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THE STABILITY OF A UV ABSORBER INCORPORATED INTO TRANSPARENT PACKAGES AND ITS EFFECT ON THE PHOTOOXIDATION OF SOYBEAN OIL

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THE STABILITY OF A UV ABSORBER INCORPORATED INTO TRANSPARENT PACKAGES AND ITS EFFECT ON THE PHOTOOXIDATION OF SOYBEAN OIL.

by

Melvin Arthur Pascall

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ABSTRACT

THE STABILITY OF A UV ABSORBER INCORPORATED INTO TRANSPARENT PACKAGES AND ITS EFFECT ON THE PHOTOOXIDATION OF SOYBEAN OIL.

by

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The stability and effectiveness of Tinuvin 326, a UV absorber, dispersed within the regrind layer of coextruded multilayered polypropylene based containers, was studied by investigating the ability of Tinuvin 326 to protect packaged, bleached soybean oil from photooxidation.

The level of Tinuvin 326 in the containers was determined using high pressure liquid chromatography and UV spectrophotometric methods respectively. No loss of Tinuvin 326 from the containers to the atmosphere was observed over a 42 day storage period at 21 and 35^{o}C.

The migration of Tinuvin 326 from the containers to the oil stored at 35^{o}C for 42 days, was greater than at 21^{o}C for 42 days. The migration level was too low to noticeably reduce Tinuvin 326 levels in the containers.

The peroxide value method used to measure lipid oxidation in the bleached oil, determined the effectiveness of Tinuvin 326. Containers with 0.3% Tinuvin 326 were found to have significantly reduced photooxidation of bleached oil exposed to fluorescent light during 35 storage days at 21 and 35^{o}C, as compared to containers with no Tinuvin 326.

To my mother Priscilla Pascall

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INTRODUCTION

An ideal package should provide adequate protection without unfavorable interaction with the product. The package should be attractive, relatively low cost and convenient to use.

Various light sensitive products cannot be packaged in transparent containers because of possible deteriorative reactions which are catalyzed by incident light. To protect such products may result in having to conceal them from purchasing consumer. If transparent packages can be made to protect light sensitive products from incident light, and still present the product attractively, then a marketing advantage would be gained.

In general, deterioration of fats, oils and foods containing lipids, can occur due to a variety of biological, chemical, and physical factors. Parker et al. (1952) reported that the chemical changes responsible for spoilage of fats and oils are basically oxidative and hydrolytic processes. Oxidation involves the addition of oxygen to unsaturated fatty acids to form hydroperoxides. These peroxides decompose and react with water to form aldehydes, ketones, and acids, some of which are responsible for disagreeable odors, flavors, color changes, and nutritive

losses. Certain metals exert profound effect on the catalytic rate of oxidation of fats and oils. Physical factors associated with fat spoilage are heat, ionizing radiation and light of certain wavelengths.

The total exclusion of light from packaged oils would be an obvious solution to photooxidation. This can be accomplished by using metal and other types of packages that are impervious to light. However, in practice this is not employed because of marketing and economic reasons. In the past, amber bottles were used for a significant portion of the vegetable oil market, but now clear glass and plastic bottles are used by most edible oil processors. Increased store lighting and transparent packaging, increase the likelihood that oils and other fatty food products will develop off flavors while on the grocery shelf. Since transparent plastic packaging is widely used for such foods, modification to improve the stability of oils, oil containing products and light sensitive foods can be beneficial to food processors and consumers.

Barriers to light such as metal, paperboard, colored glass and pigmented plastics have been shown by a number of researchers to provide protection to light sensitive foods (Hoskin, 1988; deMan, 1978; Sattar et al., 1976; Nkpa et al., 1990). In this study, however, the effectiveness of a UV absorber, Tinuvin 326, was investigated by incorporating it

into coextruded, multilayered polypropylene containers. Theoretically, this would result in a transparent package with the ability to provide protection to light sensitive foods. The stability and duration of activity, as well as the potential migration of Tinuvin 326 into soybean oil was also investigated. Soybean oil is a light sensitive product and degradation can occur due to photooxidation, leading to rancidity and the loss of fat-soluble vitamins such as vitamin E and vitamin A.

The specific objectives of this study were: (1) to develop methodology for the quantitation of Tinuvin 326 in the test containers; (2) to evaluate the stability of Tinuvin 326 in the material at various storage temperatures; (3) to investigate the migration of Tinuvin 326 from the package material to the product (soybean oil); and (4) to determine the change in product quality as a function of Tinuvin 326 (in the container wall), light intensity, exposure time, and storage temperature.

