isolated and named as α-tocopherol by Evans and Emerson in 1935, who described it as an alcohol with the activity of vitamin E [28]. Research on extracting tocopherol from varying plant sources conducted by Emerson identified β and γ-tocopherols, extracted from wheat germ and cottonseed oils respectively, in 1937 [29]. These were found to have biological activity of one-third to one-half that of α-tocopherol The last isomer of tocopherol to be identified was δ-tocopherol, which was extracted from soybean oil and characterized by Stern in 1946 [30].

Structures of tocopherol isomers

The tocopherols are a group of natural phenolic antioxidants, composed of a chroman head and a phytyl tail [15]. There are four naturally occurring tocopherols, differing only in the number and position of methyl groups on the phenolic ring (Figure 5). It is produced naturally in higher plants, with α-tocopherol concentrated in the chloroplasts and the other tocopherols found at higher concentration in non-photosynthetic tissues [31]. Natural source tocopherol concentrates are derived from the deodorizer distillate generated during the process of oil refining [32]. The relative distribution of the tocopherol isomers in these concentrates are very nearly the same as those in the parent oil.

α-Tocopherol, also known as Vitamin E, is one of the most important lipid soluble antioxidants *in vivo.* It provides a defense against oxidative stress caused by free radicals formed within the body. It has also been demonstrated that α-tocopherol serves vital biological functions in addition to its antioxidant function, including regulation of protein kinase C[33], gene regulation and prevention of atherosclerosis and other coronary diseases [34, 35]. Studies have demonstrated that γ-tocopherol reduces the proliferation of several types of cancer, including prostate and colon cancers, by down-regulation of cyclins without inducing apoptosis of the cancer cell[36].



R_1	R_2	
CH_3	CH_3	α-Tocopherol
CH_3	H	β- Tocopherol
H	CH_3	γ- Tocopherol
H	H	δ- Tocopherol

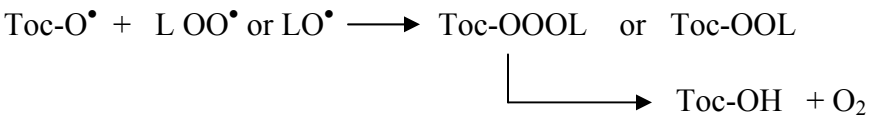
Figure 5- Structures of tocopherol isomers [15].

Antioxidant activity of tocopherol

The antioxidant activity of tocopherol was first studied by Olcott and Emerson in 1937, based on the observation that the unsaponifiable material found in vegetable oils demonstrated antioxidant properties and possessed vitamin E activity and that γ-

tocopherol exhibited the greatest protective effect towards the oxidation of both lard and olive oil. Olcott and Emerson also made inferences as to the phenolic structure of tocopherol based on the fact that all known antioxidants of the time possessed a phenolic structure. This structure was later confirmed by Fernholz in 1938 through analysis of α-tocopherol decomposition products [37].

Tocopherol acts as a chain breaking antioxidant both *in vivo* and *in vitro*. Its method of antioxidant action is by donation of the phenolic hydrogen to a reactive peroxy radical, generating a tocopheroxyl radical and a less reactive hydroperoxide (Figure 6). The tocopheroxyl radical is stabilized by conjugative electron delocalization (Figure 7) made possible by the orbital overlap of the p-orbital from the oxygen atom on the heterocyclic ring [38]. Electron delocalization reduces the redox potential of the radical considerably (Toc-O$^{\bullet}$, $E^{O'}$/mV = 500) and makes further chain propagation by the tocopheroxyl radical much less likely than by alkoxyl (RO•, EO'/mV = 1600) or peroxyl (ROO•, EO'/mV = 1000) radicals [39]. This difference in reducing potential allows donation of a hydrogen atom by tocopherol to the alkoxyl and peroxyl radicals, but prevents the tocopheroxyl radical from abstracting hydrogen from stable peroxyl and hydroxyl groups. The tocopheroxyl radical is also able to quench a second radical by a radical-radical coupling reaction, doubling its antioxidant effectiveness.



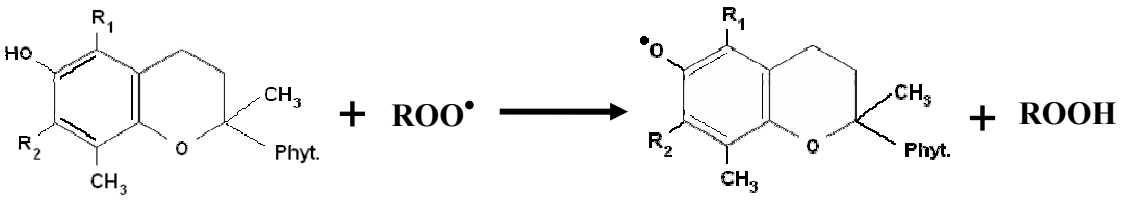


Figure 6. Hydrogen donation from tocopherol to radical.

The various isomer forms of tocopherol have different levels of antioxidant activity *in vivo* and *in vitro* [40] due to variations in the dihedral angle θ caused by the different positions of the methyl groups (Figures 8). The resonance stability of the phenoxyl radical formed by hydrogen donation to a peroxyl radical increases as the angle between the p-orbital of the methoxyl oxygen and the aromatic ring approaches perpendicular [41]. This permits the delocalization of the unpaired electron into this orbital in addition to those of the aromatic ring. Addition of methyl groups also acts to limit the number of resonance structures accessible to the tocopheroxyl radical.

Tocopherol selection as antioxidant

Current formulations of antioxidant food packaging are made using the synthetic, volatile antioxidant butylated hydroxytoluene (BHT). This is undesirable in a market where consumer trends are towards a preference for natural products. There is also concern regarding the carcinogenicity of BHT. BHT is a volatile product, so it is suitable for packages with headspace, not liquid or semi-solid products. It also has a release rate that is too fast for very long term storage applications. These concerns have led to a demand

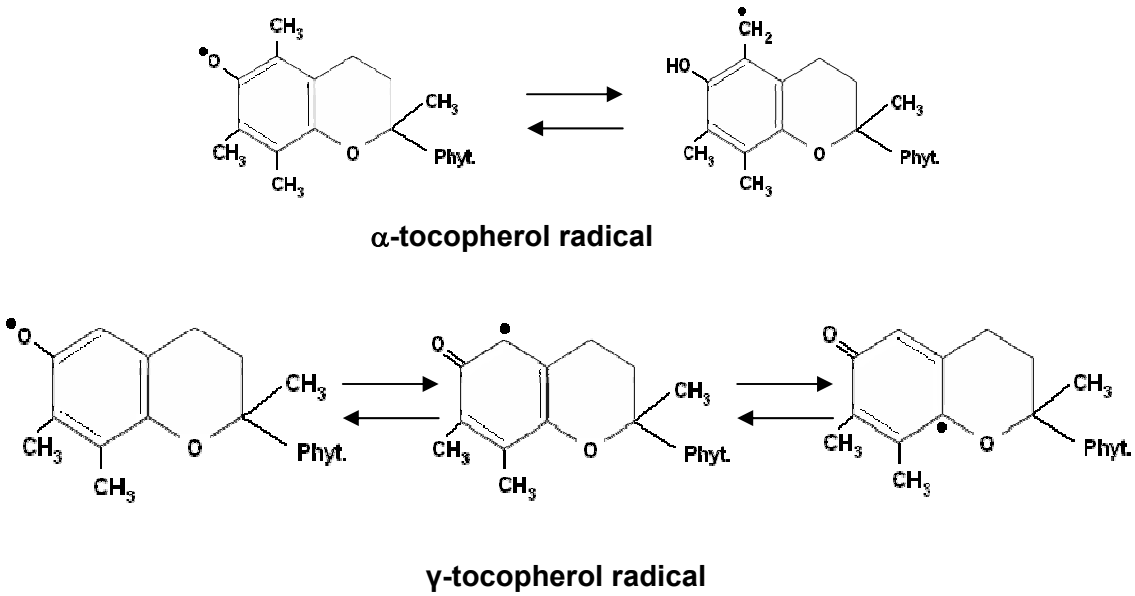


Figure 7. Resonance structures of tocopherol radicals.

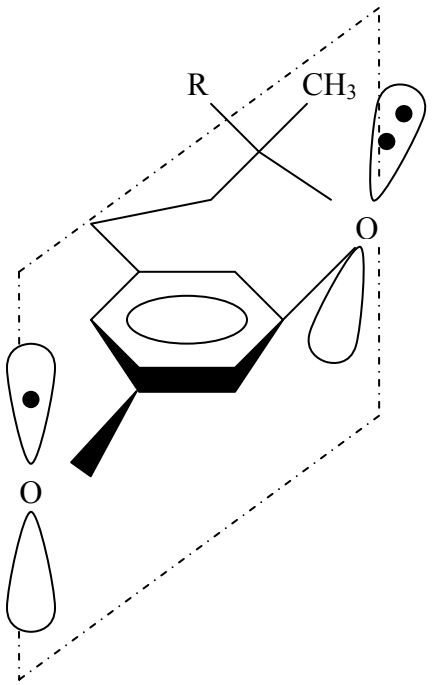


Figure 8. Electron delocalization on p-orbitals of oxygen in tocopheroxyl radicals.

for both natural antioxidant packaging, and packaging that can be applied to packages with no headspace.

Since lipid oxidation is the primary degradative reaction governing the shelf life of foods, a lipophilic antioxidant is needed. The antioxidant must be compatible with the packaging material, capable of release at appropriate rates, and also be heat stable to survive the film production process. Tocopherol meets all these criteria, and in addition, its degradation pathways are well-documented. Thus, tocopherol was selected for this initial study.

Polymer film production

Polymer materials

Plastics have taken on a leading role in the packaging of food products due to their desirable characteristics, such as oxygen and water vapor permeability, transparency, ease of processing, low density and cost, easy recyclability, and consumer appeal [7, 42, 43]. The polymers most commonly used in food packaging materials are thermoplastics, meaning that they can be subjected to repeated melting and cooling steps. This is in contrast to the thermoset polymers, which are covalently crosslinked and cannot be softened by heat once they have been formed [7].

One common class of thermoplastic polymers used in food packaging is the polyolefins, which are long chain polymers built on a carbon backbone. Two of the simplest polyolefins are polyethylene and polypropylene, produced by polymerization of ethylene and propylene monomer, respectively. The structures of the ethylene and propylene monomers are shown in Figure 9. Together, these two materials account for over 47% of the plastic consumed annually in Western Europe [42]. The physical properties of the polyolefins (Table 2) are governed primarily by chain entanglement and non-covalent van der Waals forces, allowing them to be softened into a fluid melt state by the application of heat [44].

Polyethylene Polypropylene

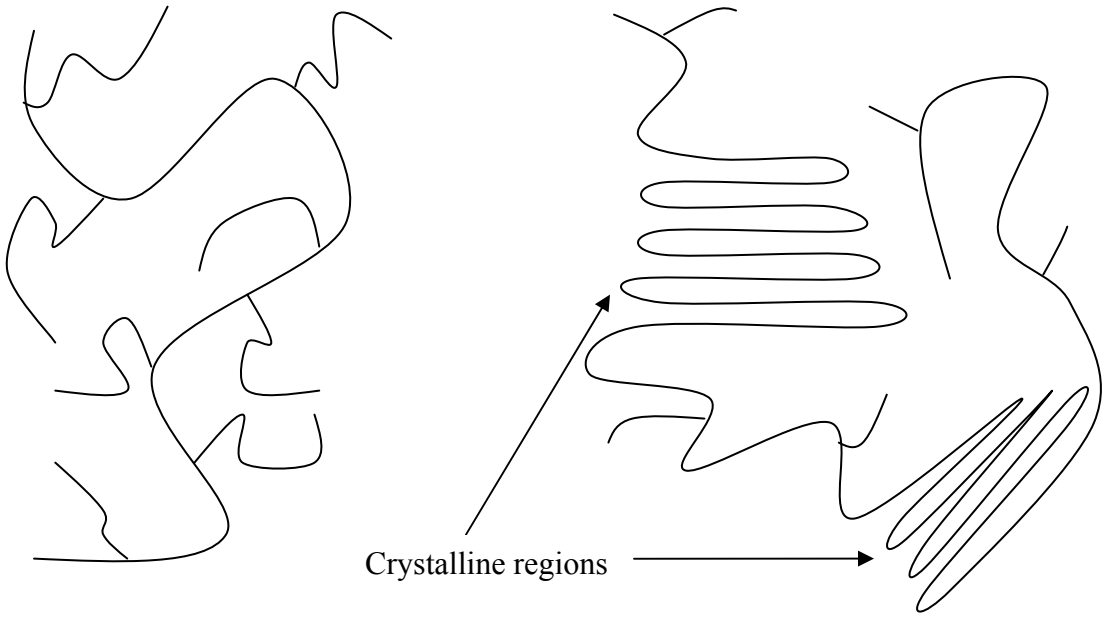
Figure 9 - Structures of polyethylene and polypropylene.

Table 2 - Some physical properties of typical polyolefins [7].

Property	**LDPE**	**HDPE**	**PP**
Molecular Weight	1-3 x 10^4	>10^5	75-200 x 10^4
Crystallinity (%)	55-70	80-95	65-70
Density (g/cm^3)	0.915-0.935	0.945-0.965	0.90
Softening Point °C	86	120-130	150
Melting Point °C	112	137	168
Tensile Strength (kg/m^2) x 10^{-3}	0.06-0.14	0.17-0.22	7.0-42.0
Glass Transition Temperature T_g °C	-20	-125	-18

The polymerization process is initiated by a radical generator such as benzoyl peroxide or 2,2'-azo-bis-isobutyrylnitrile (AIBN), which splits to form to radical fragments, that then each remove an electron from the double bond of the parent monomer, setting off a radical chain reaction that generates many monomer radicals [44]. Monomer radicals combine in radical-radical coupling termination reactions to form increasingly longer chains. Such processes are undirected, providing little control over the branching and conformation of the polymer chains. For this reason, more complex catalysts such as the Ziegler–Natta and metallocene catalysts are used to produce polymer chains with more specific chain structures [43].

Polyethylene can be produced by either high or low pressure polymerization processes, which yield highly branched or linear polymer chains respectively [44].These branches inhibit the orderly layering of polymer chains, preventing the formation of rigid crystalline regions, and decreasing the density of the solid polymer, as shown in Figure 10. For this reason, the branching characteristics of polyethylenes can be described by the density, with high density polyethylene (HDPE) having the fewest branching side chains, and low density polyethylene (LDPE) having many long side chains. Polyethylenes are also classified by molecular weight, as with ultra-high molecular weight polyethylene (UHMWPE), which is used to make prosthetic hip and knee joint replacements [45].



LDPE – Highly branched HDPE – Few branches, more crystalline regions

Figure 10 - Branching of LDPE and HDPE, and its effect on crystallinity

Polypropylene can be polymerized from the propylene monomer by either Ziegler-Natta polymerization and by metallocene catalysis polymerization, resulting in polypropylenes with differing tacticity [44]. Tacticity refers to the orientation of the pendant methyl groups on the carbon backbone, as shown in Figure 11, and can be used to alter the crystallinity and clarity of the PP, with atactic PP having little or no crystallinity, and isotactic PP forming helical crystals with the methyl groups on the outside [7].

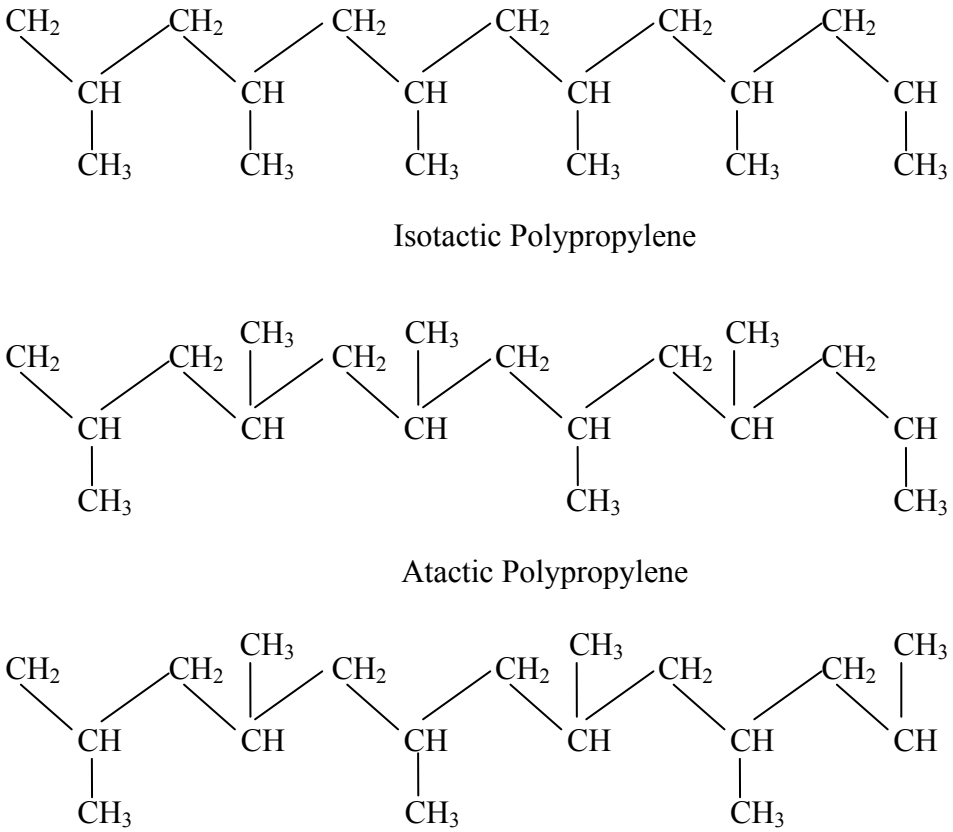


Figure 11 - Different tacticities of polypropylene

Polymer blends

One of the most important obstacles in developing CRP materials has been achieving the required release rates of active compounds. Depending on the specific application, this may not be possible using any single polymer currently available. To overcome this limitation, polymers may be blended to produce materials with more useful release characteristics. Such blending techniques have been used in other applications, such as

modifying the glass transition temperature [46] and gas transmission rates [47] of a polymer material, as well as to generate polymers with novel electrical properties [48].

Polymer blending presents its own challenges, as the miscibility of the polymers being blended must be taken into account. While some polymers are highly miscible and yield fairly homogenous blends, less miscible polymers will tend to form distinct phases, allowing the development of complex morphologies, as is the case with PE and PP [49]. Due to the differing physical properties, such as crystallinity, polarity and glass transition temperature, of the individual polymers, an active compound diffuses at different rates through the phases [50]. By modifying the relative proportion of the individual polymers and the processing methods used, film morphologies may be developed that provide optimal release of the active compound.

Extrusion

Extrusion is the process by which polymer resins are melted and formed into the desired shape by forcing the polymer melt through a die under pressure. It enables continuous production of such products as pipe, tubing, fibers, films and sheets [51]. The primary requirements of any extruder are material feeding, polymer melting and mixing, and the generation of sufficient pressure to expel the melt through the die. The most common type of extruder in use today is the single screw extruder, as shown in Figure 12, which utilizes a rotating screw enclosed within a barrel to perform these functions.

The most energy intensive operation of the extruder is polymer melting, accounting for over 90% of the total energy required [51]. Due to the poor thermal conductivity of most polymeric materials, the heat required for melting is generated almost entirely by friction of the polymer resin against the barrel surface. As the screw rotates, the solid polymer resin is rubbed against the inner surface of the barrel, generating heat through friction, which melts the polymer. This leads to the formation of a melt film along the barrel surface. As more of the polymer is melted, a growing liquid melt pool forms against the leading flight of the screw [52].

A second primary function of the extruder is the generation of pressure to pump the polymer melt through the die at an appropriate rate [52]. The rotation of the screw provides a drag force on the material contained in it, pushing it towards the die at the exit. As the die provides some degree of restriction to the melt flow, a backpressure is generated in the opposite direction, building the internal pressure in the barrel. This pressure can be modified through the use of screens and valves located inside the barrel to disrupt the polymer flow. Such screens also help to filter particulates from the melt to prevent their clogging of the die.

Adequate mixing within the extruder is also very important, particularly when the material being extruded is comprised of more than one component, as is the case with polymer blend films. Even in single component melts, mixing helps to reduce the non-homogeneity that results from the shear melting process. Several screw modifications have been developed to increase the degree of mixing, including knobs or pins places

within the screw channel, or a fluted screw section as in the Maddock or Egan mixing section. These modifications interrupt the smooth melt flow and lead to greatly increased melt uniformity [52].

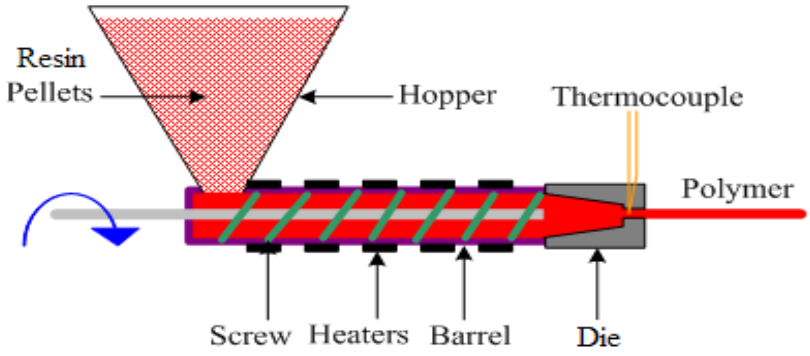


Figure 12. Schematic of single screw extruder, showing position of hopper, barrel, screw and die [53].

There are also several alternative types of extruder which are used for specialized extrusion applications, including twin screw extruders and ram extruders [52]. The twin screw extruder is designed to mix and pump the polymer melt without subjecting it to the high levels of shear found in a conventional single screw extruder, making it suitable for shear sensitive materials. Because of the lack of shear inside the extruder barrel, there is little heat energy generated by friction, so the majority of the melting energy must be provided by external heating units. The screw flights of a twin screw extruder are specifically designed to perform the desired mixing task. Screws may be positioned in either corotating or counterrotating, and intermeshing or nonintermeshing alignment, depending on the desired outcome. Ram extruders differ from screw based extruders in

that they use a rap or piston to pump the already melted polymer through a die. They are the oldest type of polymer extruder, and are still used with specific polymers that cannot handle screw extrusion, such as polytetrafluoroethylene, better known as Teflon [54].

Cast films

After the extruder has melted the polymer, it is pumped through the die, which forms it into the desired final shape. In the case of films, the die is a narrow slit with a relatively long profile. Due to machining tolerances, the die cannot be made as narrow as the desired final thickness of the film, so drawdown rolls are needed to finish thinning the films. The melt exits the die vertically and falls onto a water cooled chill roll rotating at a higher speed than the film output speed. This roll serves the dual purpose of cooling the melt to solidify it, as well as stretching the film in the machine direction to reduce its thickness. A further series of rollers are then used to further stretch the film, ending at the takeup roll which coils the finished film on a final roll. This rolling also orients the polymer in the machine direction, which improves the mechanical properties of the finished film [55].

Polymer Degradation

Polymer radical degradation under extrusion conditions

The conditions used during the extrusion process to first melt and then form the polymer subject the polymer to a high degree of stress in the form of both shear forces and thermal energy, which can cause degradation of the polymer molecules. The entanglement of the long polymer chains reduces their ability to rotate freely when exposed to shearing forces, leading to high tension on the bonds within the chain and possible chain breakage [56]. Such chain breakage generates two polymer radicals, which may initiate radical chain reactions that create many more radicals. The probability of such chain breakage is directly proportional to the melt viscosity and the temperature [57]. It is interesting to note that for a constant shear rate, the mechanical reaction temperature coefficient is negative, meaning that polymer mechanodegradation will occur more readily at lower temperatures [57]. Other radical generating reactions are also possible, such as the loss of a hydrogen atom from an unsaturated or tertiary carbon, or reaction with oxygen to form hydroperoxides, with applied shear increasing the rate of reaction. Each of these radicals is then able to enter the propagation phase of the radical chain reaction, generating many more radicals [58] (Figure 13).

$$RH \xrightarrow{\Delta} R^{\bullet} + H^{\bullet}$$

$$RH + HR \xrightarrow{\Delta} R^{\bullet} + H_2 + R^{\bullet}$$

$$R_1 - R_2 \xrightarrow{\Delta,shear} R_1^{\bullet} + R_2^{\bullet}$$

$$RH + O2 + HR \xrightarrow{\Delta} R^{\bullet} + H_2O_2 + R^{\bullet}$$

Figure 13 - Modes of polymer degradation and radical formation [58].

Polymer degradation during processing is an undesirable occurrence, as it often causes changes in the molecular mass profile of the polymer through either chain breakage or cross-linking. This can lead to both processing difficulties and loss of quality in finished products, such as changes to the polymer melt viscosity and the shrinkage and tear resistance of the final polymer film[59]. Residual radicals that remain in the finished polymer product accelerate oxidation, weakening the polymer and causing premature failure [60].

The most significant factor involved in polymer degradation during processing is the molecular weight (MW) of the polymer chains [61]. As the polymer radicals form, they can undergo either scission reactions or cross-linking reactions, both of which alter the MW distribution of the polymer. Polymer alkoxyl radicals frequently undergo β-scission reactions, cleaving the polymer chain and reducing the MW. Conversely, two polymer radicals may react with each other to form a cross-link, increasing the MW. Such shifts in the MW of the polymer lead to changes in the melt viscosity; with decreasing MW, viscosity decreases, and with increasing MW, viscosity increases. Such changes, lead to changes in the melt flow rate of the polymer, and can cause problems during extrusion.

Rex et al [62] confirmed the observation of Rideal and Padget [63] that the crosslinking reaction dominates in HDPE for processing temperatures below 290 °C, while processing at temperatures greater than 290 °C will be subject to greater scission reactions. Melt degradation of PP was found to be dominated by chain scission reactions, as measured by decreasing melt viscosity, by da Costa et al [64]. Gugumus [65] also notes that PP has a greater tendency towards intramolecular reactions, leading to increased chain scission. A summary of the degradative reactions of polymer radicals is presented in Figure 14.

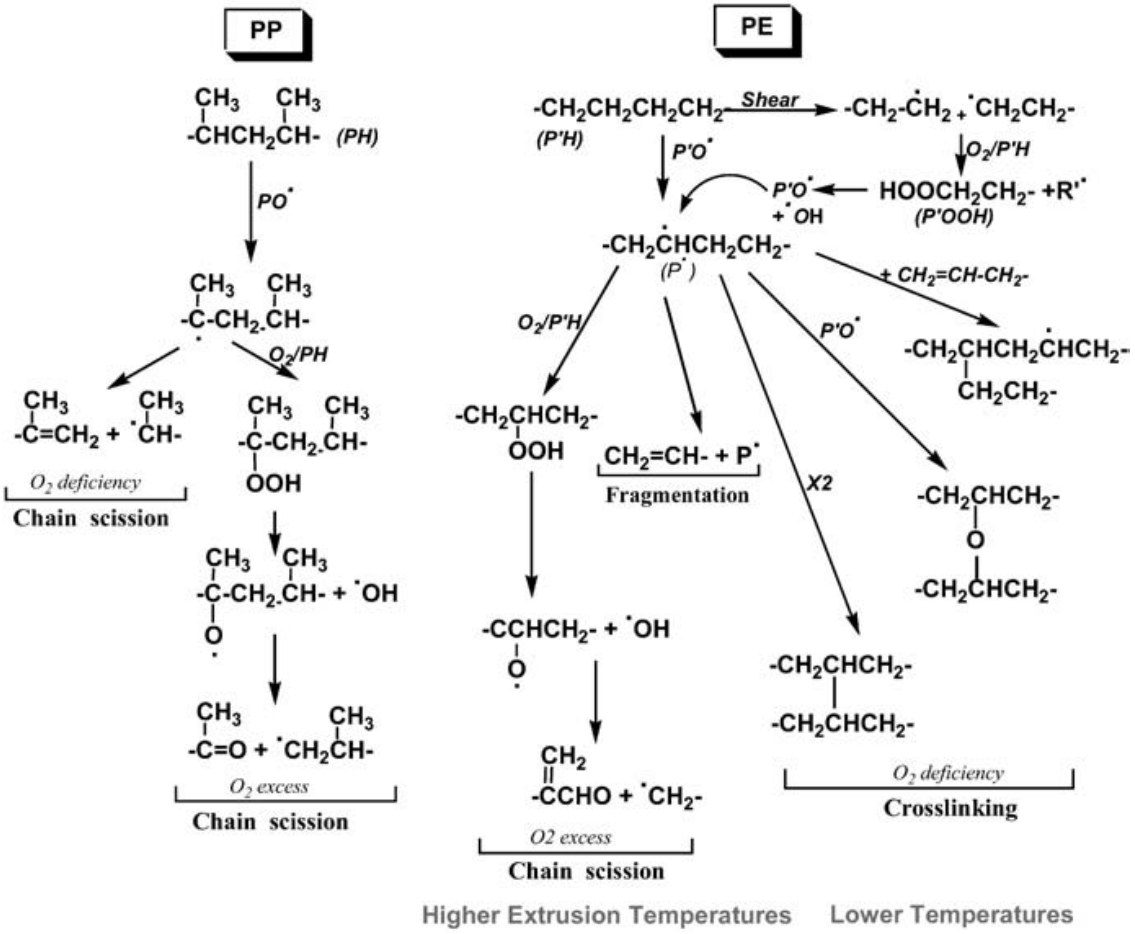


Figure 14. Degradative reactions of polymer radicals [66].

Antioxidants in polymers

Antioxidant additives are frequently added to the polymer resin to minimize the negative effects of radical formation during polymer processing. Primary antioxidants such as hindered phenols quench the polymer radicals before they are able to form degradation products or initiate radical chain reactions within the polymer [67]. Synthetic phenolic antioxidants such as Irganox (Figure 15) are one of the most commonly used types of polymer antioxidant [61]. The physical properties of Irganox 1010 are given in Table 3. Secondary antioxidants containing phosphorus or sulfur which act to decompose polymer hydroperoxides formed by the reaction between polymer radicals and oxygen are also used for polymer stabilization to minimize the formation of off-color degradation products in the finished polymer.

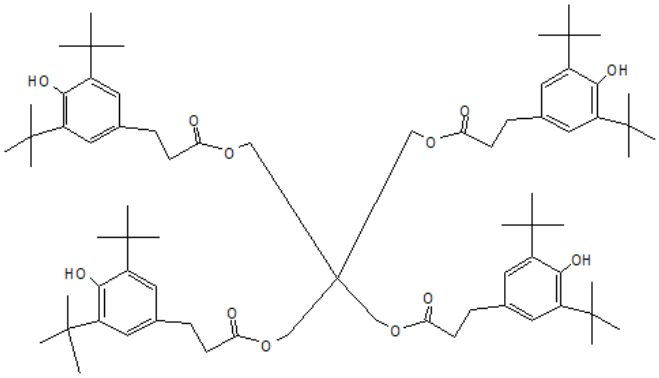


Figure 15 - Structure of Irganox 1010, an example of a synthetic hindered phenolic antioxidant used as polymer stabilizers [68].

Table 3 - Physical properties of Irganox 1010 [69].

Molecular Weight	1176
Melting Point	115-118C
Boiling Point	1130.4C

As noted above, due to concerns regarding the safety of synthetic antioxidants for food packaging and to consumer preference for natural products, there has been a movement towards the use of natural antioxidants such as tocopherols for the stabilization of polymers [70]. As a sterically hindered phenolic antioxidant, tocopherol serves the same quenching function as primary synthetic hindered phenol antioxidants. Al-Malaika et al [71] demonstrated that α-tocopherol acts as a better melt flow stabilizer than Irganox 1010 in PP, with a lower concentration required to produce greater stabilization (Figure 16). In addition to facilitating processing, tocopherol also helps to improve the characteristics of the finished product, such as reducing off-odor and flavor caused by volatile polymer degradation products in beverage bottles [72]. Tocopherol has also been demonstrated to be an effective antioxidant against oxidation induced by γ-radiation, enabling it to be used in the production of materials to be radiation sterilized [73].

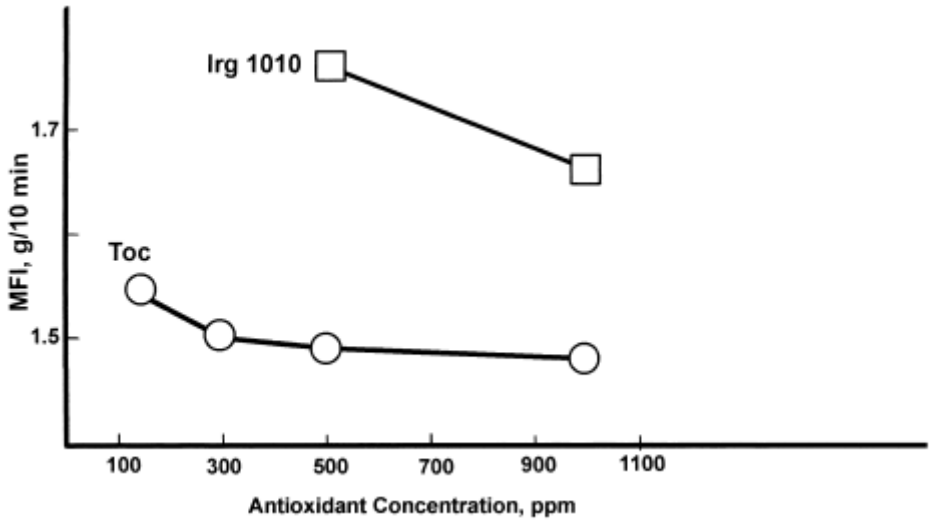


Figure 16. Effect of antioxidant concentration and type on melt flow index of PP, demonstrating the superiority of tocopherol over Irganox 1010 [71].

Tocopherol Degradation and Stability

Basic mechanism of tocopherol degradation

Tocopheroxyl radicals left after radical quenching are unlikely to propagate radical chain reactions, but they are nevertheless reactive and undergo a number of degradative reactions that may or may not result in the loss of antioxidant activity. In aprotic systems, such as lipids or polymer melts, the tocopherol radicals will react primarily by radical-radical coupling reactions. These reactions may occur with lipid or polymer peroxyl, alkoxyl or hydroxyl radicals to form molecular products [74], or in their absence, another tocopherol radical [75]. When tocopherol radicals self-react, they form dimers, trimers, epoxides, and quinones [15], with the structure and relative concentration being dependent on both the nature of the solvent and the isomeric form of the tocopherol. The general degradation scheme for α-tocopherol in aprotic solvents is shown in Figure 17.

The degradation of γ-tocopherol follows a similar mechanism as that of α-tocopherol, with the formation of the diphenol dimer in the absence of peroxyl radicals, and quinonoid dimers and the γ-tocopheryl orthoquinone, referred to as tocored in some literature, in the presence of peroxyl radicals (Figure 18). For all tocopherols, the rate of reaction between the tocopherol radical and a peroxyl radical is always greater than the bimolecular coupling of two tocopherol radicals, leading to the conclusion that in the presence of peroxyl radicals, the formation of tocopherol adducts will predominate over the formation of dimeric tocopherol products [76]. Experiments conducted by Ha and Igarashi [77] confirmed the fact that, due to their different reactivity with lipid peroxy

radicals, two different isomers of tocopherol will not form mixed dimers; α-tocopherol will be completely consumed before γ-, and then δ-tocopherol react to form radicals

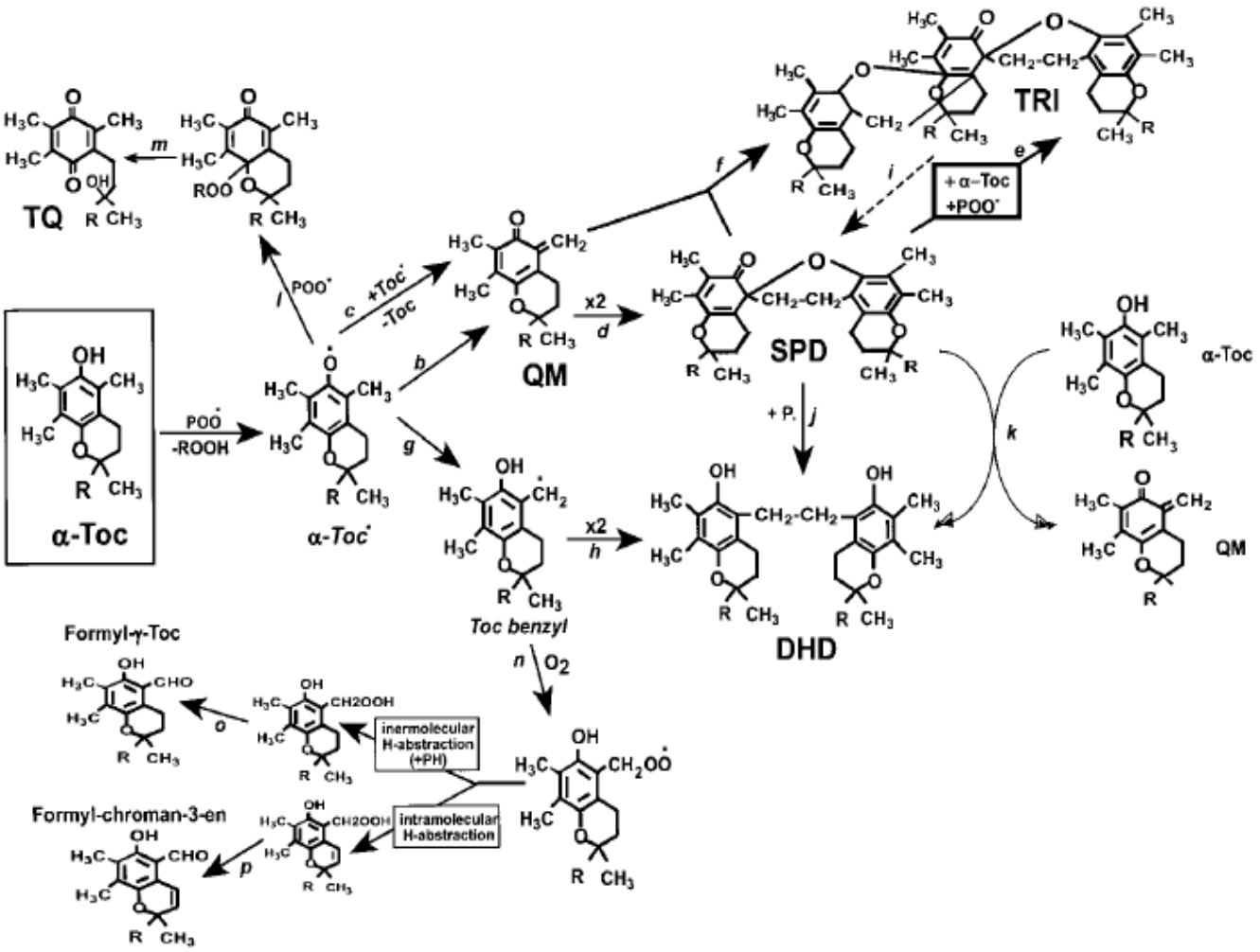


Figure 17 - Degradation pathways of a-tocopherol in aprotic solvents [78].

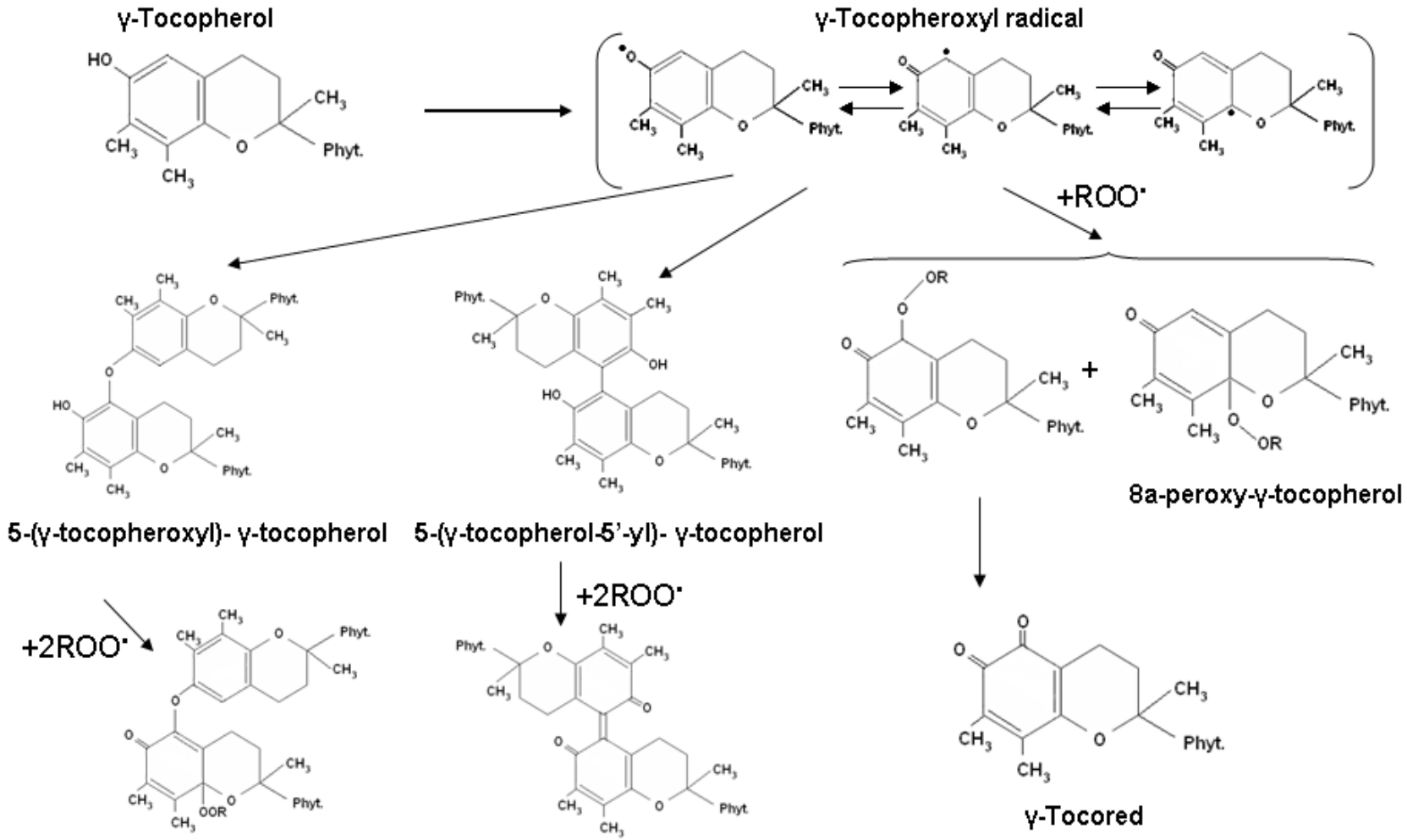


Figure 18. Degradation pathway for γ-tocopherol [15].

Many α-tocopherol degradation products possess antioxidant activity, as demonstrated in the polymer melt, measured by change in the melt flow index [71], including α – tocopherolquinone (QM), α-tocopherol-5-ethane dimer (DHD), the aldehydes formyl-γ-tocopherol and formyl-chroman-3-en (ALD), and by Diels-Alder polymerization, the α-tocopherolspirodimer (SPD) and α-tocopherolspirotrimer (TRI) (Figure 19) [78].

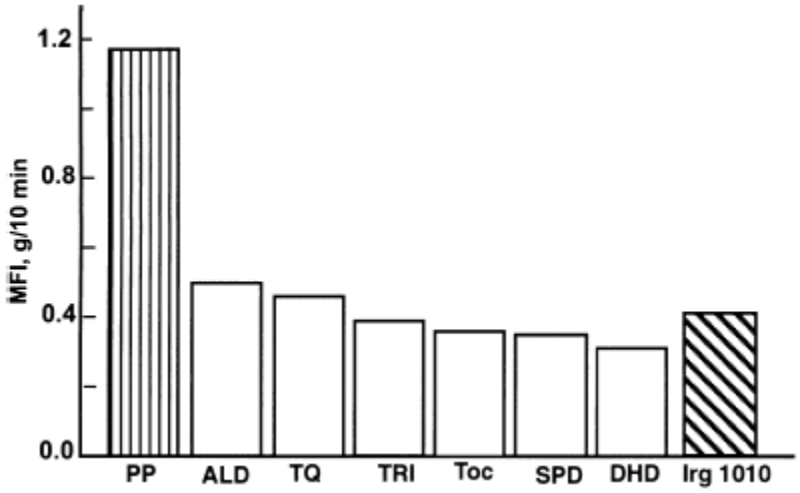


Figure 19 - Melt stabilizing effect of a-tocopherol and its degradation products (2000 ppm) in PP extruded at a die temperature of 260°C and screw speed of 100 RPM [71].

Tocopherol degradation during frying

During frying, tocopherols in the frying oil are rapidly degraded by both heat and oxidation of the oil. Loss of tocopherol leaves the oil unprotected and subject to oxidation, resulting in poor oil quality that may affect the food fried in it. In a study on simulated frying, Barrera-Arellano et. al. used a Rancimat to demonstrate the effects of simulated frying on tocopherol loss first in model triacylglycerols (TAGs) with added tocopherols [13], and then in natural oils [79]. They found that in the model TAG

systems, α-tocopherol was lost more quickly than δ-tocopherol, with more rapid loss in the more unsaturated trilinolein than in the monounsaturated triolein. They also found that addition of a mixture of all four tocopherol isomers not only extended the time before tocopherols were lost, but also provided greater protection against TAG oxidation and polymerization. In later experiments with natural oils stripped of antioxidants, they demonstrated that α-tocopherol addition had a greater effect in the prevention of oil degradation in monounsaturated oils. Surprisingly, they found that in native oils, tocopherols were lost more rapidly from monounsaturated oils than polyunsaturates, most likely due to the lower levels and different types of tocopherol naturally present in less saturated oils. This is supported by the finding that in stripped oils, tocopherols were lost more rapidly in the more unsaturated oil.

Tocopherol degradation during oil storage

Even under relatively mild conditions, tocopherol may undergo degradation when present in oils due to its radical quenching action on the autoxidizing lipids. In a study of stored olive oils, Okogeri [80] found that even during storage in the dark at average room temperatures ranging from 6-18°C, 62% of the α-tocopherol was lost in 12 months. An even higher level of tocopherol loss was observed when the oils were stored under diffuse light conditions, with α-tocopherol levels decreasing by 79% in only 6 months. This much more rapid degradation was attributed to the singlet oxygen quenching effect of α-tocopherol during lipid photooxidation [80], but may also derive from increased LOOH decomposition by uv light, with increased generation of $LO^{\bullet}$ that react even faster with tocopherol than $LOO^{\bullet}$.

Tocopherol degradation during polymer processing

Due to increased interest in using tocopherol as an antioxidant stabilizer for extruded polymers, studies were conducted on the stability and degradation of α-tocopherol during polymer processing. Al-Malaika et al [78] performed a series of studies to evaluate both the efficacy of α-tocopherol as a polymer stabilizer, and the degradation of α-tocopherol during said polymer processing. They found that in LDPE extruded at 180°C on a single screw extruder, α-tocopherol degrades to form trimers (TRI), dihydroxy dimers (DHD), spirodimers (SPD), tocoquinone (TQ) and a series of aldehydes (ALD) (Figure 20), with TRI being the most prevalent product formed. They also found that product formation was concentration-dependant, with increasing tocopherol concentrations leading to greater formation of the DHD at the expense of both TRI and SPD (Figure 21). It was observed that the prevalence of TQ remained relatively constant regardless of initial tocopherol concentration. Increased processing severity, simulated by repeatedly extruding the polymer at 180°C, resulted in an increase in TRI, TQ and ALD, at the expense of SPD. In the case of PP, extruded at 260°C on a twin-screw extruder, no SPD was detected, while the distribution of the other products was similar to that found in

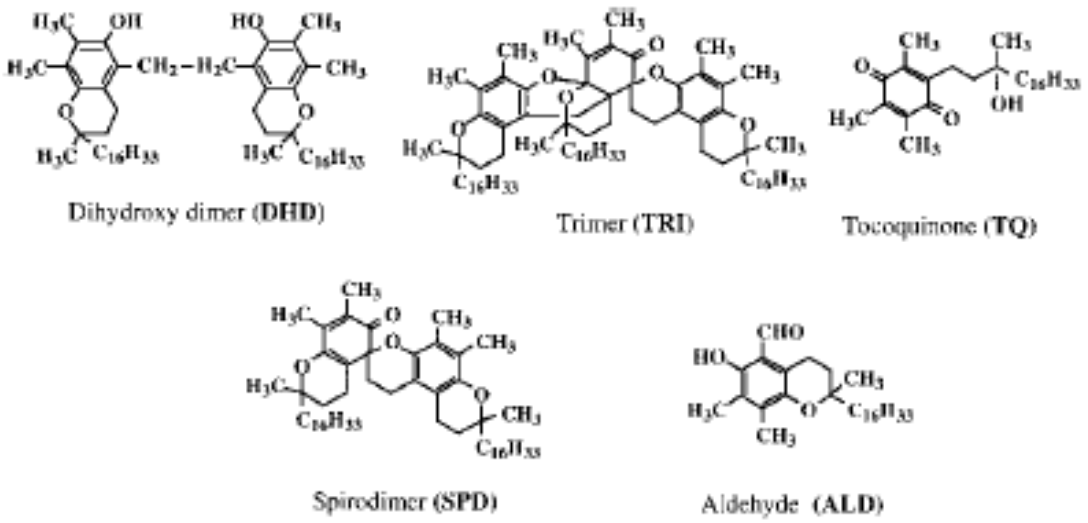


Figure 20 - Structures of α-tocopherol degradation products found in polymers after extrusion

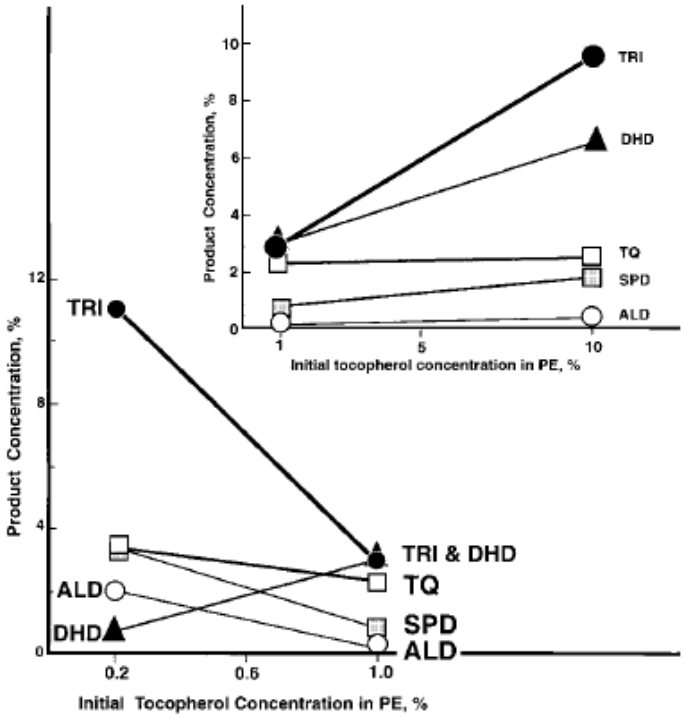


Figure 21 - Effect of initial α-tocopherol concentration on degradation product formation in LDPE extruded at 180°C [78].

LDPE. It was also observed that increasing extrusion temperature above 290°C led to a dramatic increase in the number of degradation products formed, particularly the highly colored aldehyde.

The products formed by degradation of α-tocopherol during polymer extrusion were further characterized in a later study by Al-Malaika and her group [81]. This work established that the degradation products formed are found in all possible stereoisomeric conformations and that SPD is highly susceptible to oxidation so it is formed only under conditions of restricted oxygen.

Toxicity of tocopherol degradation products

In order to satisfy the requirements of the mixture doctrine [21], it must be demonstrated that the tocopherol added to the polymer material does not degrade to form any toxic or carcinogenic compounds. This is unlikely, as many of the observed degradation products, such as α-tocopherylquinone, 8a-hydroxytocopherone, and epoxy-α-tocopherylquinone, are known to be present as natural metabolites of vitamin E in human plasma [82]. Mackenzie et al [83] even found that α-tocopherylquinone possesses vitamin E activity, and can be used to treat the symptoms of vitamin E deficiency in rabbits. The mode of action for this was discovered by Infante et al [84], who found that α-TQ serves as an enzyme cofactor in mitochondrial fatty acid desaturases. Csallany and Draper [85] found that an α-tocopherol dimer was produced naturally as a metabolite of α-tocopherol in the liver of the rat. Due to their natural presence *in vivo,* it can be concluded that these oxidation products of tocopherol are unlikely to prove harmful to the health of consumers.

Methods for Analyzing Tocopherol Degradation

The separation and purification of the various isomers of tocopherol is a subject that has received much attention due to both their function as lipid antioxidants and their biological activity [86]. While thin-layer and column chromatography can be used as low-cost separation methods, they require long run times and can not provide sufficient precision for studies requiring precise quantitation [87, 88]. In cases where greater speed and precision are required, newer methods such as gas chromatography (GC) and high-performance liquid chromatography must be employed.

Gas chromatography - Mass spectrometry

Gas chromatography utilizes a column containing material to which the analytes are differentially retained. Only compounds that are volatile or can be volatilized can be analyzed using GC methods. If an analyte is not volatile, it may be possible to volatilize it by derivatization. In the case of tocopherol, which is marginally volatile (boiling point 200-220°C), derivatization to form trimethylsilyl (TMS) derivatives for GC–MS can be accomplished by heating the substances with N-methyl-N-trimethylsilyltrifluoroacetamide for 20 min at 60 °C [89].

Commonly used detection methods for GC analysis are flame ion detection (FID) and mass spectrometry (MS). While FID can be used to provide quantitative information about compounds with established retention times, unknown compounds may be identified by observing the mass fragments presented by MS.

Liquid chromatography - Mass spectrometry

High performance liquid chromatography (HPLC) methods are commonly used to analyze materials that are non-volatile and cannot be volatilized, or may suffer degradation caused by the high temperatures involved in GC. HPLC separation is similar to GC in that analytes are passed through a column containing material to which they are differentially retained, but in HPLC the analyte partitions from the liquid phase rather than the gas phase.

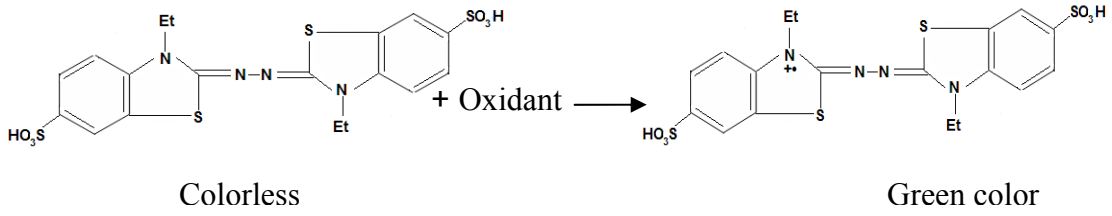
There are two main types of HPLC analysis, normal phase and reversed phase. The first HPLC separations of tocopherols were performed on normal phase systems which utilize silica as the column material and a non-polar mobile phase such as hexane [90]. While normal phase methods provide the best separation of the β and γ tocopherol isomers [91], the mobile phases employed are volatile and may pose health hazards, making them less desirable from a safety perspective. The separations are also technically difficult to control since even traces of water make critical difference in separations.

An alternate method, reversed phase HPLC (RP-HPLC), uses columns composed of silica to which nonpolar 18-carbon alkyl chains have been esterified to make a hydrophobic phase along with an aqueous mobile phase. Advantages of the reversed phase method are less volatile mobile phases and greater peak reproducibility [86]. By modifying the column material, the polarity characteristics, and thereby the retention characteristics of the column may be tailored to the specific application at hand. For example, by increasing the length of the alkyl chains from 18 to 30 carbons, Strohschein

was able to achieve separation of the β and γ isomers of tocopherol, because the longer chains possess greater rigidity and shape specific selection [92]. Obinata [50] in our group improved this separation by increasing the polarity of the C30 column. For the separation of more polar compounds, such as polyphenols, the addition of polar endcap groups to the carbon chains is used to increase retention times and sensitivities. Whelan et al demonstrated that in the separation of polycarboxylic acids and polyphenols, a polar endcapped column provided significantly improved peak shape as compared to the traditional C18 column [93].

Trolox Equivalent Antioxidant Capacity

First reported by Miller et al [94] the Trolox Equivalent Antioxidant Capacity (TEAC) assay is used to measure the ability of an antioxidant molecule to quench 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radicals through either donation of a hydrogen atom, or through electron transfer. Loss of the aqua-green colored radical (Figure 22) is easy to follow optically. The most intense absorption peak is at 415 nm, but at this wavelength there is often interference by pigments in natural extracts, so the reaction is most commonly followed at 734 nm where the extinction coefficient is lower but there is no interference. Quenching by the test antioxidant is compared to the quenching ability of the water-soluble tocopherol homologue Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) in order to determine the TEAC value, which can be used to compare the antioxidant efficiency of different materials. Prior et al [95] published a thorough review of the history, pros and cons of this assay, along with many other assays commonly used for the analysis of antioxidants.



Colorless Green color

Figure 22. Structure of $ABTS^{+\cdot}$ radical.

Several pitfalls complicate interpretation of antioxidant behavior using this method. $ABTS^{+\bullet}$ can simultaneously be neutralized by reduction as well as hydrogen donation so antioxidant mechanism cannot be unambiguously determined. Bulky antioxidants sterically have limited access to the radical located in the center of the molecule, resulting in artificially low antioxidant values for larger antioxidants. Protic solvents such as ethanol are hydrogen donors and quench $ABTS^{+\bullet}$ even in the absence of antioxidants, so solvents must be selected with care and proper controls run. This assay has also been criticized on grounds that hydrogen donation to the nitrogen-centered $ABTS^{+\bullet}$ radicals by phenols is not the same as to lipid peroxyl radicals, so results of this assay may not accurately reflect the actual antioxidant activity of a material in a real food system. The method thus has limitations for comparing antioxidants of different structure and for predicting mechanism, but it can be used to monitor changes in activity when a single antioxidant is modified, as in processing.

Hypothesis

Hypotheses

- Tocopherol is suitable for use as an antioxidant in CRP applications since it will both stabilize the polymer film and be retained sufficiently to allow release into the food product.
- Polymer films produced with a sufficient amount of tocopherol for controlled release applications will retain physical properties suitable for use as packaging materials.
- Degradation products will be limited to previously identified products of tocopherol oxidation, and there will be no formation of toxic or carcinogenic products.

Necessary Tasks

- Produce tocopherol-containing polymers films under a variety of conditions, including ‘worst-case’ processing scenarios.
- Develop methods to extract, separate and identify volatile and non-volatile tocopherol degradation products from polymer films.
- Evaluate the effect of polymer composition and processing conditions on the stability of polymers and tocopherol in tocopherol-containing films.

Experimental

Materials

Polymer resins

The polymer resins used for film production -- low density polyethylene (LDPE) and polypropylene (PP) -- were selected based on their current acceptance for use as food packaging materials, common use in food contact layers (i.e. adhere well to other polymers), and hydrophobic nature compatible with tocopherol, the active compound.

Polymer resins with few or no additives were used to minimize any potential side reactions between tocopherol and the additives and also to simplify analysis. LDPE was a barefoot resin (one without any additives: ExxonMobil LGA 105). PP is not available in barefoot form due to stability issues, so a minimally treated resin was used: Dow DX5E66. Additives present in the PP resin used include the hindered phenolic antioxidant Irganox 1010 (structure presented in Figure 15, physical properties presented in Table 3), and the secondary phosphite antioxidant Irgafos 168 (structure presented in Figure 22, physical properties presented in Table 4). Physical properties of both resins are given in Table 5. Polymer resins were provided by Pliant Corporation.

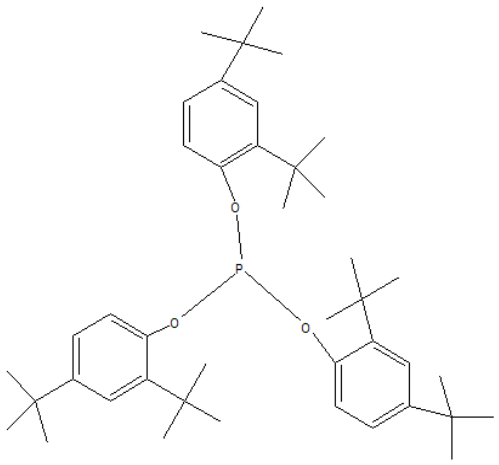


Figure 23 - Structure of the phosphite stabilizer Irgafos 168.

Table 4 - Physical properties of Irgafos 168 [96].

Molecular Weight	646.5
Melting Point	181-184°C
Boiling Point	619.8°C

Table 5 - . Physical properties of polymer resins used for film production.

Sample	Type	ΔHf (j/g)	ΔHf (j/g) theoretical	%X	Tm_1 max(°C)
Dow DX5E66	PP	85.6	207	41	163.6
ExxonMobil LGA 105	PE	129.4	293	44	108.9

Mixed Tocopherols

Mixed tocopherols extracted from soybeans containing α, β, γ and δ isomers were provided by Cargill in the form of highly viscous deep amber liquid containing over 90% tocopherol. This tocopherol concentrate is rich in the γ isomer [97]; the typical composition given in

Table 6. The boiling point for mixed tocopherols is 200-220°C, and melting point is 2.5-3.5°C.

Table 6. Isomer composition of Cargill Mixed Tocopherols

α-tocopherol	< 20%
β-tocopherol	< 5%
γ-tocopherol	50-70%
δ-tocopherol	15-30%

Film production

Extruder

All films were produced on a single screw Filmmaster extruder with a 2.5" barrel diameter and L/D of 24:1. The screw was a linear low density type, with standard configuration and a Maddox mixing head. The die was a 36"coathanger die (Figure 24) Films were cast on a Parkinson winder (Figure 25).

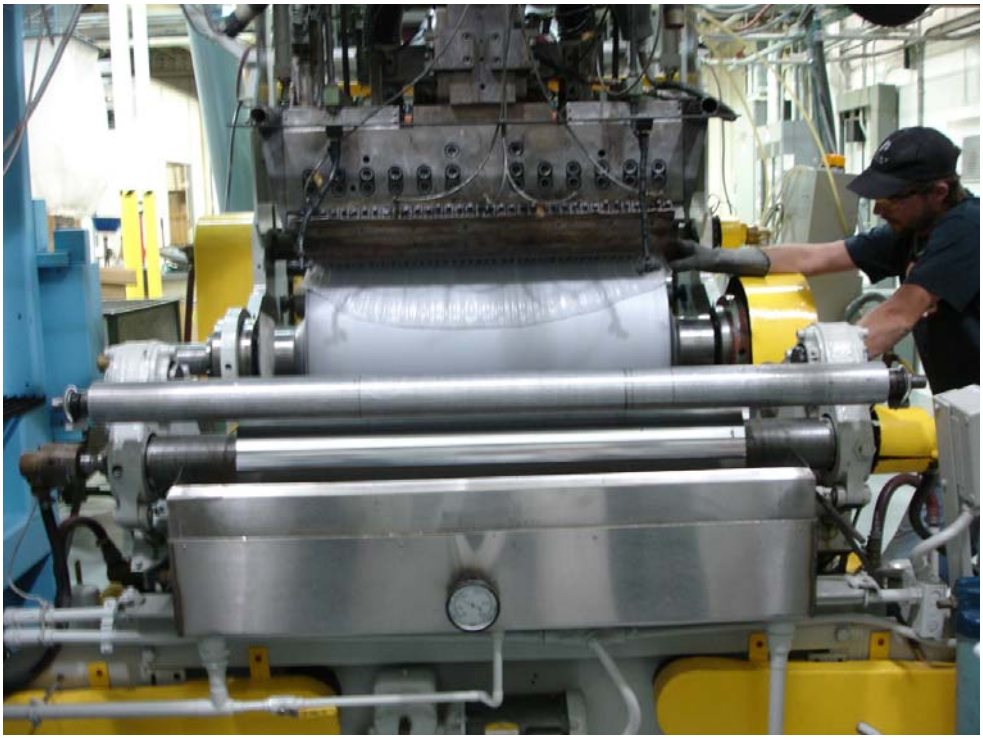


Figure 24 - Photo of die and takeup roll used for film production.

Figure 25 - Parkinson winder used in film production.

Selection of processing variables

Since the link between film production parameters and tocopherol release has not yet been fully developed, tocopherol stability must be evaluated for a wide range of processing parameters. The parameters chosen as variables in this experiment were extrusion temperature and screw speed. By varying these parameters, the polymer resin is subjected to varying levels of thermal and shear stress, both of which influence degradation of the polymer and therefore also the tocopherol within it.

Processing parameters were selected to fall within the range of typical extrusion conditions for the polymer resins selected, as it is expected that these are the conditions under which any future controlled release films will be produced. Both literature values and the expert advice of the Pliant production staff were considered in the selection of these values. Extrusion conditions were chosen to represent both ideal and worst case scenarios, to ensure that minor production errors would not result in potentially dangerous situations. All films were assigned a logical reference number to simplify record keeping. Control films containing no added tocopherol are coded with even numbers (86.0, 86.2, 86.4), and tocopherol containing films are coded with odd numbers corresponding to their polymer composition. Typical extrusion conditions were assigned no S code, while more stressful conditions were assigned a code preceded by the letter S (for severe). Details of the composition and extrusion conditions for each film are shown in Table 7.

Table 7. Film production parameters.

	Film composition			Extruder conditions	
Reference Code	LDPE (%)	PP (%)	Tocopherol (ppm)	Barrel temperature (˚F/˚C)	Screw speed (RPM)
86.0	100	0	0	430/221	75
86.2	50	50	0	430/221	75
86.4	0	100	0	430/221	75
86.5	0	100	3000	430/221	75
86.5 S1	0	100	3000	430/221	150
86.5 S2	0	100	3000	460/238	75
86.3	50	50	3000	430/221	75
86.3 S1	50	50	3000	430/221	150
86.3 S2	50	50	3000	500/260	75
86.3 S3	50	50	3000	500/260	150
86.3 S4	50	50	3000	400/204	100
86.1	100	0	3000	430/221	75
86.1 S1	100	0	3000	430/221	150
86.1 S2	100	0	3000	500/260	75
86.1 S3	100	0	3000	500/260	150
86.1 S4	100	0	3000	400/204	100
86.5 S3	0	100	3000	500/260	150
86.5 S4	0	100	3000	400/204	100

Film production method

Tocopherol addition

Tocopherol was added to polymer resins using a dry blending method. For each batch of resin, 3000 ppm of tocopherol, plus an additional 5% to account for mixing loss, was weighed in a beaker with a small amount (~200g) of the polymer resin (Figure 26), and then plated onto 10% of the total resin by manually stirring in a plastic bucket (Figure 27). This was then added to the remainder of the resin and mixed in a ribbon blender for 5-10 minutes to evenly distribute the tocopherol on the resin (Figure 28). The batch size for each film was 100 lbs of resin.

Figure 26 – Mixed tocopherols being weighed onto a small amount of polymer resin.

Figure 27- Mixed tocopherols being manually plated onto 10% of the polymer resin.

Figure 28 - Ribbon mixer used to blend tocopherol coated resin and remaining polymer resin.

Film storage

All films were stored under inert conditions to minimize post-production degradation of tocopherol. Films were stored under argon gas in opaque laminated pouches at room temperature for the duration of the experiment.

Tocopherol Extraction

Tocopherol and any degradation products present in the films must be extracted before analysis. A variety of extraction methods can be used for the extraction of small molecules from a plastic matrix, including supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), Soxhlet extraction, headspace emission and dissolution-precipitation [98], sonication-assisted extraction [99], and simple shake-flask extraction [100]. However, previous work by members of this group concluded that a shake-flask method utilizing 100% methylene chloride (MC) is the most appropriate based on efficiency and equipment availability [50]. This method was demonstrated to extract over 90% of added tocopherols from all polymers within 24 hours.

Approximately 1 gram of the film to be extracted was cut into small pieces, ~ 1cm^2, to improve solvent contact. The pieces were placed into a 250 ml Erlenmeyer flask with 40 ml MC, flushed with argon, and capped. Flasks were shaken at 100 RPM at room temperature (25°C) for at least 24 hours. The solvent was then decanted and stored frozen until analyzed for tocopherol content. After initial concentration analysis was performed, samples were flushed with argon, sealed and stored in the freezer (-4°C).

More concentrated samples were required for LC-MS analysis of degradation products found in very low quantities. For these, approximately 10 grams of film were cut into

small pieces, ~ 1cm^2, to improve solvent contact. The pieces were placed in a 500 ml Erlenmeyer flask with 400 ml MC, flushed with argon, and capped. Flasks were then shaken at room temperature (25°C) at 100 RPM for at least 24 hours. The solvent was decanted and filtered to remove any remaining film pieces or other foreign matter, then transferred to a 500 ml round boiling flask and evaporated to dryness using a Buchi Rotavapor rotary evaporator with water bath at 40°C. After evaporation, flasks were placed uncapped in a vacuum oven at room temperature for 1 hour to complete drying. The residue was dissolved in 10 ml methanol, flushed with argon and stored in the freezer (-4°C) until analyzed.

Tocopherol Recovery Analysis

Tocopherol concentrations in film extracts were measured using an optical method developed by members of our group, based on the optical absorbance of tocopherol at 295 nm [50]. Approximately 1 ml of MC film extract was transferred to a semi-microcuvette and optical absorbance at 295 nm was recorded using a Cary 50 spectrophotometer (Varian Inc., Palo Alto, CA, USA) operated with Cary WinUV software. Total concentration of mixed tocopherols was calculated by extrapolation from a standard curve prepared from prepared solutions of pure tocopherols in a range of concentrations.

For the standard curve, 50 mg of α-tocopherol (5,7,8-trimethyltocol), β-tocopherol (5,8-dimethyltocol), γ-tocopherol (7,8-dimethyltocol), and δ-tocopherol (8-methyltocol) (EMD Biosciences, San Diego, CA, USA) were dissolved separately in 50 ml methanol

to yield a final concentration of 1000 μg/ml for each isomer. Individual standard stock solutions were combined in the ratio of α : β : γ : δ = 10 : 5 : 65 : 20 to match the Cargill Mixed Tocopherols incorporated in the CRP films. This master solution was diluted with methanol to generate a series of calibration standard solutions ranging from 0 to 110 mg/ml. All solutions were sparged with argon gas to prevent tocopherol oxidation and stored at -20ºC. The standard curve is shown in Figure 29.

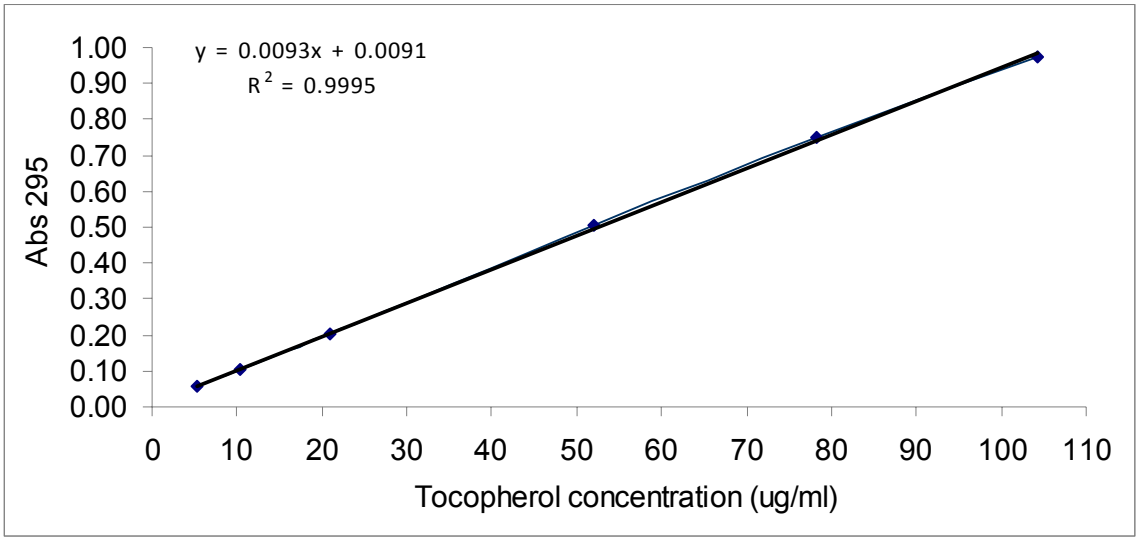


Figure 29 - Standard curve for mixed tocopherols measured by UV spectrophotometer at 295 nm.

Tocopherol oxidation

In order to develop and validate analytical methods for use in measurement of tocopherol degradation of in CRP polymer films, samples of tocopherol degradation products were required. Since these products are not readily available commercially, it was necessary to synthesize them in the lab. The method for oxidation was based on that of Al-Malaika and Issenhuth [101], using lead dioxide (PbO_2) and tocopherol in different molar ratios to generate the tocopherol spirodimer, dihydroxydimer, trimer, and 5-formyl-tocopherol.

Modifications to the method were required since the earlier studies had been carried out using normal phase HPLC which employs a non-polar mobile phase, and this research was being conducted on a reversed phase HPLC which requires a polar mobile phase. Because of this, the solvent used for tocopherol oxidation was changed from hexane to methanol.

A stock solution of 4.64 mmol Cargill mixed tocopherols was prepared in methanol, flushed with argon, and stored in an opaque flask at –20 °C between experiments to minimize thermal and photooxidation effects. PbO_2 was added to 100 ml portions of this stock solution at an equimolar ratio and at 10 and 40 times molar excess. The reaction was carried out at ambient temperature with constant argon bubbling for 6 hours. The solution was then filtered to remove unreacted PbO_2 and stored under argon in opaque vials in the freezer.

GC-MS

Gas chromatography analysis was performed on a Varian 3400 gas chromatograph using a 30 m MDN 5S column with ID of 0.32 mm. Mass analysis was performed on a Finnegan MAT 8230 with Finnegan MAT SS300 data system and MassLynx software for data analysis.

GC analysis of film extracts was performed to determine if any volatile compounds would be released from the films into solvent. MC extracts of polymer films, prepared using the shake-flask method, had an internal standard of 1µg deuterated toluene /1µg deuterated naphthalene added to aid in quantification. Injection volume for these samples was 1 µL. Samples were introduced by splitless injection, with an injector temperature of 210°C and head pressure of 10 psi. The GC temperature program was started at 50°C and held for 3 min, then increased by 10°C/min to 320°C. The GC-MS Interface Line Temperature was 320°C and the carrier gas was helium. Ionization was by electron impact at 70eV in the positive ion mode. The ion source temperature was 250°C and the filament emission current was 0.5 mA. Masses were scanned over the range of 35-650 with a scan rate of 0.6s and an interscan time of 0.8s.

In order to analyze volatile compounds released directly from the films into package headspace, direct thermal desorption analysis was performed on film samples. A 10 cm^2 sample of film was placed into the desorption tube loaded with Tenax trapping material. An internal standard of 1ug each deuterated toluene and napthalene was included to allow approximate quantification. The tube was loaded into the heating block, held at -20°C for

5 minutes as a cryotrap, then heated at a rate of 10°C/min to 340°C, and the volatiles generated were injected into the GC interfaced with a MS detector. Ionization was by electron impact at 70eV in the positive ion mode. The ion source temperature was 250°C and the filament emission current was 0.5 mA. Masses were scanned over the range of 35-650 with a scan rate of 0.6s and an interscan time of 0.8s.

LC-MS

In order to identify and quantify the degradation products formed from tocopherol in polymer films, a separation method was needed. While most earlier work on the separation and analysis of tocopherol and its degradation products was performed using normal phase HPLC due to its better ability to resolve the identically massed β- and γ- tocopherol isomers [91], such methods are not suitable for this lab, due to the toxicity of the mobile phase, the difficulty in controlling moisture, and the lack of available instrumentation. Thus, a reversed phase HPLC method for the separation of tocopherol and its degradation products was developed.

HPLC experiments with PDA detection were conducted on a Shimadzu SCL-10Avp System (Kyoto, Japan) equipped with a LC10ADVP solvent delivery unit, FCV-10ALvp Low Pressure Gradient Unit, SIL-10Ai Autoinjector, and SPD-M10Avp Photodiode Array Detector. Data were processed using Class VP EZ Start Version 7.3 data acquisition software. A Phenomenex® Synergi Fusion-RP column (250 x 4.6 mm, particle size 4 μm, pore size 80 Å) preceded by a Phenomenex® SecurityGuard™ pre-column, with both non-polar C18 groups and bound polar groups was chosen for this method. The polar endcapping provides some of the characteristics of normal phase separation by increasing the retention of more

polar compounds. Increased retention is critical for detection and separation of polar oxidation products of tocopherol in the film extracts. To minimize the further degradation of compounds being analyzed by metals, a non-metallic PEEK column enclosure was used.

HPLC method development began using a gradient program based on the method used by Koskas for the separation of α-tocopherol, α-tocopherolquinone and α-tocopherol dimer and γ-tocopherol [102]. The Koskas method utilized a two-solvent system, the first being an 85:15 v/v methanol-water system, and the second 100% methanol. The gradient program was set to begin with 100% of solvent 1, increasing to 100% solvent 2 over a period of 18 minutes. This separation was performed on a stainless-steel Spherisorb ODS column (20 x 0.47 cm, particle size 5 μm). This method was able to completely separate the compounds of interest within a reasonable short 30 minute runtime.

Unfortunately, implementation of a gradient program on the Shimadzu system proved difficult and very unreliable. Frequent leaks in the solenoid mixing unit led to inconsistent pressure throughout the run, yielding results that were nearly impossible to interpret correctly. Because of these difficulties, an alternate isocratic method was developed.

Development of an isocratic method began with a mobile phase of 10% HPLC grade water in methanol. Due to very high column backpressure, and very long (> 60 minutes) retention time for tocopherol isomers, the amount of water was reduced, until 2% water in methanol mobile phase was selected. The mobile phase flow rate was 0.5 ml/min, and the run time was 50 minutes.

HPLC analysis was performed on standards of the Cargill mixed tocopherols in order to confirm the isomer composition provided by Cargill. Because the amount of β-tocopherol present in the mixed tocopherols was very low and separation of β and γ-tocopherols is somewhat difficult, these two isomers were quantified together. Standard curves were developed for each isomer by using the 1000 µg/ml stock solutions to prepare solutions of each isomer with concentrations ranging from 10-120 µg/ml, which were used to develop ismer specific standard curves. Standards were run a total of six times, and the isomer concentrations were calculated based on the peak area at 295 nm and averaged to give the final ratios shown in Figure 30.

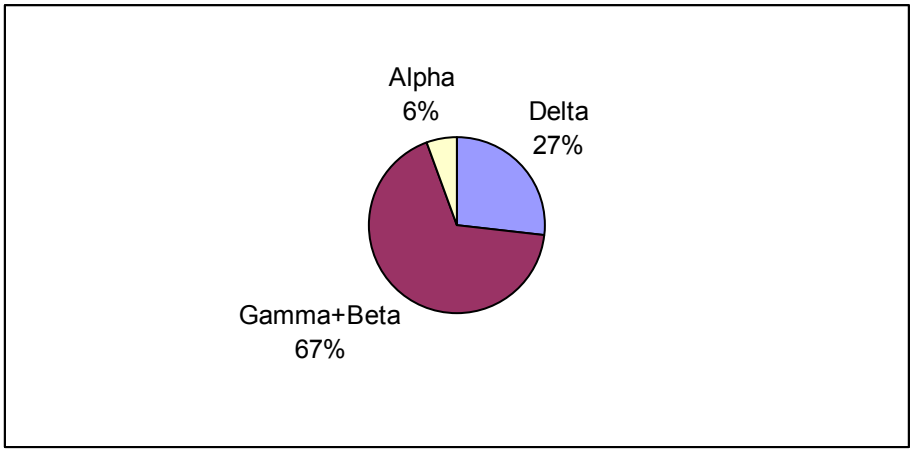


Figure 30 - Isomer ratio in Cargill Mixed Tocopherols as determined by HPLC.

Because of the differing reactivities of tocopherol isomers in radical containing systems, the degree of degradation taking place during polymer film production will likely vary between them. In order to determine if, and to what extent, isomer-specific degradation was taking place, isomer separation and quantification was performed using HPLC with PDA detection at 295 nm.

Analysis by LC-MS was performed using two different methods, on two different systems, in order to provide the most thorough analysis. Difficulties encountered with the first series of analysis led to the repetition on a second system. Analyses were performed at Rutgers University Department of Plant Science, and at Chemic Laboratories, Canton, Massachusetts.

Rutgers Plant Science

Samples were analyzed with a Waters liquid chromatography system (Milford, MA) consisting of a solvent delivery system with a W616 pump and W600S controller, W717plus auto-sampler, W996 photodiode array detector, and a Varian 1200L (Varian Inc., Palo Alto, CA) triple quadrupole mass detector with electrospray ionization interface (ESI), operated in positive ionization mode. The electrospray voltage was 5 kV, heated capillary temperature was 280 ^{o}C, sheath gas nitrogen; mass detector scanning from 65 to 1500 atomic mass units. Data from the Varian 1200L mass detector was collected and compiled using Varian's MS Workstation, v. 6.41, SP2, and UV data were collected and analyzed with the Waters Millennium® v. 3.2 software. Substances were separated on a Phenomenex® Synergi Fusion-RP column (250 x 4.6 mm, particle size 4 μm, pore size 80 Å) preceded by a Phenomenex® SecurityGuard™ pre-column. The mobile phase was single-component 2% HPLC grade water (Fisher Scientific) in methanol. The mobile phase flow was 0.5 ml/min, in isocratic mode for all analyses. Injection volume was 25 μL. An adjustable flow splitter, model 600-PO10-06 (Analytical Scientific Instruments, El Sobrante, CA) was installed in the mobile phase flow path after the photodiode array detector and before the mass spectrometer, so that a 0.25 ml/min flow rate was fed to the mass spectrometer for optimal ionization.

Chemic Laboratories

Samples were analyzed on an Agilent 1100 Series HPLC equipped with quaternary pump, automatic sampler, temperature controlled column heater, diode array and single quadruple mass selective detectors. An isocratic separation was performed using 2% water in methanol as the mobile phase. The injection volume was 20 µL for all analyses, and the flow rate was 0.5 mL/minute. Samples were separated using a Waters Symmetry C18 column (4.6 x 150 mm 3.5-µm particle size), which was maintained at 40°C. The run time was 35 minutes. Ionization was performed by APCI in both the positive and negative modes. The dry gas flow rate was 8 L/minute, and the drying gas temperature was 350°C. The vaporizer temperature was 450°C, and the nebulizer pressure was 35 psig. Ionization energy (Vcap) was 3kV for both positive and negative ionization modes. In the positive mode, the corona was 4 µA, while in the negative mode it was 15 µA. The mass scan range was 100 – 1300 Da with a step of 0.35 Da.

Film properties

All films produced at Pliant were analyzed by the lab at Pliant to determine gauge, density, clarity, haze, coefficient of friction, water vapor and oxygen transmission rates, as well as several stress and strain measurements. Single experiment data was provided by Pliant for analysis.

Antioxidant Assays

The ultimate goal of producing controlled release tocopherol containing films is to provide antioxidant protection to food products. This requires that tocopherols included in the films do not undergo any degradative reactions that may reduce their antioxidant efficacy. Antioxidant activity assays thus were performed to determine loss of functionality during film production.

The method used is based on that given by Re et al [103]. ABTS ammonium was dissolved in water to a 7 mmol concentration. This solution was then oxidized by addition of an equal volume of 2.45 mmol potassium persulfate solution. This mixture was stored at room temperature in the dark for 12-16 hours to allow the development of $ABTS^{+\bullet}$ radicals, which have a deep blue-green color. For reaction, this solution was diluted with ethanol or saline buffer to an absorbance of 1.0 at 734 nm, a modification of Re's method which recommends dilution to 0.7 absorbance units.

To assay ability to quench ABTS radicals, 950 µl of the working $ABTS^{+\bullet}$ reagent (OD~1.0) was pipetted into a semi-microcuvette and absorbance at 734 nm was recorded using a Cary 50 Bio UV/visible spectrophotometer with Carey Kinetics software (Varian, Inc., Palo Alto, CA, USA). 50 µl tocopherol extract was then added, quickly mixed by re-aspirating the mixture into the pipette, and recording of absorbance at 734 nm was started immediately. The reaction was followed for six minutes to observe the quenching of ABTS radicals by the sample. Because reactions with $ABTS^{+\bullet}$ are very fast in the absence of steric hindrance, the instantaneous drop in absorbance was determined as a measure of innate reactivity, and the six-minute ΔOD was determined as a measure of

extended reactivity which includes slow access to $ABTS^{+\bullet}$ by larger molecules. The molar extinction coefficient of 1.5×10^4 determined by Re was used for calculations of $ABTS^{+\bullet}$ concentration [103].

Results and Discussion

Film Physical Properties

Selected data on the physical properties of CRP films produced at Pliant are summarized in the following graphs. For all graphs, the first column shown for each series is the control film containing no tocopherol. All other columns represent films containing 3000 ppm tocopherol and processed under different conditions.

Figure 31 shows the effect of film composition and processing on film density. As expected from polymer properties, 100% PP films have the lowest density (0.89 ± 0.002 g/cc), 100% LDPE films the highest density (0.92 ± 0.001 g/cc), and the 50:50 blend falling in between (0.91±0.002 g/cc). In the absence of non-tocopherol containing polymer control films or each processing condition, it is difficult to establish whether the variation observed is due to the presence of tocopherol or the processing method itself. It appears that addition of tocopherol had little effect on film density under mild conditions, and that changing processing conditions has little effect on film density.

Figure 32 shows the effect of film composition and processing on film clarity, which is a measure of the ability of light to pass through the film. Single polymer films (PP, LDPE) showed higher clarity than blended films, and LDPE films had higher clarity than PP films. In CRP films with tocopherol, LDPE exhibited a change (decrease) in clarity, from 98% to 93%, only under the extreme condition of low temperature/high RPM, which generates much shear energy. In contrast, clarity of PP films increased markedly, from 87.7% in the control without tocopherol to 98.1% in tocopherol containing high

temperature/low RPM, under most extreme conditions except low temperature/high RPM, where there was only a very slight increase, to 88.4%, over the control clarity. LDPE/PP blends showed changes (increases) in clarity only under conditions of 75 rpm and 221 and 260 °C.

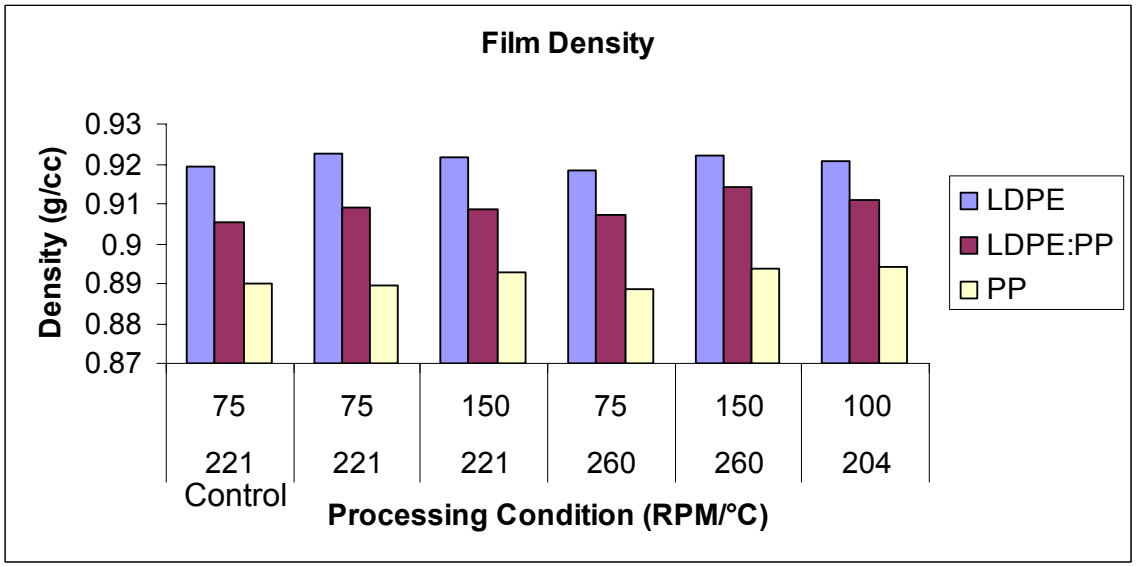


Figure 31- Film density

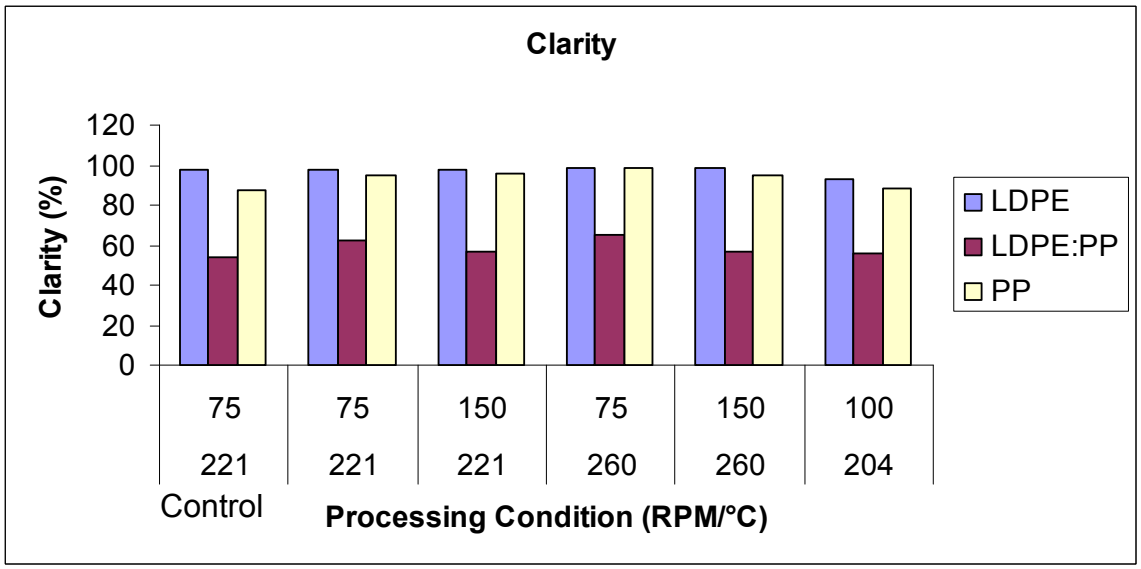


Figure 32 - Film Clarity

Figure 33 shows the effect of film composition and processing on film haze, which describes the amount of light scattered at diffuse angles rather than being reflected directly back to its source. Under all conditions, the heterogeneous blended LDPE:PP film has the greatest percentage haze due to the formation of separate polymer phases that act to scatter the light. 100% LDPE film shows the lowest haze, with a slight increase seen with increasing processing severity, including both high temperature and high screw speed conditions. The most unusual behavior is observed for 100% PP films, which have low haze when produced without tocopherol under standard conditions, but with tocopherol addition, haze increases nearly twofold, and fluctuates widely depending on processing condition. The highest haze is seen under the high screw speed conditions, implying that increased screw speed may lead to the formation of surface microstructures that scatter incident light.

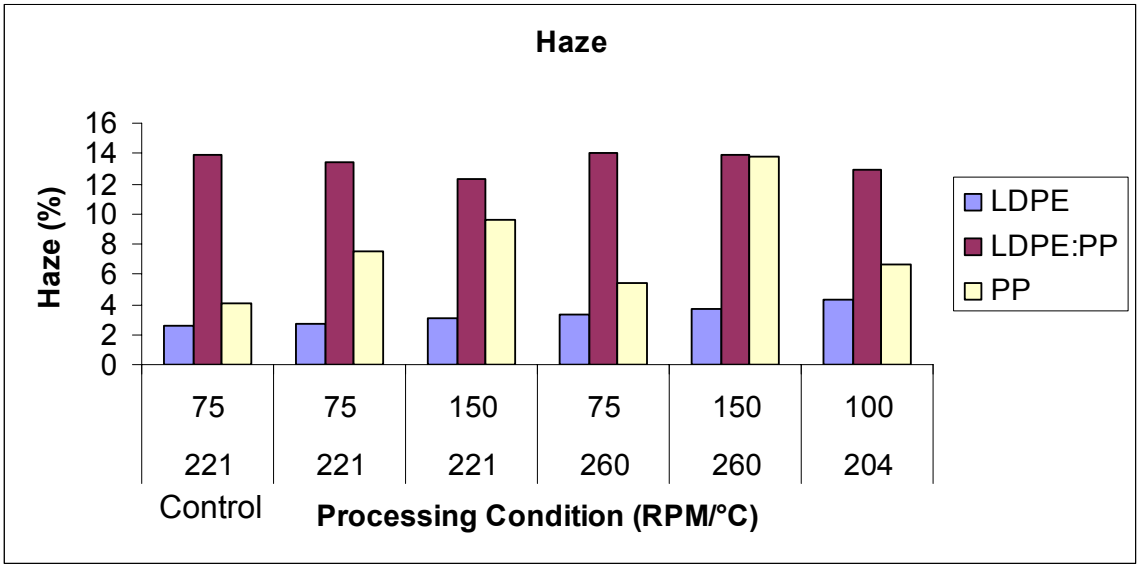


Figure 33 - Film haze

Figures 34 and 35 show the effects of film composition and processing on the water vapor and oxygen transmission characteristics of the films, respectively. LDPE films show rather high water and oxygen transmission rates under all processing conditions; PP reduced transmission of both gasses, while blended films show values between those of the single polymer. Addition of tocopherol has little effect on the transmission rate when compared to the control produced under the same conditions. Modifying processing conditions gave some variation, both increasing and decreasing the rate, with the highest transmission rates being observed from films produced under the high temperature/low RPM condition.

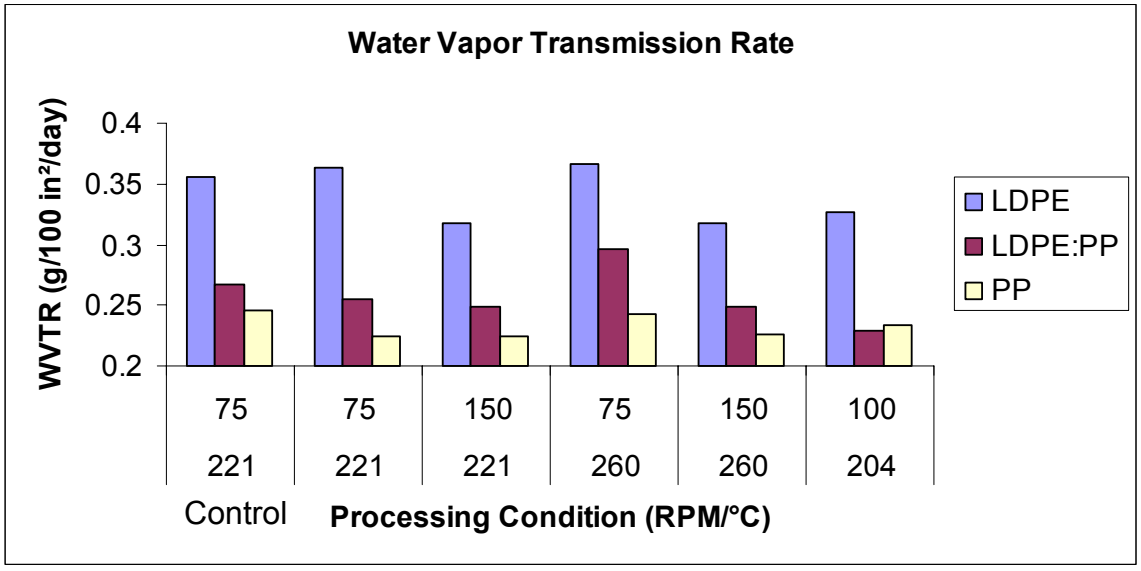


Figure 34 - Water vapor transmission rate

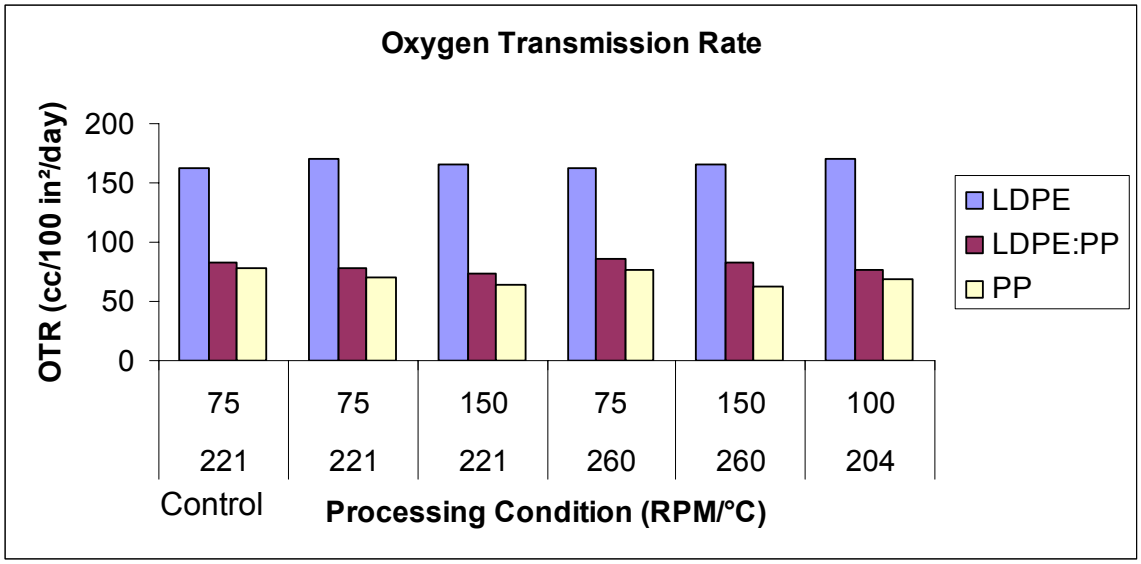


Figure 35 - Oxygen transmission rate

Figure 36 shows the effects of film composition and processing on the peak seal strength of the films. Seal strength is an important quality of food packaging materials, as a secure seal ensures that contamination of the contents does not occur. PP films exhibited stronger seal strength than LDPE films under all conditions. Interestingly, under the moderate temperature/high RPM extrusion condition (150 RPM, 221°C), seal strength increased in LDPE containing films but decreased in PP film to such an extent that the blended film exhibits the highest seal strength under this processing condition.

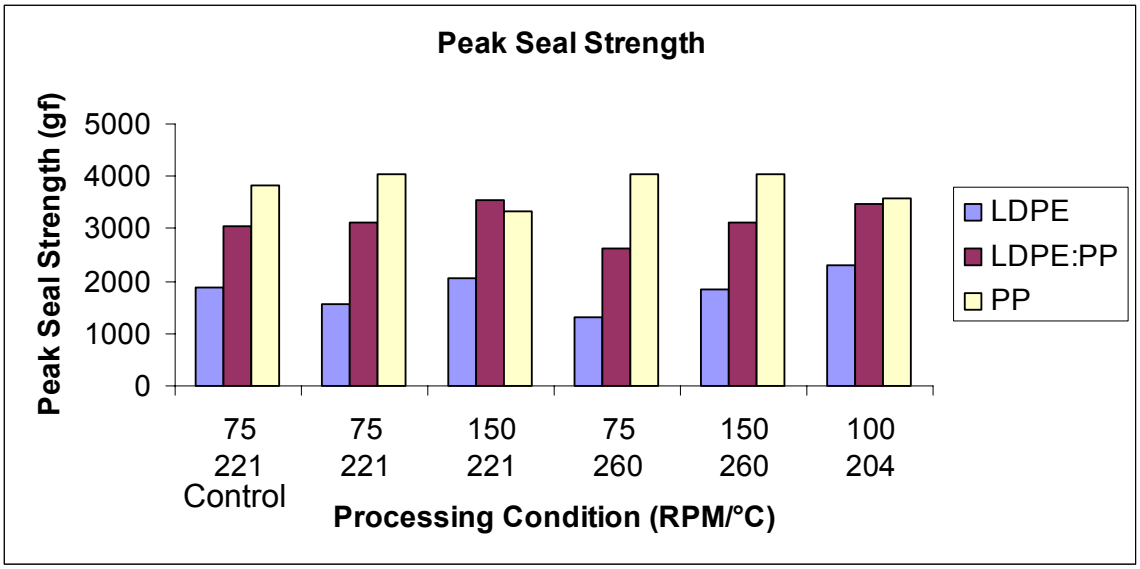


Figure 36 - Peak seal strength

Tocopherol Recovery

Results of total film extraction in methylene chloride (means of four replicates) are shown in Figure 37. Tocopherol recovery from the films was nearly total, ranging from over 100% for some LDPE films to 92% in the case of the most severely processed PP film. In 100% LDPE films recovery was consistently greater than 100%. This can be explained by the additional of 5% extra tocopherol to the resins to compensate for losses during mixing and transfer to the extruder. However, much less tocopherol than expected was actually lost during processing. Thus, recoveries of 105% tocopherols from the PE films indicate no loss of PE in either handling or extrusion under typical conditions. Losses of a few percent tocopherols were seen under extreme extrusion conditions.

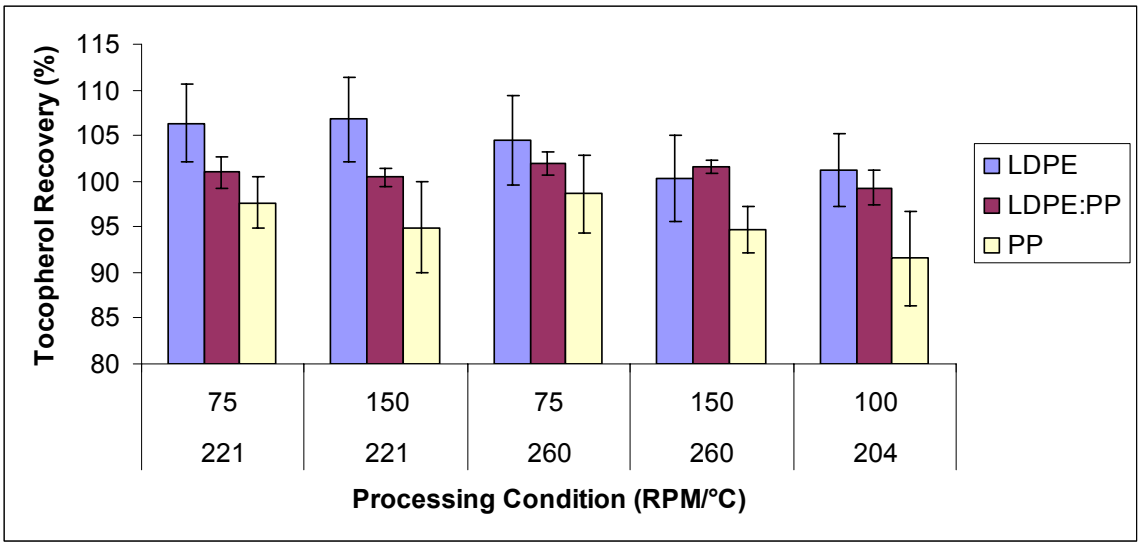


Figure 37. Tocopherol recovery from polymer films.

The greatest effect of extrusion conditions on tocopherol recovery was seen in PP films. As an overall trend, total tocopherol release was reduced by increasing concentration of PP in the films. The most likely explanation for this is that the PP polymer has a much

greater crystallinity, trapping some tocopherol in the crystalline regions of the polymer film. It is also possible that tocopherol radicals are more likely to form adducts with PP radicals, binding the tocopherol within the polymer. Tocopherol recovery was lowest for PP films produced with extrusion condition 5 (400°F, 100 RPM). PP has a greater viscosity than PE at lower temperature, and is therefore more heavily impacted by the shear energy of the extruder. Shear generates polymer radicals, which react with the tocopherol to form either tocopherol degradation products, or tocopherol-PP recombination products. Also, at low temperatures, a greater level of crystallinity is retained in PP, trapping more of the tocopherol between impermeable crystalline regions. Such rigid crystal structures cannot form in LDPE, as the long branching side chains prevent tight alignment of the molecular backbone of the polymer.

Antioxidant Activity by ABTS

Antioxidant activity of tocopherols extracted from CRP films was assayed by reaction with $ABTS^{+\bullet}$ to determine whether extrusion in polymer films alters tocopherol antioxidant capabilities. Figure 38 shows a typical kinetic curve of film extracts measured at 734 nm from immediately after mixing to 6 minutes. This curve shows that the reaction with $ABTS^{+\bullet}$ is >95% complete within seconds of mixing.

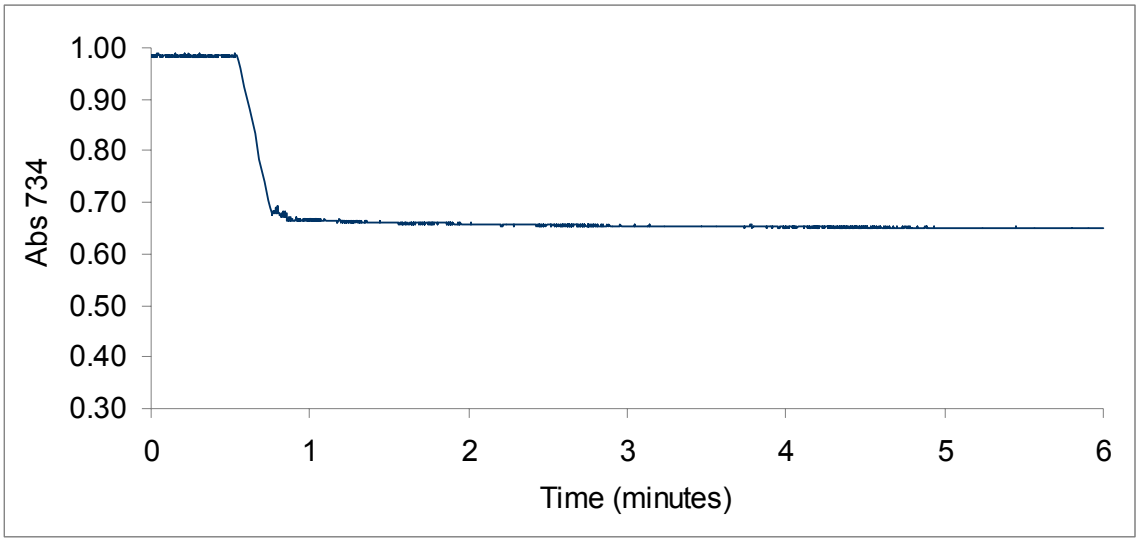


Figure 38 - Kinetics of ABTS radical quenching by mixed tocopherol standard (150 µM).

The ΔOD of this initial drop was measured for a series of extracts diluted to generate a range of concentrations, and this data was used to generate percentage-based concentration curves for reaction of all three films under mild (S3 : 260 °C/150 rpm) and high (S4 : 204 °C/100 rpm) stress conditions (Figure 39). Tocopherol concentrations were determined from absorbance at 295 nm.

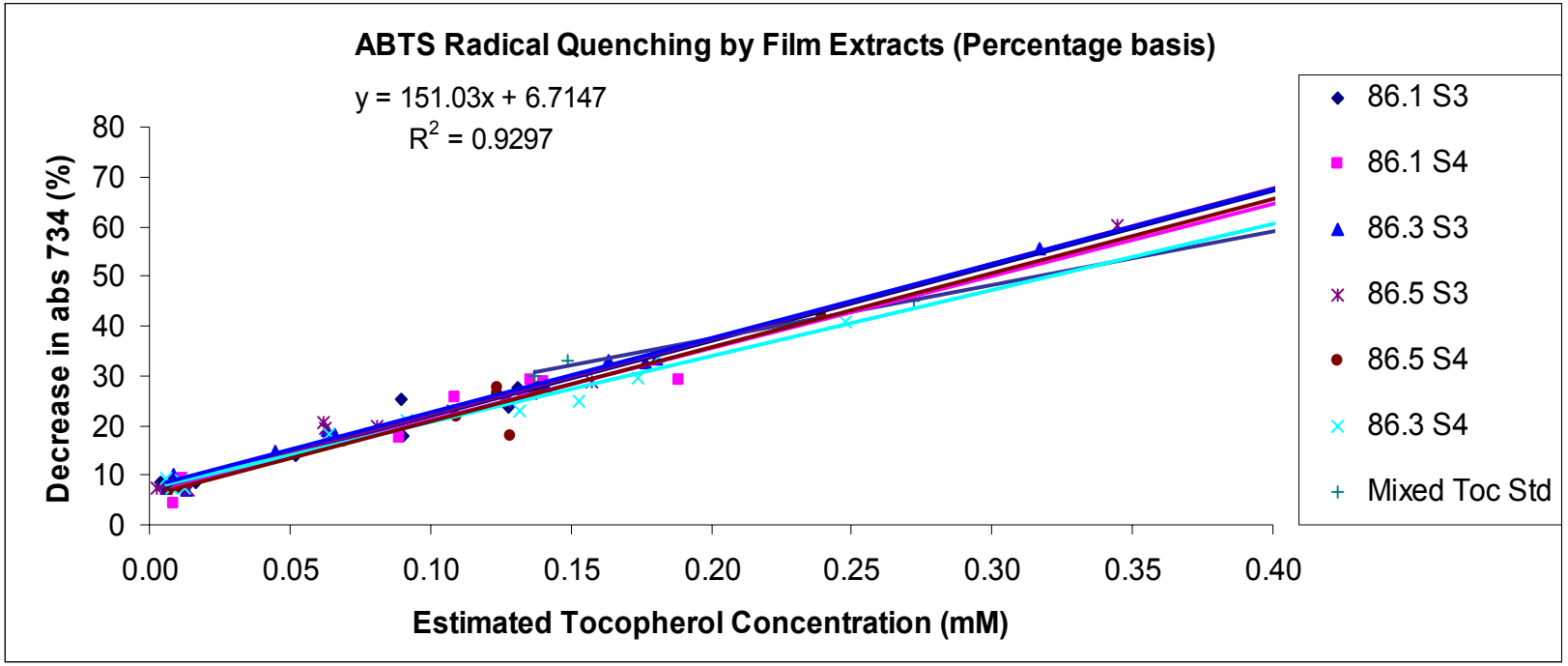


Figure 39 - Antioxidant activity of film extracts compared to the standard of mixed tocopherols, as measured by the percent decrease in absorbance at 734 nm, corresponding to loss of the ABTS radical.

Table 8 - Coefficient 'C' and R-values for linear interpolation of ABTS data, with equation of the form I=Cx+b.

Film #	C	R value
86.1 S3 (LDPE)	151.03	0.9297
86.1 S4 (LDPE)	145.12	0.9218
86.3 S3 (LDPE:PP blend)	149.48	0.991
86.3 S4 (LDPE:PP blend)	133.06	0.9764
86.5 S3 (PP)	151.70	0.9816
86.5 S4 (PP)	148.98	0.9401
Mixed Tocopherol Standard	107.51	0.9836

If linear regressions are calculated for the data sets presented in Figure 39, equations of the form I=Cx+b are generated, where I is the percent inhibition of the ABTS radical, and x is the concentration of tocopherol in the sample determined by measuring absorbance at 295 nm, and can be interpreted as films with higher C values have greater antioxidant activity. The values for the coefficient C for each film and the mixed tocopherol standard are presented in Table 8, along with the R^2-values for the equations. From these C values, it is observed that film extracts demonstrate nearly identical antioxidant characteristics, regardless of polymer type. A small decrease is observed in all films under processing condition 4 (100 RPM/204°C), which further supports the assertion that the low

temperature, high RPM condition causes the greatest degradation due to the high shear forces generated.

In comparing the film extracts to the mixed tocopherol standard, it is observed that all extracts exhibit a greater ability to quench the $ABTS^{+\bullet}$ radical. There are two possible explanations for this. The first is that antioxidant stabilizers present in the polymer resins are not being detected by measurement of absorbance at 295 nm, but are still present and acting to quench ABTS radicals. However, this cannot be the entire explanation since there is little difference between the coefficients of LDPE films, produced with unstabilized barefoot resin, and those of PP films containing several stabilizers. Thus, a more likely explanation is that tocopherol degradation products such as the quinone and dihydroxydimer, present in the extract but not quantified at 295 nm, not only retain antioxidant activity but have increased reactivity due to altered mechanisms. Native tocopherol quenches $ABTS^{+\bullet}$ entirely by hydrogen abstraction, but many of the quinone and other oxidation products of tocopherol are capable of electron transfer, which is a much faster process with $ABTS^{+\bullet}$. In addition, some quinones have been shown to react with oxygen to produce superoxide anion, $O_2^{\bullet-}$, which reacts very rapidly with $ABTS^{+\bullet}$. This type of reaction of tocopherol products needs to be explored further.

An alternate method of interpreting the data from the ABTS assays is to calculate the moles of $ABTS^{+\bullet}$ quenched by each mole of tocopherol in the sample, using extinction coefficients of $\varepsilon_{734} = 16000\ Lmol^{-1}cm^{-1}$ for $ABTS^{+\bullet}$ and $\varepsilon_{295} = 4100\ Lmol^{-1}cm^{-1}$ for tocopherol. Data from this calculation is presented alongside the results for the

tocopherol standard in Figure 40. This shows the subtle differences between the antioxidant activities of the extracts more clearly. LDPE films processed at high temperature and high RPM show the greatest activity, while blended films show the lowest activity overall. Interestingly, PP films produced under the low temperature/high shear condition show higher antioxidant activity, despite these films having the lowest tocopherol recovery of all films. Overall, PP films showed the least variability due to processing conditions. Even under the condition of greatest loss (Blend film, low temperature/high shear) the activity is only 12% less than that of the tocopherol standard. This high activity in PP resins may be due to the presence of other antioxidants present in the polymer resin, including Irganox 1010, which do not absorb at 295 nm, but are capable of quenching the $ABTS^{+\bullet}$ radical. This may also explain the lower activity of blended films, as they would contain less of these polymer antioxidants.

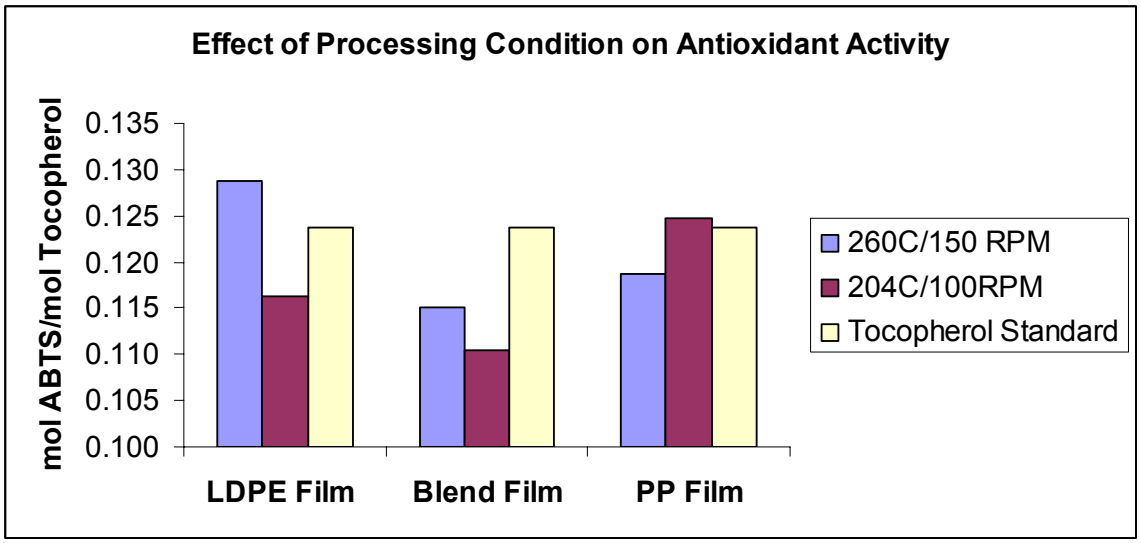


Figure 40 - Antioxidant activity of film extracts expressed as moles of ABTS quenched per mole of tocopherol as measured by absorbance at 295 nm.

GC-MS

Figure 41 shows the GC-MS chromatogram for the mixed tocopherol standard. Peaks of the δ, γ+β, and α-tocopherol isomers can be clearly seen at 23.8, 24.5, and 24.9 minutes, respectively. The small peaks visible at the start of the run are the internal standards of deuterated toluene and deuterated napthaline. No other peaks are visible.

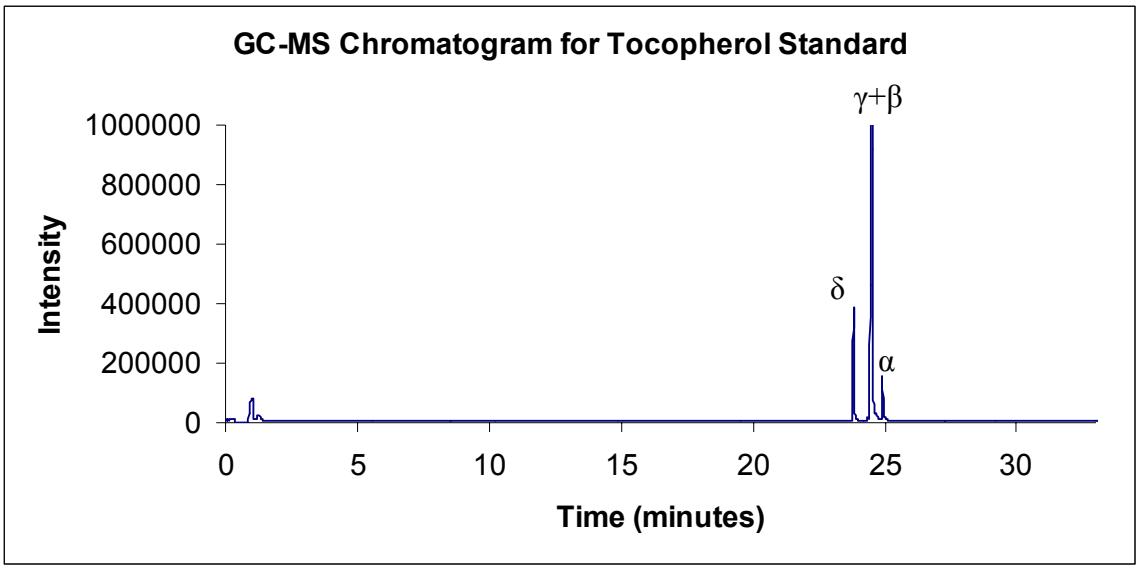


Figure 41 - GC-MS Chromatogram for Mixed Tocopherol Standard

The mass spectra for the detected peaks are shown in Figures 42 - 44, along with the structures of the parent tocopherol molecule and the fragment ions corresponding to the detected masses. Spectra align very well with projected fragmentation of tocopherol isomers.

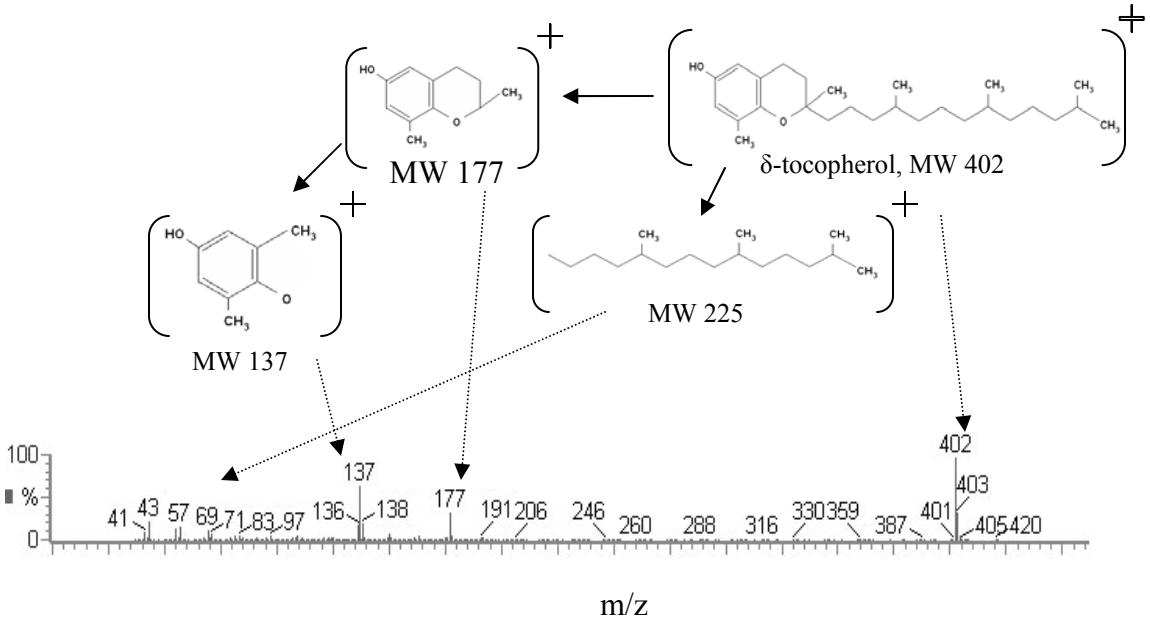


Figure 42- EI-MS spectra of delta tocopherol, scan 1020 (23.8 minutes)

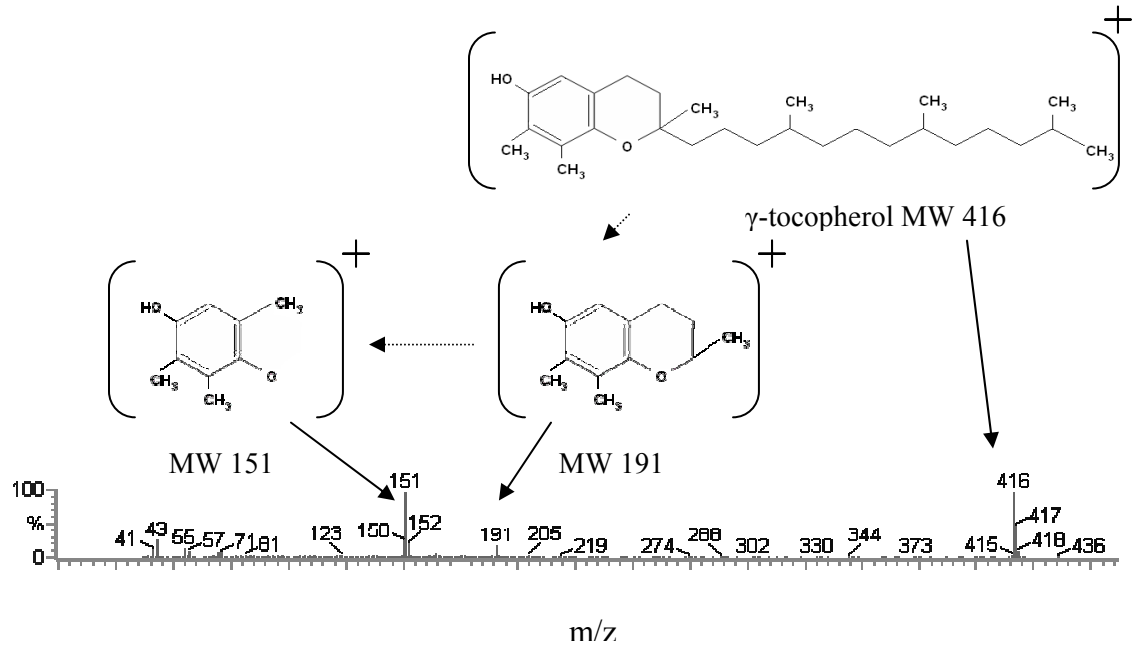


Figure 43- EI-MS spectrum of gamma tocopherol, scan 1048 (24.5 minutes)

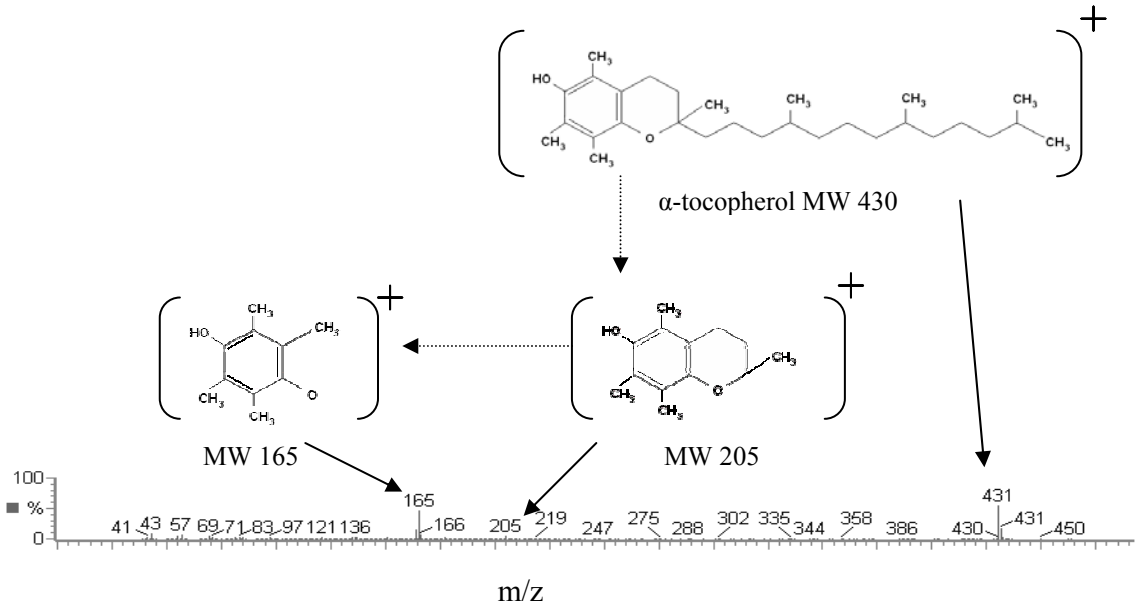


Figure 44 - EIMS spectrum of alpha tocopherol, scan 1068 (24.9 minutes)

Samples of mixed tocopherols oxidized with PbO_2 were also analyzed by direct injection GC-MS. Figure 45 shows the total ion chromatogram for a sample of tocopherol that was oxidized with an equimolar amount of PbO_2. No additional peaks are detected that were not present in the unoxidized tocopherol standard, shown expanded in Figure 46. Peaks corresponding to the tocopherol isomers elute at the same times and possess the same mass spectra as those in the standard. From this it may be concluded that the oxidation products of tocopherol by PbO_2 are not volatile, and by extension, that any tocopherol degradation products formed in the tocopherol containing films should be non-volatile.

γ+β

δ

α

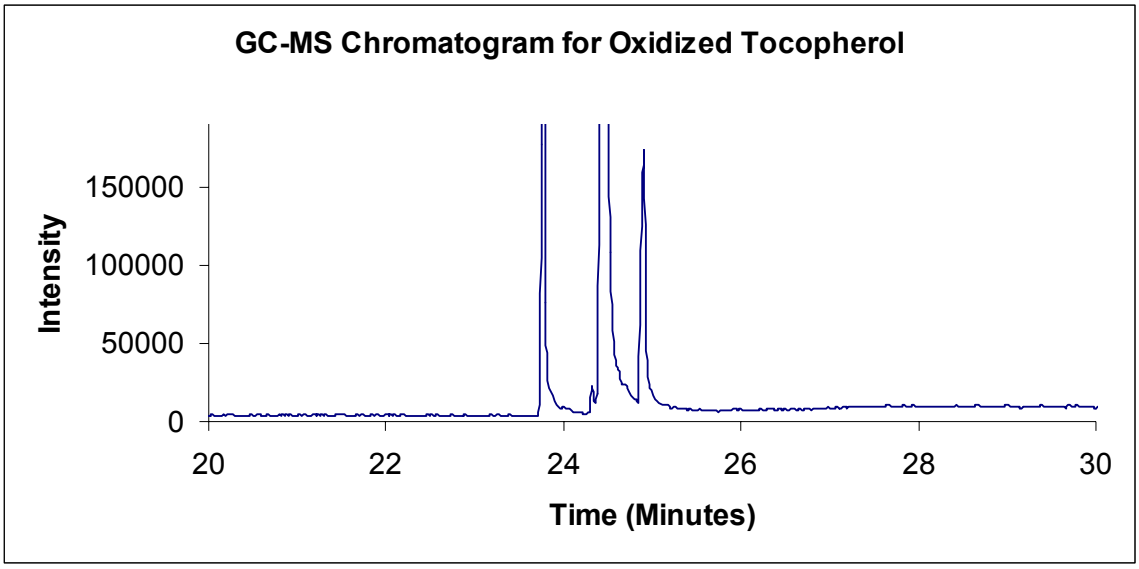


Figure 45 - Total ion GC-MS chromatogram for direct injection of mixed tocopherols oxidized with an equimolar concentration of PbO_2

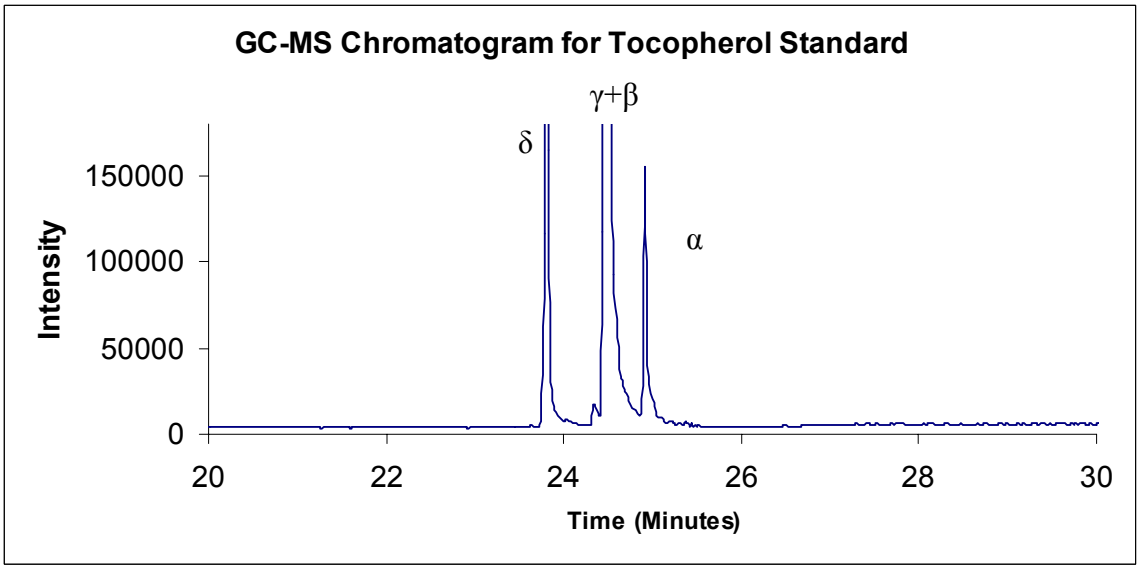


Figure 46 - Total ion chromatogram for tocopherol standard, expanded in to facilitate comparison to oxidized tocopherol.

The results of direct injection of methylene chloride extracts of tocopherol-containing polymer films are shown in figures 47-49. Figure 47 shows the total ion chromatograms for LDPE films produced with and without the addition of 3000 ppm tocopherol (86.1S1 and 86.0, respectively). All other tocopherol-containing LDPE films exhibited similar chromatograms to 86.1S1, and are presented in the appendix. That several volatile monomeric product peaks from the polymer were found at much higher intensity in the LDPE film without tocopherol is expected since tocopherol acts as a polymer stabilizer, reducing the extent of polymer degradation during processing. Only one peak is observed that does not correspond to either the tocopherol standard or the polymer control, and is labeled '1' in Figure 47. The mass spectrum for this peak is provided in Figure 48. Upon comparison to the polymer control at the same time (Figure 49), it can be seen that they have the same mass spectra, indicating that they are the same compound, identified as the plasticizer Bis(2-ethylhexyl)phthalate (DEHP), a common polymer additive.

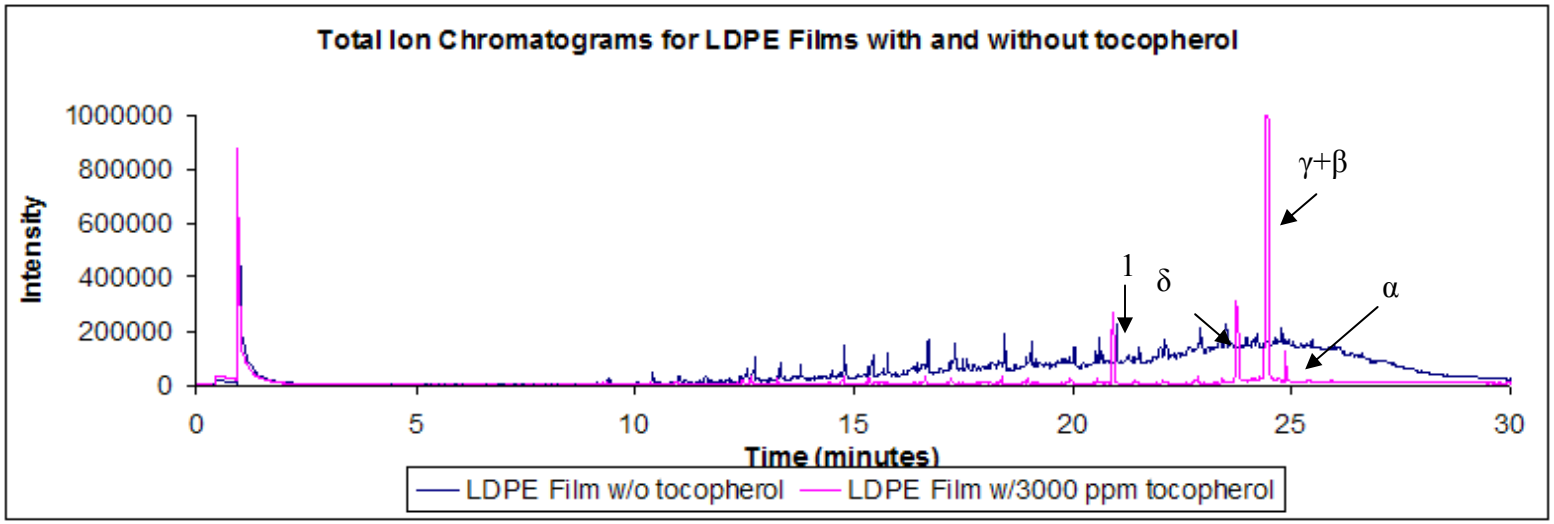


Figure 47 - Total ion chromatograms for direct injection of MC extract of LDPE films with and without tocopherol

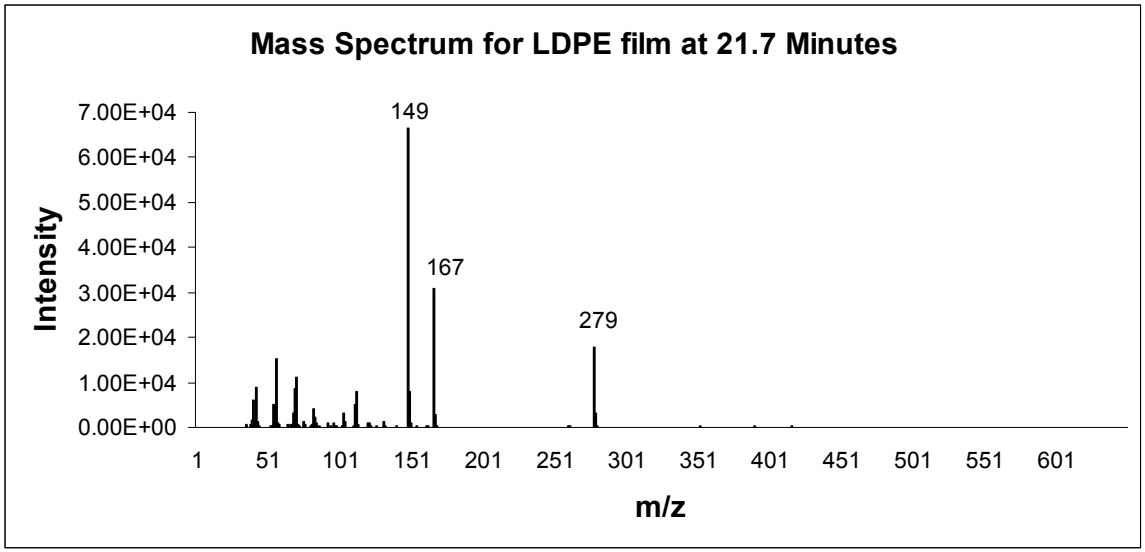


Figure 48 - Mass spectrum for peak "1" observed at 21.7 minutes in tocopherol containing LDPE films, identified as the plasticizer DEHP.

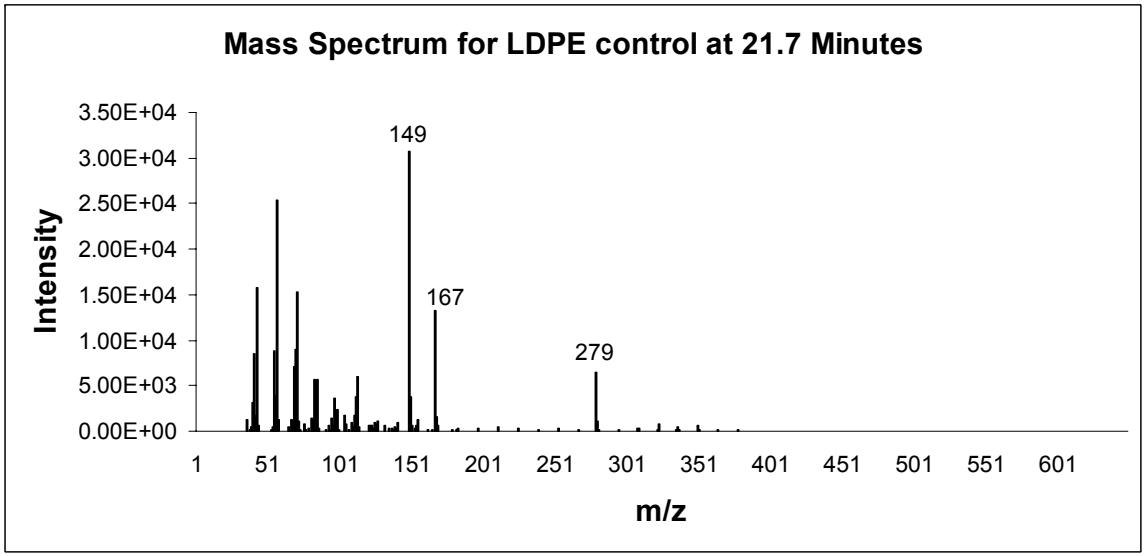


Figure 49 - Mass spectrum of LDPE control sample at 21.7 minutes, corresponding to peak "1" in tocopherol containing LDPE films.

Figure 50 shows the total ion chromatogram for PP films produced with and without the addition of 3000 ppm tocopherol (86.5S3 and 86.4, respectively). All other tocopherol-containing PP films exhibited similar chromatograms to 86.5S3, and are presented in the appendix. One peak is observed that does not correspond to the polymer control, labeled "2", at 22.9 minutes. Based on its mass spectrum (Figure 51), it has been identified as squalene, a natural product produced by animals and plants [104]. It may have been present in the natural mixed tocopherols, or introduced by handling the film samples, as it is present in skin oil. The structure of squalene is shown in Figure 52.

The peaks labeled '3' and '4' at 26.5 and 27.6 minutes respectively are believed to arise from additives present in the PP resin since they present identically in both tocopherol containing film and film without tocopherol. Compound '3' (mass spectrum shown in Figure 53) has been identified as the phosphate stabilizer Irgafos 168, as the mass spectrum corresponds exactly to that provided by Christie [105]. Compound '4' (mass spectrum shown in Figure 54) is believed to be oxidized Irgafos 168, as the mass spectrum corresponds to that provided by Waters [106]. Proposed fragmentation for Irgafos 168, corresponding to the mass spectrum, is shown in Figure 55.

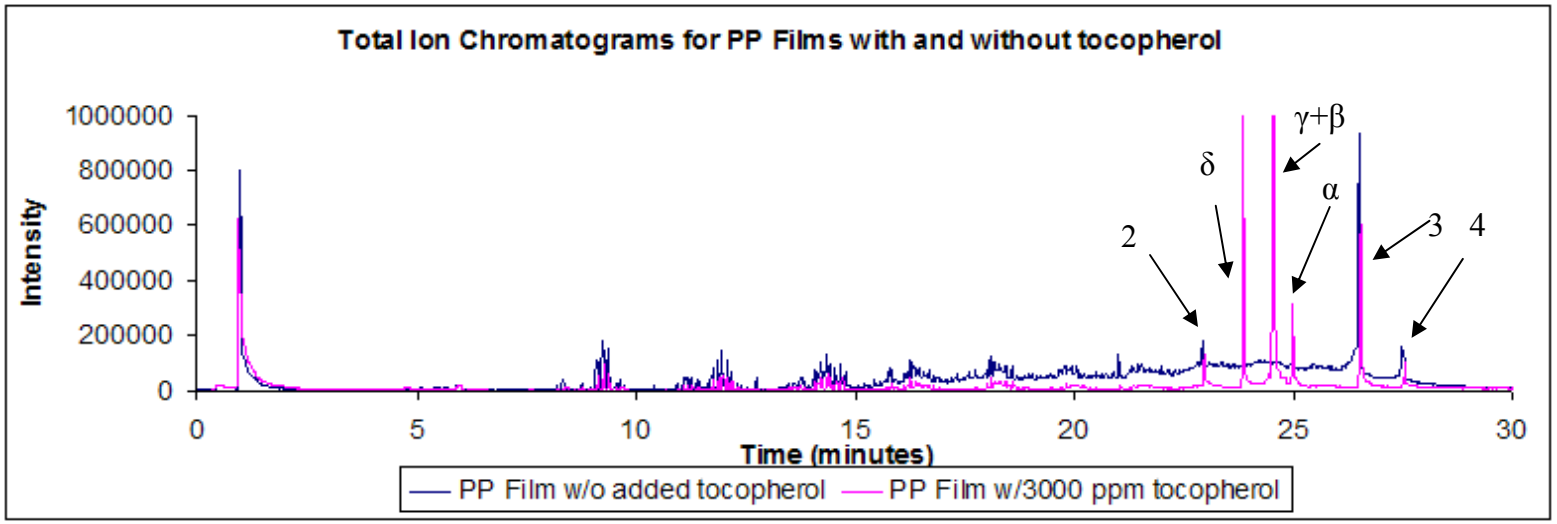


Figure 50 -Total ion chromatograms for direct injection of MC extract of PP films with and without tocopherol.

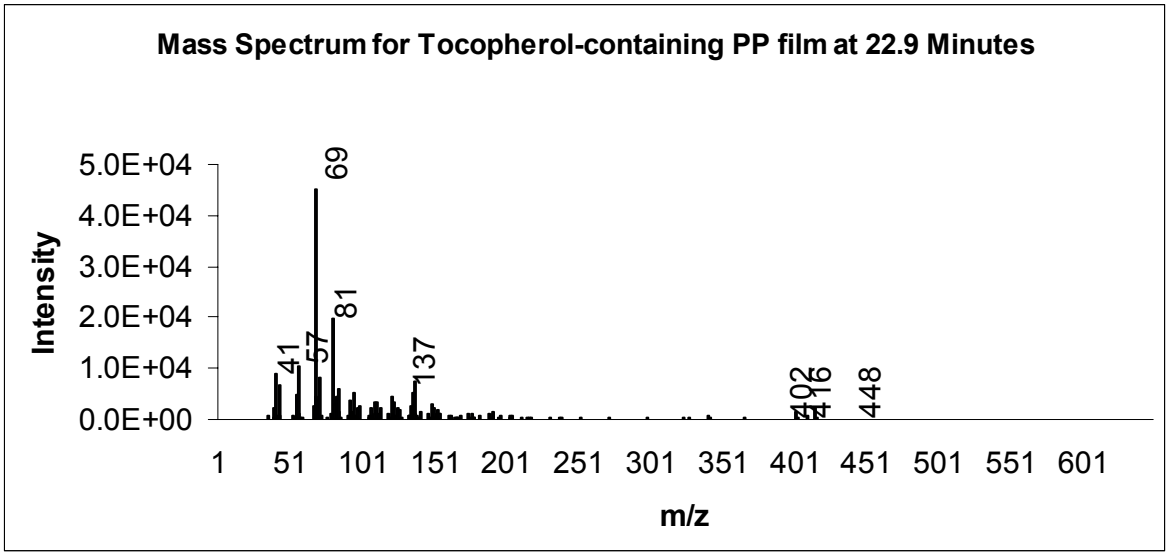
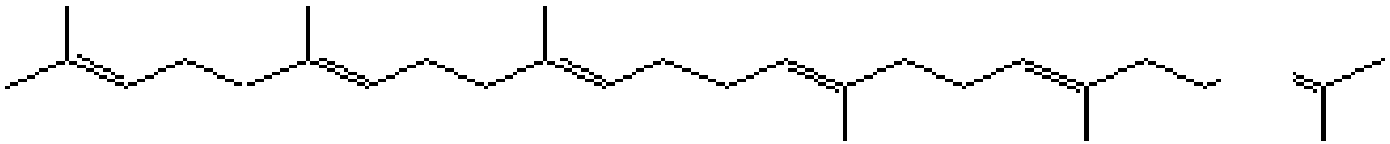


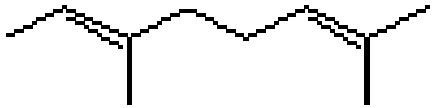
Figure 51 - Mass spectrum of peak "2" observed in tocopherol-containing PP films at 26.5 minutes, identified as squalene.



Parent Molecule squalene
MW 410.4

Fragments

MW 136.1



MW 80.0

MW 68.0

Figure 52 - Structure of squalene, the compound identified at peak ”2”, with fragments corresponding to mass peak s.

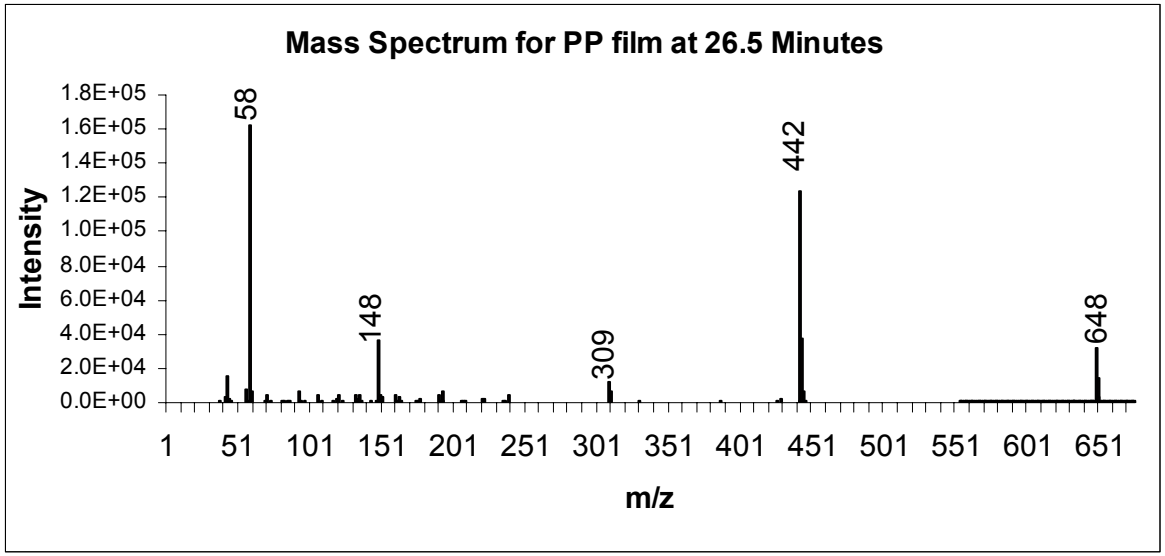


Figure 53 - Mass spectrum of peak "3" observed in all PP containing films at 26.5 minutes, proposed to be the phosphite stabilizer Irgafos 168.

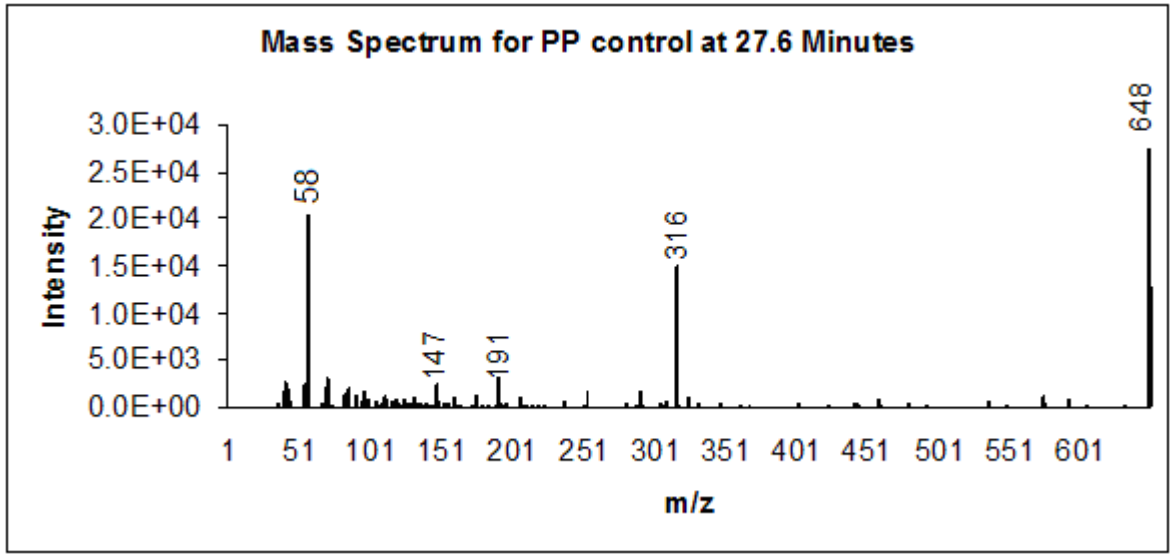
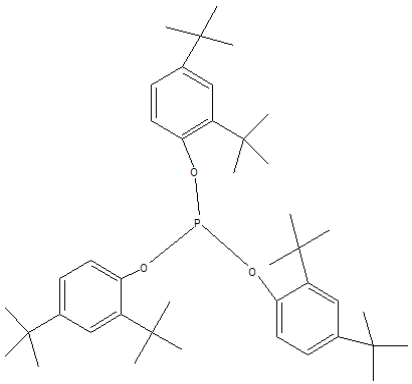


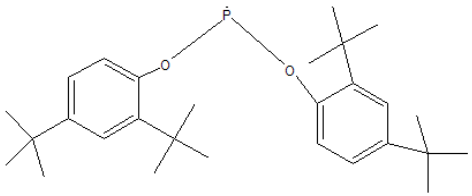
Figure 54 - Mass spectrum of peak "4" observed in all PP containing films at 27.6 minutes, proposed to be oxidized Irgafos 168.



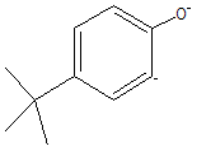
Parent molecule Irgafos 168
MW – 646.45

Fragments

MW 441.29



MW 148.09



Isopropyl group
MW 57.07

Figure 55 - Proposed fragmentation of Irgafos 168, corresponding to the masses observed in peaks 3 and B.

Figure 56 shows the total ion chromatogram for PE:PP blend films produced with and without the addition of 3000 ppm tocopherol (86.3S3 and 86.2, respectively). All other tocopherol containing blended films exhibited similar chromatograms to 86.3S3, and are provided in the appendix. No peaks are observed that do not correspond to either a tocopherol isomer present in the tocopherol standard or a polymer additive found in the polymer control film. The same peaks corresponding to compounds '3' and '4' observed in PP films are present, though less intense than in PP films, on account of the lower level of PP present. Since peak '3' in blended films is only 31% of its expected value based on the intensity observed in 100% PP films, lower levels of PP alone cannot account for the difference in peak area between PP and blend films. It is also observed that peak "4" increases in intensity, supporting the theory that it corresponds to oxidized Irgafos 168. This shift is most likely explained by oxidation of Irgafos in the otherwise unstabilized LDPE resin.

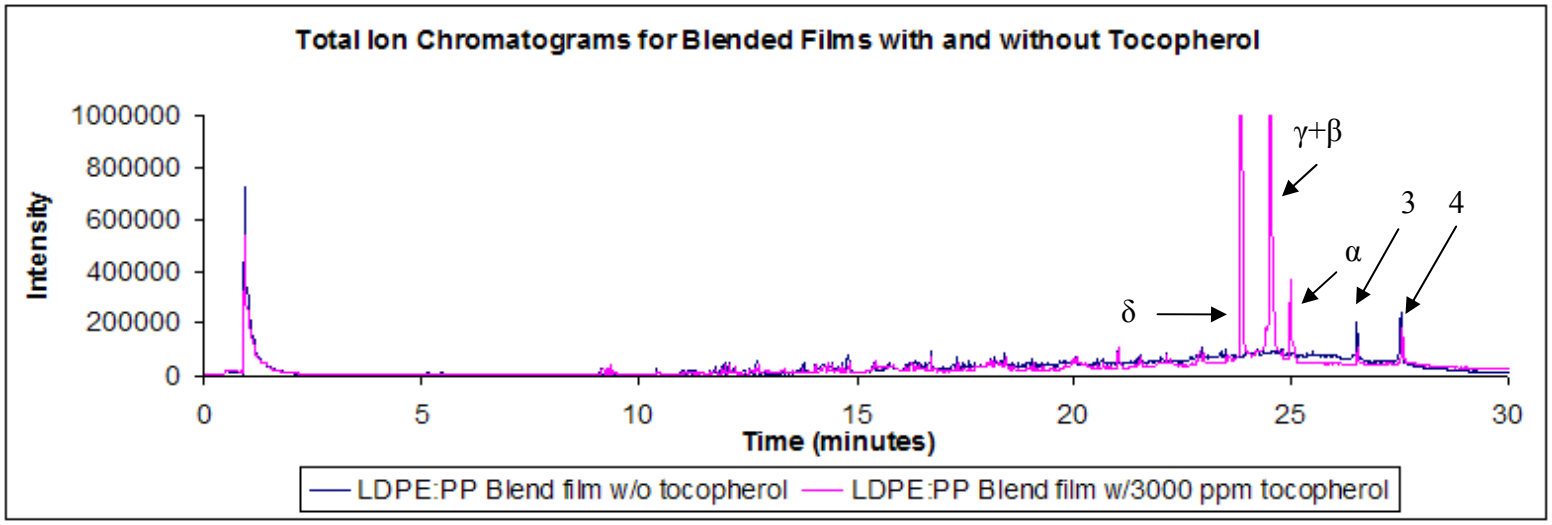


Figure 56 - Total ion chromatograms for direct injection of MC extract of LDPE:PP blend films with and without tocopherol.

While GC-MS analysis of film extracts provides information about volatile compounds that may leach from the packaging material, in order to evaluate volatile compounds that release directly into the headspace of a package, direct thermal desorption (DTD) analysis is required. Direct thermal desorption of PE control film (86.0) and tocopherol-containing PE film (86.1) yielded the chromatograms shown in Figure 57. The only observed discrepancies between the tocopherol-containing film and a combination of the control film and tocopherol standard are peaks at scans 1089 (25.4 minutes) and 1307 (30.5 minutes), designated peaks A and B respectively. The mass spectra for these peaks are shown in Figures 58 and 59. The spectrum of Peak B matches that of peak 3 found in PP films under direct injection so this peak is believed to be the phosphite stabilizer Irgafos 168. While differences between the direct injection method and DTD method alter retention times, preventing direct comparison, the relative position of this peak to the tocopherol peaks is similar to that of peak 3, further supporting its identification. How this came to be present in the LDPE DTD sample, and not in the LDPE extract, especially as the LDPE resin was a barefoot, unstabilized resin, is unknown, but likely due to contamination by contact with Irgafos-containing PP films. Peak A has yet to be identified.

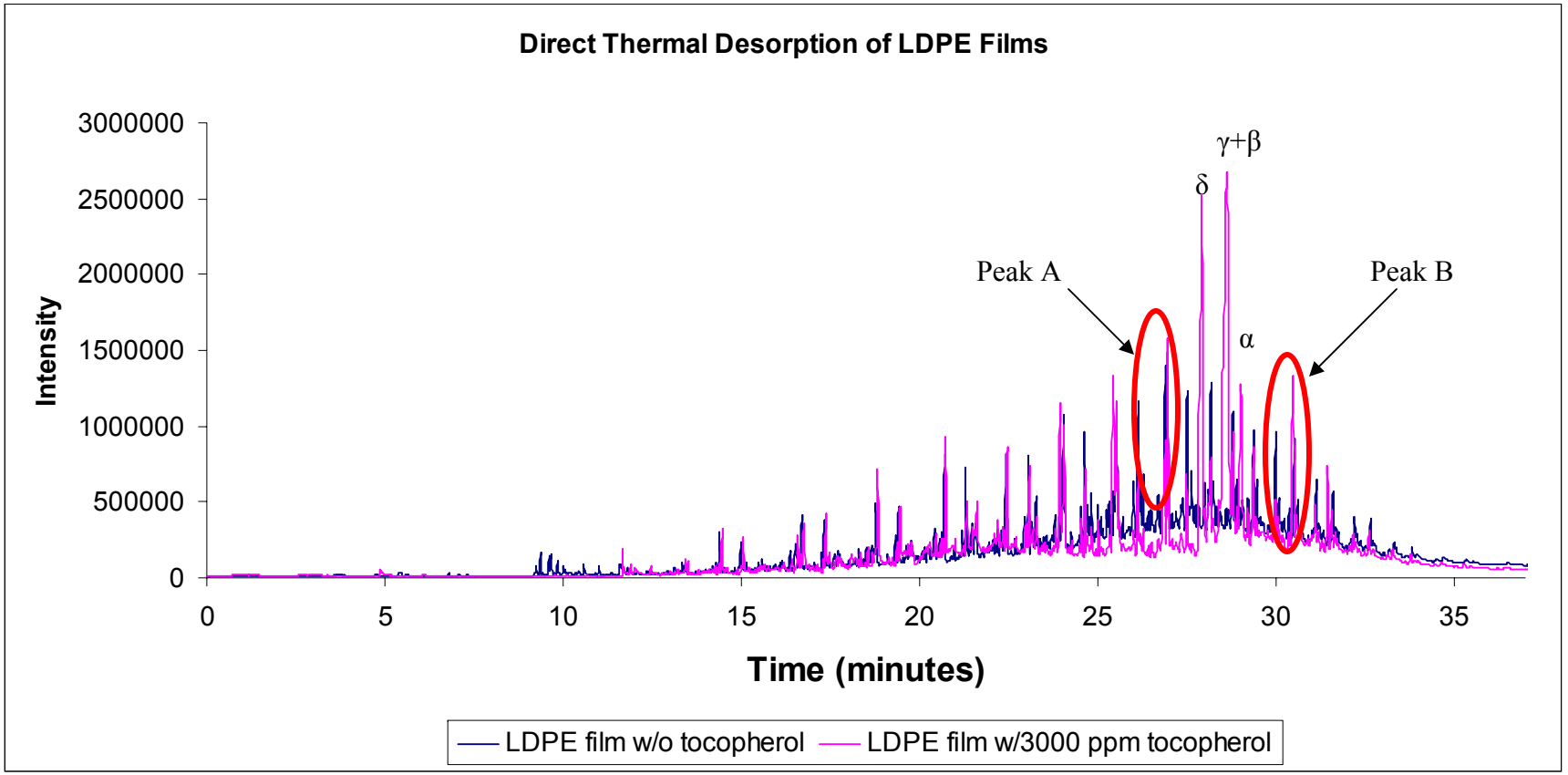


Figure 57 - Direct Thermal Desorption Chromatogram for PE films.

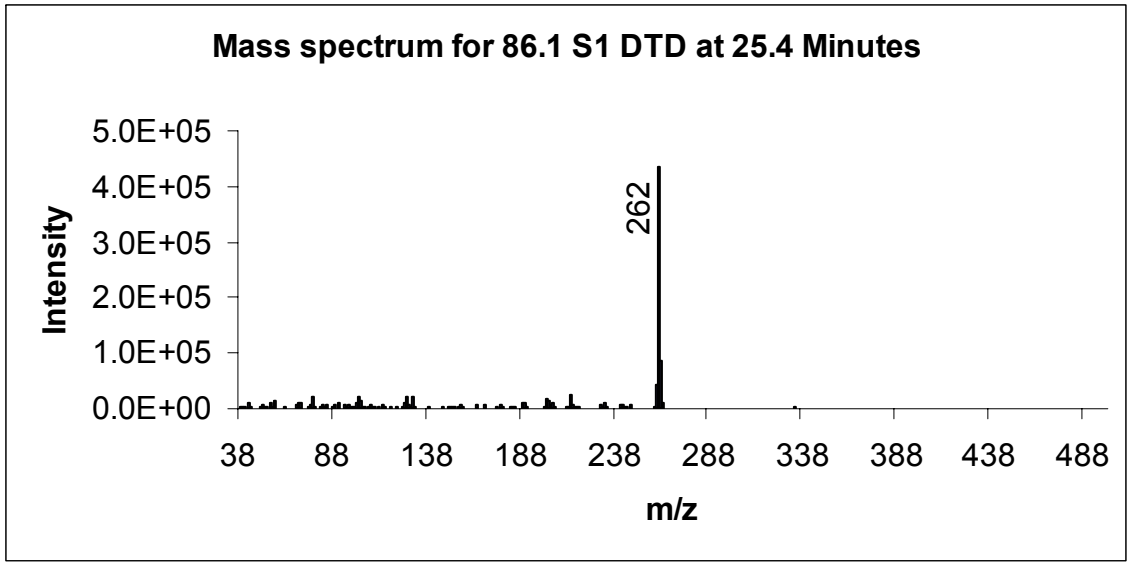


Figure 58 - Mass spectrum for unknown at peak A in DTD analysis of LDPE film.

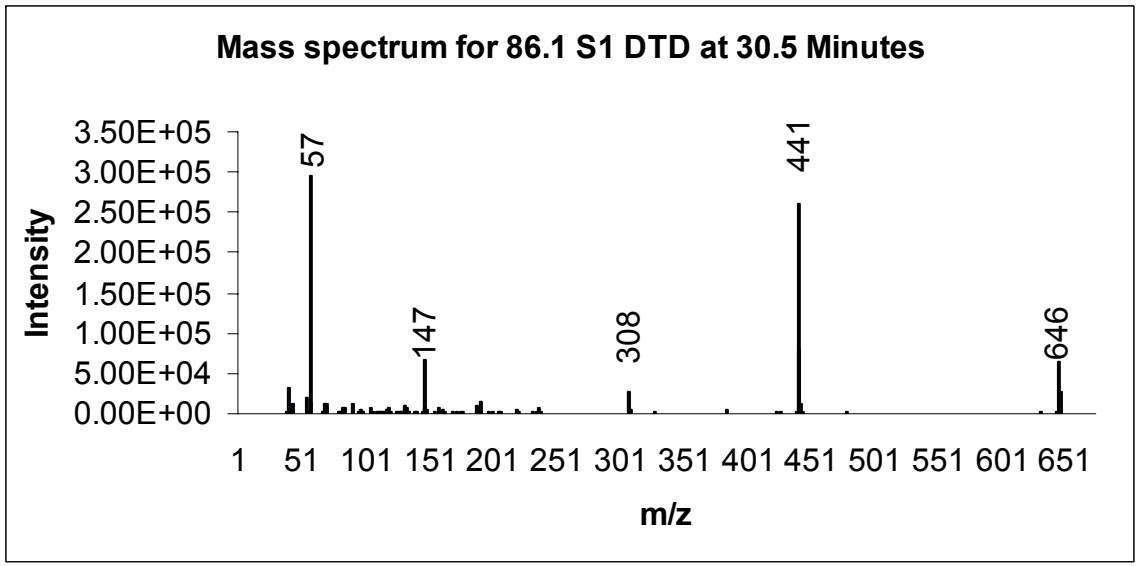


Figure 59 - Mass spectrum for compound at peak B in DTD analysis of LDPE film, proposed to be the phosphite stabilizer Irgafos 168.

HPLC Data

Since GC analyses showed clearly that most oxidation products of tocopherols were not volatile, extracts were analyzed by HPLC-MS to detect non-volatile products. Figure 60 shows the HPLC chromatogram of the mixed tocopherol standard (400 ug/ml) detected at 295 nm. Three peaks corresponding to the parent δ (18.7 minutes), γ+β (21.0 minutes) and α-tocopherol (23.7 minutes) are clearly visible, with three small peaks eluting before tocopherol. These peaks are presumed to be natural impurities from the soybean present in the mixed tocopherols.

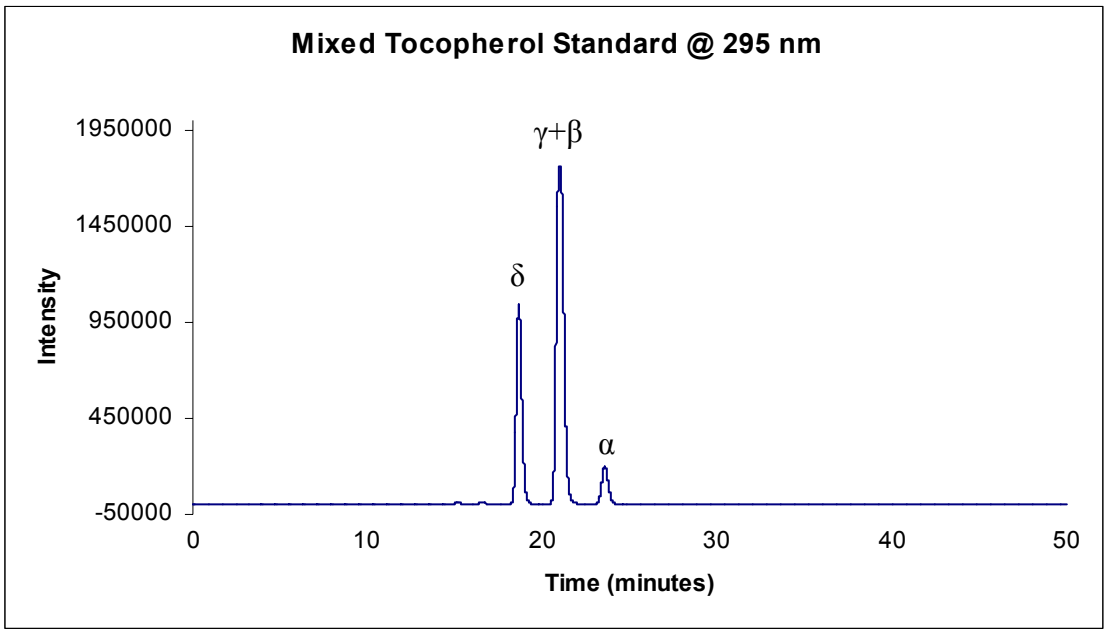


Figure 60- HPLC chromatogram of mixed tocopherol standard by photodiode array (PDA) detection at 295 nm.

Figure 61 shows the same tocopherol standard (400 ug/ml) detected at the wavelength of maximum absorbance for each peak scan, which makes the small peaks eluting ahead of the tocopherols are more clearly visible. Table 9 provides the retention times and wavelengths of maximum absorbance for the small leading peaks.

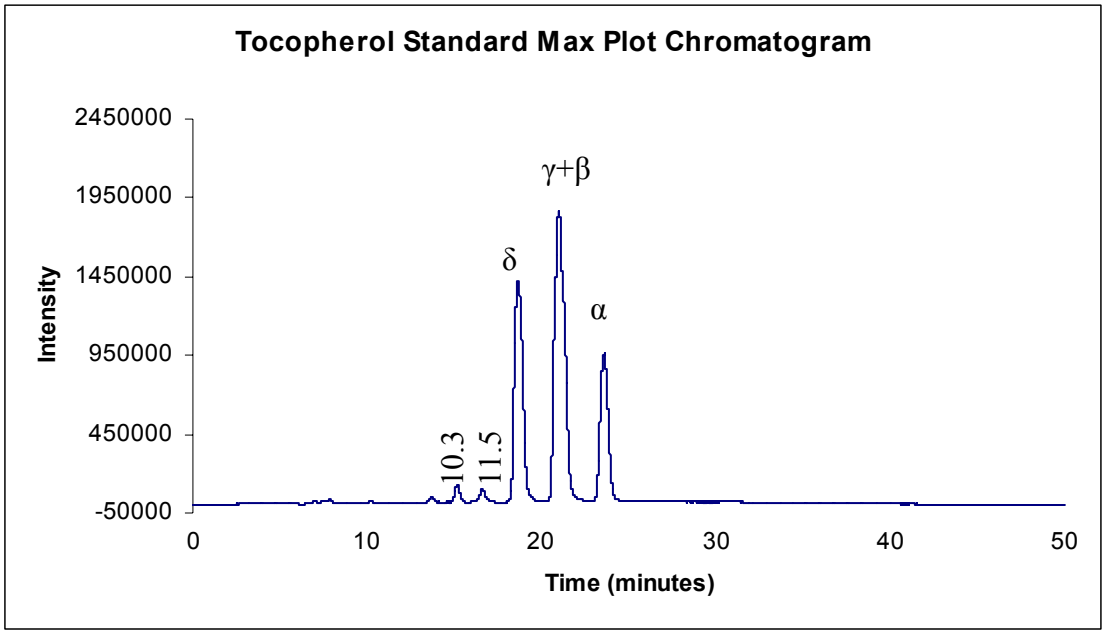


Figure 61 - HPLC chromatogram of mixed tocopherol standard by PDA detection at maximum absorbance

Table 9 - Retention times and frequency of maximum absorbance for small peaks in mixed tocopherol standard.

Retention Time (minutes)	Maximum Absorbance (nm)
10.3	205, 296
11.5	205, 296

Figures 62 and 63 show the chromatograms for samples of the tocopherol standard oxidized with an equimolar and ten times excess of PbO_2, respectively. Oxidation products, labeled A-E were observed. Retention times and wavelengths of maximum absorbance for these products are given in Table 10. These figures clearly show that peaks A, B and C increase under stronger oxidizing conditions, while peaks D and E are eliminated. These later peaks may correspond to the tocopherol dihydroxydimer (DHD), which was reported by al-Malaika [101] as observed under equimolar reaction of tocopherol with PbO_2, but not under ten times excess. This would imply that peak C is the tocopherol spirodimer (SPD), as al-Malaika reported this to be present at higher levels of oxidant. It also matches the predicted elution pattern, as the DHD would have greater polarity than the SPD due to its alcohol groups. Absorbance at 299 nm is also consistent with the absorbance of the SPD.

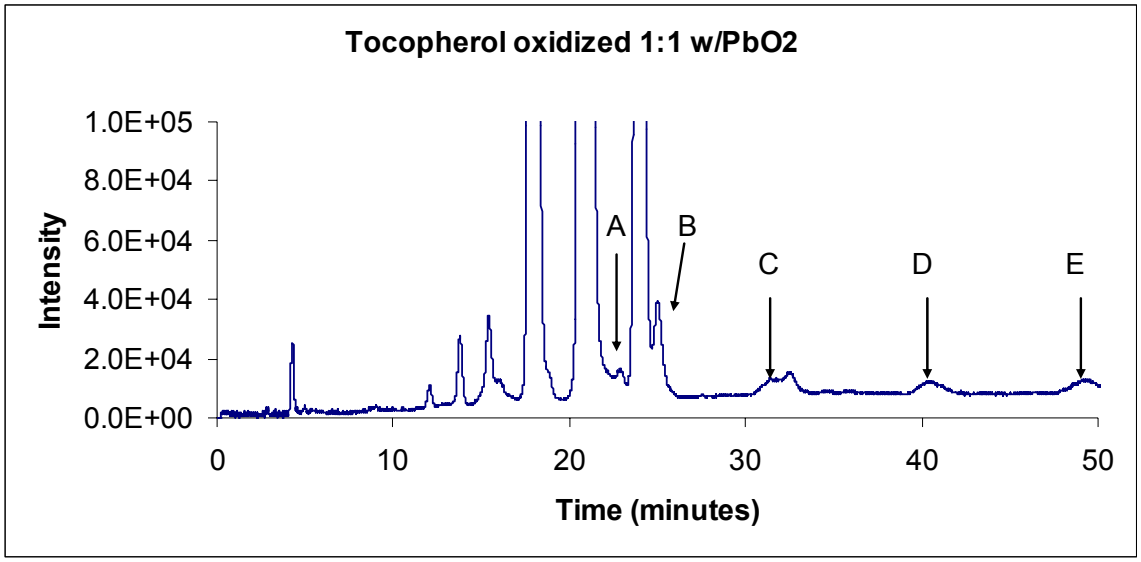


Figure 62 – HPLC chromatogram of tocopherol oxidized with PbO_2 in an equimolar ratio by PDA detection at maximum absorbance.

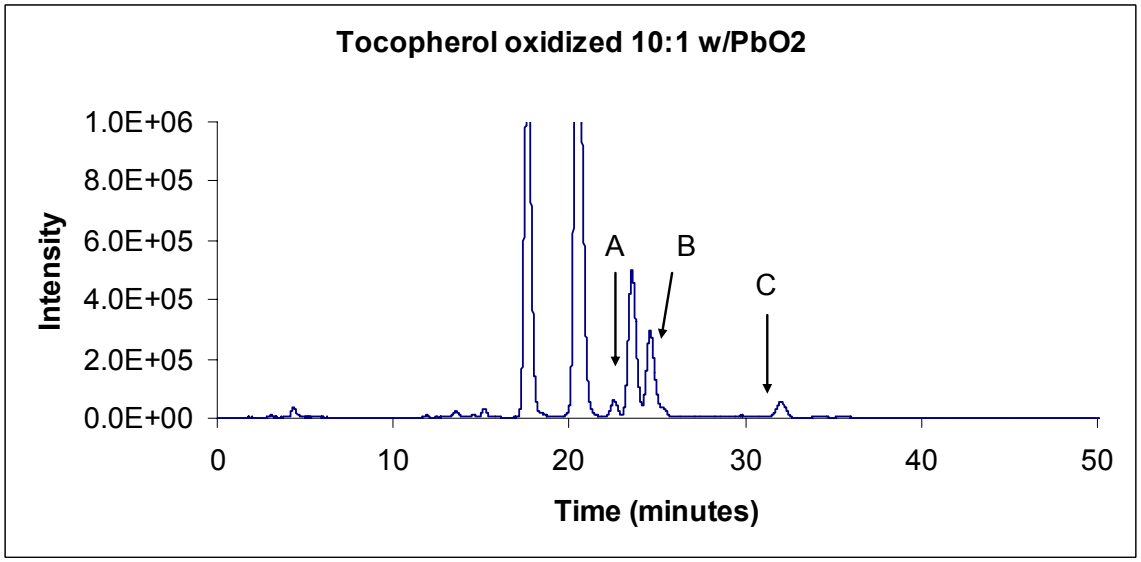


Figure 63 - HPLC chromatogram by PDA detection at maximum absorbance of tocopherol oxidized with PbO2 at 10 times molar excess.

Table 10 - Retention times and wavelengths of maximum absorbance for oxidation products of tocopherol upon reaction with an equimolar amount of PbO2.

Retention Time (minutes)	Maximum Absorbance (nm)
23.1 (A)	205, 301
25.1 (B)	206, 298, 227 (shoulder)
32.1 (C)	205, 299
40.7 (D)	205
49.7 (E)	205

LDPE film chromatograms

Figures 64 and 65 show the HPLC chromatograms for the 100% PE control film (86.0), containing no tocopherol, at 295 nm and maximum absorbance respectively. Note that the chromatogram at 295 nm has been scaled in by a factor of ten compared to the maximum absorbance chromatogram, and that even the maximum absorbance chromatogram has been zoomed in to amplify very small peaks (indicated in Figure 62). These very small peaks most likely arise from polymer degradation products, such as short-chain ethylene oligomers, that fragment from the polyethylene backbone during processing. Table 11 shows the retention times and wavelengths of maximum absorbance for peaks found in LDPE polymer control film.

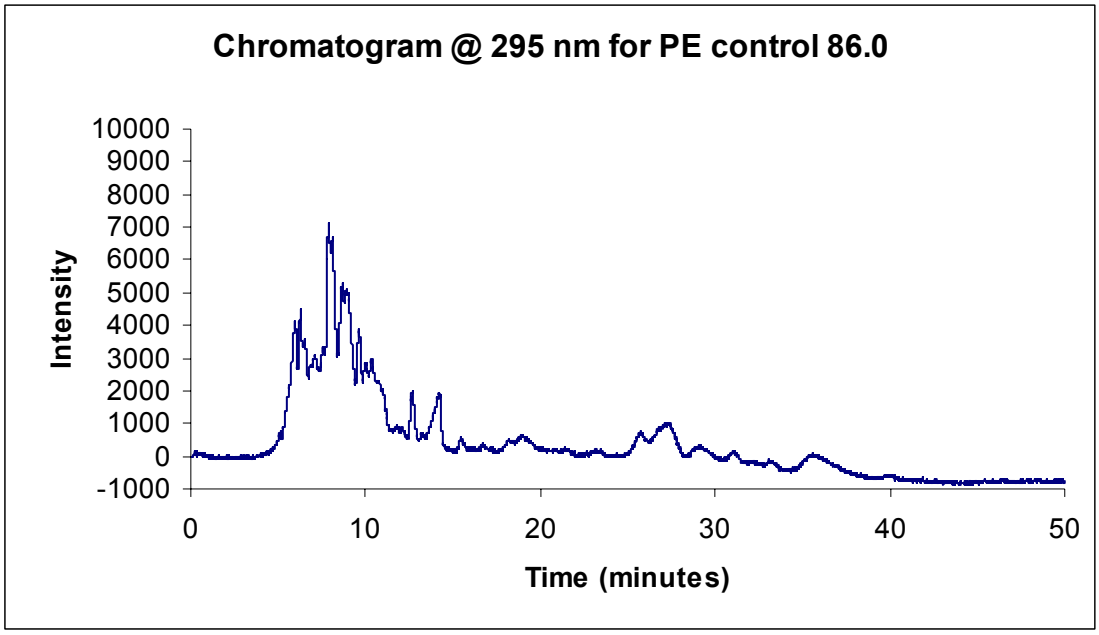


Figure 64 - HPLC chromatogram of PE control film extract by PDA detection at 295 nm

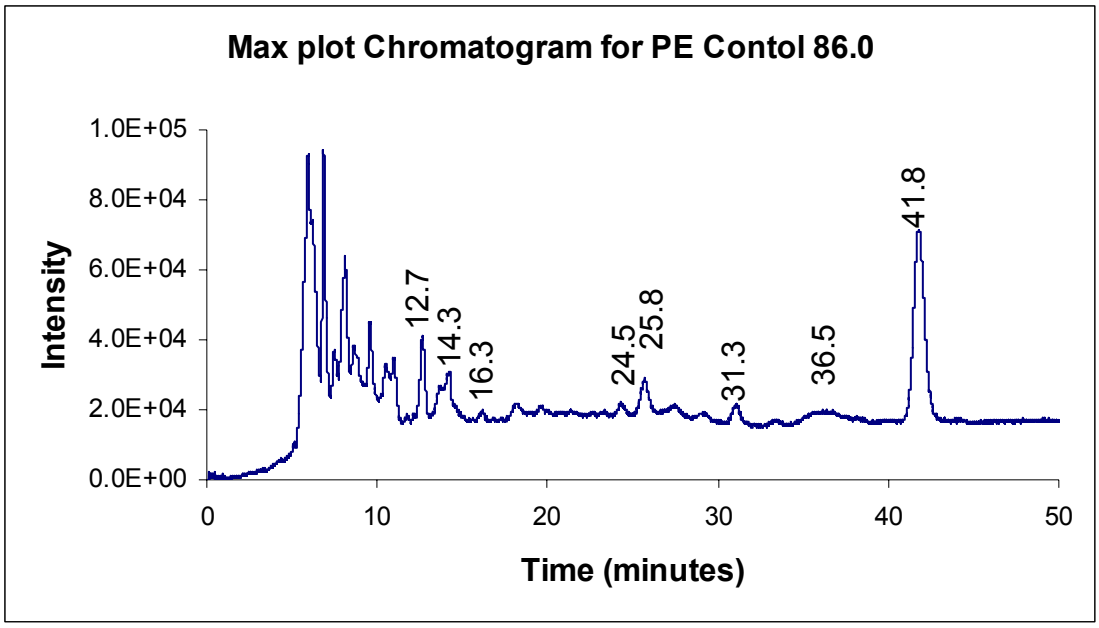


Figure 65 - HPLC chromatogram of PE control film extract by PDA detection at maximum absorbance.

Table 11- Retention times and frequency of maximum absorbance for peaks found in LDPE polymer control

Retention Time (minutes)	Maximum Absorbance (nm)
12.7	205, 227 shoulder, 275
14.3	204, 225, 272
16.3	205
24.5	205, 235 shoulder
25.8	204, 235 shoulder
31.3	205, 236 shoulder
36.5	204, 235
41.8	205, 270

Figures 66 and 67 show the HPLC chromatograms for the 100% PE film containing 3000 ppm tocopherol produced under typical production conditions (86.1), at 295 nm and maximum absorbance respectively. The scale for these chromatograms has been expanded so that the smaller peaks of concern are more clearly visible. Peaks from tocopherol isomers are clearly visible under both conditions. Other peaks match components of either tocopherol or polymer standards (Figure 67), with the exception of two small peaks eluting at 35.8 and 39.9 minutes respectively. The frequencies of maximum absorbance for all peaks are given in Table 12. Figure 68 shows the overlaid chromatograms for the tocopherol standard, the LDPE film standard without tocopherol and the tocopherol containing LDPE film.

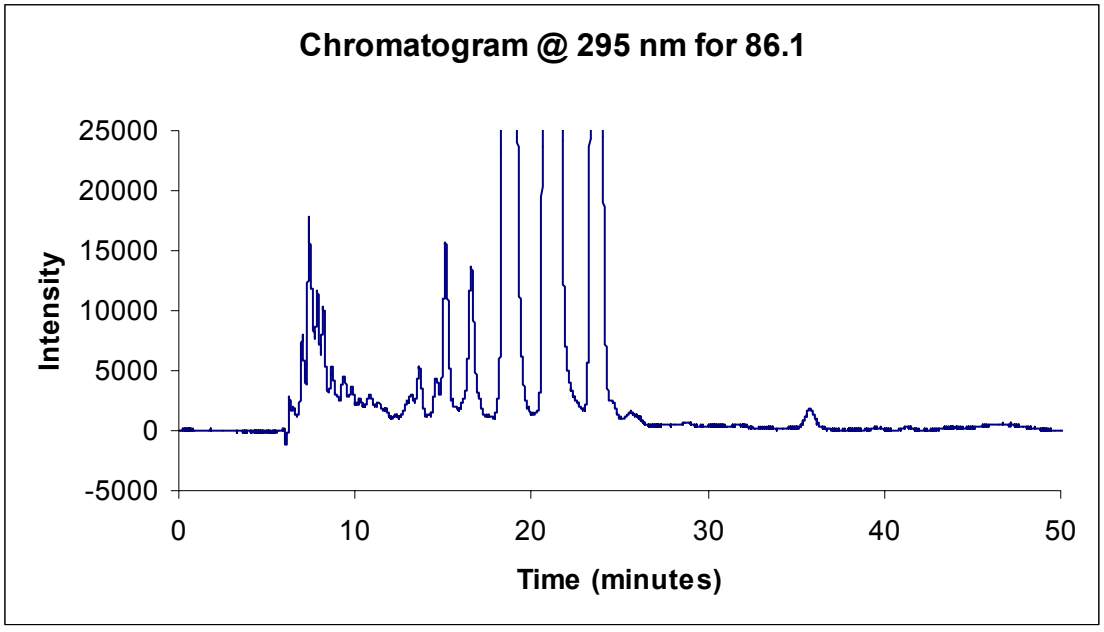


Figure 66 - HPLC chromatogram of tocopherol containing PE film (86.1) extract by PDA detection at 295 nm.

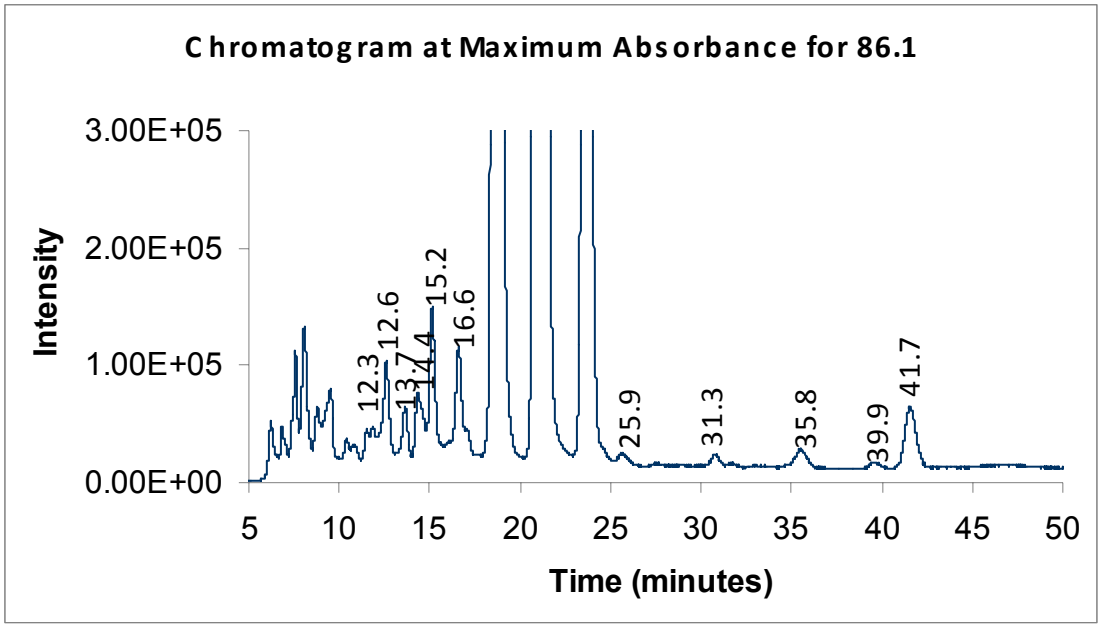


Figure 67-HPLC chromatogram of tocopherol containing PE film (86.1) extract by PDA detection at maximum absorbance.

Table 12 - Retention times and frequencies of maximum absorbance for peaks in tocopherol containing LDPE film

Retention Time (minutes)	Maximum Absorbance (nm)
12.3	205 227 shoulder, 275
12.6	206, 225 shoulder, 296
13.7	204
14.4	207, 296
15.2	205
16.6	206, 296
25.9	205
31.3	205, 296
35.8	205, 227 shoulder, 281
39.9	205, 226, 270
41.7	205, 270

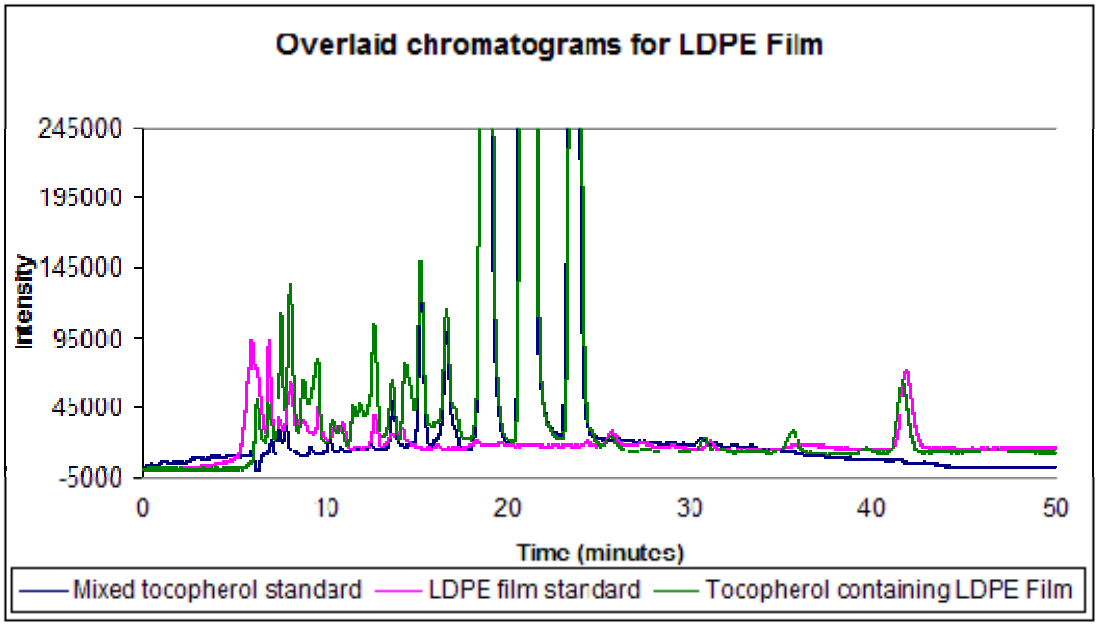


Figure 68 - Overlaid chromatograms of LDPE films with and without tocopherol, along with tocopherol standard.

PP film chromatograms

Figures 69 and 70 show the HPLC chromatograms for the 100% PP control film (86.4), containing no tocopherol, detected at 295 nm and maximum absorbance respectively. Note that the scale of the maximum absorbance chromatogram has been expanded by 100x to increase detection of small peaks. As with the blended film, detected peaks are from polymer degradation products as well as stabilizers present in the PP resin. The wavelengths of maximum absorbance for major peaks are given in Table 13.

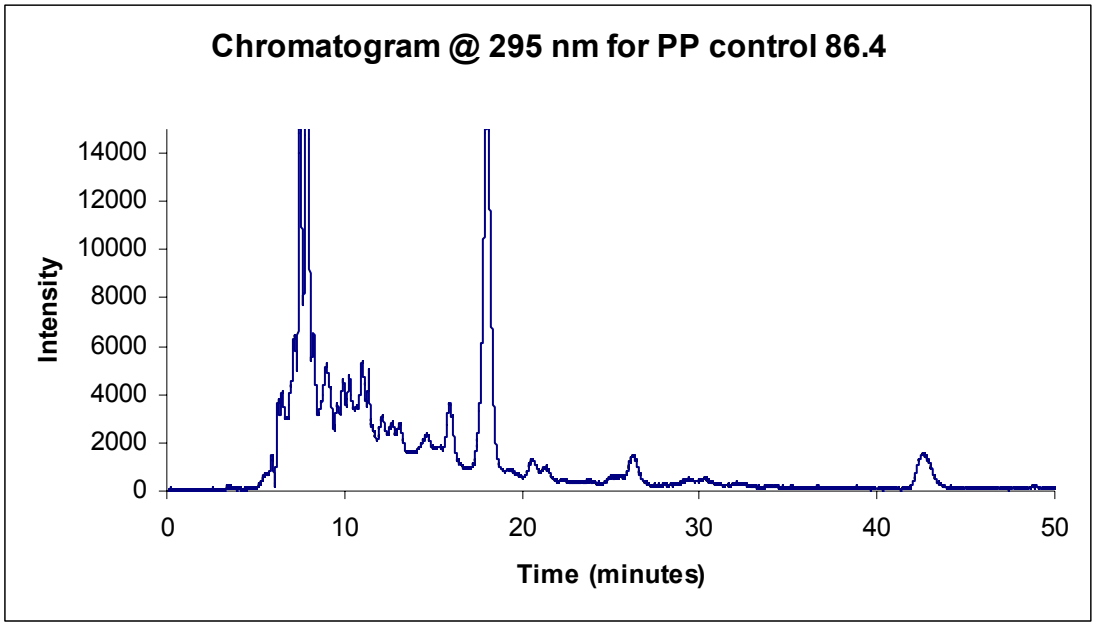


Figure 69 - Chromatogram of PP control film 86.4 detected at 295 nm.

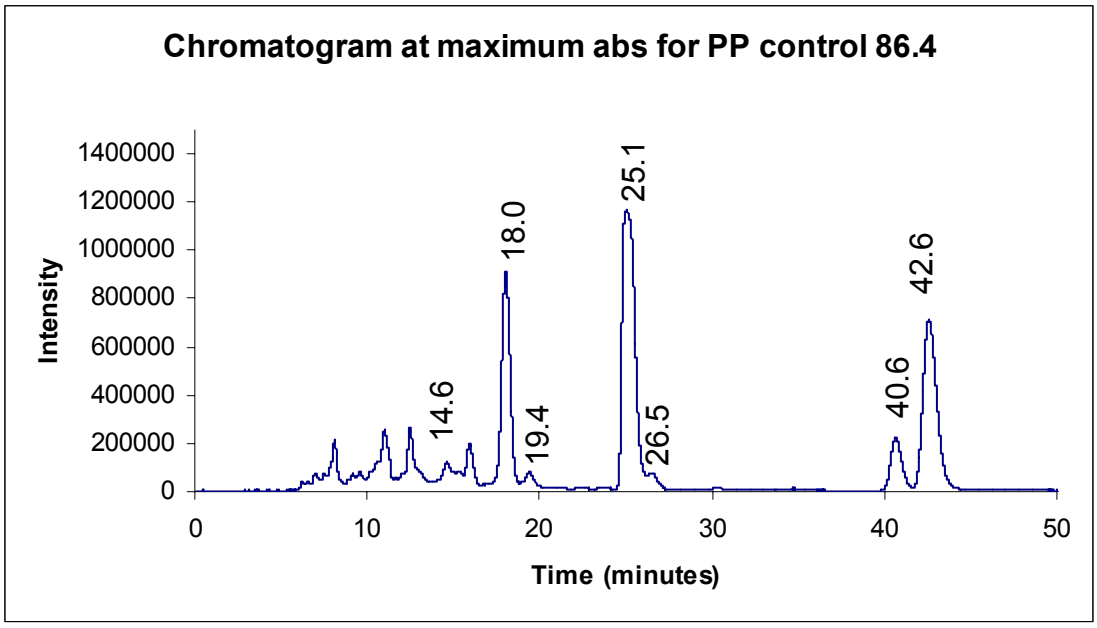


Figure 70 - Chromatogram of PP control film 86.4 detected at maximum absorbance.

Table 13 - Retention times and frequencies of maximum absorbance for peaks in PP film control.

Retention Time (minutes)	Maximum Absorbance (nm)
14.6	205, 275, 225 (shoulder)
18.0	208, 228 shoulder, 275
19.4	205, 234 shoulder, 275
25.1	211, 223, 265, 273
26.5	205, 274
40.6	206
42.6	207, 224, 270

Figures 71 and 72 show the HPLC chromatograms for the 100% PP film containing 3000 ppm tocopherol produced under typical production conditions (86.5), at 295 nm and maximum absorbance respectively. Peaks from tocopherol isomers are clearly visible under both detection conditions. No additional peaks are seen at 295 nm, but under maximum absorbance, a small peak is observed at 48.3 minutes that does not correspond to a peak in either the tocopherol standard or the polymer control. The wavelengths of maximum absorbance for these peaks are given in Table 14. Figure 73 shows the overlaid chromatograms for the tocopherol standard, the PP film standard without tocopherol and the tocopherol containing PP film.

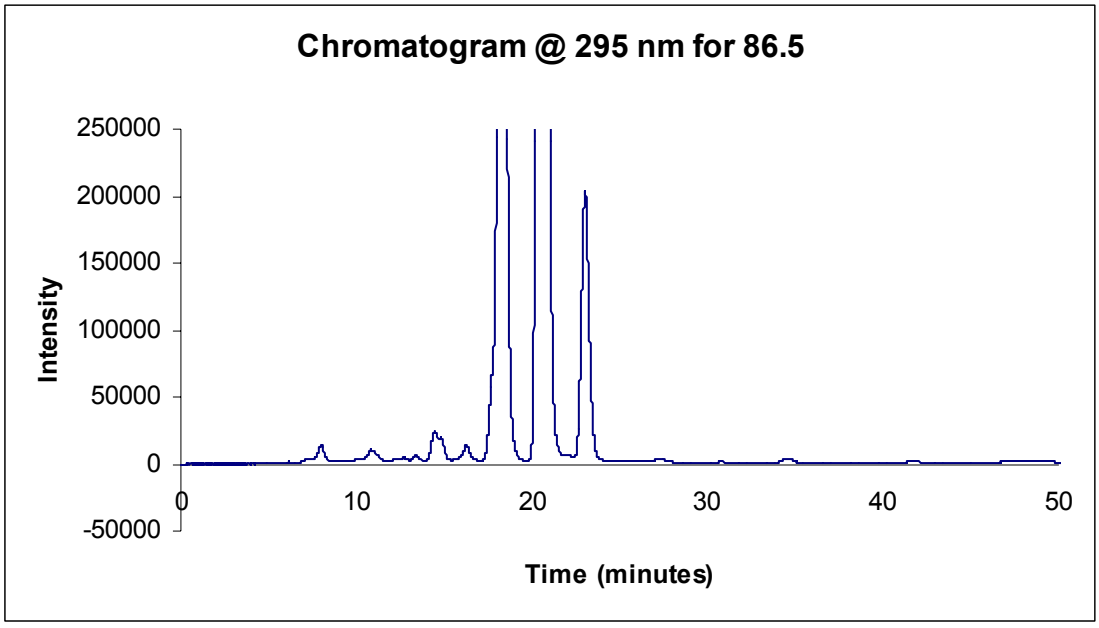


Figure 71 - HPLC chromatogram of tocopherol containing PP film (86.5) extract by absorbance at 295 nm.

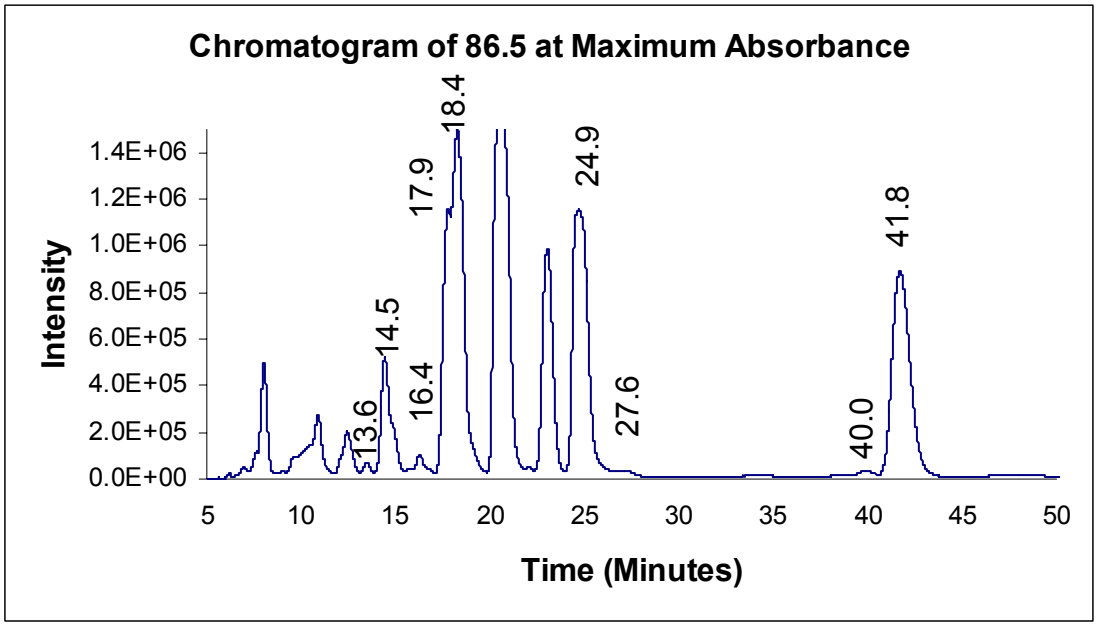


Figure 72 - HPLC chromatogram of tocopherol containing PP film (86.5) extract by PDA detection at maximum absorbance.

Table 14 - Retention times and frequencies of maximum absorbance for peaks in tocopherol containing PP film.

Retention Time (minutes)	Maximum Absorbance (nm)
13.6	205, 293
14.5	205, 275, 225 (shoulder)
16.4	206, 295
17.9	211, 275, 320
18.4	219, 293
24.9	210,223, 266, 273
27.6	207, 293
40.0	206
41.8	208, 224, 270

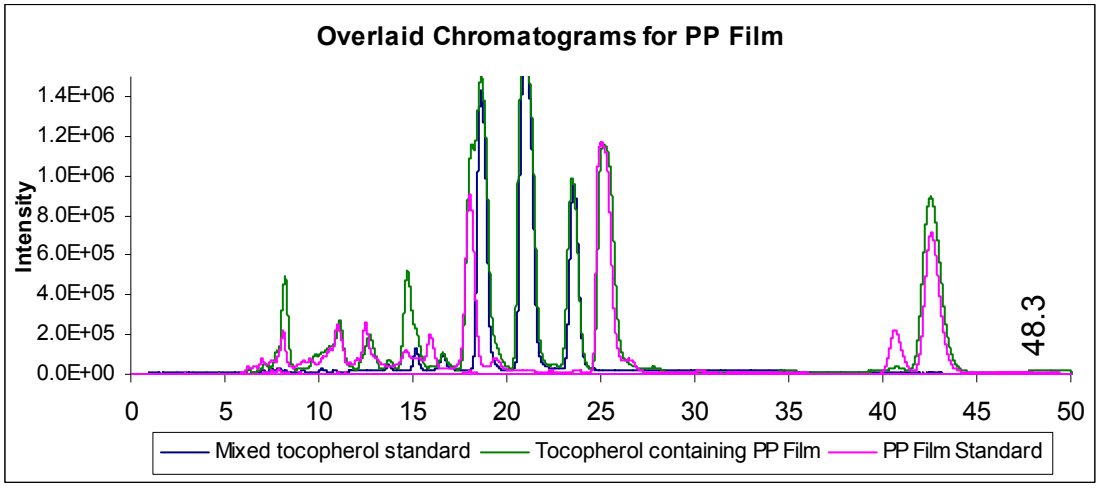


Figure 73 - Overlaid chromatograms of PP films with and without tocopherol, against tocopherol standard.

PE:PP Blend film chromatograms

Figures 74 and 75 show the HPLC chromatograms for the blended 50% PE 50% PP control film containing no tocopherol (86.2), at 295 nm and maximum absorbance respectively. Several peaks are observed, which likely arise from both polymer degradation products, and stabilizers present in the PP resin. The wavelengths of maximum absorbance for these peaks is given in Table 15.

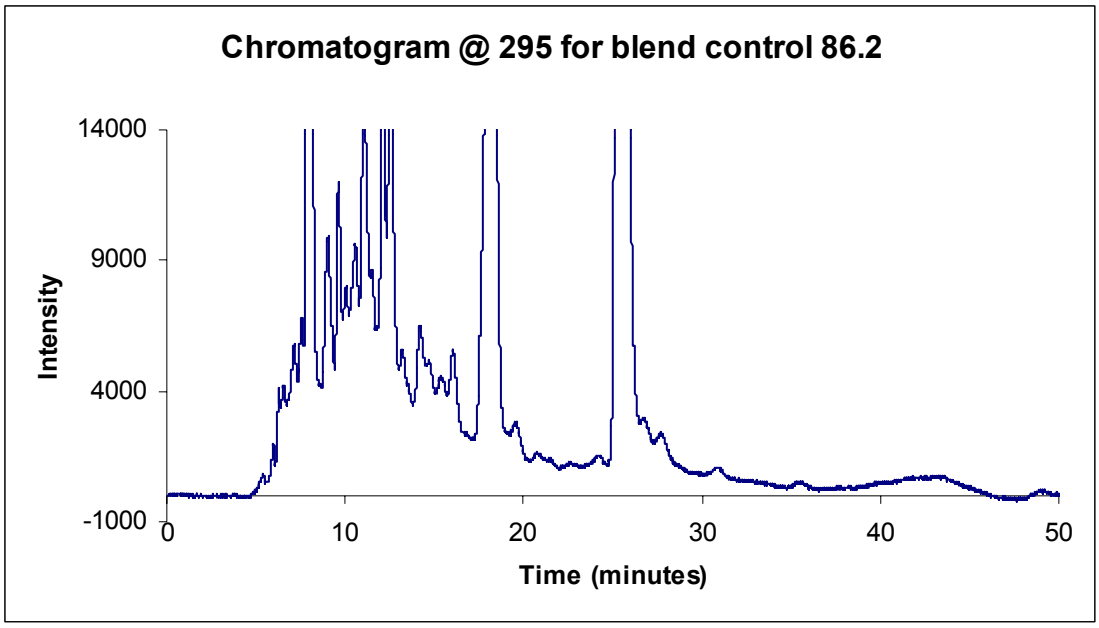


Figure 74 - HPLC chromatogram for PE:PP blend control film by PDA detection at 295 nm.

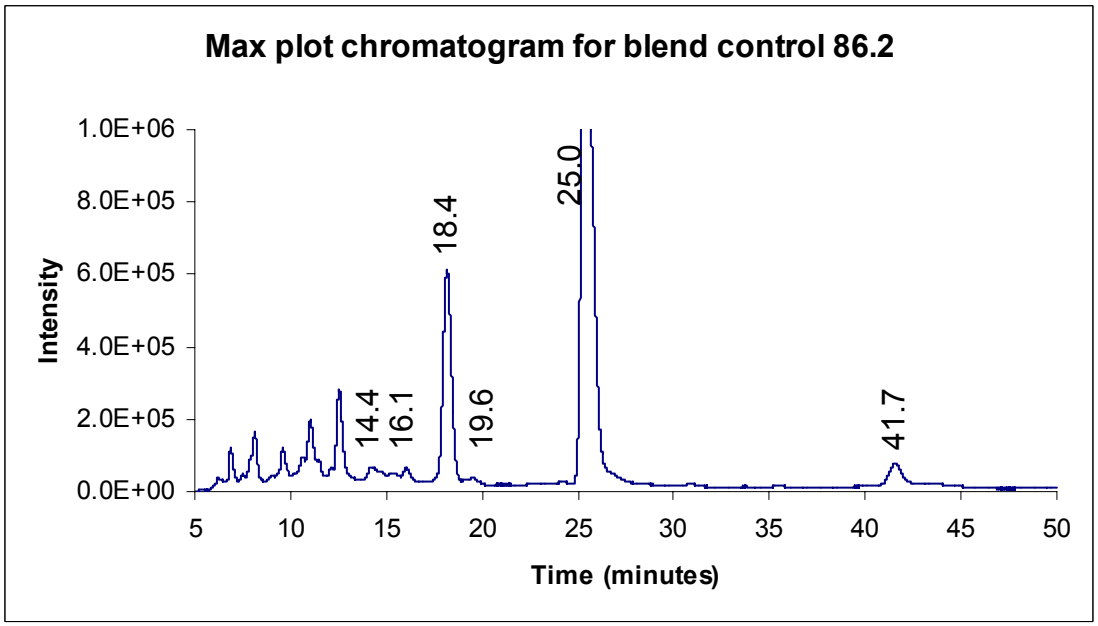


Figure 75-HPLC chromatogram for PE:PP blend control film by PDA detection at maximum absorbance.

Table 15 - Retention times and frequencies of maximum absorbance for peaks in LDPE:PP blend film control

Retention Time (minutes)	Maximum Absorbance (nm)
14.4	205, 228 shoulder
16.1	205, 275
18.4	206, 275
19.6	205, 275
25.0	210, 223, 266, 273
41.7	206

Figures 76 and 77 show the HPLC chromatograms for the 50% PE 50% PP film containing 3000 ppm tocopherol produced under typical production conditions (86.3), at 295 nm and maximum absorbance respectively. Figure 78 shows the overlaid chromatograms for the tocopherol standard, the LDPE:PP blend film standard without tocopherol and the tocopherol containing blended film. Peaks corresponding to tocopherol isomers are clearly visible under both conditions. No additional peaks are seen at 295 nm, but under maximum absorbance, new peaks are observed at 14.7 and 43.6 minutes that do not correspond to peaks in either the tocopherol standard or the polymer control. The frequencies of maximum absorbance for all peaks are given in Table 16.

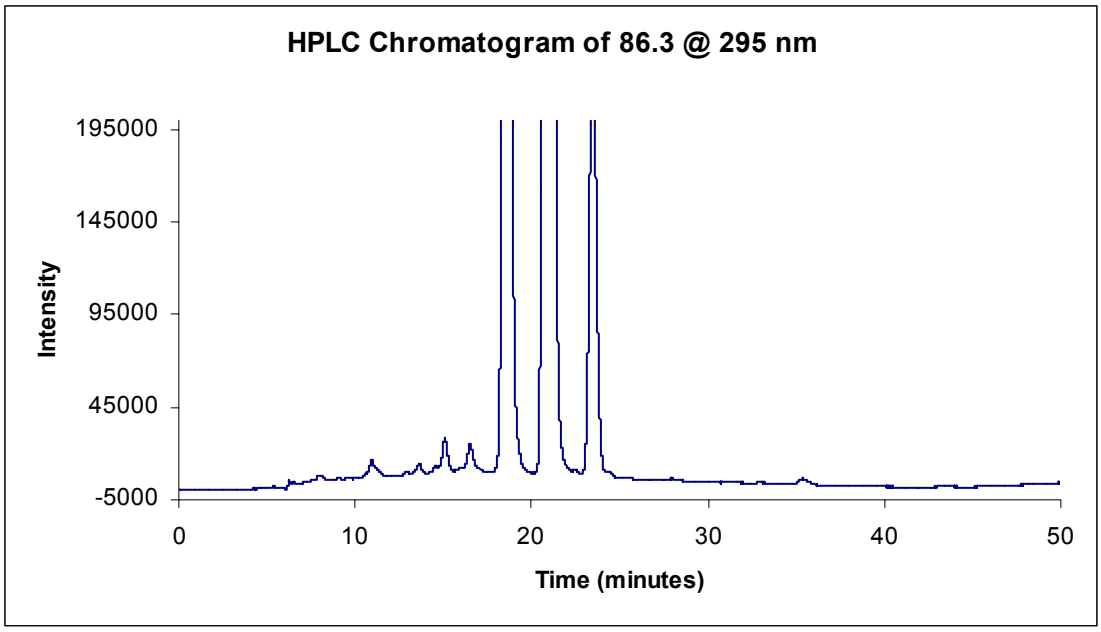


Figure 76-HPLC chromatogram for tocopherol containing PE:PP blend film by PDA detection at 295 nm.

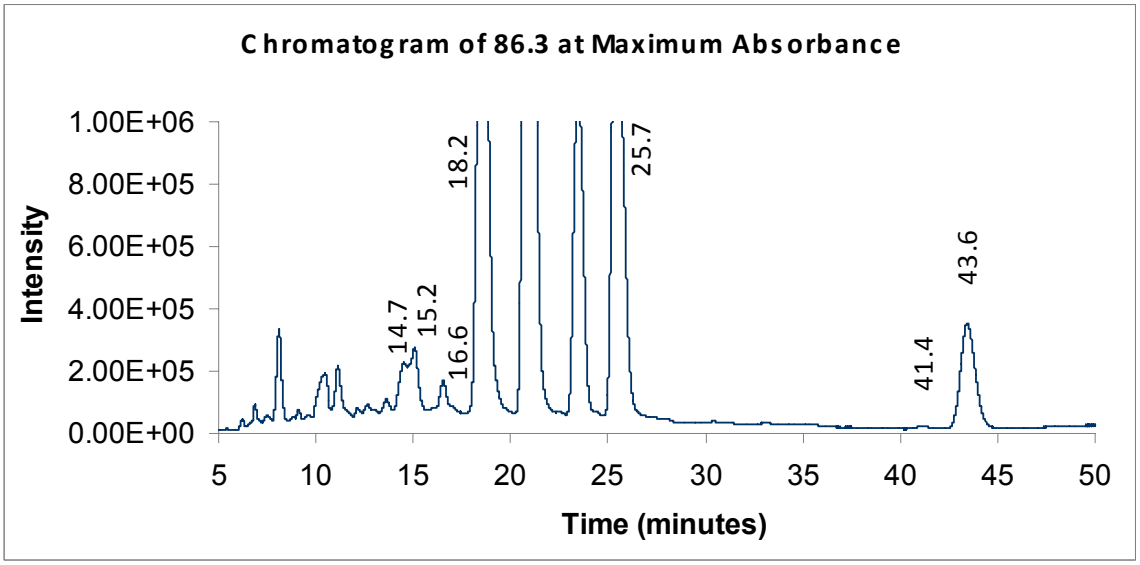


Figure 77-HPLC chromatogram for tocopherol containing PE:PP blend film 86.3 by PDA detection at maximum absorbance.

Table 16 - Retention times and frequencies of maximum absorbance for peaks in tocopherol-containing LDPE:PP blend film.

Retention Time (minutes)	Maximum Absorbance (nm)
14.7	205, 227 shoulder, 277
15.2	206, 295
16.6	206, 295
18.2	221, 294
25.7	210, 223, 266, 273
41.4	206, 263
43.6	205, 225, 270

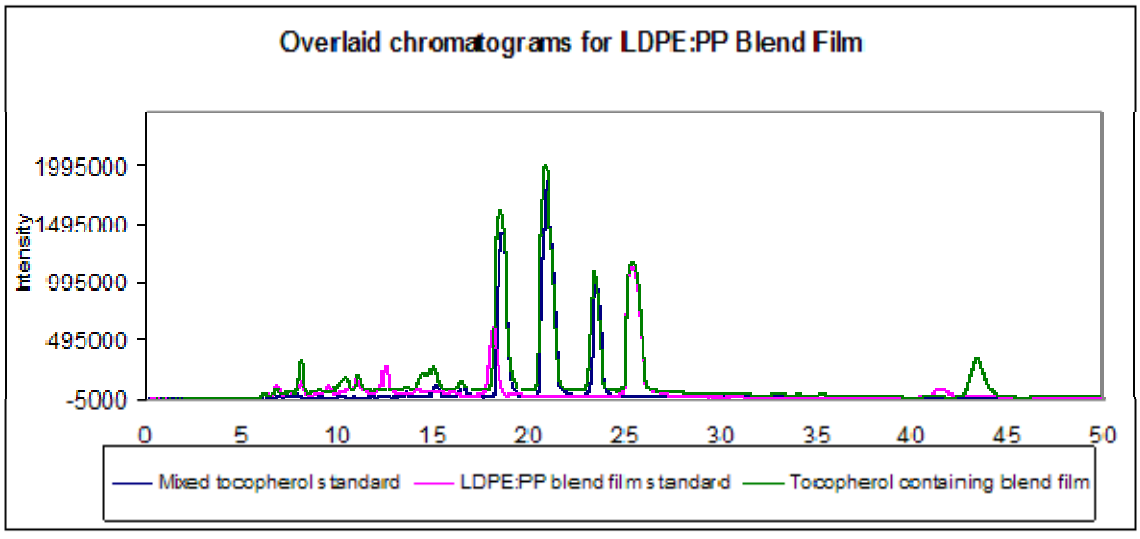


Figure 78 - Overlaid chromatograms for LDPE:PP blended films with and without tocopherol, along with tocopherol standard.

LC-MS Data

The following figures show the chromatograms and mass spectra for samples run at Rutgers University Department of Plant Science. Due to a series of system malfunctions, the reproducibility of these samples was compromised, making direct analysis between samples and controls very difficult. Ionization also proved difficult, with unusual spectra observed.

Figure 79 shows the chromatogram for the mixed tocopherol standard (4.64 mM), run in the negative mode, and filtered for the ions corresponding to the negative ionization of tocopherol (401, 415, and 429). The peaks corresponding to the tocopherol isomers are observed, with δ-tocopherol eluting at 23.8 minutes γ and β-tocopherol eluting together at 27.2 minutes, and α-tocopherol eluting at 30.9 minutes. The filtration for specific ions of interest makes it easier to observe the peaks of interest. Figures 80, 81 and 82 show the mass spectra under negative ionization for the tocopherol isomers. Each shows the expected [M-1] ion for the tocopherol isomer, as well as small amounts of larger masses corresponding to the addition of a methylene (CH_2) group [M-1+14], and addition of oxygen (O_2) [M-1+32].

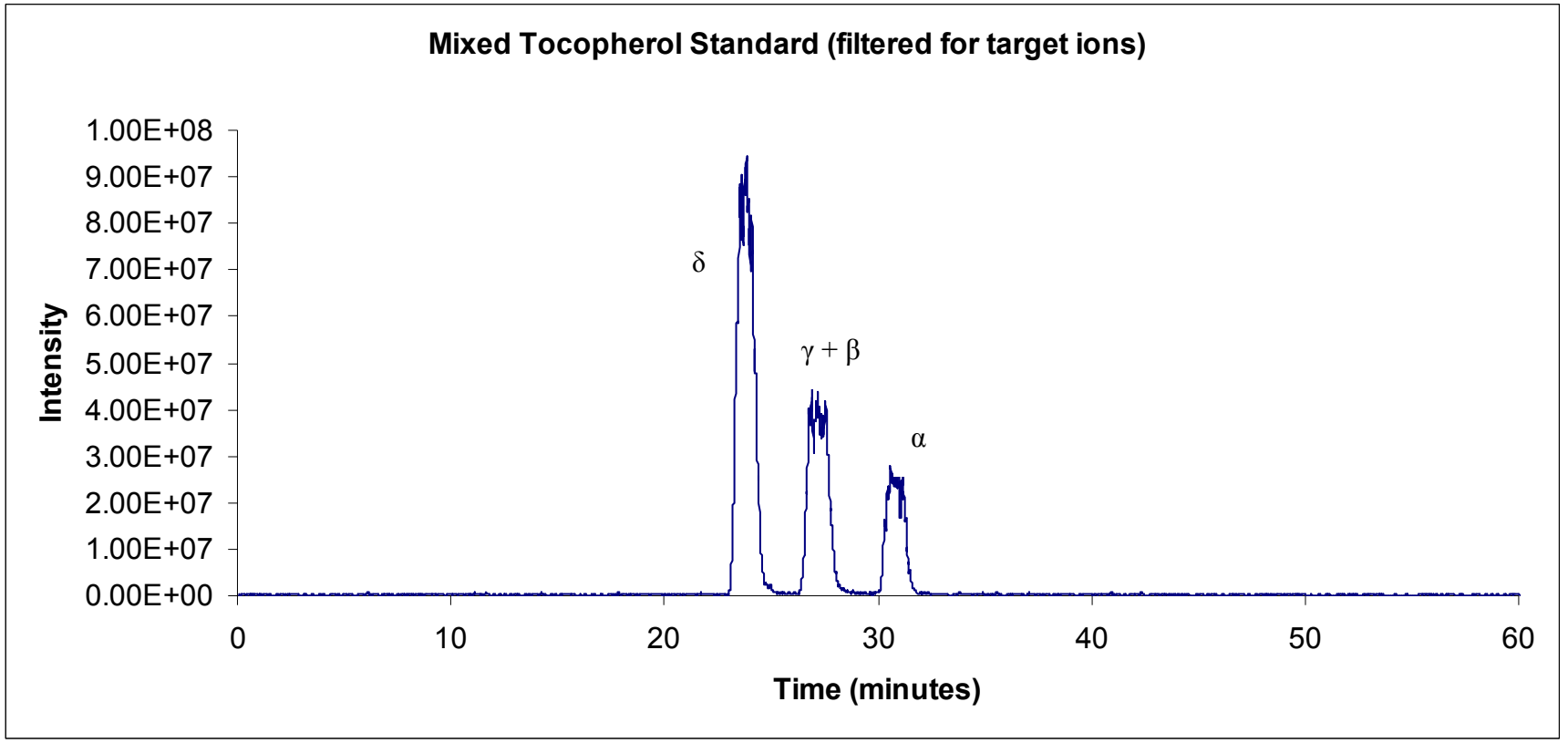


Figure 79 - Chromatogram of mixed tocopherol standard under negative ionization, filtered for ions 401, 415 and 529, showing δ (23.8 minutes), γ and β (27.2 minutes), and α (30.9 minutes) tocopherols.

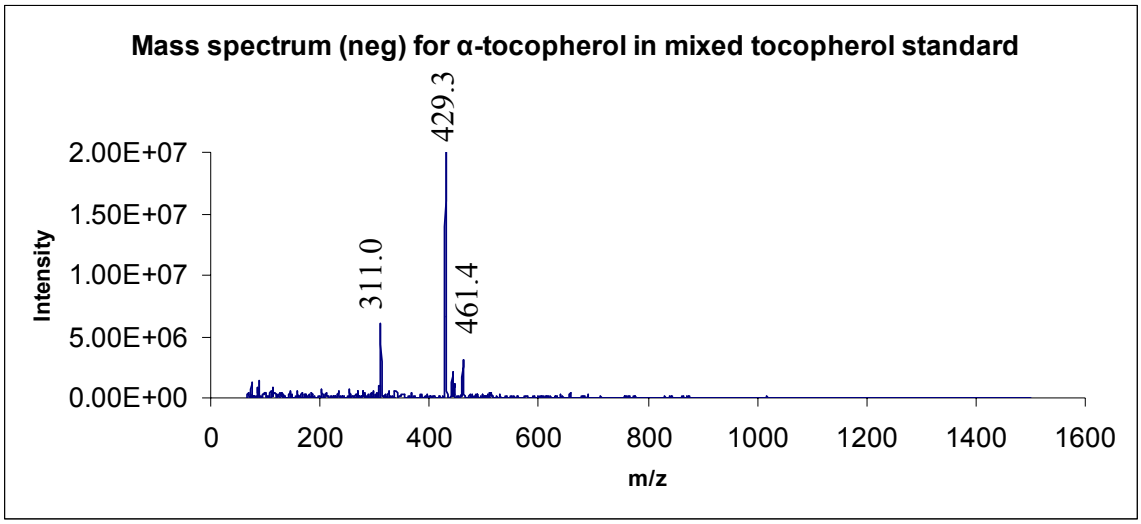


Figure 80 - Mass spectrum (-ESI) for a-tocopherol in mixed tocopherol standard, as observed at 30.9 minutes.

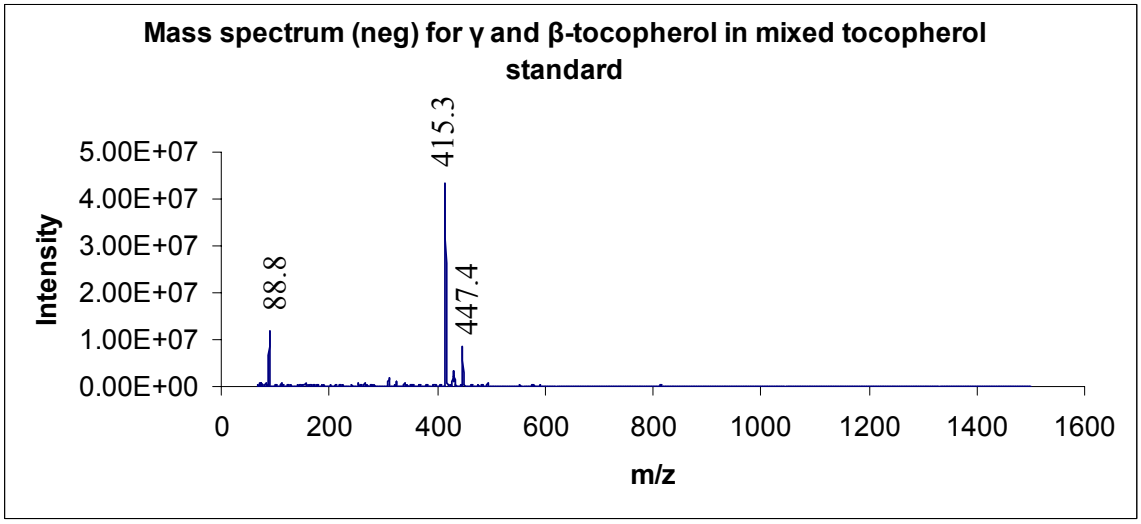


Figure 81 - Mass spectrum (-ESI) for γ and β-tocopherol in mixed tocopherol standard, as observed at 27.2 minutes.

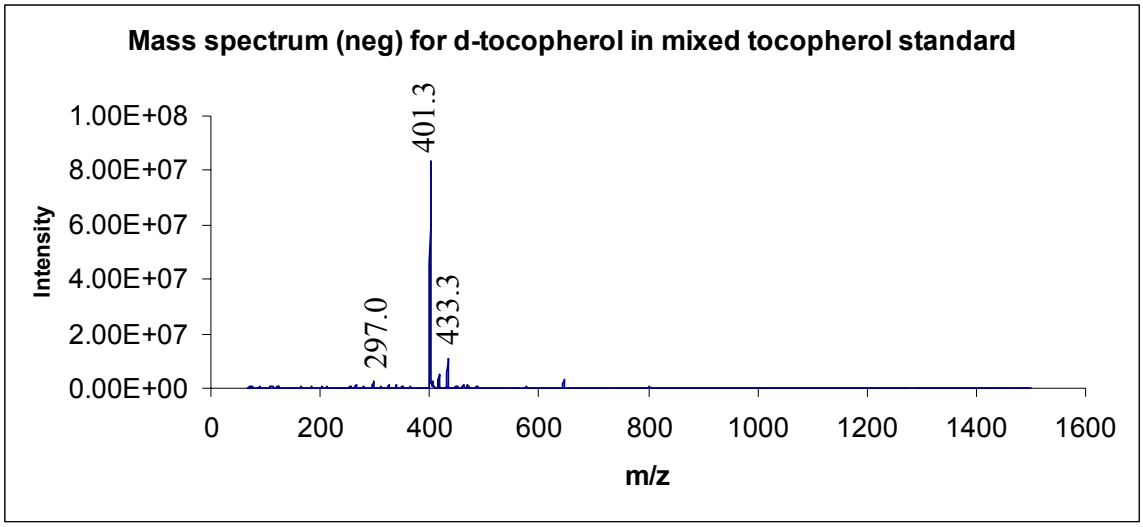


Figure 82 - Mass spectrum (-ESI) of delta tocopherol in tocopherol standard.

Figures 83, 84 and 85 show the overlaid total ion chromatograms (TICs) for tocopherol containing PE, LDPE:PP blend, and PP films overlaid with their respective polymer control films. The tocopherol-containing films shown here were produced under typical processing conditions, and only minor differences were observed in films produced under more severe conditions. Chromatograms for all films are provided in the appendix.

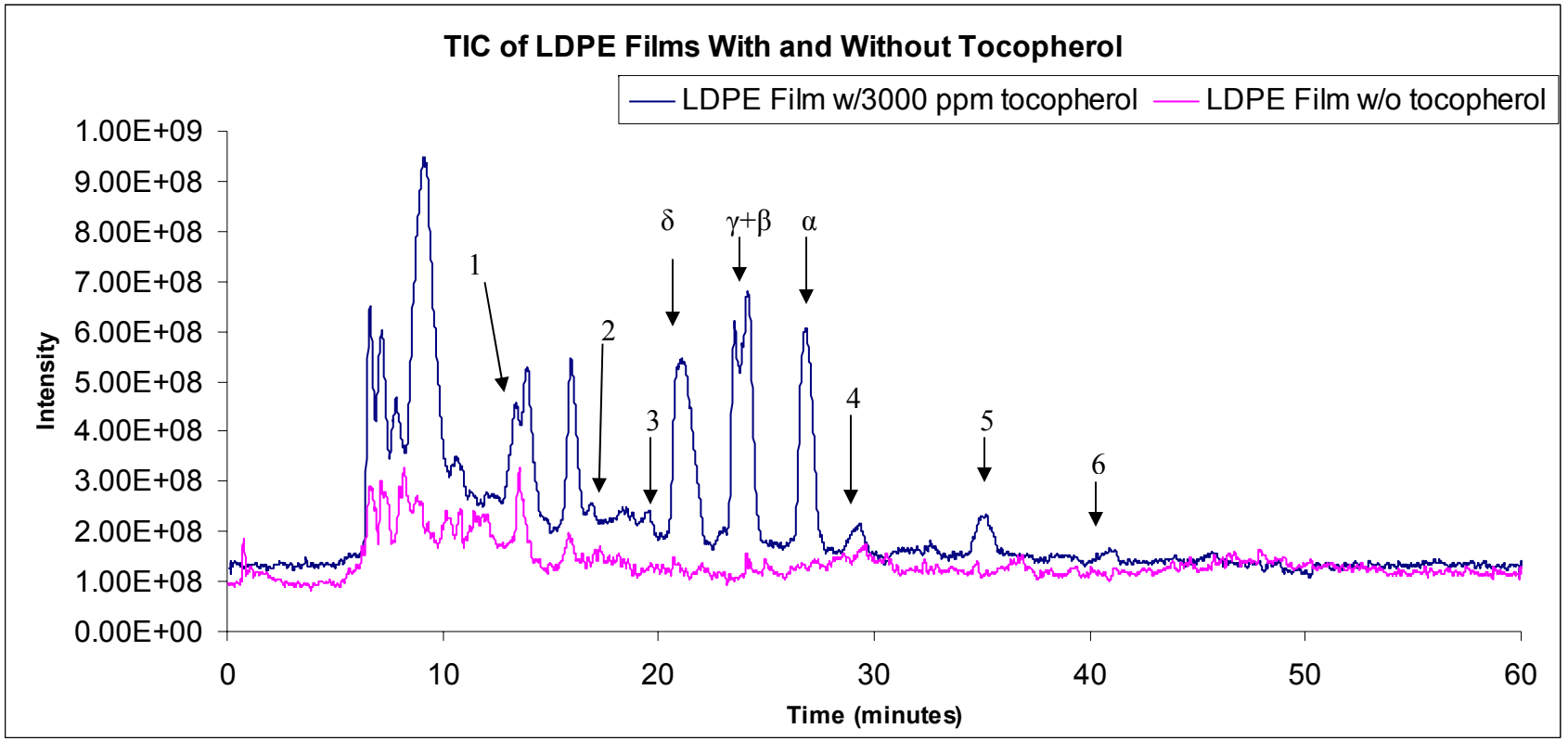


Figure 83 - Total Ion Chromatogram for LDPE film with and without tocopherol.

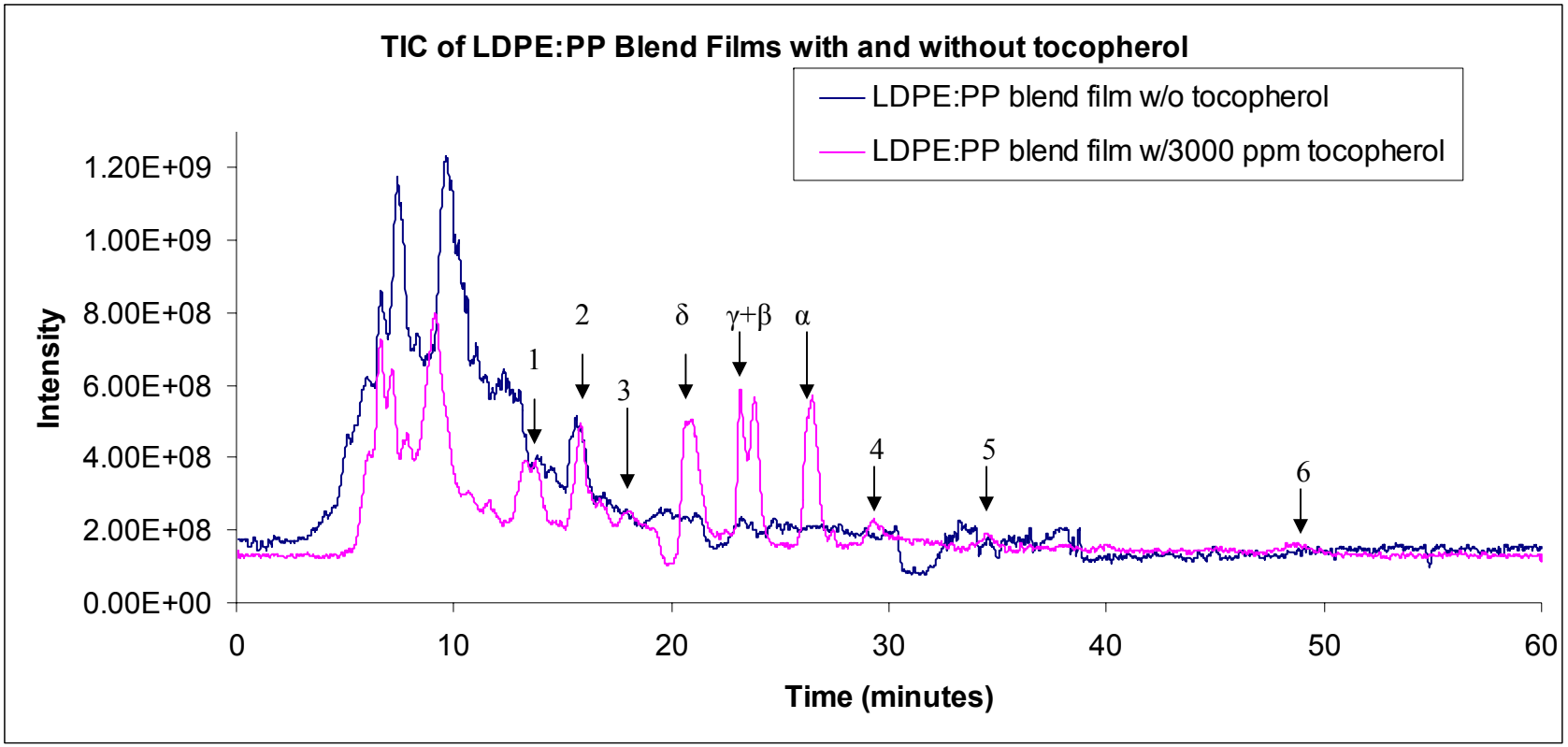


Figure 84 - Total ion chromatogram for LDPE:PP blend film with and without tocopherol.

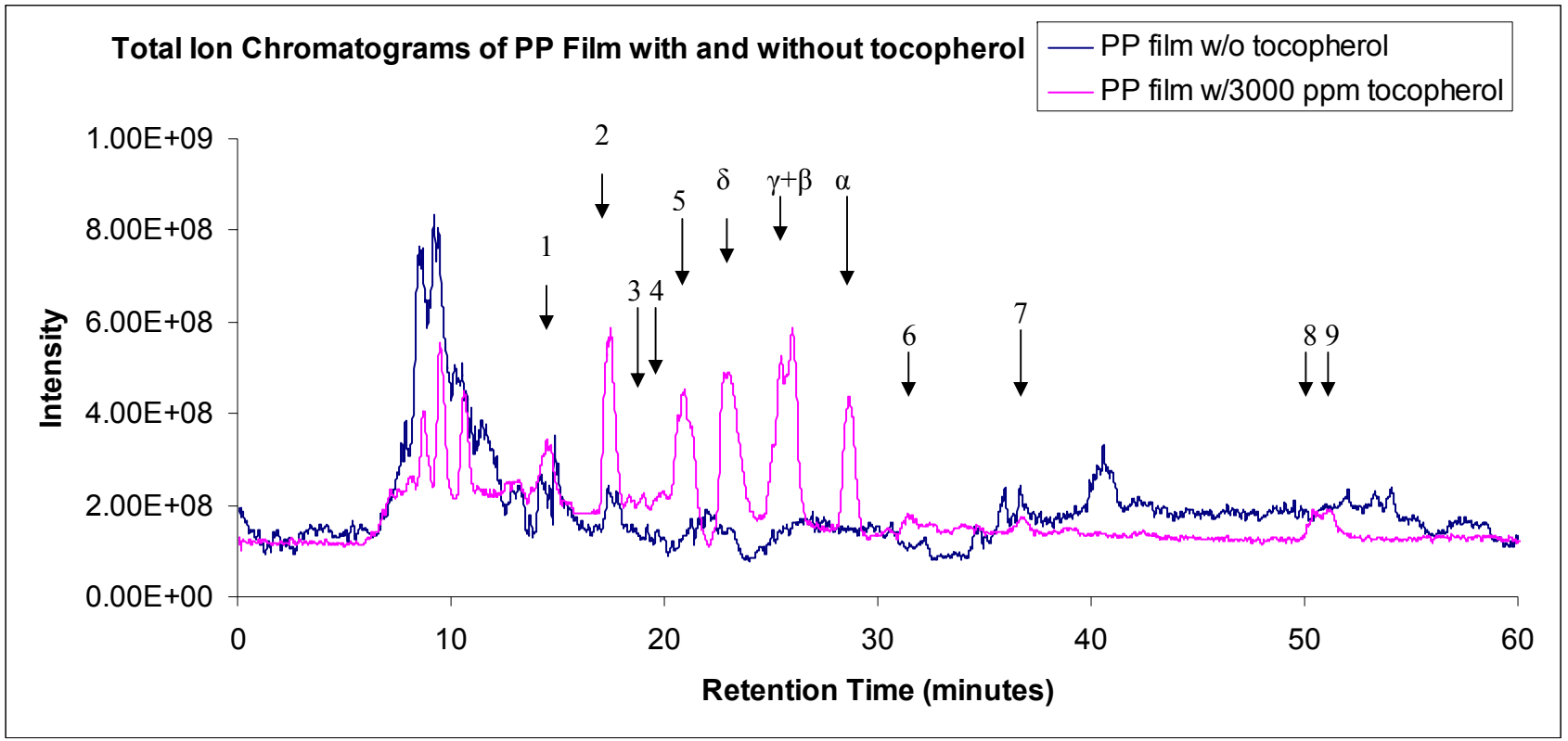
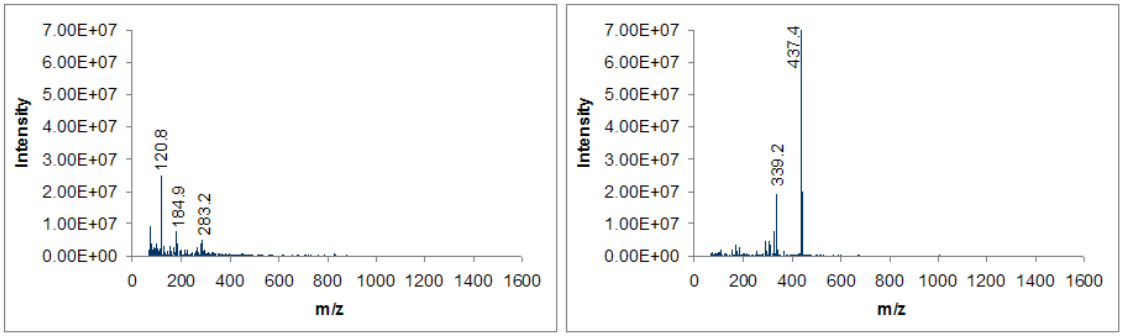


Figure 85 - Total Ion chromatogram for PP film with and without tocopherol.

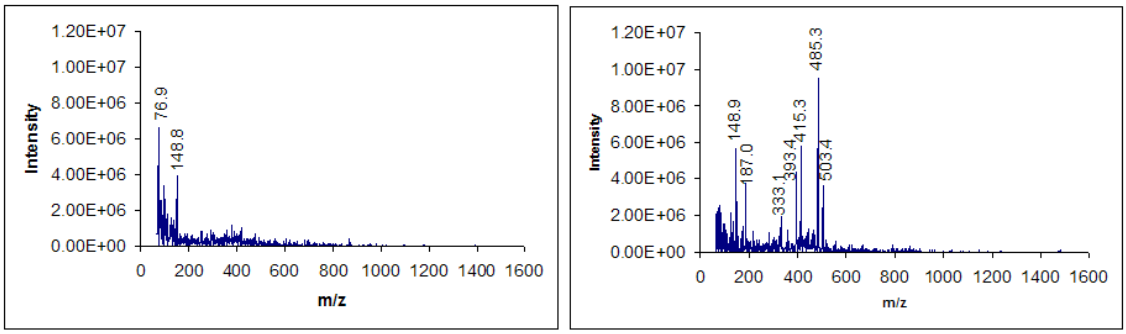
In LDPE films, peaks not corresponding to any found in the tocopherol or polymer standard are observed at 13.7, 17.0, 19.6, 29.4, 35.3 and 41.3 minutes, designated peaks 1, 2, 3, 4, 5 and 6 respectively in Figure 83. Mass spectra for these peaks are shown in Figure 86 alongside spectra for the polymer control eluting at the same time. As the mass spectra for the tocopherol containing peaks show ions that are not found in the polymer control, these products must arise from the tocopherol.

LDPE Polymer Control **Tocopherol Containing LDPE Film**

Peak 1 – 13.7 minutes



Peak 2 – 17.0 minutes



Peak 3 – 19.6 minutes

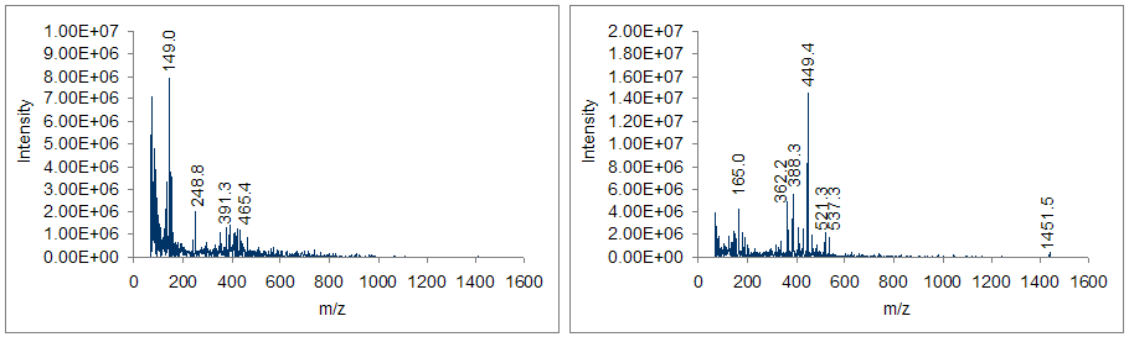
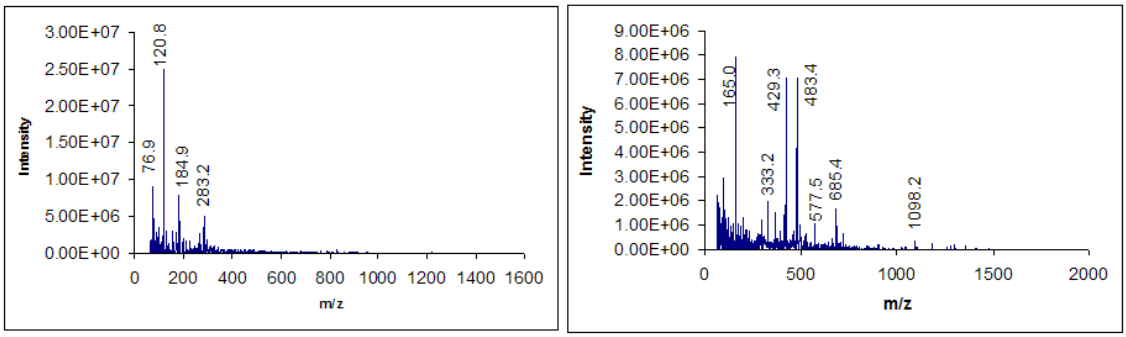


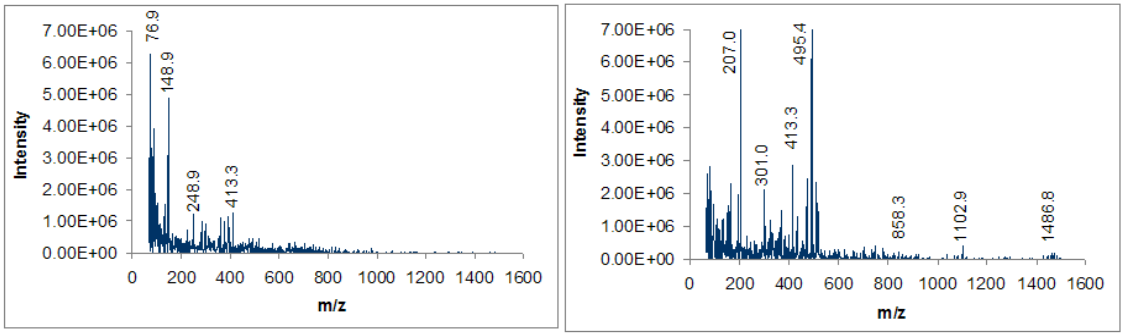
Figure 86 - Comparison of mass spectra from matching retention times in LDPE polymer controls and films containing tocopherol.

LDPE Polymer Control **Tocopherol Containing LDPE Film**

Peak 4 – 29.4 minutes



Peak 5 – 35.4 minutes



Peak 6 – 41.3 minutes

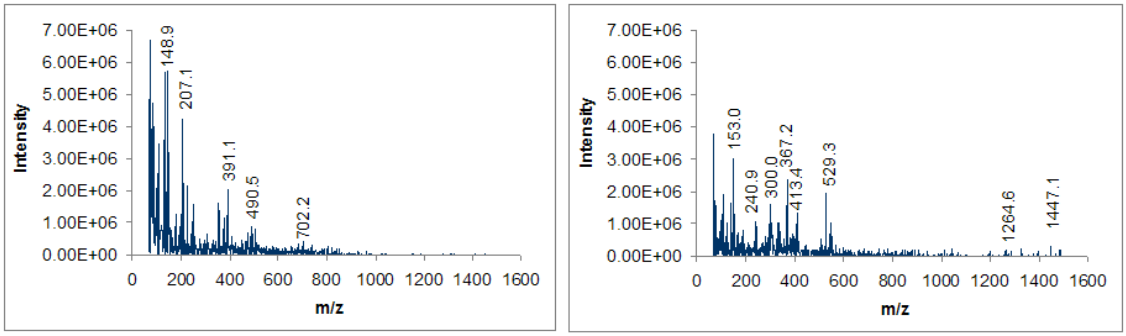
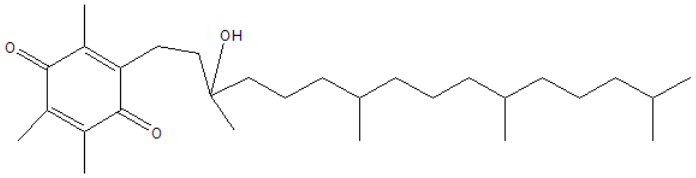


Figure 86 (continued) - Comparison of mass spectra from matching retention times in LDPE polymer controls and films containing tocopherol.

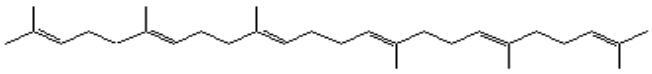
Tentative identification is proposed for peaks 2, 3 and 5, as α-tocopherol quinone, squalene, and α-tocopherol spirodimer respectively. These identifications are based on the mass fragmentation pattern observed in the mass spectra for these compounds. It is proposed that potassium salts present in trace amounts lead to the formation of the [M+39] ions for these compounds.

The structure for α-tocopherol quinone is shown in Figure 87. The structure for squalene is shown in Figure 88. The structure of the α-spirodimer is shown in Figure 89, along with the structure of the fragments observed in peak 5. The long retention time for the spirodimer is to be expected, as it has double the molecular weight of the tocopherol monomer.



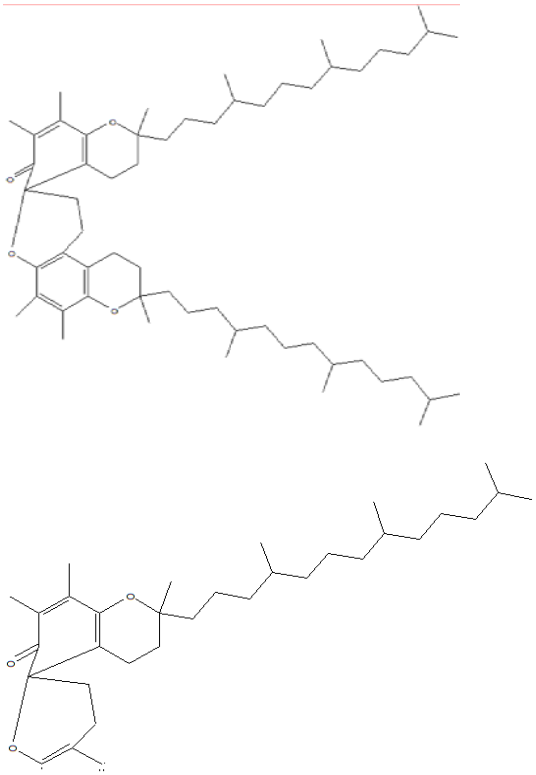
MW 446
[M+39] 485

Figure 87 - Structure of α-tocopherol quinone, proposed as the compound eluting at peak 2 (17.2 minutes).



MW 410
[M+39] 449

Figure 88 - Structure of squalene, proposed as the compound eluting at peak 3.



Parent Molecule
MW 856.7

Fragment
MW 495.8

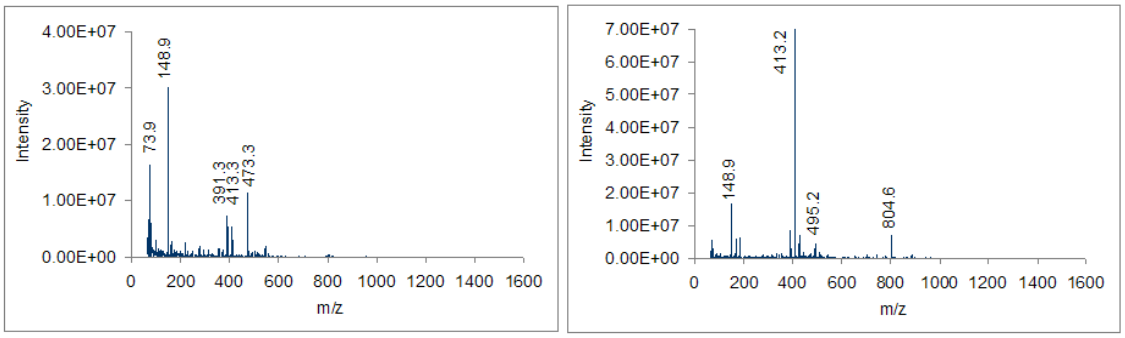
Figure 89 - Structure of α-tocopherol spirodimer, tentative identification of the compound eluting in peak 5.

In PP films, new peaks are observed at 14.7, 17.5, 19.1, 20.2, 21.0, 31.8, 37.0, 50.8, and 51.3 minutes (designated peaks 1, 2, 3, 4, 5, 6, 7, 8 and 9 in Figure 85). Mass spectra for these peaks are shown alongside those of the corresponding time from the control film sample in Figure 90.

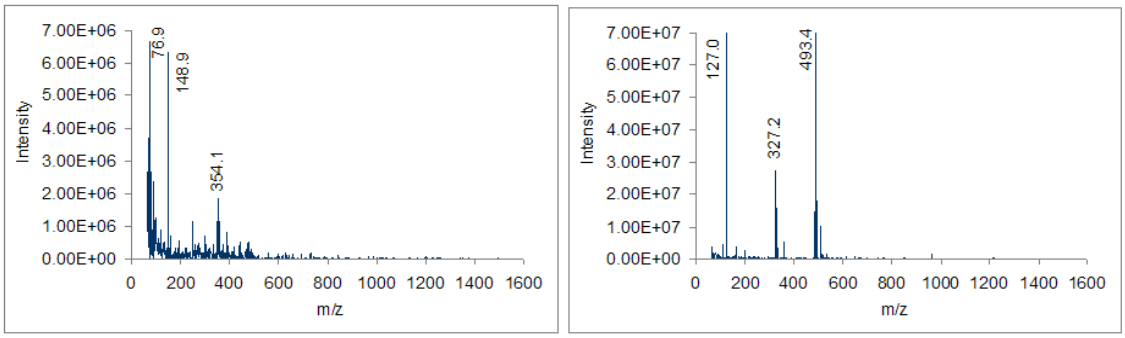
As no peaks are observed in blended LDPE:PP films that are not present in either the 100% LDPE film or 100% PP film, it is assumed that no additional products are formed under this condition. The peaks eluting at 13.7, 15.8, 18.2, 29.4, 34.8 and 48.9 minutes, labeled 1, 2, 3, 4, 5 and 6, correspond to PP peaks 1, 2, 4, 6, 7 and 8+9.

PP Polymer Control **Tocopherol Containing PP Film**

Peak 1 – 14.7 minutes



Peak 2 – 17.5 minutes



Peak 3 – 19.1 minutes

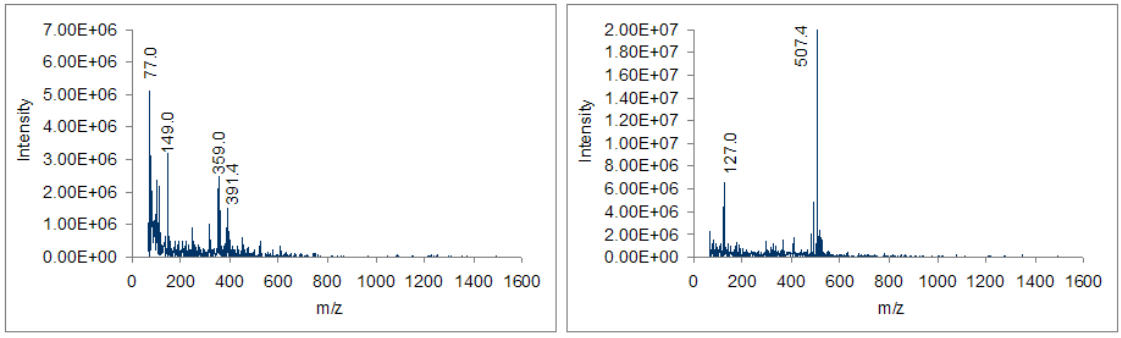
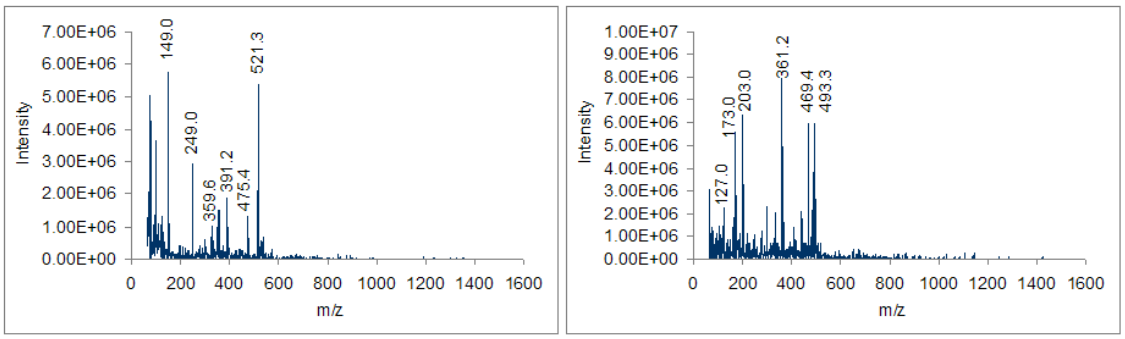


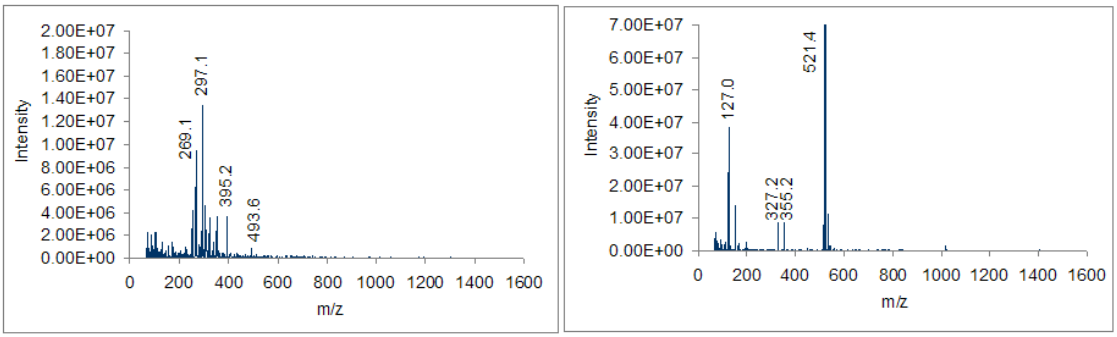
Figure 90 - Comparison of mass spectra from matching retention times in PP polymer controls and films containing tocopherol.

PP Polymer Control **Tocopherol Containing PP Film**

Peak 4 – 20.2 minutes



Peak 5 – 21.0 minutes



Peak 6 – 31.8 minutes

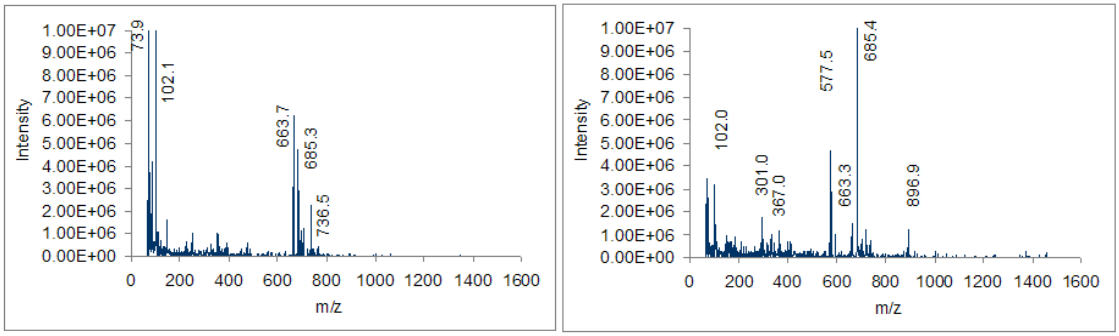
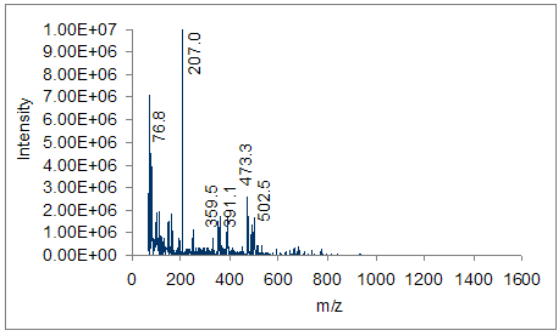
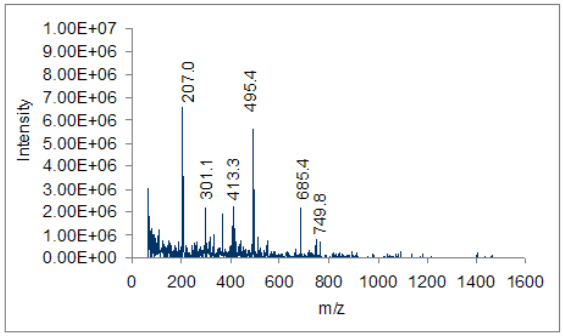


Figure 90 (continued) - Comparison of mass spectra from matching retention times in PP polymer controls and films containing tocopherol.

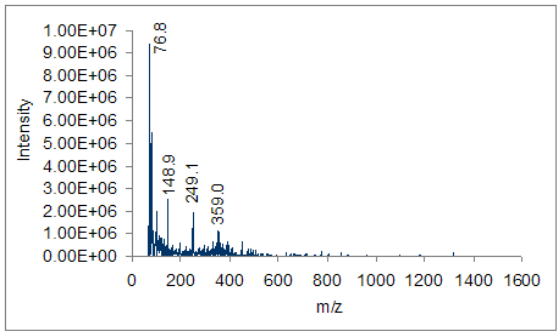
PP Polymer Control **Tocopherol Containing PP Film**

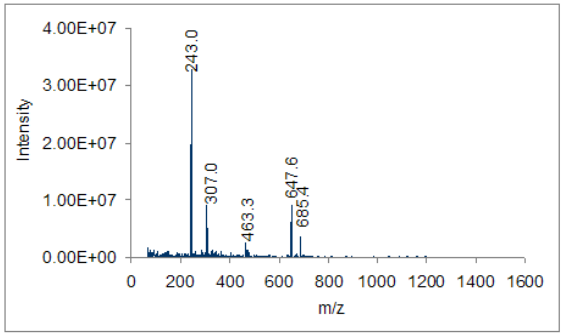
Peak 7 – 37.0 minutes



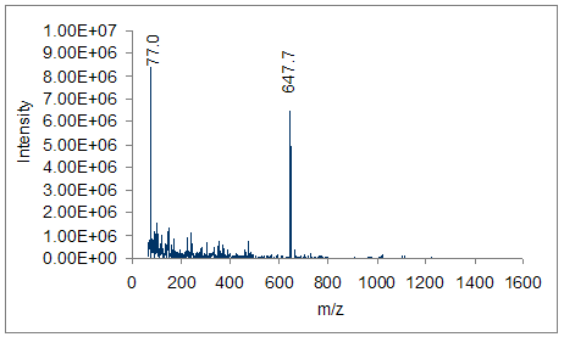


Peak 8 – 50.8 minutes





Peak 9 – 51.3 minutes



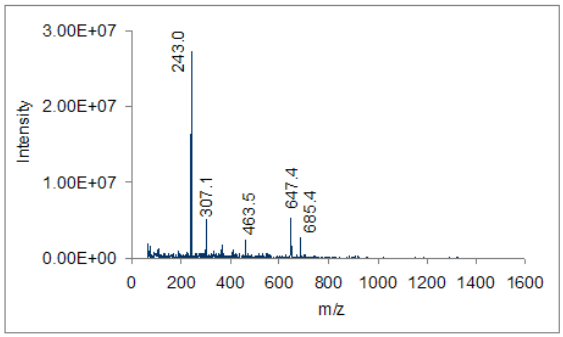
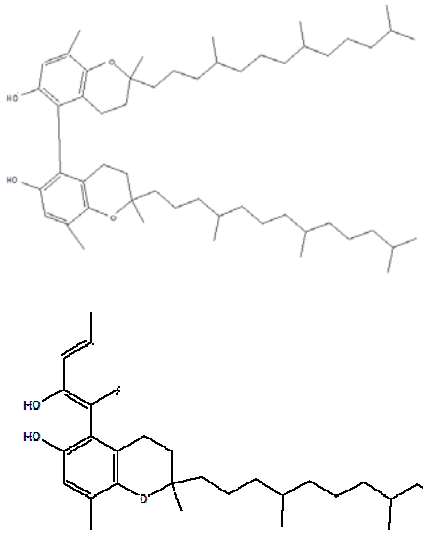


Figure 90 (continued) - Comparison of mass spectra from matching retention times in PP polymer controls and films containing tocopherol.

In PP films, an unusual thing is observed in peak 1(14.7 minutes). The mass fragments observed in this peak correspond well to a dimer of δ-tocopherol (Figure 91), but this seems unlikely, as such a dimer would have a high molecular weight, and therefore not elute ahead of the tocopherol monomers. It is possible that dimerization affects the polarity of the molecule such that it is able to elute at this position.



Parent Molecule
MW 803.3

Fragment
MW 494.4

Figure 91 - Structure of δ-tocopherol dimer, which has mass fragmentation pattern possible for PP peak 1.

PP peak 6 is likely to be the same as LDPE peak 4, due to the relative position, and presence of similar mass fragments (577, 685). This compound has not yet been identified. Similarly, PP peak 7 appears to be the same α-spirodimer as LDPE peak 5. PP peaks 8 and 9 have mass spectra consistent with Irgafos 168, with the two peaks likely corresponding to the unoxidized and oxidized molecule.

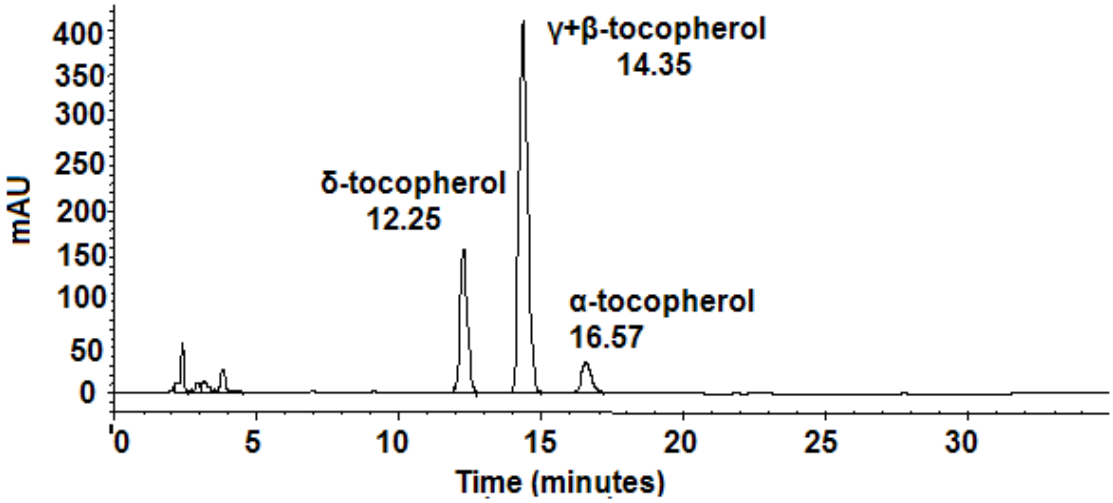


Figure 92 - UV Chromatogram at 225 nm for tocopherol containing LDPE film 86.1S4.

Figure 92 shows the UV chromatogram at 225 nm for tocopherol containing LDPE film run at Chemic Laboratories. Tocopherol has been separated into the δ, γ+β, and α isomers, with retention times as indicated. Three small peaks with retention times of 2.38, 2.91 and 3.80 are also observed near the front of the chromatogram.

Figure 93 shows the (+)APCI TIC for the same run of the tocopherol containing LDPE film. The tocopherol isomers are still visible at the same retention times as in the UV chromatogram, and several new peaks are detected, with retention times as marked on the figure. All new peaks elute before the α-tocopherol peak at 16.63 minutes, which makes it highly unlikely that any of these peaks is a dimer or trimer of tocopherol, as these would have much higher molecular weights, and therefore higher retention times. Figure 94 shows the (-)APCI TIC for the same film. Under this ionization condition, only the tocopherol peaks are observed. This indicates that the tocopherol degradation products and polymer-based compounds are not easily deprotonated under APCI conditions.

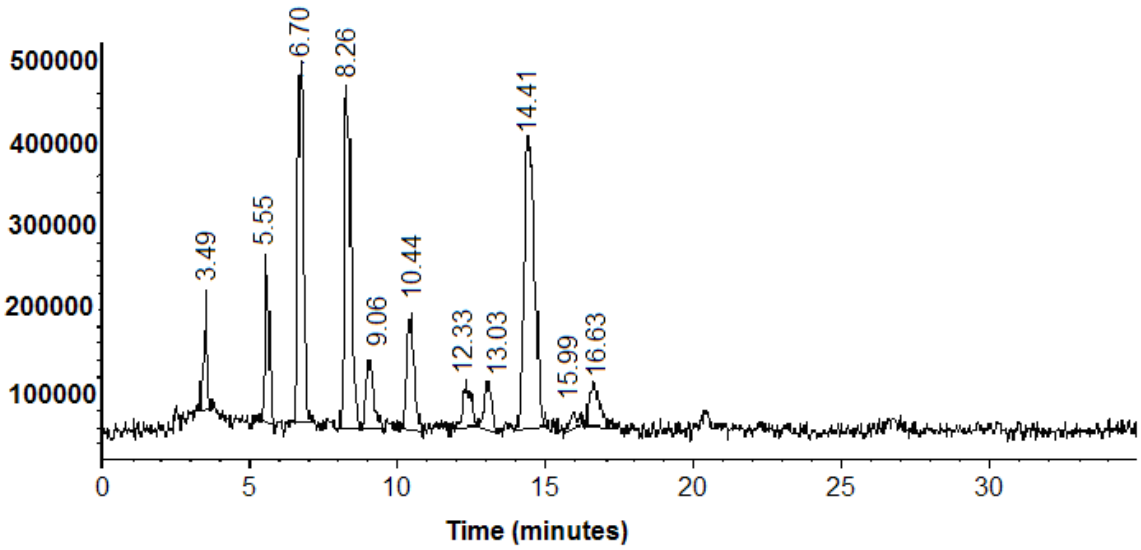


Figure 93 - Total ion chromatogram for tocopherol containing LDPE film 86.1S4, under +APCI condition.

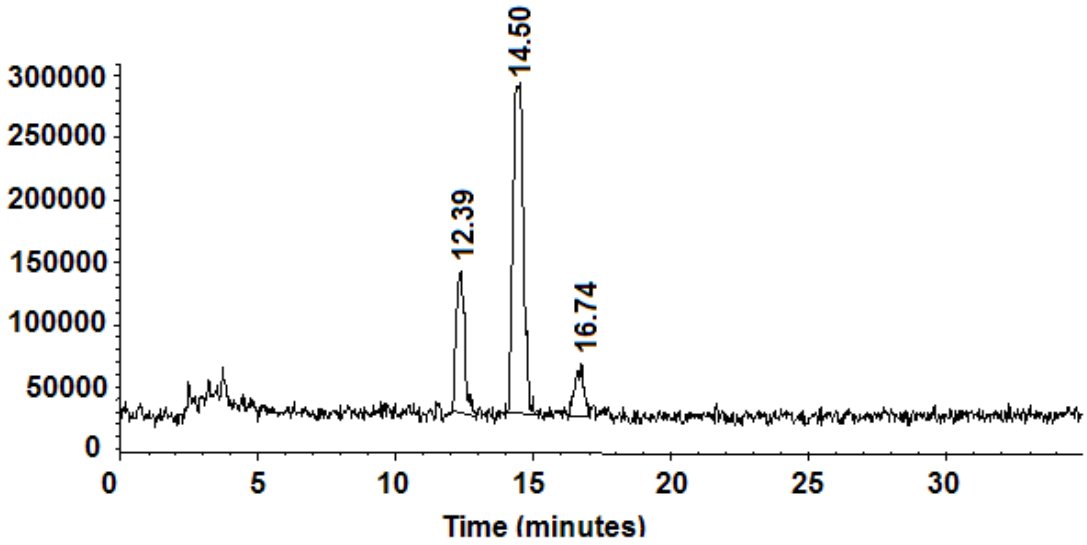


Figure 94 - Total ion chromatogram for tocopherol containing LDPE film 86.1S4, under -APCI condition.

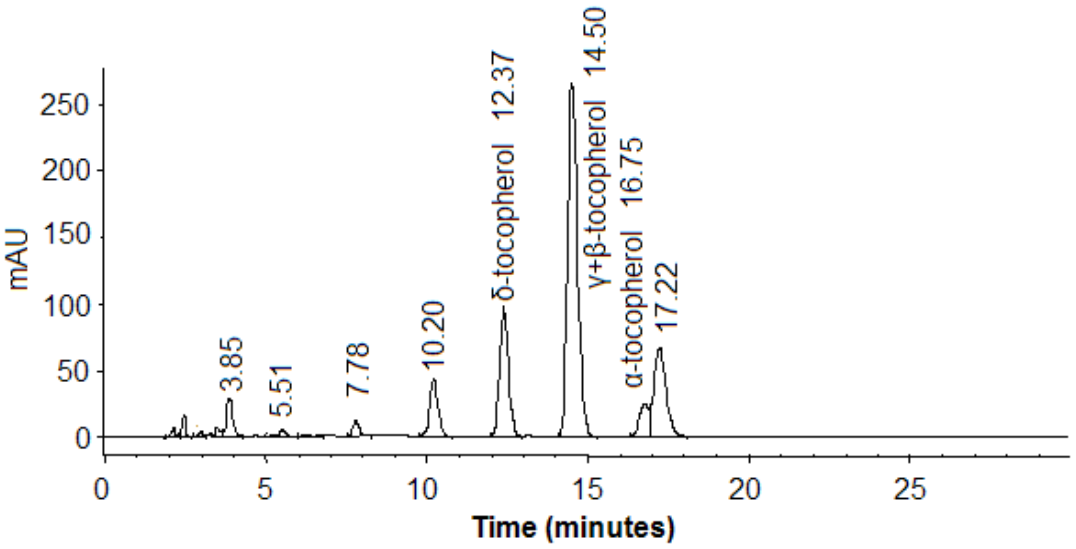


Figure 95 - UV chromatogram for tocopherol containing PP film 86.5S4 at 225 nm.

Figure 95 shows the UV chromatogram for the tocopherol-containing PP film 86.5S4 as detected at 225 nm. In contrast to the LDPE film, not only are the tocopherol peaks detected, but 5 additional peaks can be observed at this wavelength. These include four peaks eluting ahead of the tocopherols, and one peak nearly overlapping that of α-tocopherol.

Figures 96 and 97 show the (+)APCI and (-)APCI total ion chromatograms for the tocopherol-containing PP film 86.5S4. In Figure 96, 8 peaks are seen in addition to the tocopherol peaks, with retention times as shown in the figure. In contrast to the LDPE film, additional peaks are also observed under (-)APCI, as shown in Figure 97. These peaks correspond to the peaks visible at 225 nm on the UV chromatogram.

Mass spectra for the tocopherol isomers are as expected under (-)APCI conditions, showing only a single peak at (M-1), along with the expected small isotopic peaks at +1 intervals. Mass spectra for the non-tocopherol peaks are shown in the Figures 98-103.

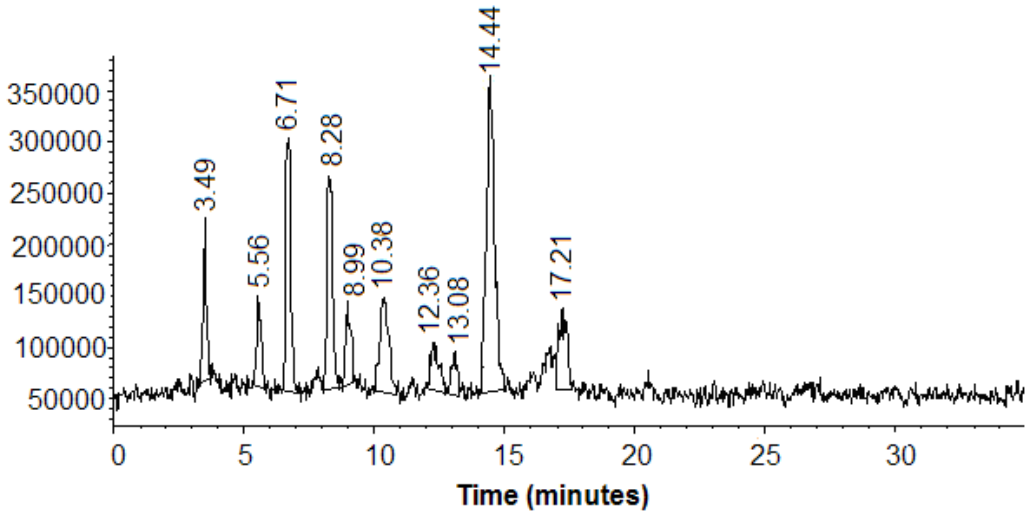


Figure 96 - TIC for tocopherol containing PP film 86.5S4 under (+)APCI conditions.

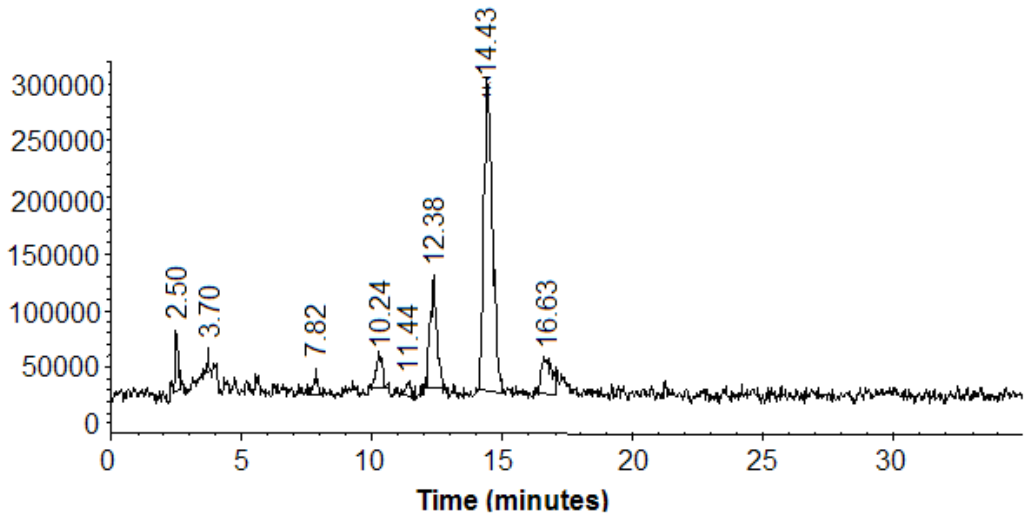


Figure 97 - TIC for tocopherol containing PP film under (-)APCI conditions.

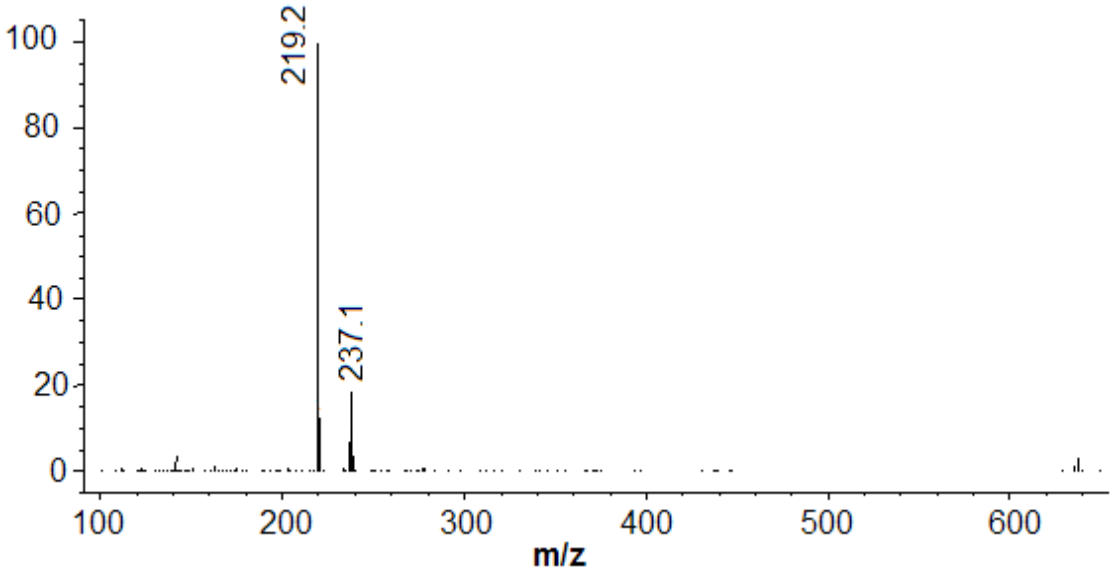


Figure 98 - Mass spectrum for 3.5 minute peak from tocopherol containing PP film sample, under (+)APCI.

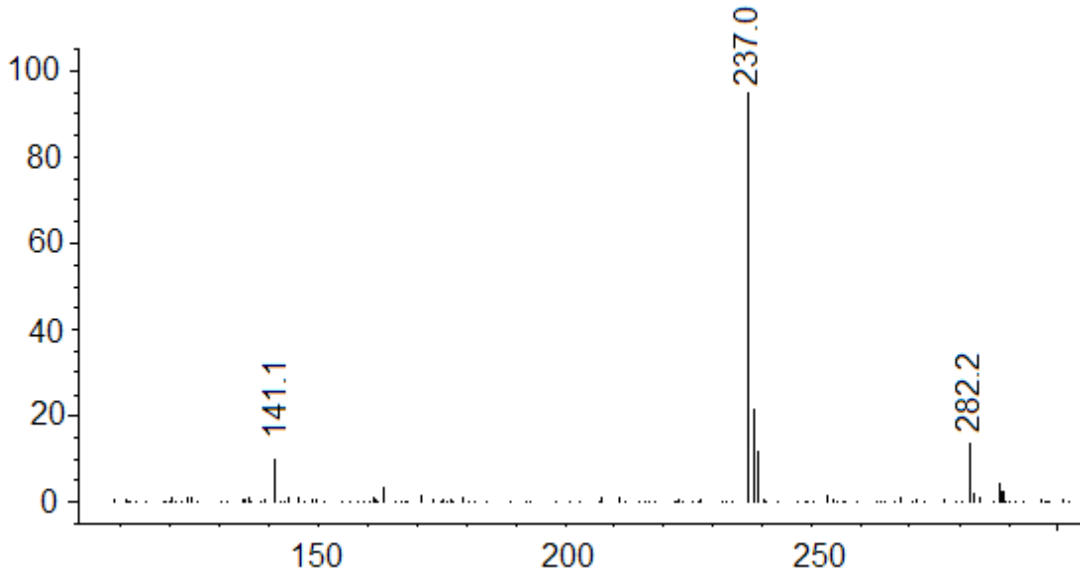


Figure 99 - Mass spectrum for 5.5 minute peak from tocopherol containing PP film sample, under (+)APCI.

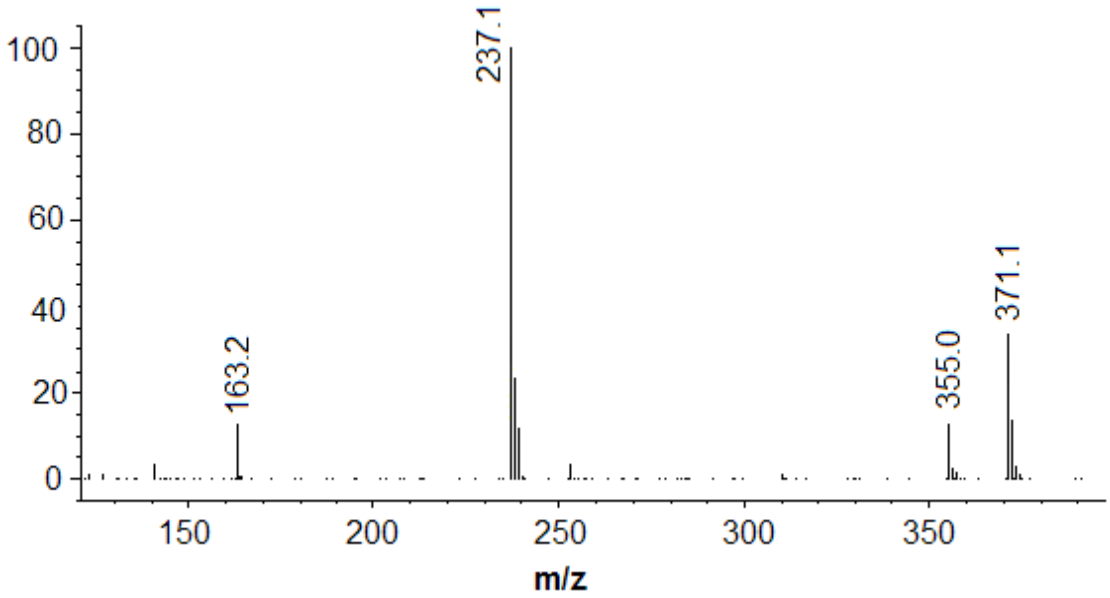


Figure 100 - Mass spectrum for 6.7 minute peak from tocopherol containing PP film sample, under (+)APCI.

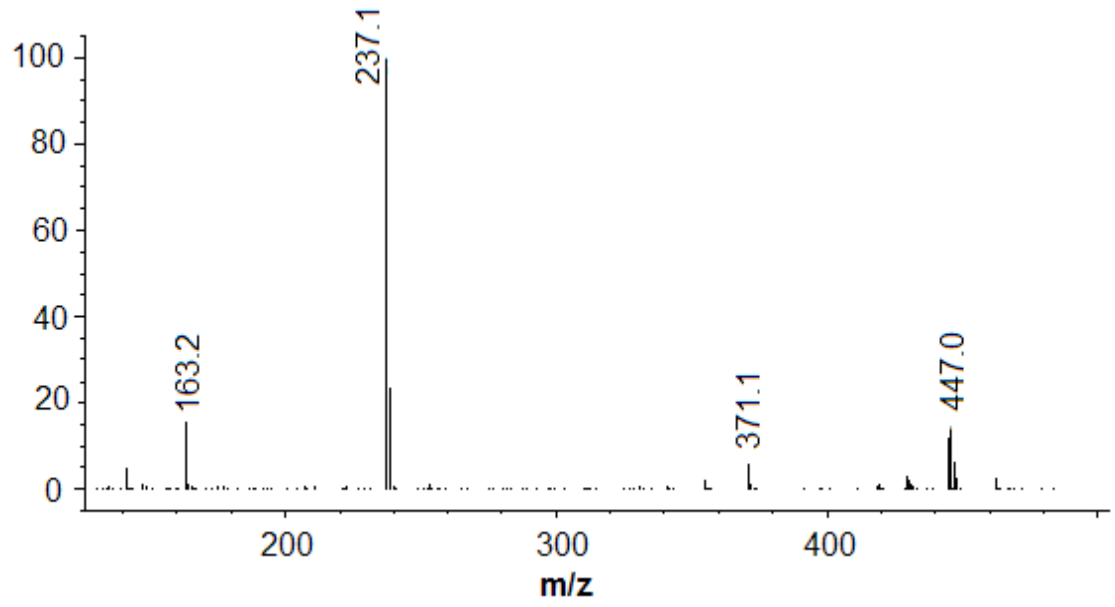


Figure 101 - Mass spectrum for 8.3 minute peak from tocopherol containing PP film sample, under (+)APCI.

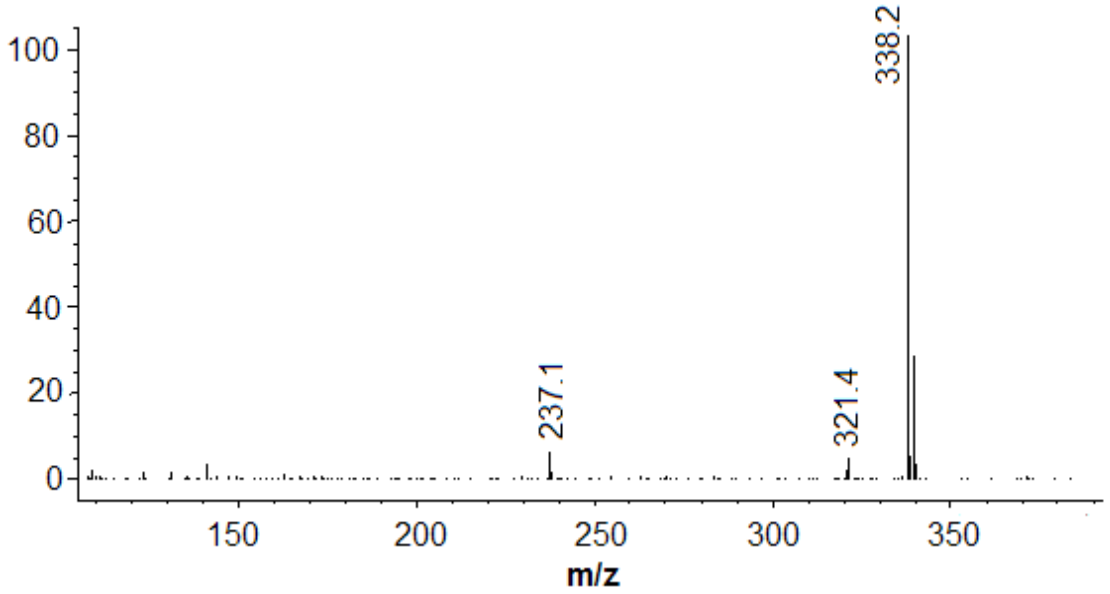


Figure 102 - Mass spectrum for 8.9 minute peak from tocopherol containing PP film sample, under (+)APCI.

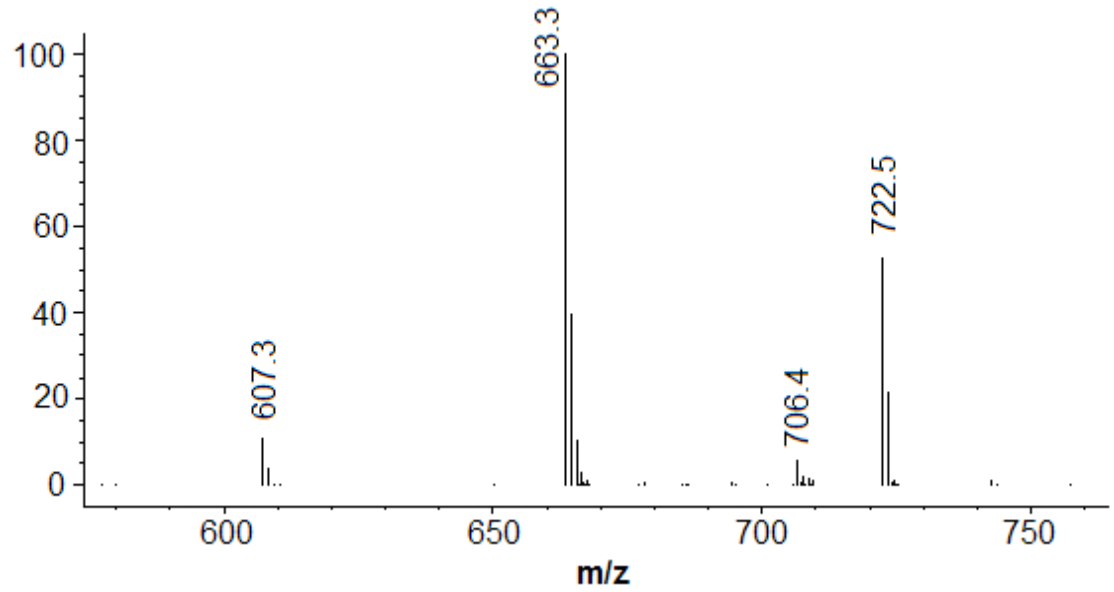


Figure 103 - Mass spectrum for 17.2 minute peak from tocopherol containing PP film sample, under (+)APCI.

Based on these spectra, identification is proposed for several of these compounds. The peak eluting at 5.5 minutes (Figure 99) is believed to be the polymer additive oleamide, the structure of which is presented in Figure 104. Oleamide (9-octadecenamide) is a polymer slip agent, used to provide surface lubrication to films. It has a molecular weight of 281.3, which corresponds exactly to the (M+1) ion seen in Figure 91. McDonald et al [107] observed oleamide leaching from PP labware, which is of concern in microbiological research, as it is an enzyme inhibitor.

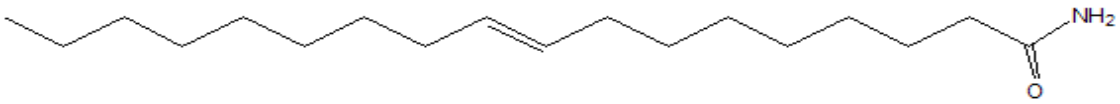


Figure 104 - Structure of oleamide (MW 281.3), proposed to be the compound eluting at 5.5 minutes in PP film chromatogram.

The peak observed at 8.9 minutes (Figure 102) is believed to be the polymer additive erucamide, the structure of which is shown in Figure 105. Erucamide is used as a polymer slip agent, as well as an anti-static agent, germicide and insecticide [108]. It is a fatty acid amide, which can be enzymatically produced from natural oils, including olive oil [109]. It has a molecular weight of 337.6, corresponding exactly to the (M+1) observed in Figure 102.

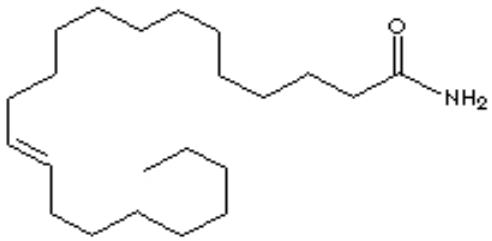
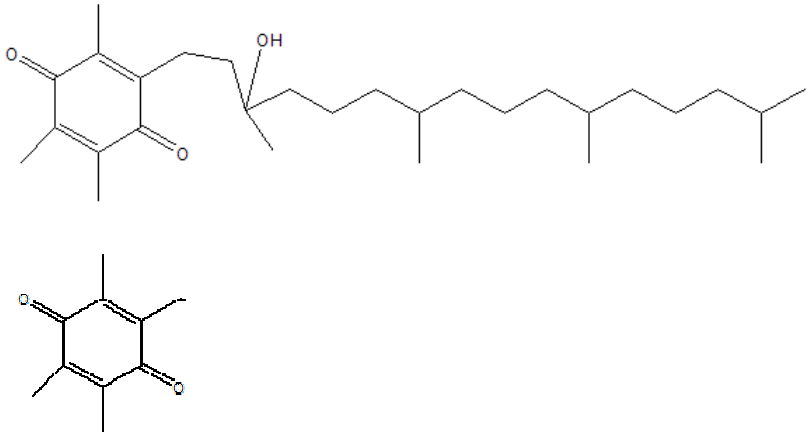


Figure 105 - Structure of erucamide (MW 337.6), proposed as the compound eluting at 8.9 minutes in PP film sample.

The peak at 8.3 minutes (Figure 101) is consistent with the tocopherol degradation product α-tocoquinone, which has the structure shown in Figure 106. Identification is based on the mass spectrum, as it matches both the molecular ion and a possible fragment. It is not observed by UV detection at 225 nm, as the wavelength of maximum absorbance for α-tocoquinone is 268 nm [101]. This is an expected product of tocopherol degradation in the presence of oxygen. As the α-tocoquinone is fully methylated, it is considered a non-arylating quinone, which were determined by Wang et al [110] to have little cytotoxic effect, and should be considered non-toxic.

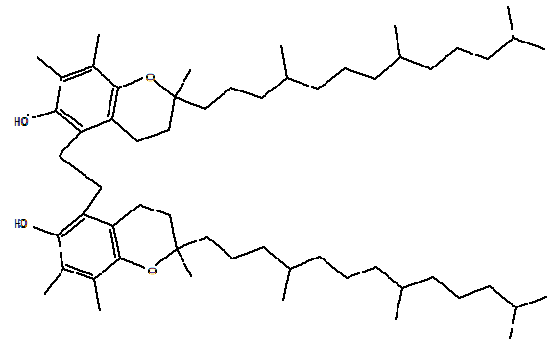


MW – 446.4

MW – 163.2

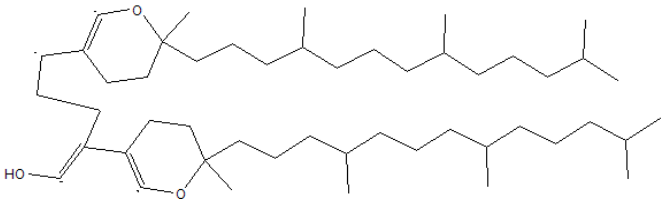
Figure 106 - Structures of α-tocoquinone, proposed as the compound eluting at 8.3 minutes in PP film sample, with fragment corresponding to fragment ion detected.

The peak at 17.2 minutes (Figure 103) has a mass spectrum that is consistent with the dihydroxy dimer of α-tocopherol, the structure of which is shown in Figure 107 along with the fragments corresponding to the peaks observed in the spectrum. The molecular (M+1) peak of 859.8 was not observed, as the large size of the molecule may make it less stable under ionization conditions. This dimer is an expected degradation product of tocopherol, formed by the coupling of two α-tocopheroxyl radicals.

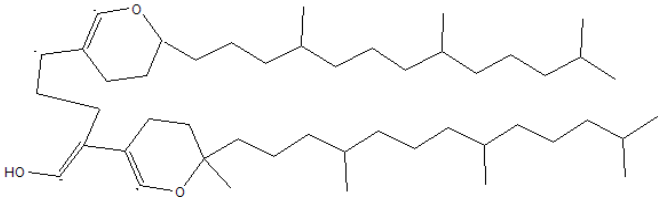


Parent Molecule
MW 858.8

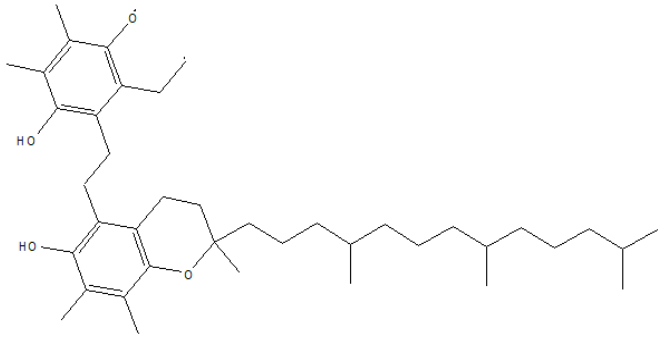
Fragments



MW 721.7



MW 706.6



MW 606.5

Figure 107 - Structures of α-tocopherol dihydroxy dimer, along with fragments as detected in peak at 17.2 minutes from PP film sample.

Effect of processing conditions

Figures 108, 109 and 110 show the variation in products observed between different processing conditions for LDPE, PP and LDPE:PP blend films respectively. In all samples, the tocopherol isomers remain the most prevalent compound detected. In LDPE films, some variation is seen between processing method for peaks 3, 4 and 6. Interestingly, the blended film shows far less variability between processing conditions than either the 100% LDPE or 100% PP film. These results align well with the data observed from the tocopherol recovery studies, as LDPE films show the highest level of tocopherol relative to other products, and PP films show the lowest.

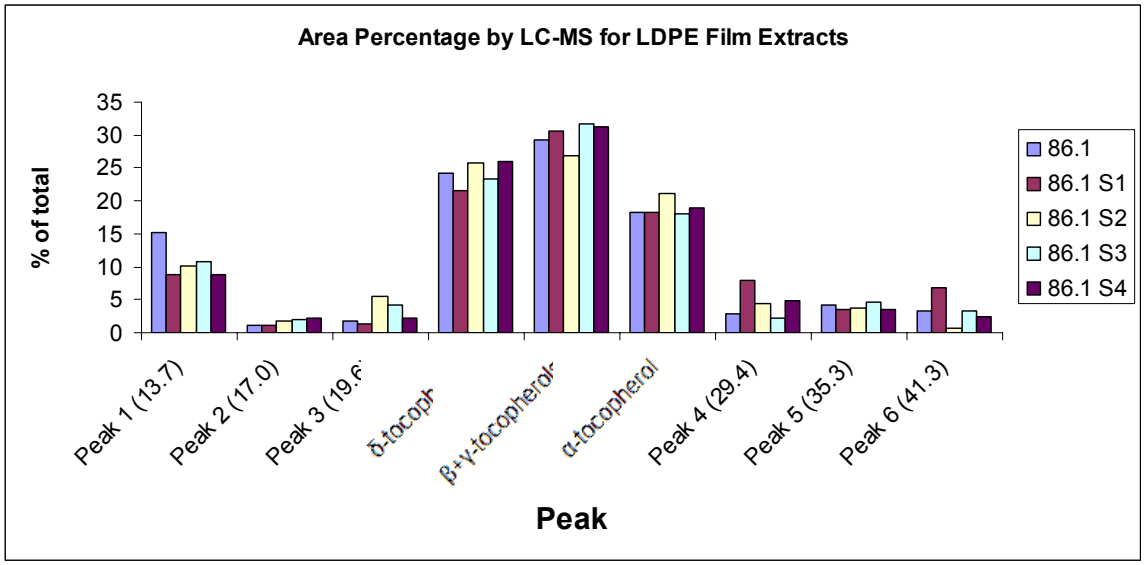


Figure 108 - Relative size of each peak from LDPE films, expressed as percent of the sum of all peaks.

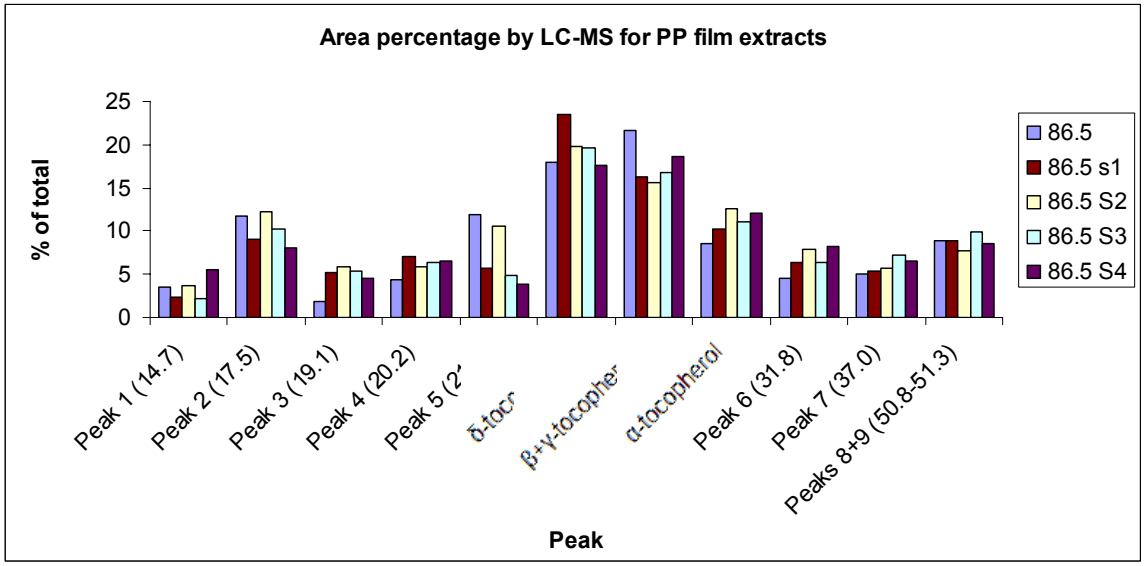


Figure 109 - Relative size of each peak from PP films, expressed as percent of the sum of all peaks.

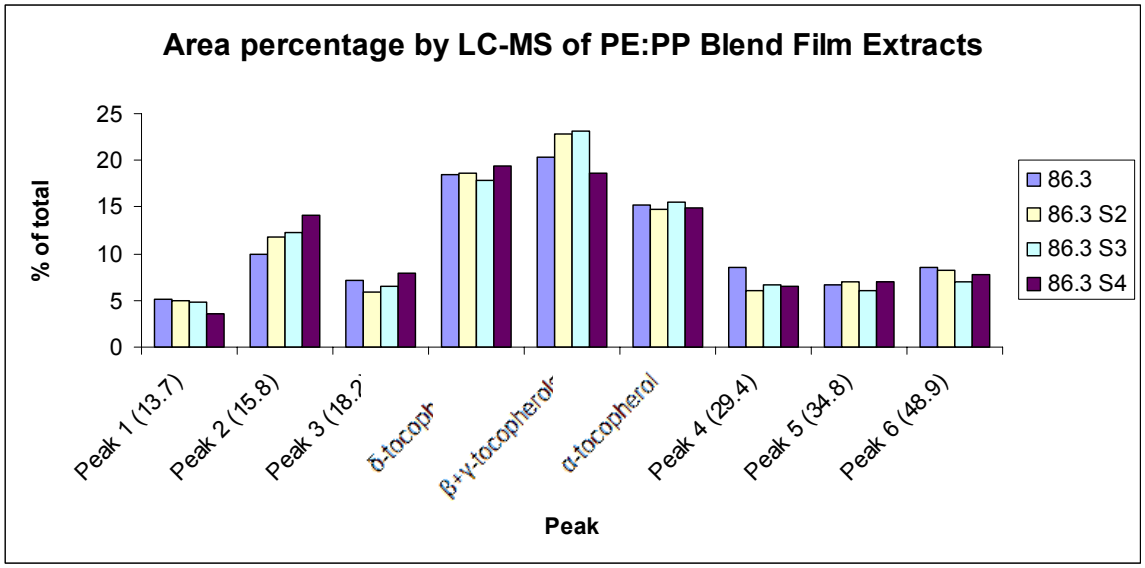


Figure 110 - Relative size of each peak from LDPE:PP blend films, expressed as percent of the sum of all peaks.

Conclusions

Film properties

The data received from Pliant shows that addition of 3000 ppm tocopherol to polymer films has much less effect on the final film properties of density, clarity, gas transmission rate and seal strength than the choice of polymer resin. Variations that do occur with tocopherol addition are minimal, and occasionally even have different effects in different film polymers. Since the ultimate intended use for these controlled release packaging materials is in the food contact layer of a laminated multi-layer package, issues of gas permeation and clarity are minimized by the inclusion of opaque barrier layers with very low gas permeability characteristics. Potential problems with seal strength remain, and it is hoped that this does not prove a stumbling block for the development of a practical tocopherol containing controlled release package.

Interpreting these results is complicated by the lack of control films without tocopherol for each of the production conditions. Without these controls (not produced by Pliant), it is not possible to determine whether the variations in film physical properties observed are due to the addition of tocopherol or to the changes in temperature and screw speed.

Tocopherol recovery

Data regarding tocopherol recovery from the tocopherol containing films shows that nearly all of the tocopherol added is extractable from the final film. The addition of PP to the films reduces the recovery somewhat, but as greater than 90% is recoverable from even the 100% PP films, they should still prove sufficient for controlled release antioxidant packaging applications. Processing conditions has a slight effect on the recovery, with PP films being the most affected by changes in processing conditions. The greatest loss of tocopherol recovery was seen in films extruded under conditions of low temperature and high screw rotation rate. This may be because at lower temperature, the polymer melt is more viscous, leading to greater shear forces, which lead to the formation of polymer radicals, which degrade and possibly bind the tocopherol to the polymer.

Should the fate of the non-extractable tocopherol become a concern, future experiments could be performed to locate it. If the tocopherol has not formed radical coupling products with the polymer, and is simply unextractable because it is trapped in the crystalline structure of the polymer matrix, a more complete picture could be gained by dissolving the film in a solvent such as toluene. This would free any trapped tocopherol, and show that it was not covalently bound to the polymer. If 100% of the tocopherol still cannot be accounted for, it my have bound to the polymer. In this case, it will be impossible to extract, and would have to be detected in another manner. Graciano-

Verdugo et al developed a method that uses FTIR to measure the concentration of α-tocopherol in LDPE films [111], which could possibly be adapted to this purpose.

This loss in PP containing films may also be explained by the formation of α-tocopherol dimers, as observed in the PP film sample analyzed at Chemic Laboratories. The peak believed to be the α-tocopherol dimer eluting immediately after α-tocopherol at 17.2 minutes is not observed in the LDPE film sample. This likely means that tocopherol is being degraded to a greater extent in the PP films, leading to the formation of the dimers with absorbance maxima at wavelengths other than that of α-tocopherol, leaving fewer tocopherol molecules to be detected by UV spectrophotometry.

Antioxidant activity

The results from the ABTS assay show that antioxidant activity of the material extracted from the films equals or exceeds that of the mixed tocopherols alone. This verifies that tocopherol antioxidant efficacy is not lost in the film extrusion process and that tocopherols released from films retain full capability to protect foods contained in CRP packages. That there was no significant difference in antioxidant activity observed for the extracts from films of different polymer composition or processing indicates that polymer selection and processing variables do not affect antioxidant activity.

Tocopherol Degradation Products

GC-MS analysis of the films shows that few volatile degradation products of tocopherol are formed during film production. Some volatile compounds are observed from the polymer materials themselves, but as these materials have already been approved for use

as food contact materials by the FDA, they should not pose a concern for CRP applications. While there were differences observed between polymer materials, corresponding to different additives and polymer breakdown patterns, no differences were detected in the tocopherol between different polymers. No difference was observed between films produced under different extrusion conditions, beyond the slight variation in the actual amount of tocopherol present in the extracts.

It is difficult to identify structures of non-volatile degradation products of tocopherols from the LC-MS analyses performed at Rutgers Plant Science due to the poor reproducibility of retention times and the highly fragmented spectra generated by use of excessively high ESI powers. Compounds tentatively identified as α-tocoquinone, squalene and the α-spirodimer were observed, but further analysis will be required to confirm these assignments.

Results from the LC-MS analyses performed at Chemic Laboratories indicate that tocopherol degrades to form α-tocoquinone and α-tocopherol dihydroxydimer during the production of PP films. The quinone is also produced in the LDPE films, but the dihydroxydimer is not.

In order to confirm the identities of the compounds detected, including both tocopherol degradation products and polymer additives, standards of these materials must be obtained and subjected to analysis by the same methods as the polymer film extract samples to determine retention times and mass spectra for each standard, which can then be compared to the unknowns in the samples for final confirmation of their identity. Standards will also provide standard curves for accurate quantification of the products.

The majority of products observed are well-known safe degradation products of tocopherol. Additional studies must be performed to determine the amount of these compounds actually released into a more realistic food simulant, since this work used a powerful solvent to effect complete extraction of tocopherols.

Overall Conclusions

Based on the results presented here, cast films can be produced containing high (3000 ppm) levels of mixed tocopherols. These films retain necessary physical properties to permit their use as food packaging materials, without compromising the structural integrity of the package. Even under severe processing conditions of high temperature and high shear, physical properties are retained. It would serve especially well if the tocopherol-containing polymer film is incorporated as a food contact layer in a multilayer package that includes a high gas barrier material, such as aluminum, to compensate for the high gas permeability of polyolefin films, which is unrelated to tocopherol inclusion.

Based on antioxidant assay data, it can be conclude that the compounds present in the tocopherol-containing polymer films retain antioxidant activity. While some slight variation is observed based on polymer composition and processing method, even under worst-case situations, nearly 90% of the activity of unmodified tocopherol is retained. This should be sufficient to prevent lipid oxidation, especially considering the fact that tocopherol and related compounds will migrate into the food product slowly over time, preventing the buildup of high radical loads.

Based on GC-MS and LC-MS analyses, no unknonwn tocopherol degradation products are detected in any of the films. Identification is still pending for some of the non-volatile peaks observed. The identity and origin of these compounds must be resolved before a final recommendation can be made to the FDA for approval of tocopherol-containing controlled release packaging.

Future Work

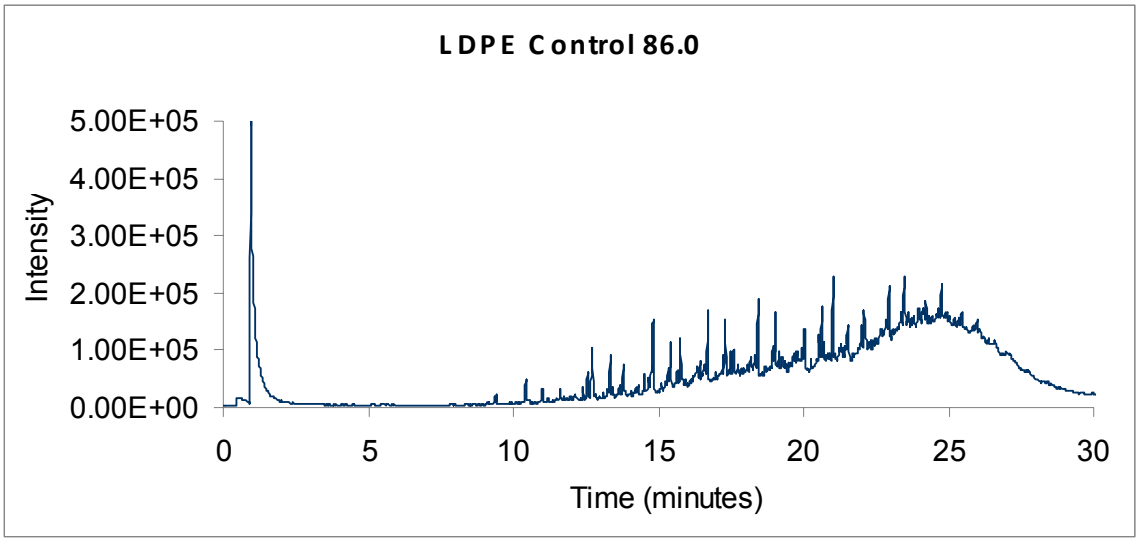
Based on the work presented here, the following future experiments are proposed:

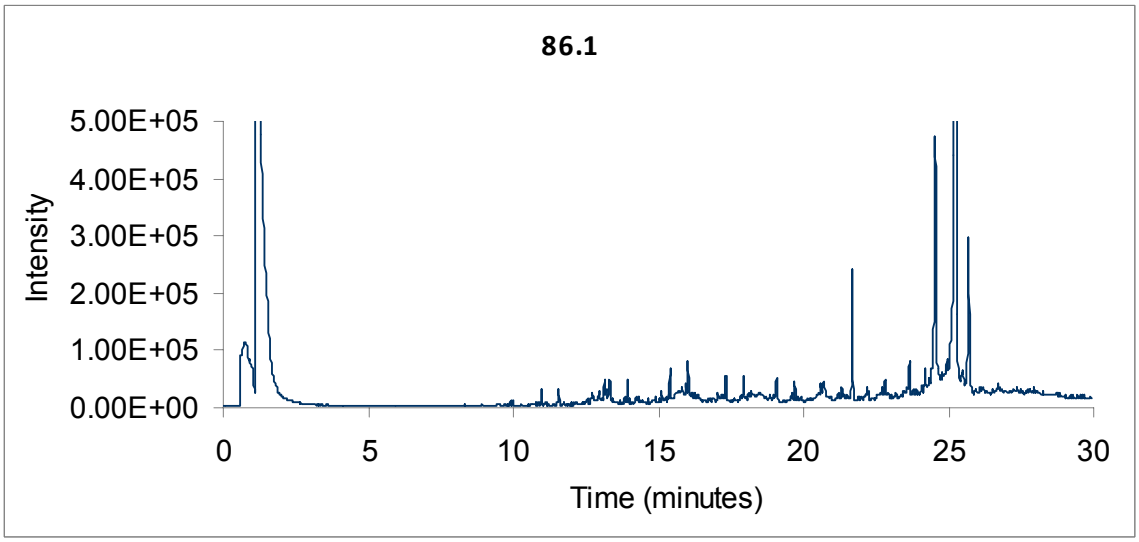
- Production of polymer control films, containing no tocopherol, under the range of severe processing conditions used for the tocopherol-containing films. These films would be analyzed for physical properties, as well as subjected to GC and LC analysis, to more definitively establish the effect of tocopherol inclusion on physical properties, as well as volatile and non-volatile products.
- Perform accelerated aging studies on tocopherol containing CRP films in order to present an additional "worst case" scenario. Films can be stored under high oxygen conditions at elevated temperature to simulate long term storage, and accelerate the degradation of both tocopherol and the polymer.
- Obtain standards of tocopherol oxidation products, such as α-tocoquinone, tocopherol spirodimer and dihydroxydimer, to be analyzed by GC and LC methods. This would allow confirmation of the identities of the compounds proposed to be these tocopherol degradation products, as well as enabling the development of standard curves to quantify the amount of these additives migrating from the film.
- Migration studies using a solvent that is a more accurate food simulant, such as 95% ethanol or Miglyol oil. This would provide a more accurate picture of the polymer additives and tocopherol degradation products that would actually be found in food products stored in contact with the controlled release tocopherol containing film.

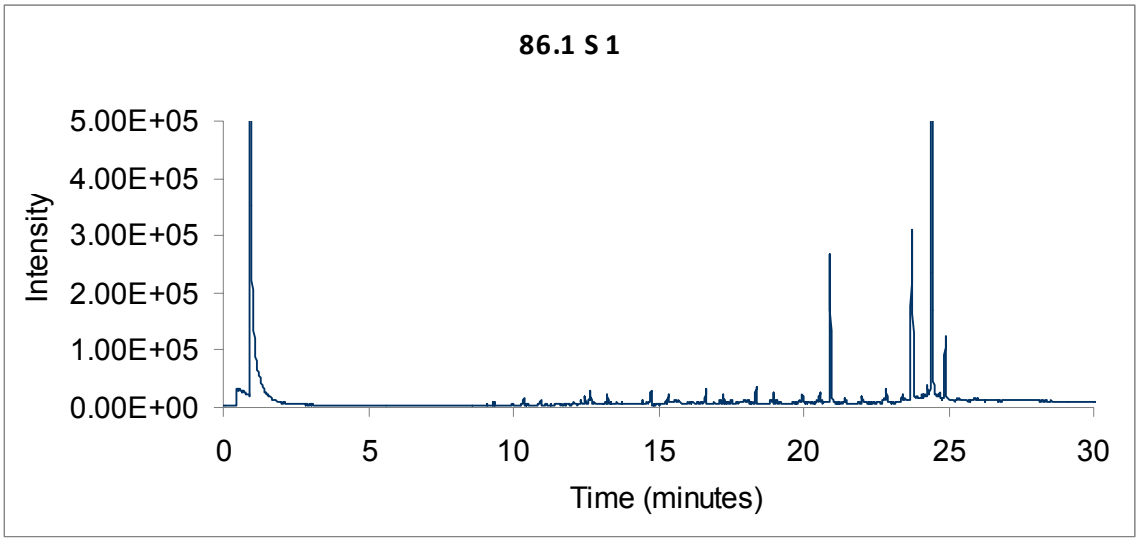
Appendices

GC-MS Chromatograms

LDPE Films



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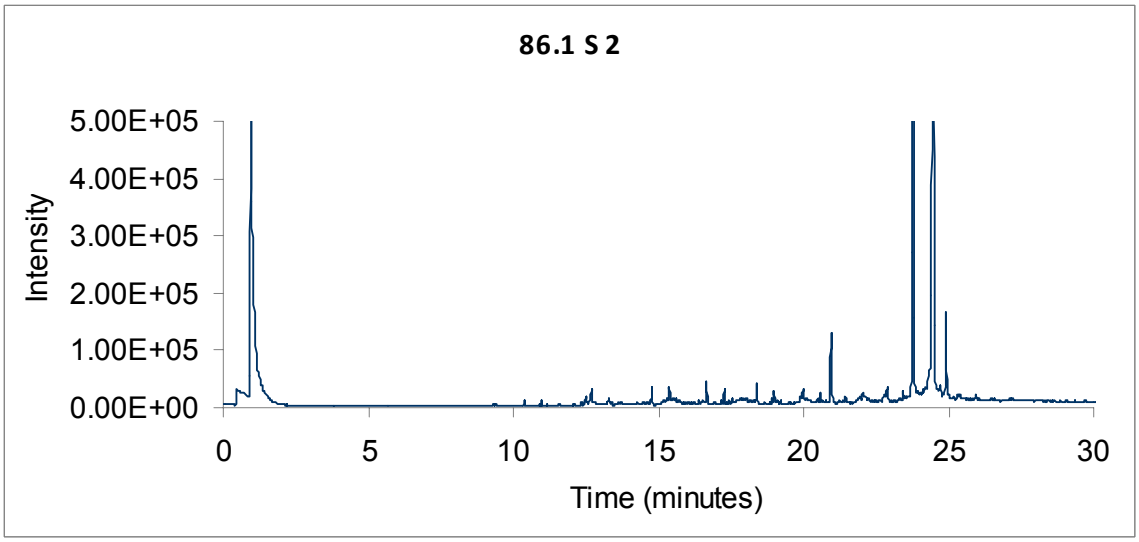
Time (minutes)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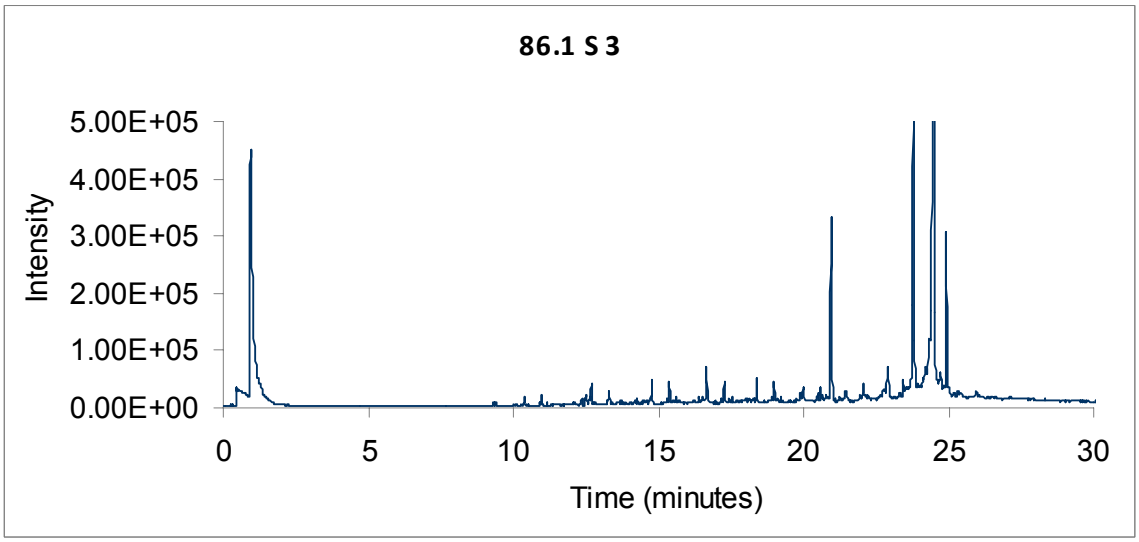
Time (minutes)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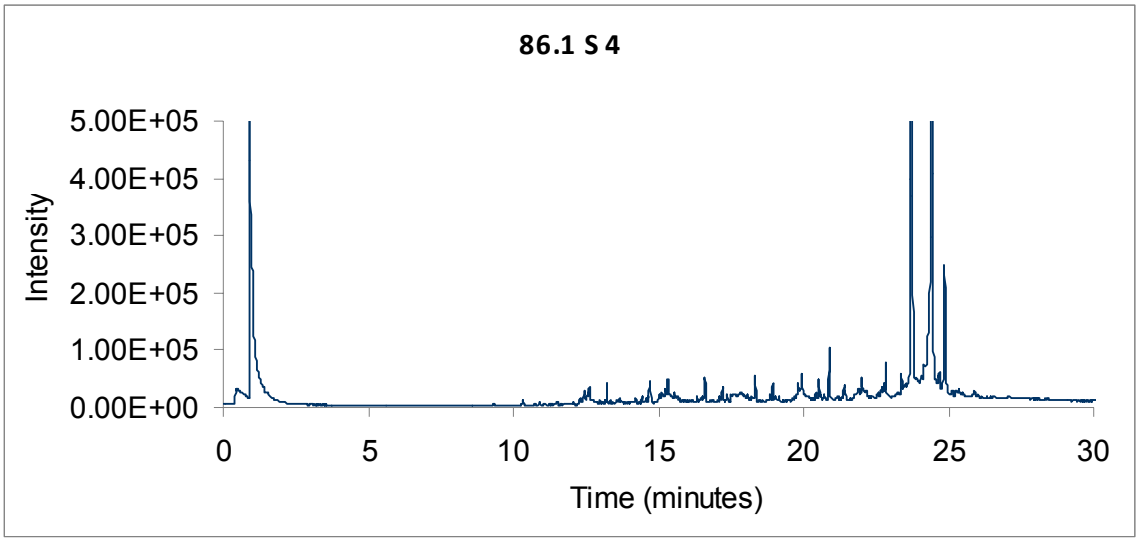
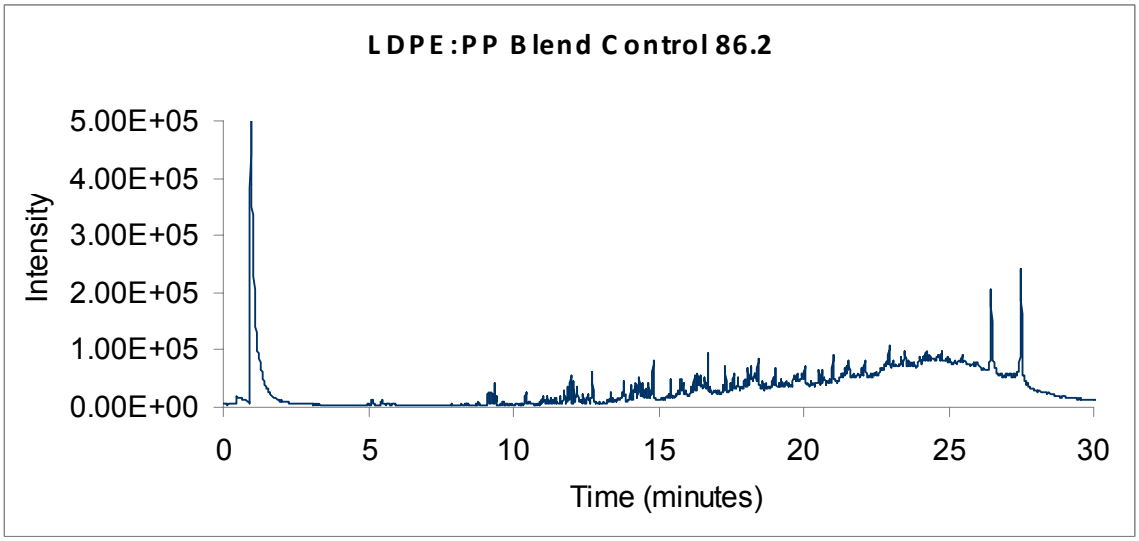
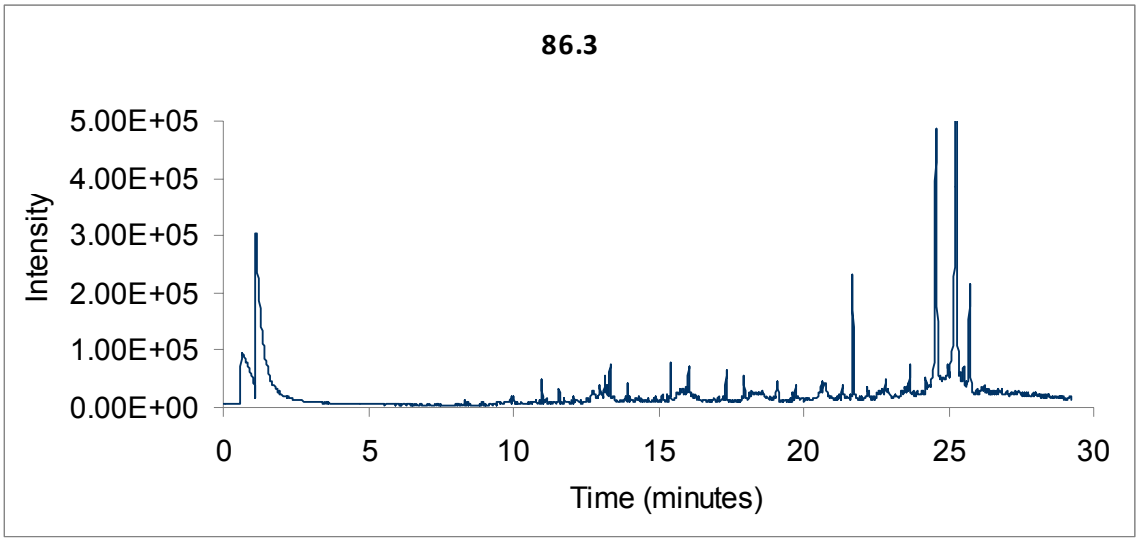
Time (minutes)



LDPE:PP Blend Films

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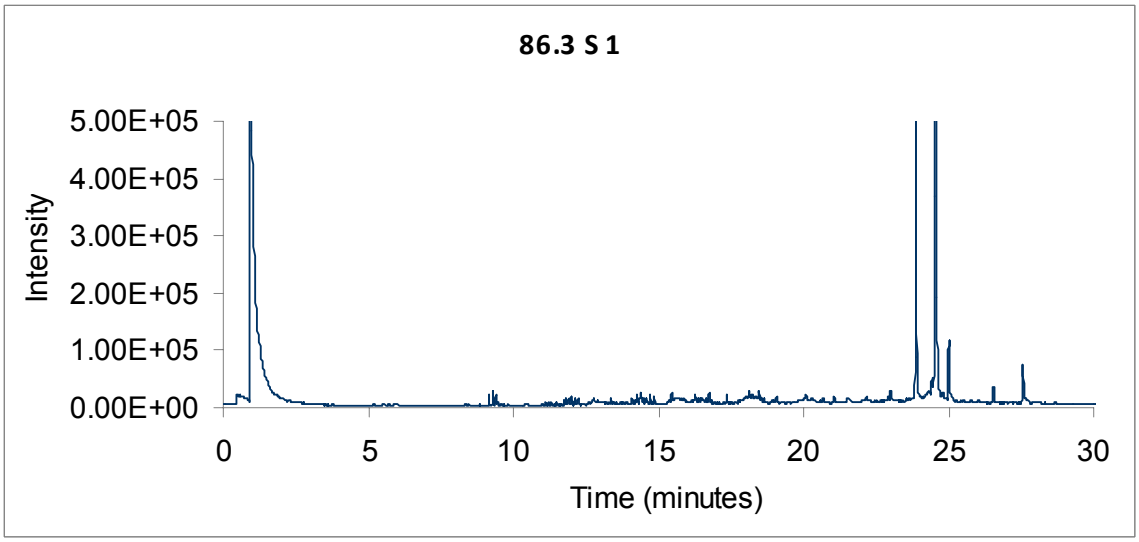
Time (minutes)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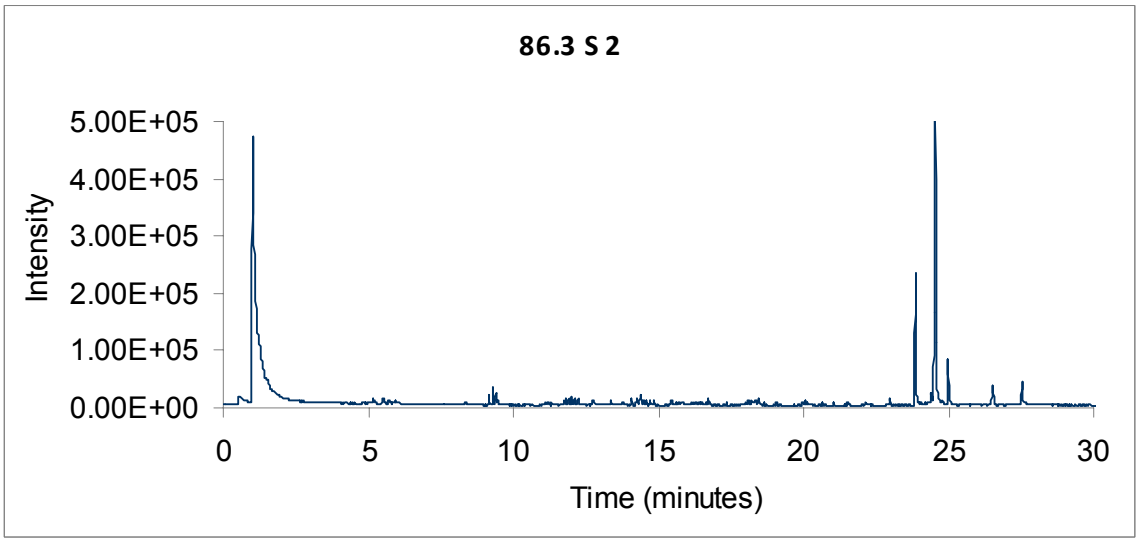
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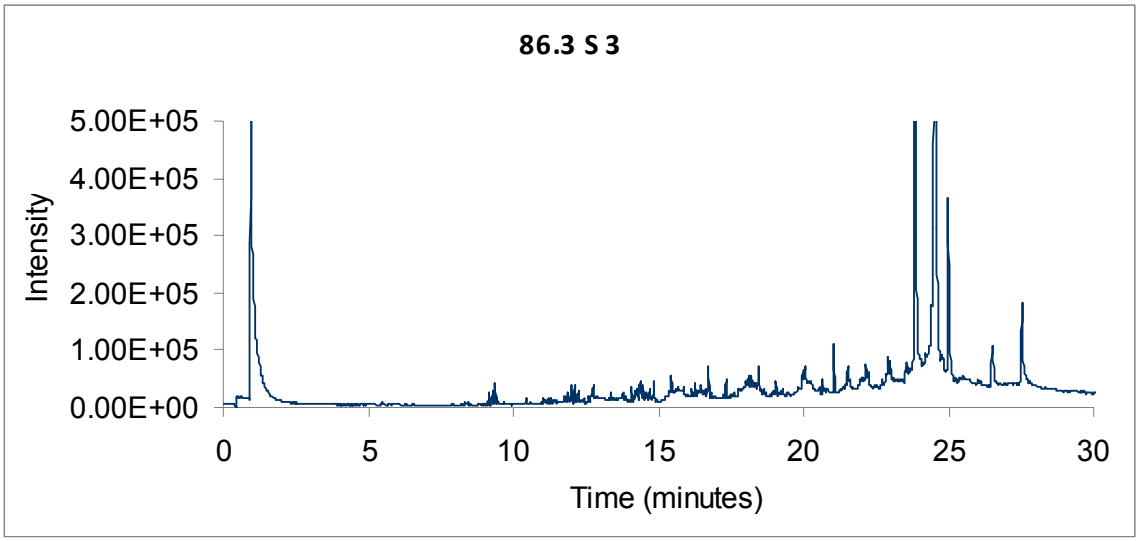
Time (minutes)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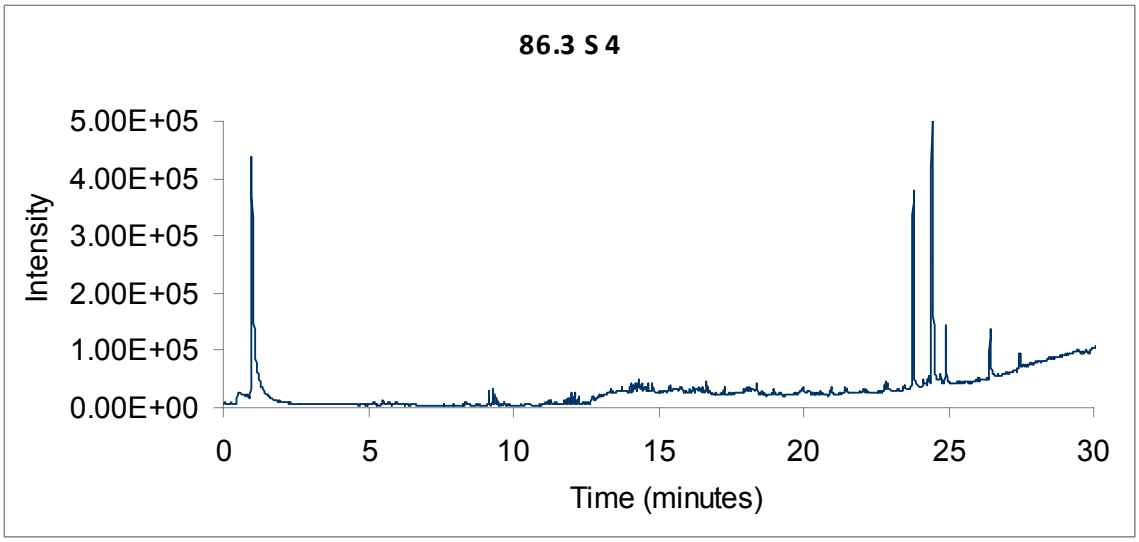
Time (minutes)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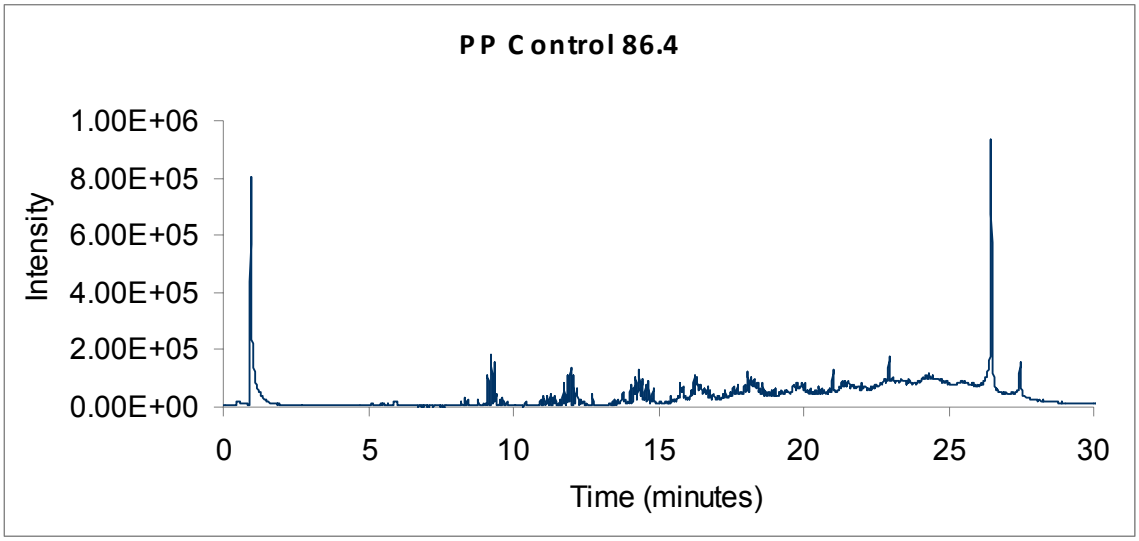
Time (minutes)

PP Films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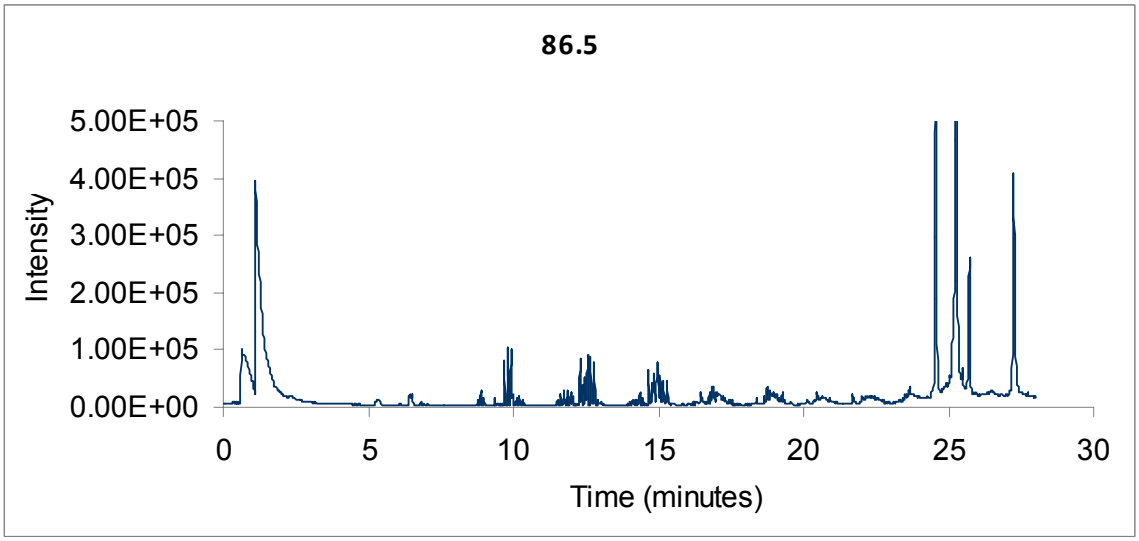
Time (minutes)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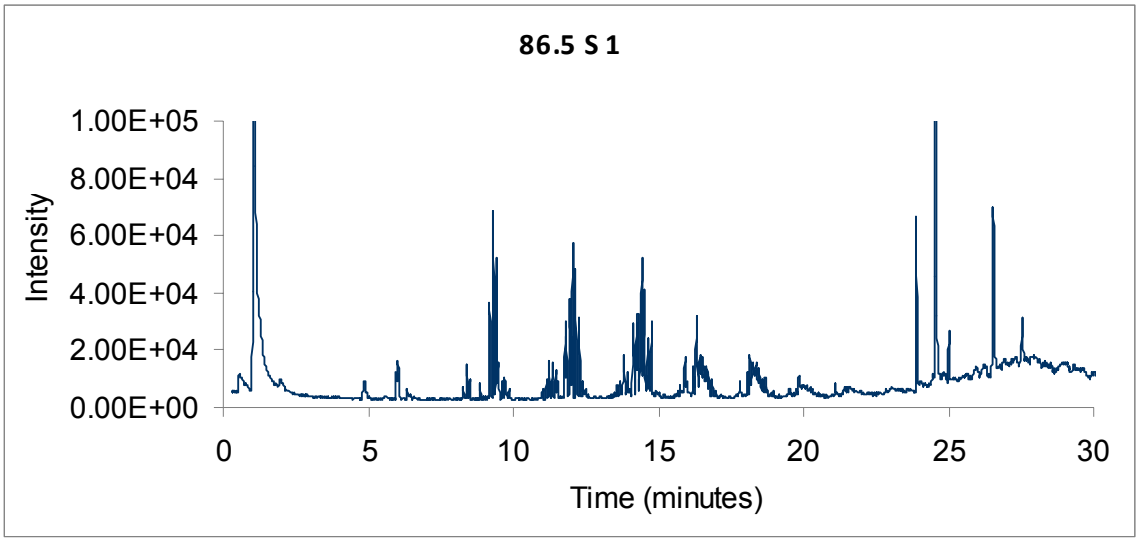
Time (minutes)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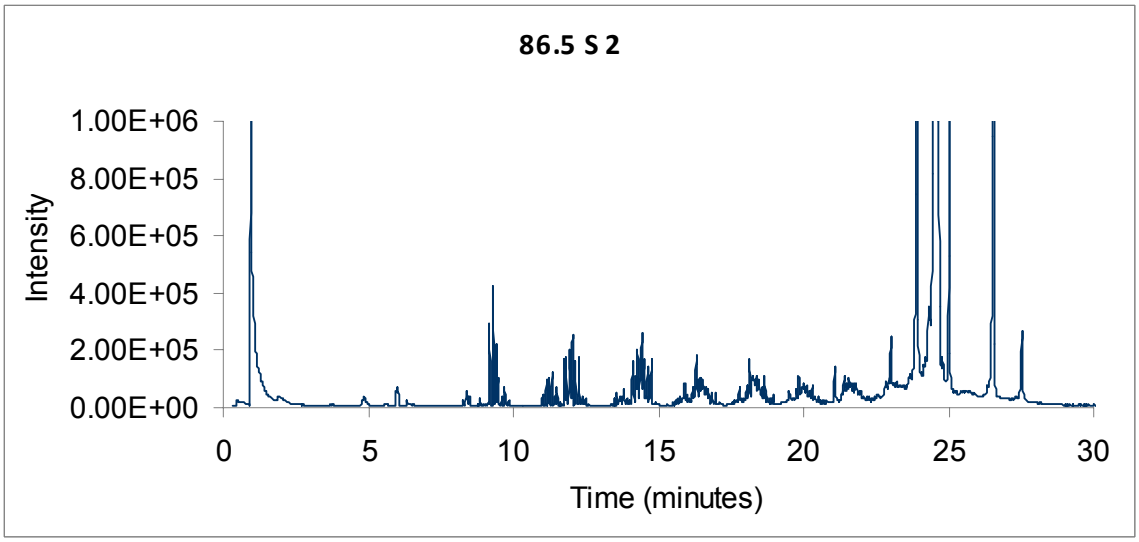
Time (minutes)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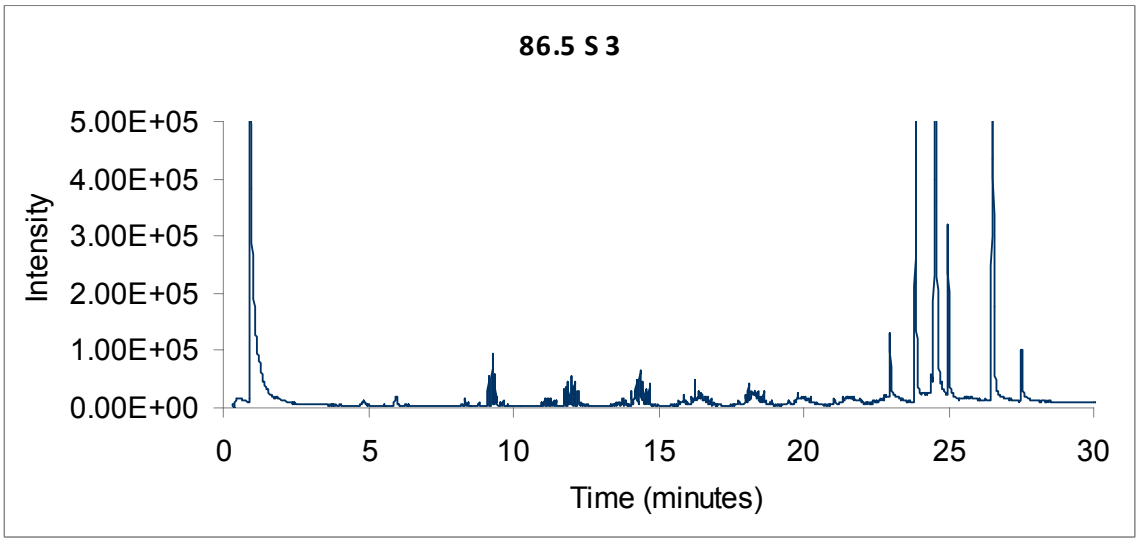
Time (minutes)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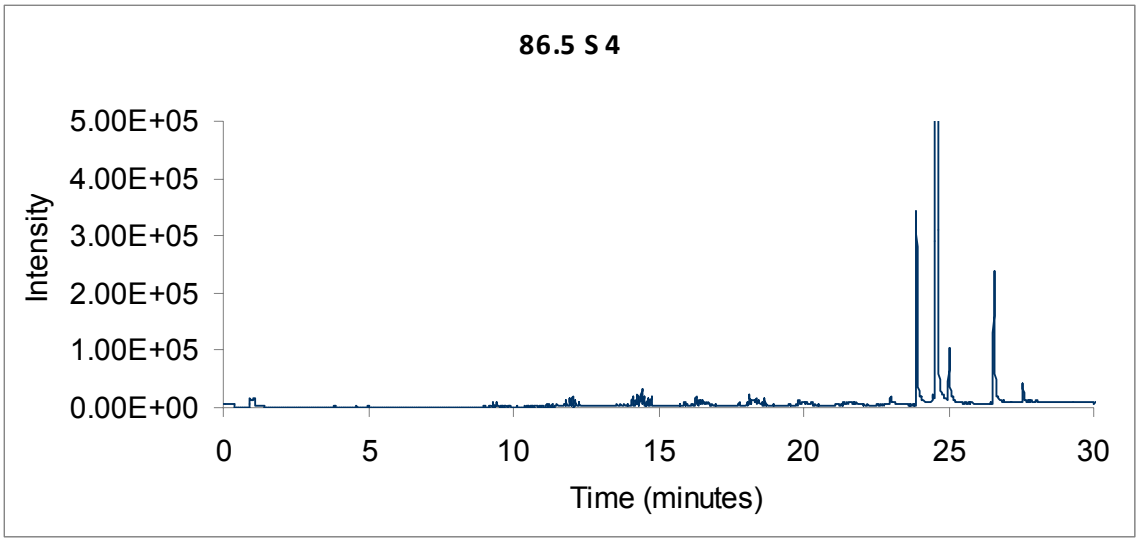
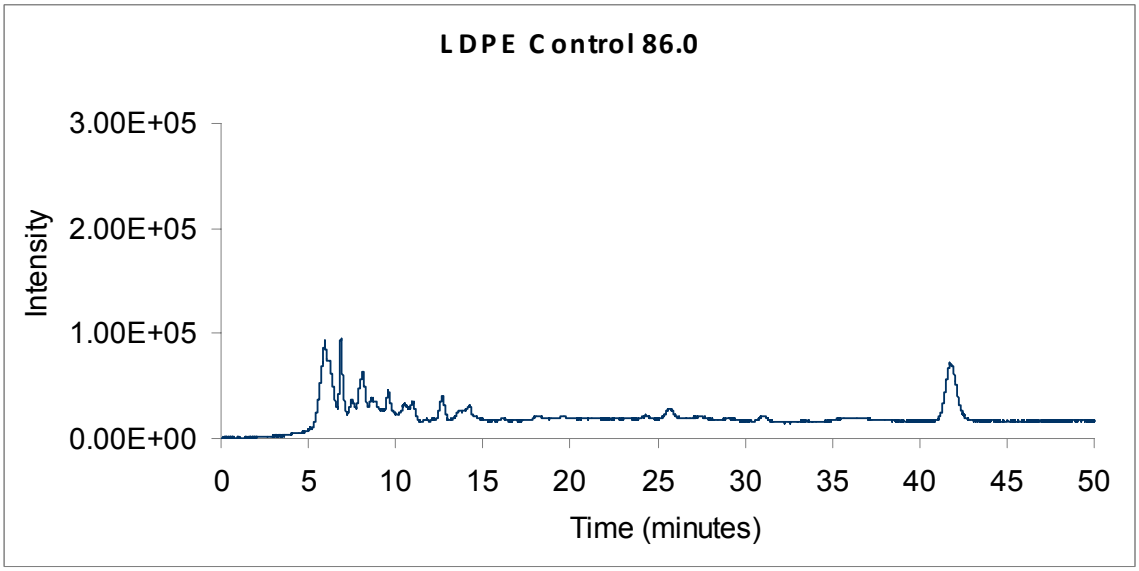
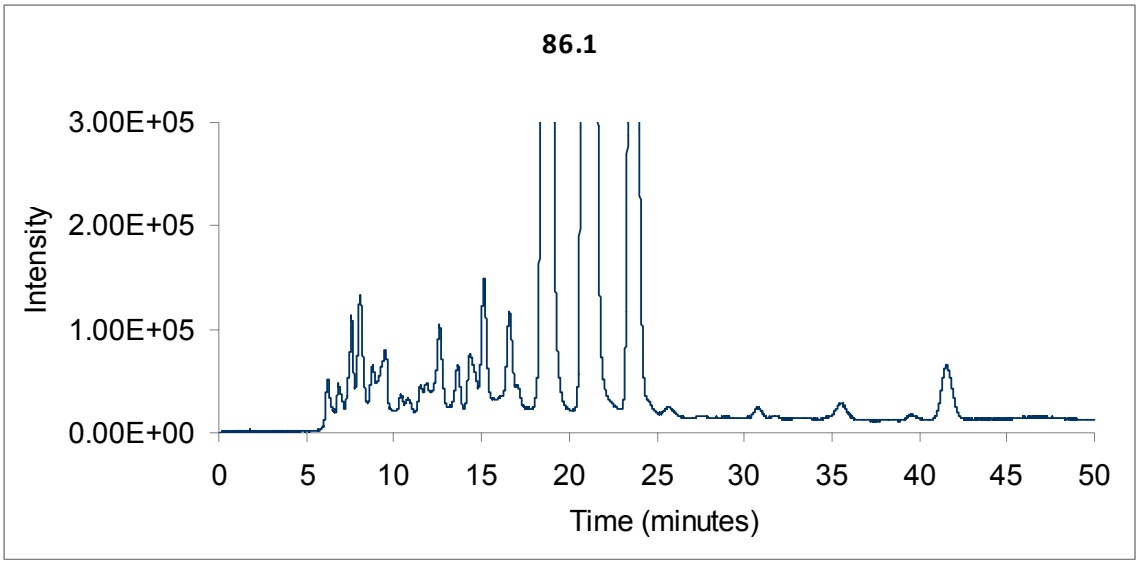
Time (minutes)



HPLC Chromatograms by PDA Detection

LDPE Films

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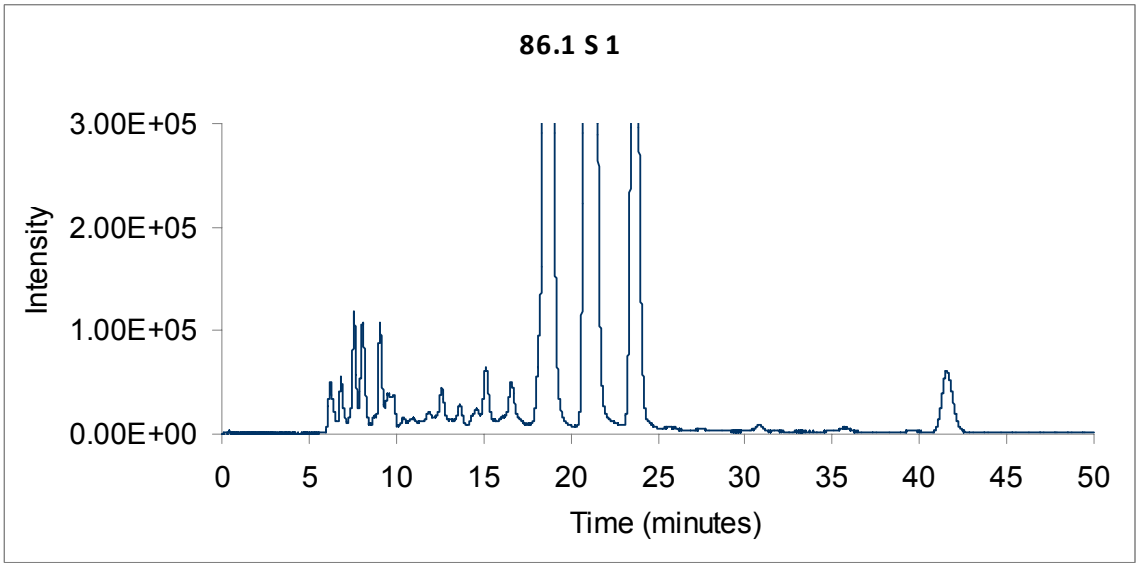
Time (minutes)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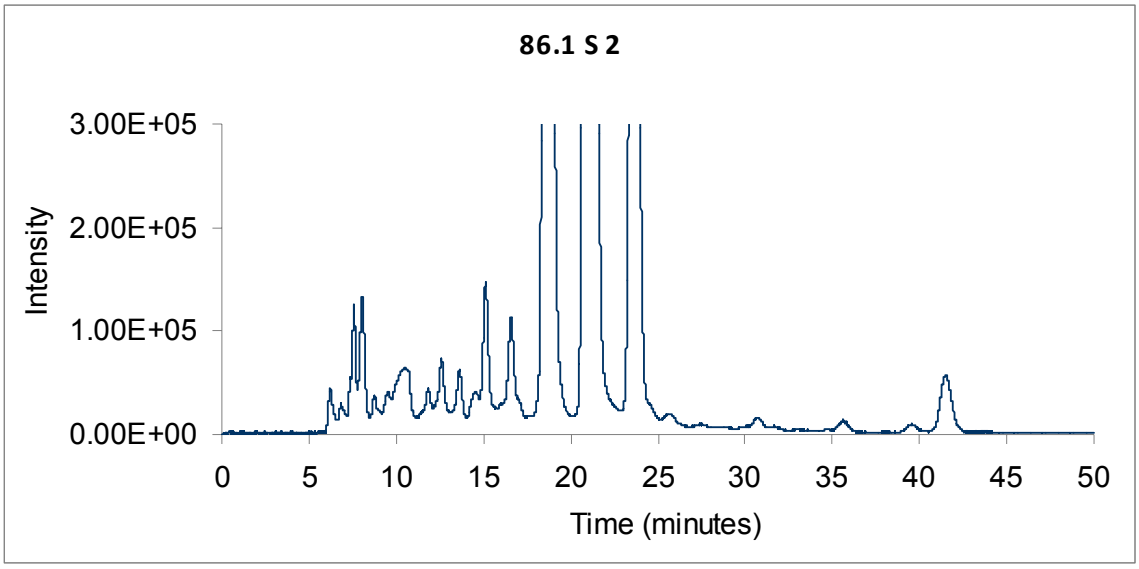
Time (minutes)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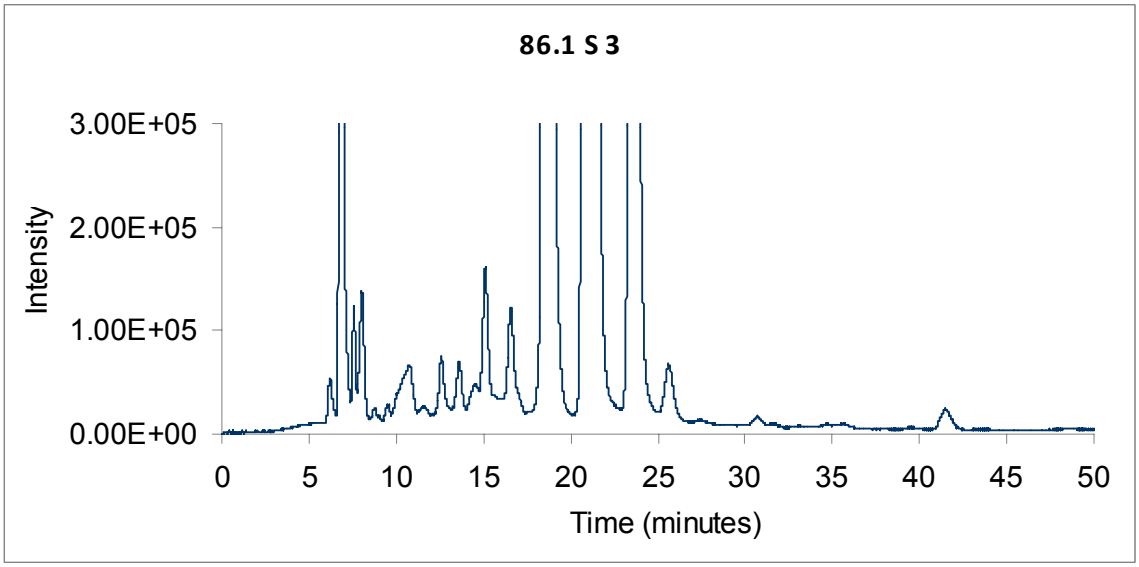
Time (minutes)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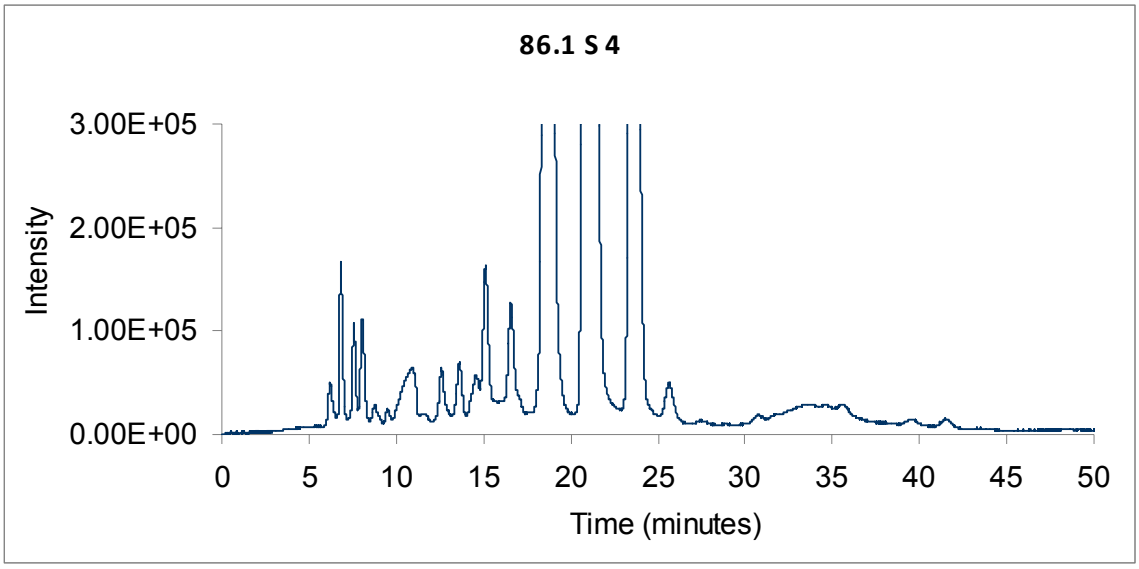
Time (minutes)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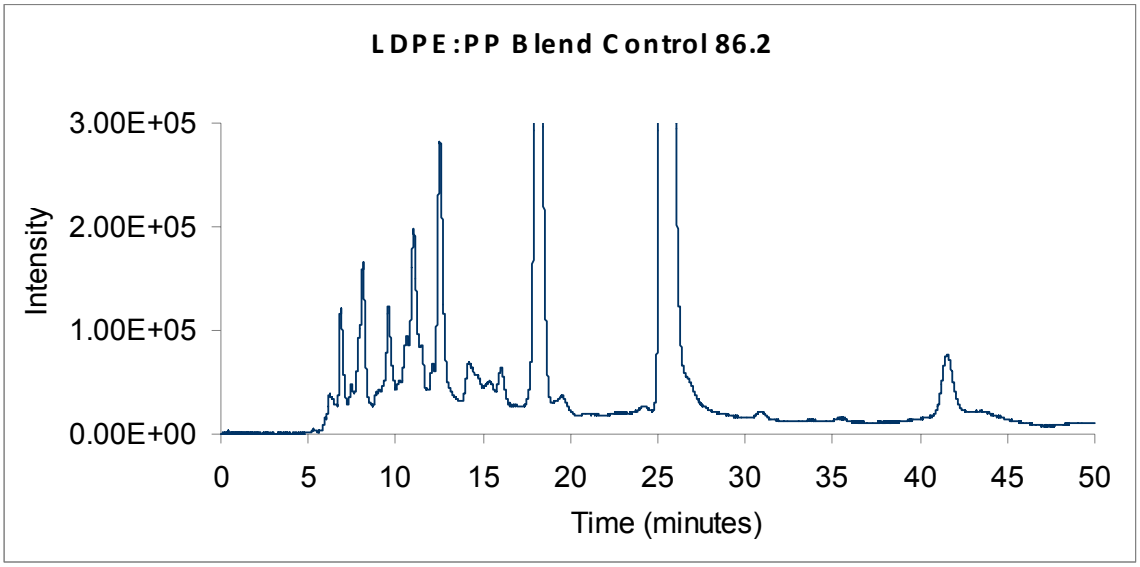
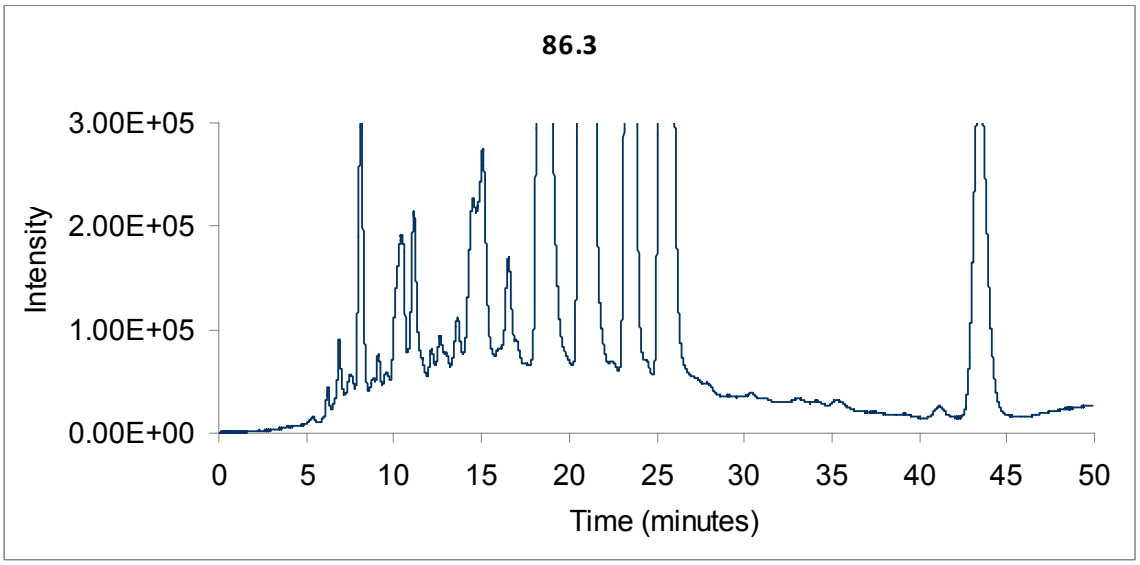
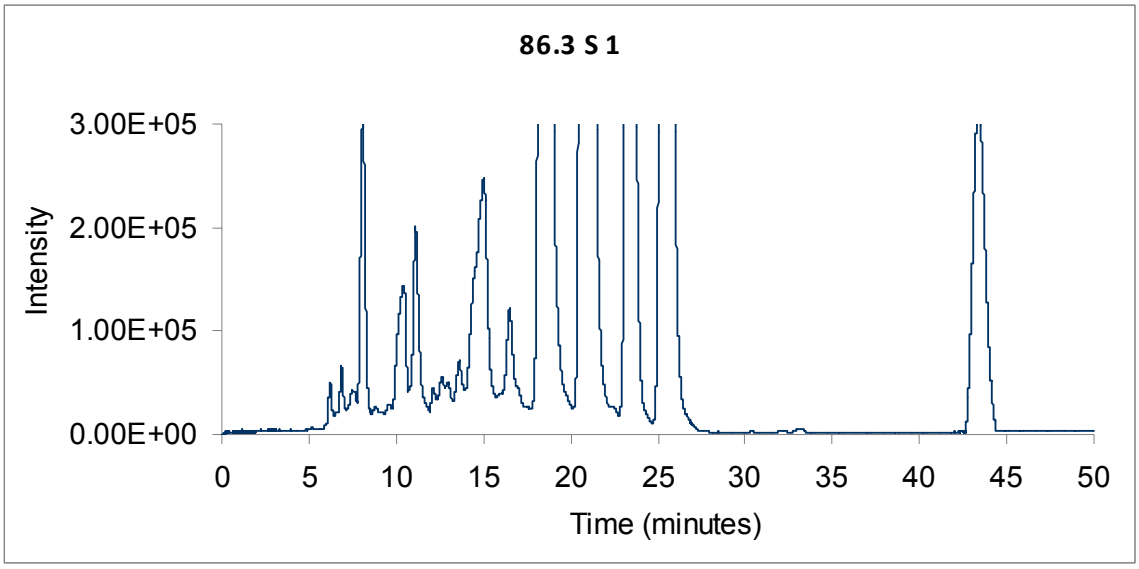
Time (minutes)

LDPE:PP Blend Films



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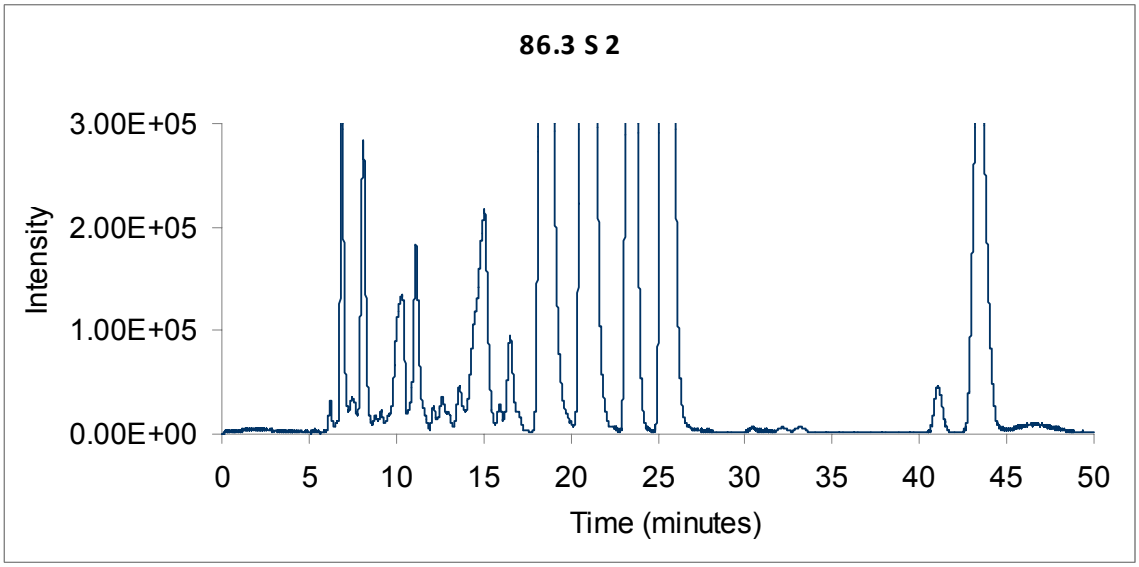
Time (minutes)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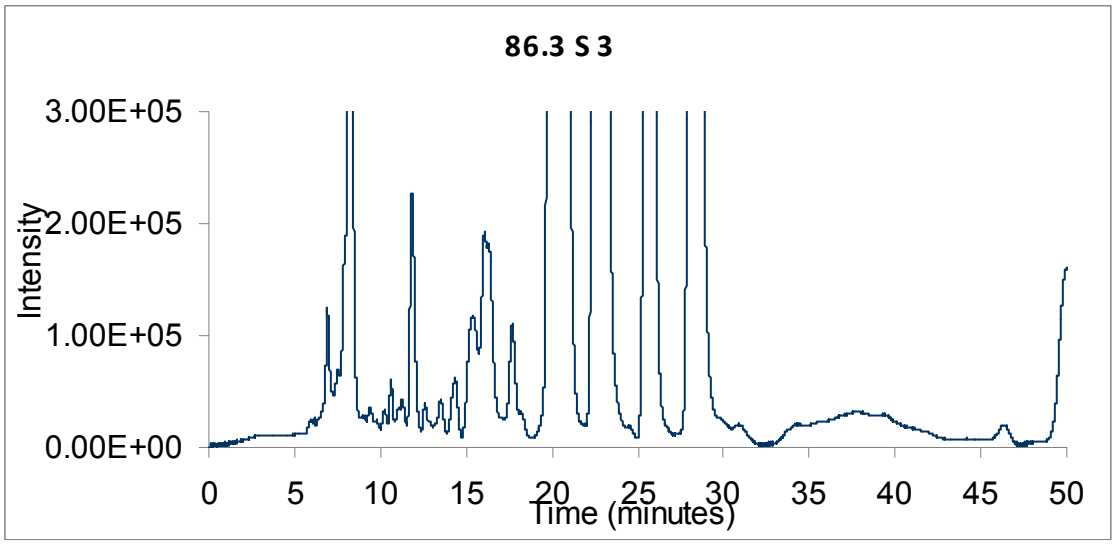
Time (minutes)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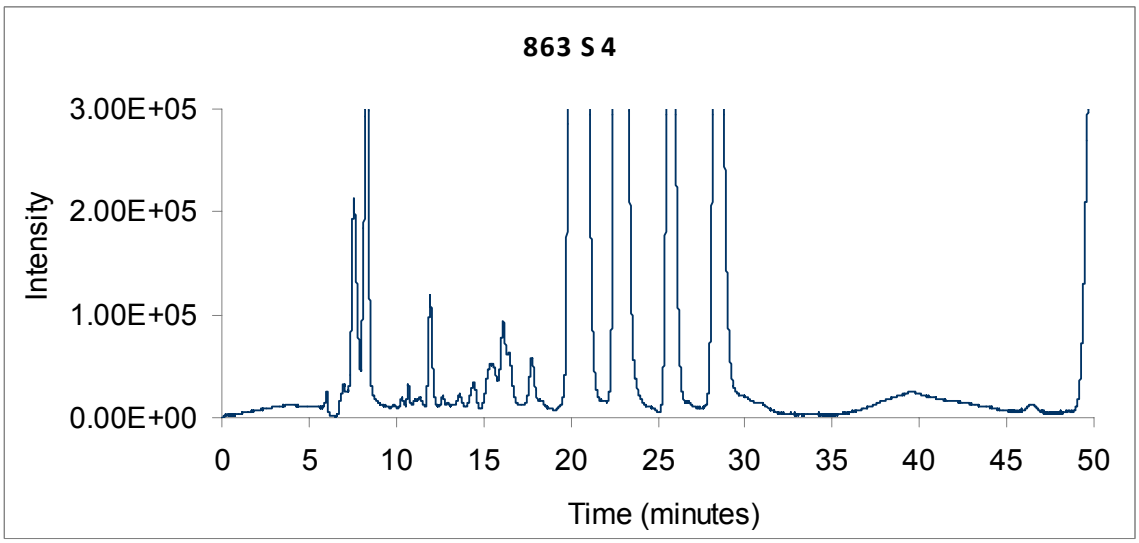
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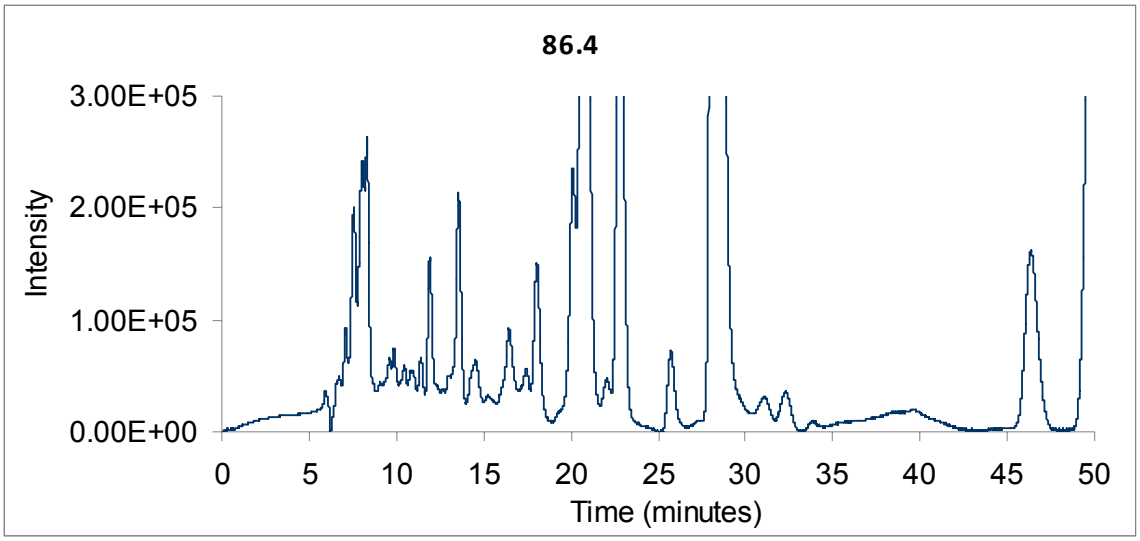
PP Films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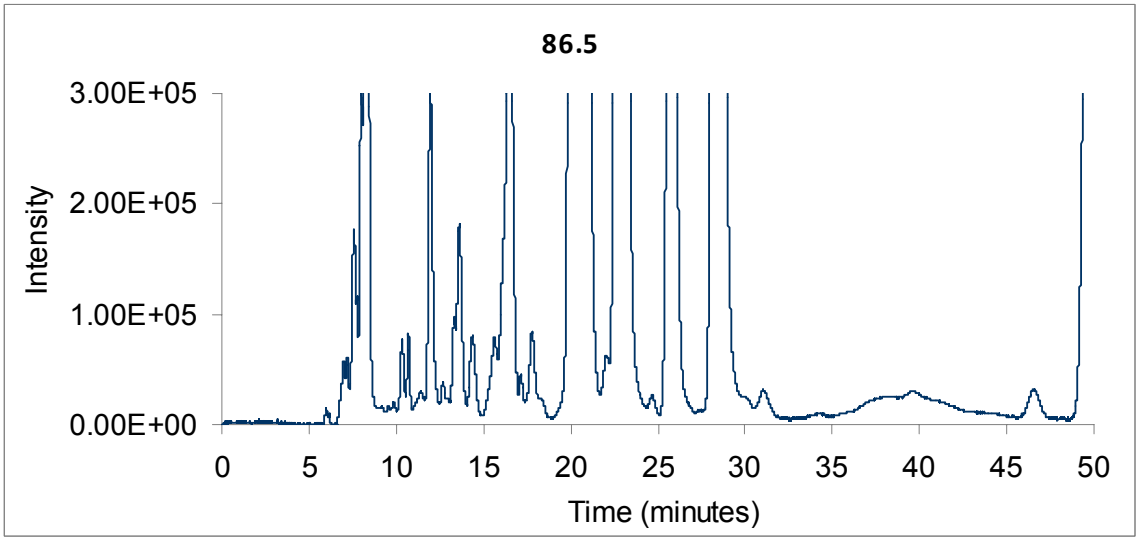
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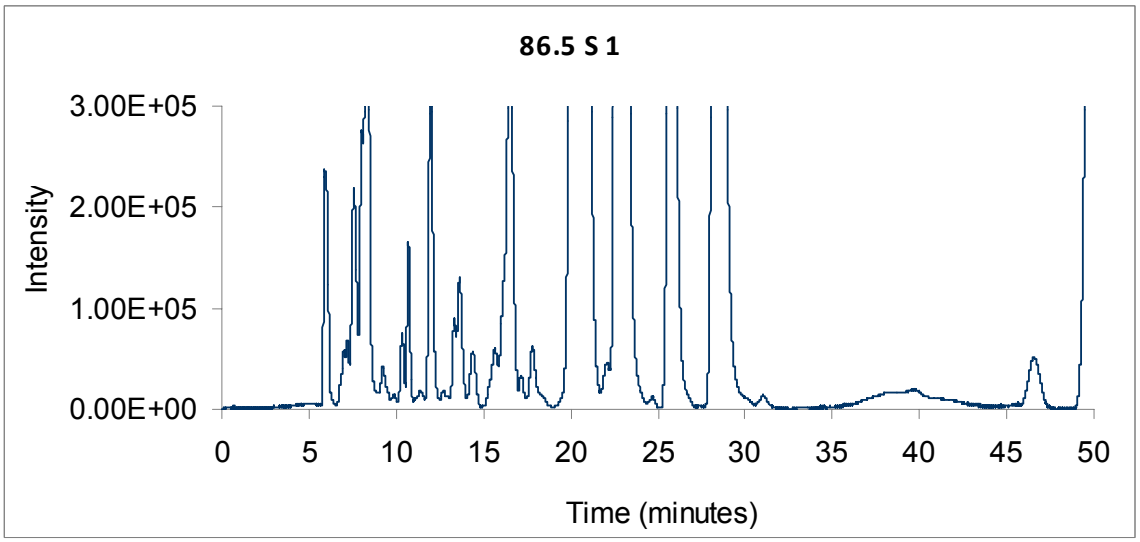
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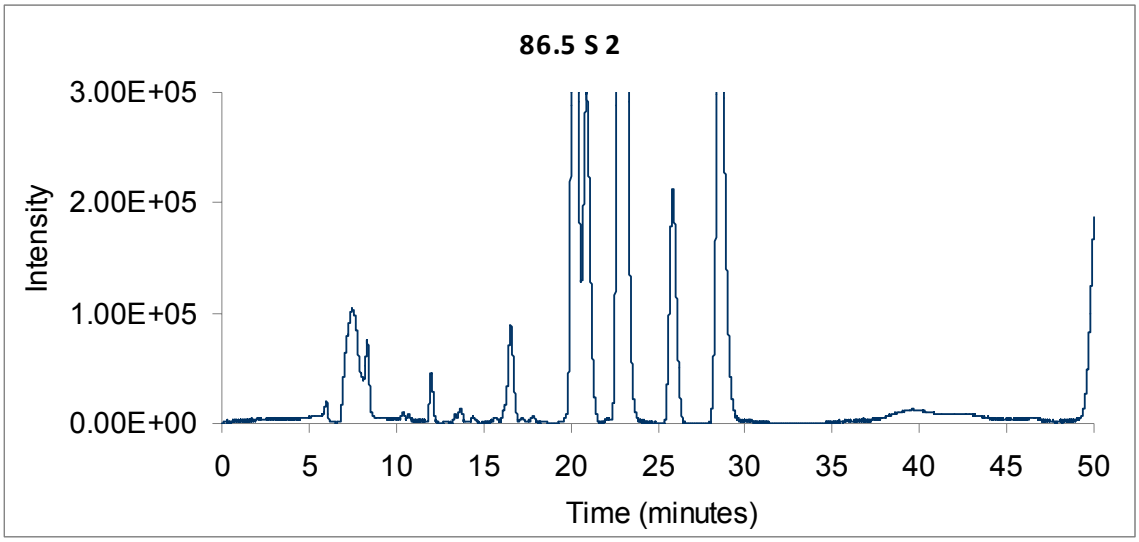
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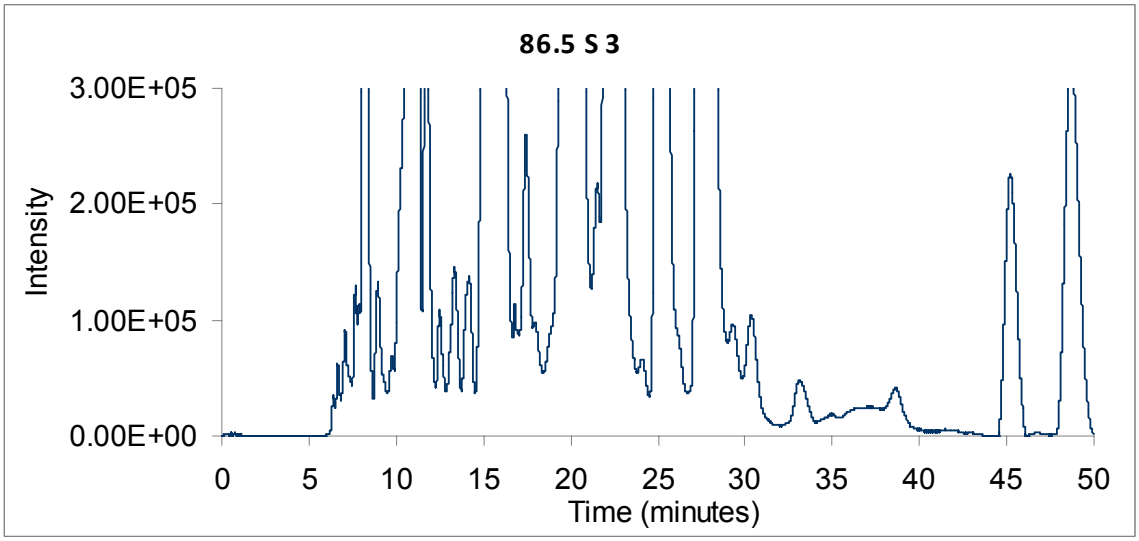
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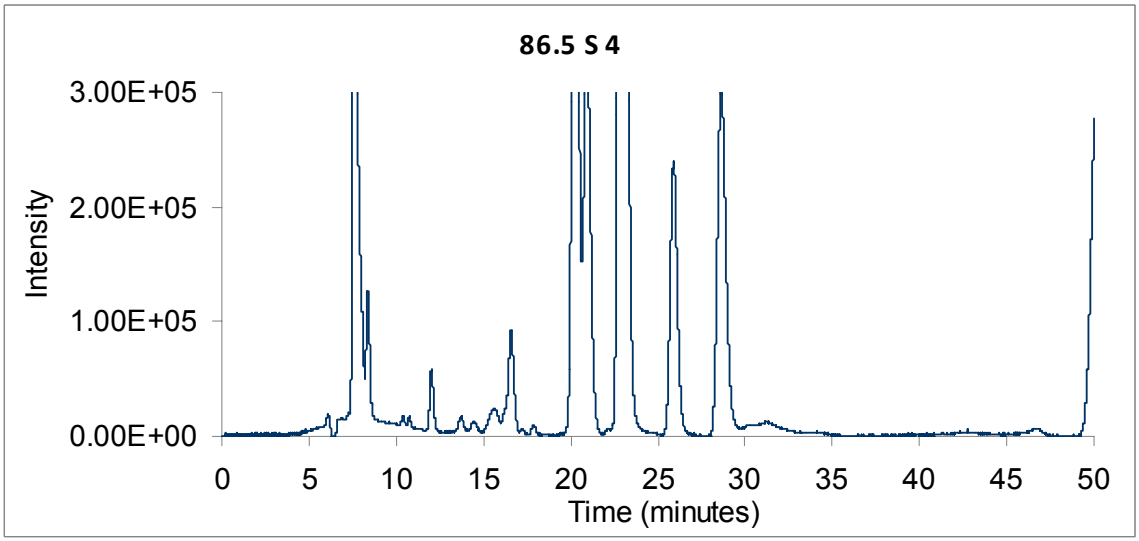
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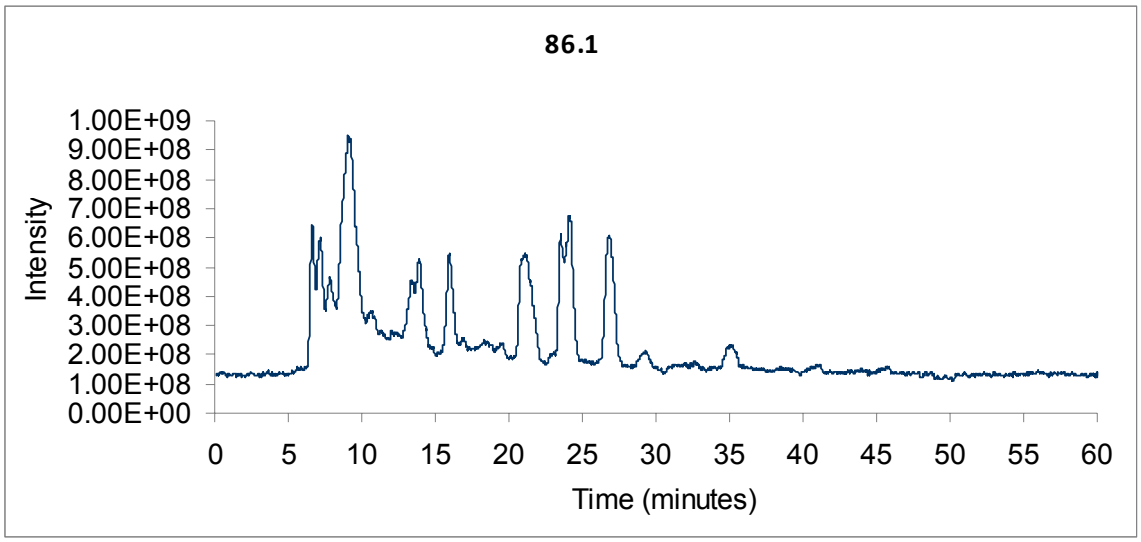
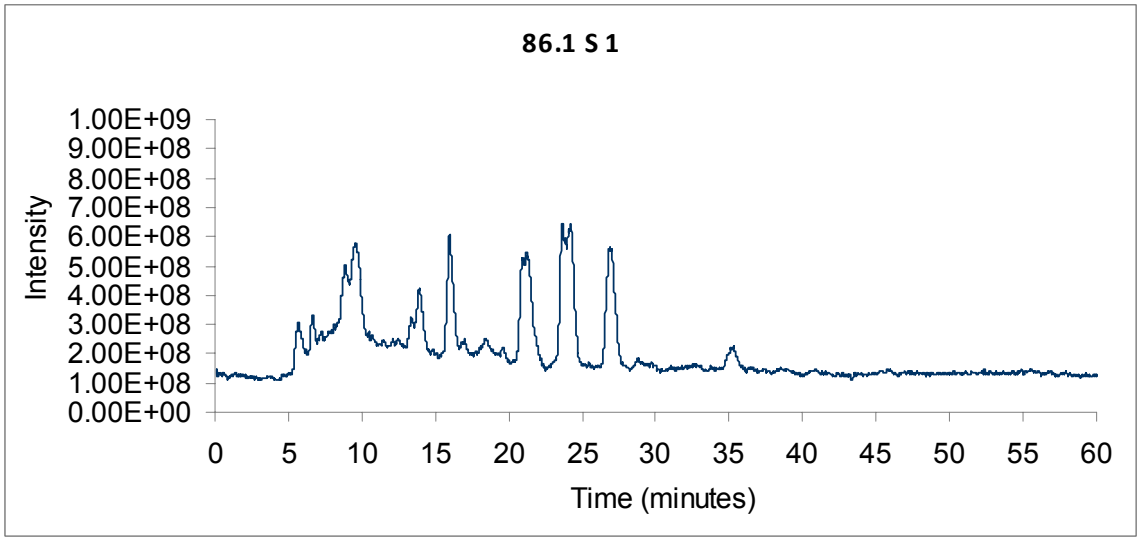
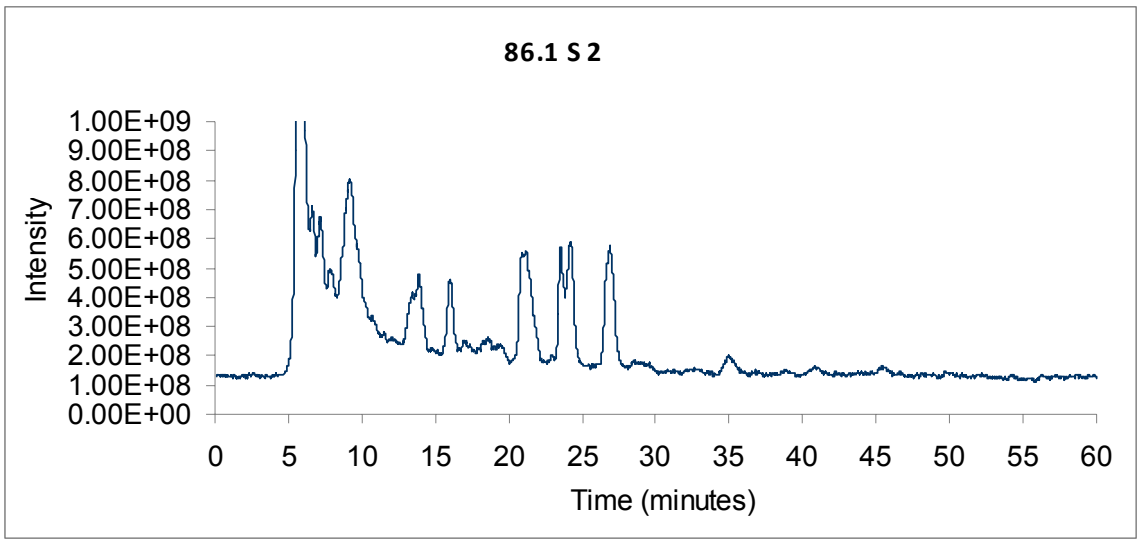
Time (minutes)

LC-MS Chromatograms

LDPE Films



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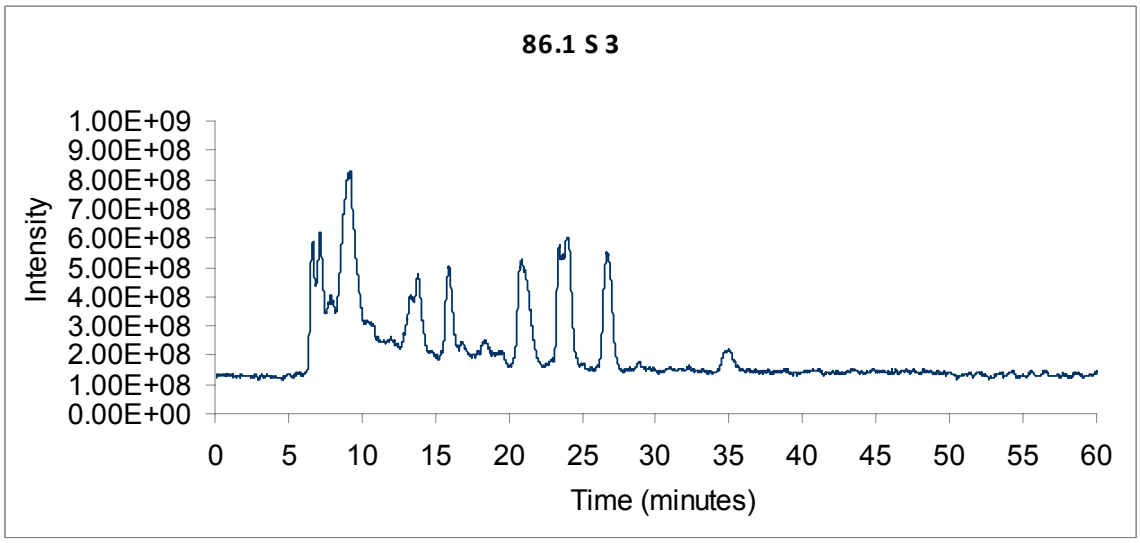
Time (minutes)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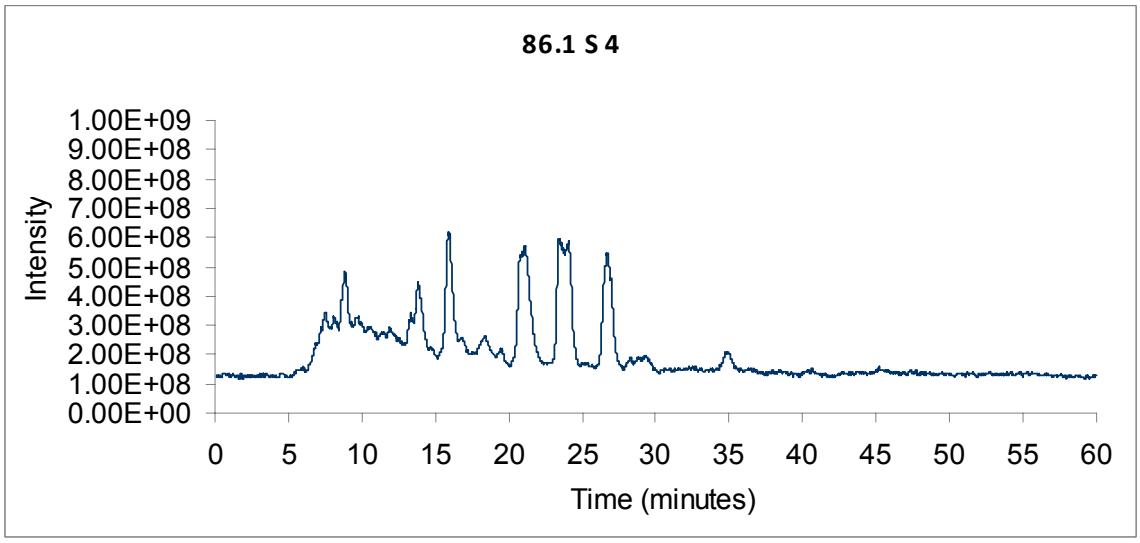
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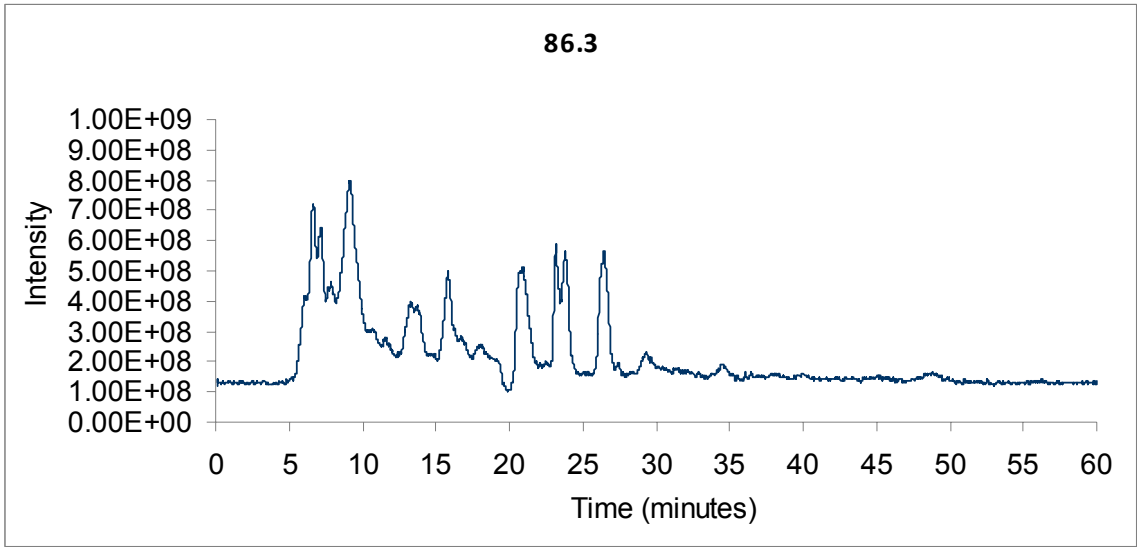
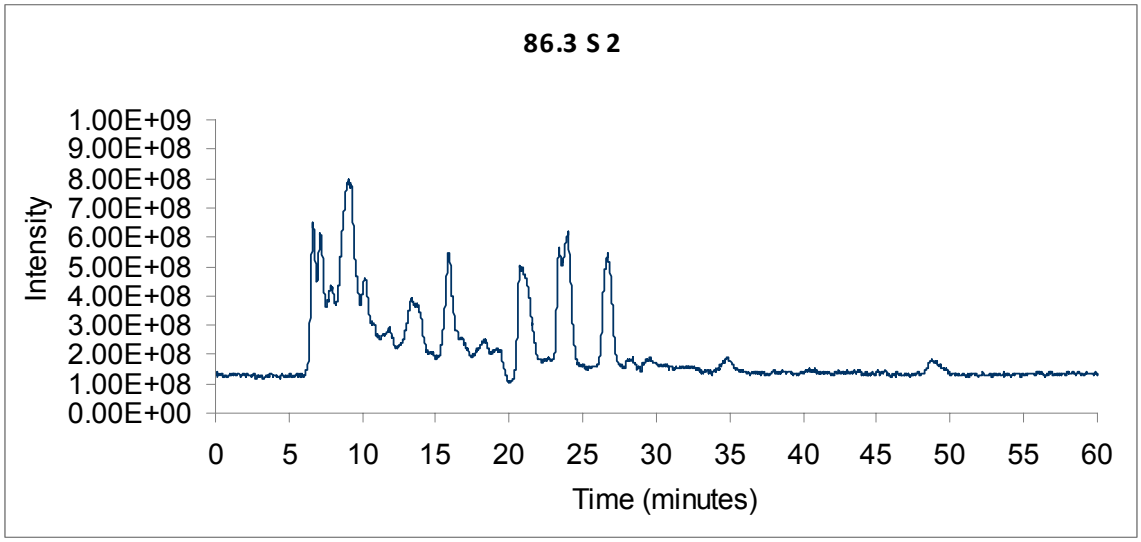
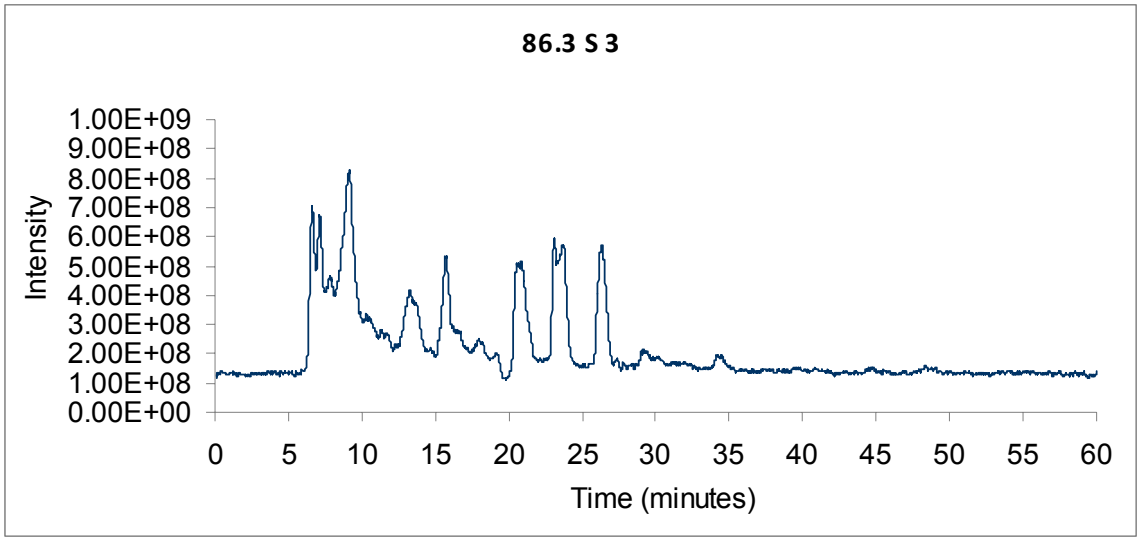
Time (minutes)

LDPE:PP Blend Films



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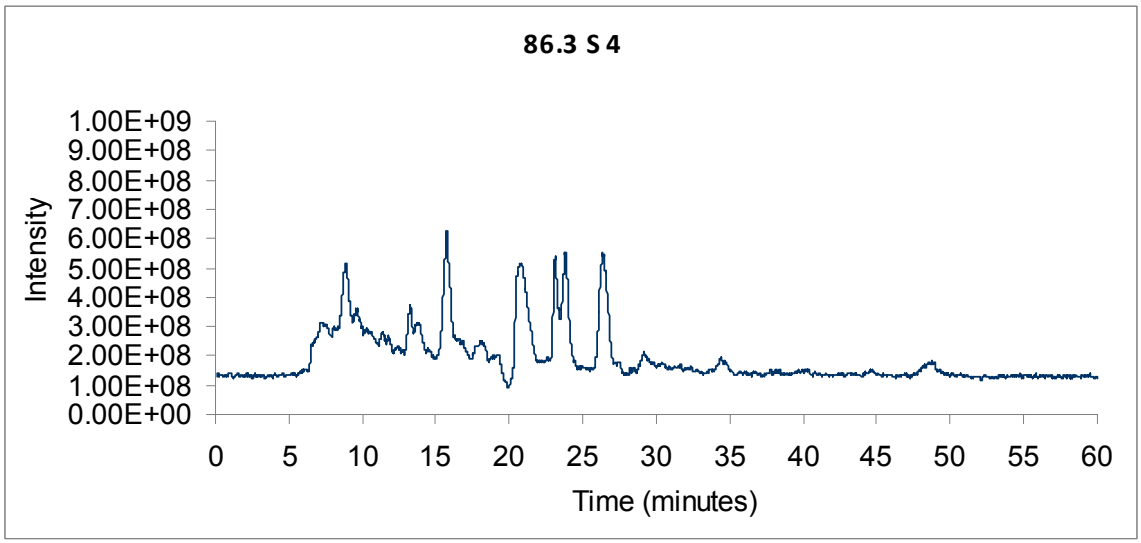
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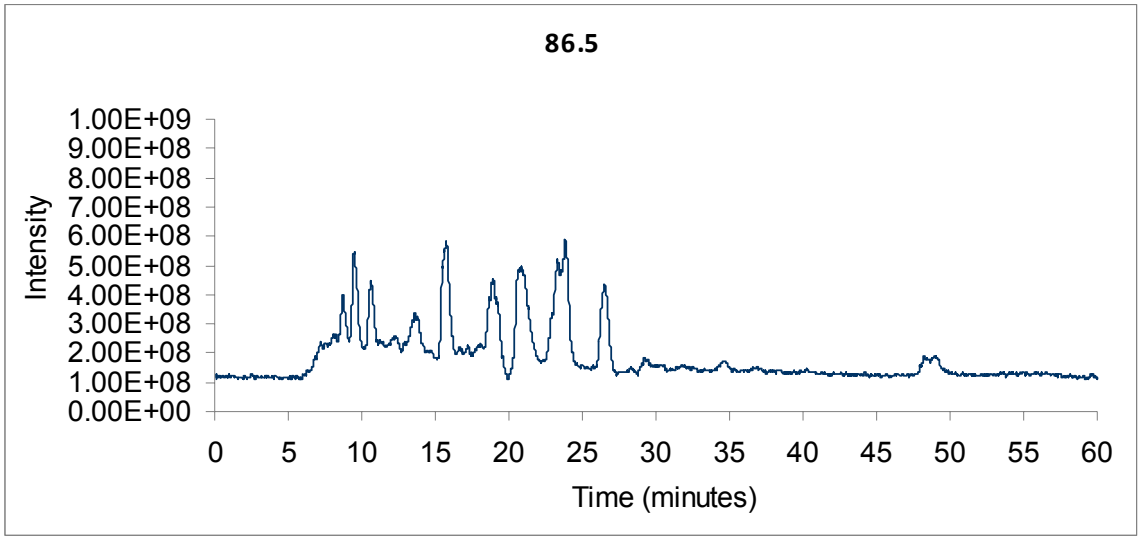
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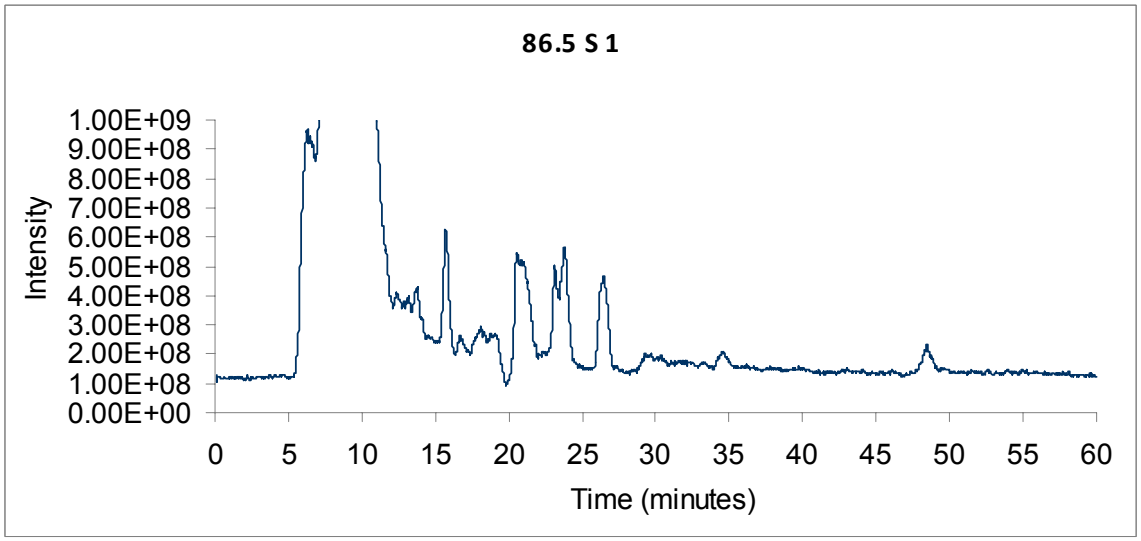
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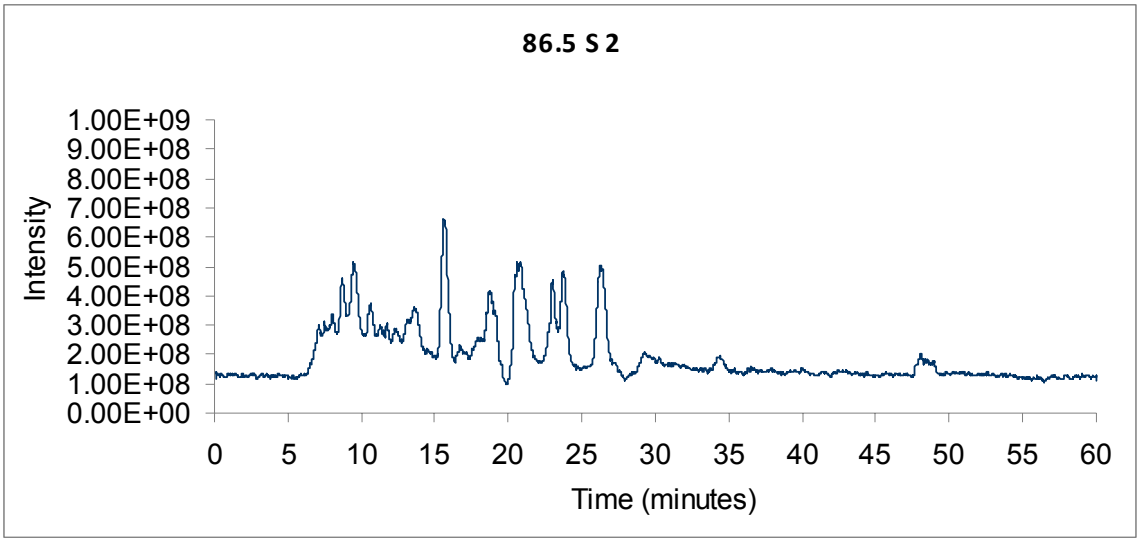
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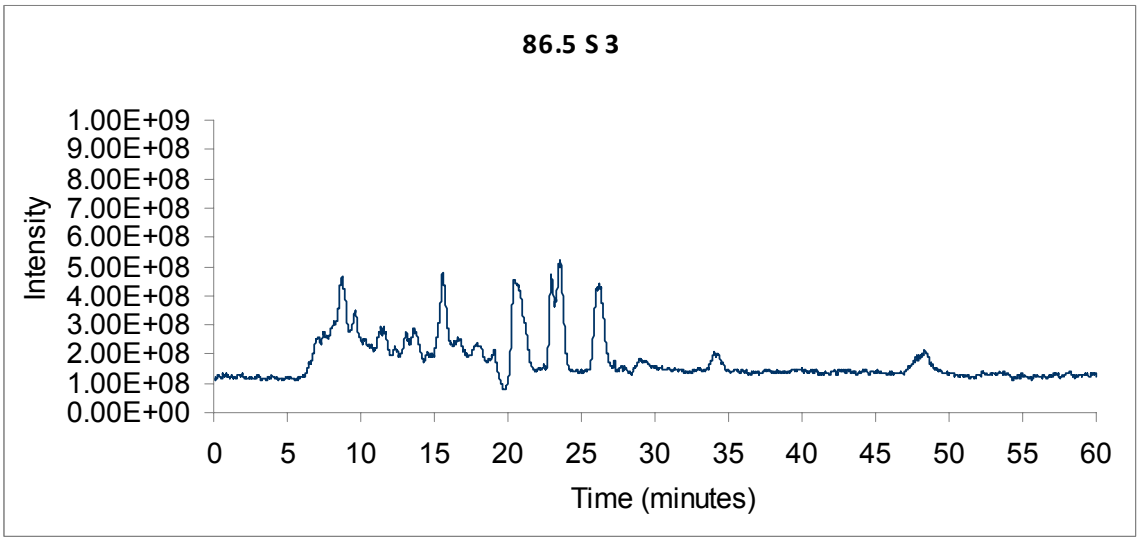
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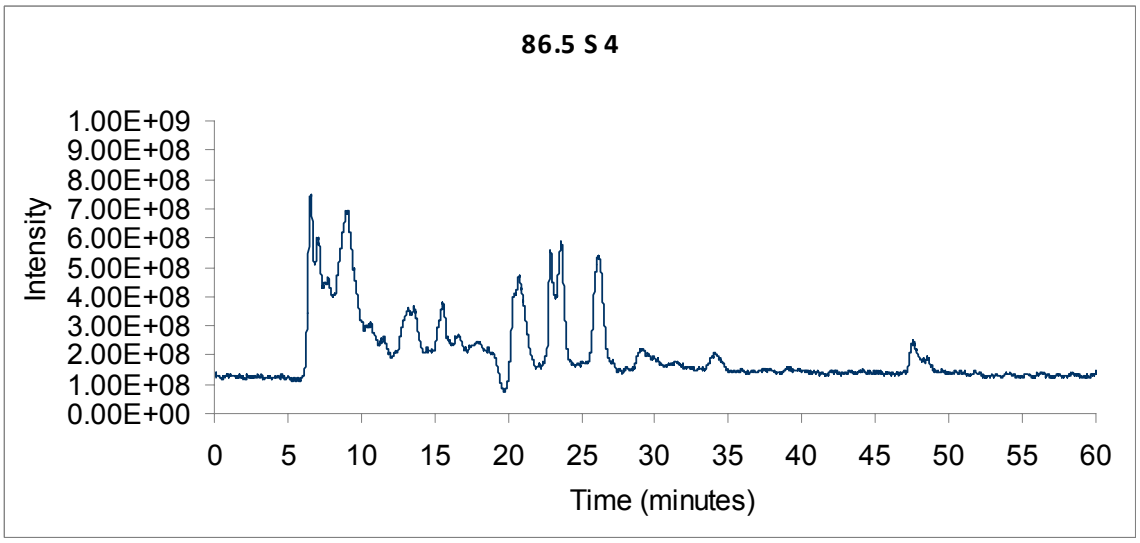
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Time (minutes)

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Curriculum Vita

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Education

1999-2003 Rutgers University – Rutgers College, BA

- Major - Biological Sciences
- Minor – Computer Science

2004-present Rutgers University – Graduate School New Brunswick

- Candidate for MS degree in Food Science

Employment

2003-2004 Flavour-Tec International, Little Ferry, NJ

Product Development

- Develop spice blends and marinade systems for the commercial food market.
- Maintain product development laboratory, including ingredient inventory.

2006-present The Tec-Team, Branchburg, NJ

Food Technologist

- Savory and sweet flavor development and application
- Interface with customers to establish product requirements
- Raw material and finished flavor QC and documentation
- Involved in all aspects of flavor production, including purchasing, compounding, and shipping