LITERATURE REVIEW

Mechanism of Lipid Oxidation

Lipid oxidation is a primary cause of food spoilage. In edible oils, and food products containing lipids, this can lead to the development of off flavors, off-odors, decreased nutritive quality and the production of certain potentially toxic, oxidative products. It is generally accepted that autoxidation is the main reaction involved in oxidative deterioration of lipids. Although photochemical reactions have been known for a long time, only recently has the role of photosensitized oxidation and its interaction with autoxidation begun to be realized (Nawar, 1985). At conditions that normally exist during oxidation of food fats, the primary products of autoxidation are hydroperoxides (Sattar et al., 1976). The reaction is often referred to as autoxidation due to apparent self-catalytic properties. The generalized mechanism reviewed by Gray 1978, is deplicted as:

1. Initiation

$$RH + O_2 \rightarrow R. + .OH$$

2. Propagation

$$R. + O_2 \rightarrow ROO.$$

$$RH + ROO. \rightarrow ROOH + R.$$

$$ROOH \rightarrow RO. + .OH$$

3. Termination

$$R. + R. \rightarrow RR$$

$$R. + ROO. \rightarrow ROOR$$

$$ROO. + ROO. \rightarrow ROOR + O_2$$

RH is any unsaturated fatty acid in which the H is labile due to its position on a carbon atom adjacent to a double bond. R. is a free radical formed by removal of a labile hydrogen (Gray, 1978; Luby, 1982).

Frankel (1984) reported that decomposition of lipid hydroperoxides is a very complicated process. Decomposition proceeds by homolytic cleavage of RO-OH to form alkoxy radicals (RO.). Lipid hydroperoxides can react with oxygen to form secondary products as epoxyhydroperoxides, ketohydroperoxides, dihydroperoxides, cyclic peroxides and bicyclic endoperoxides. These secondary products decompose like monohydroperoxides to form volatile breakdown products. Lipid hydroperoxides can condense into dimers and polymers that can also breakdown and produce volatile materials (Hildebrand et al., 1984).

The initiation step may occur due to hydroperoxide decomposition, metal catalysis, or by exposure to light (Nawar, 1985). The source of hydroperoxide can be from another system undergoing oxidation. More recently, it has been postulated that singlet oxygen can be the active species involved, with plant and tissue pigments such as chlorophyll

or myoglobin, acting as sensitizers (Nawar, 1985). In this process, oxygen becomes activated to the singlet state by transfer of energy from the photosensitizer. The resulting singlet oxygen is extremely reactive (Frankel, 1984). Linoleate is reported to react at least 1500 times faster with singlet oxygen ($^1O_2$) than with normal oxygen in the triplet ground state ($^3O_2$)

Two pathways have been proposed for photosensitized oxidation (Nawar, 1985). In pathway 1, the sensitizer presumably reacts, after light absorption, with substrate (A) to form intermediates, which then react with ground state (triplet) oxygen to yield the oxidation products as shown:

$$\text{Sensitizer} + A + h\nu \rightarrow \text{intermediates - I}$$

$$\text{Intermediates - I} + O_2 \rightarrow \text{products} + \text{sensitizer}$$

In pathway 2, molecular oxygen rather than the substrate is the species that reacts with the sensitizer due to light absorption.

$$\text{Sensitizer} + O_2 + h\nu \rightarrow \text{intermediates - II}$$

$$\text{Intermediates - II} + A \rightarrow \text{products} + \text{sensitizer}$$

(Nawar, 1985).

Braun and Oliveros (1990) reported that besides sensitization, certain chemical reactions, electric discharge and photolytic decomposition of ozone may produce singlet oxygen. They summarized the sensitization mechanism as

follows:

$$\text{Sensitizer} \xrightarrow{hv} {}^1\text{Sensitizer}^*$$

$${}^1\text{Sensitizer}^* \xrightarrow{isc} {}^3\text{Sensitizer}^*$$

$${}^3\text{Sensitizer}^* + O_2 \xrightarrow{et} \text{Sensitizer} + {}^1O_2$$

When the sensitizer is excited by absorption of light (hv), the singlet state of the sensitizer results (^{1}Sensitizer*). This singlet state can be converted to triplet state by intersystem crossing (isc). Depending on the energy (et) of the triplet state, both singlet states of oxygen ($^1\Sigma^+$ and $^1\Delta$) can be generated, and the sensitizer is deactivated without chemical alteration. However, in practice, the $^1\Sigma^+$ state is rapidly deactivated, and only the $^1\Delta$ state acts as the intermediate referred to as singlet oxygen ($^1O_2$) (Braun et al., 1990).

There is also increasing evidence that singlet oxygen can react directly with the double bonds of unsaturated fatty acids in fats and oils to produce peroxides. This suggests that photosensitized oxidation may be important in initiating or propagating normal free radical autoxidation of unsaturated fats and oils (Vianni, 1980). Sherwin (1976) reported that the greater the unsaturation of a vegetable oil, the more susceptible it is to oxidative deterioration. Oils containing substantial amounts of linolenic acid, or certain other fatty acids with more than two double bonds in their molecular structures, may undergo oxidative degradation known as "flavor

reversion." In commercial edible oils, the reversion problem is most likely encountered with soybean and rapeseed oils, which have high levels of linolenic acid (Sherwin, 1976).

MEASUREMENT OF LIPID OXIDATION

Various methods have been developed to measure lipid oxidation. These tests may utilize chemical, physical or organoleptic evaluation. Gray (1978) reported that the method chosen depends on the type of information required, the speed of the method, the nature and quantity of the sample, and the test conditions.

In sensory evaluation, the method can be as simple as individual tasting or smelling of the product. To minimize bias and human error, a multimembered panel is usually employed. A statistically designed sampling experiment is then conducted. The disadvantages of this type of analysis are the length of time required and poor reproducibility (Gray, 1978).

Various chemical methods are used to measure lipid oxidation. Among these methods the thiobarbituric acid (TBA) test is one of the most popular (Sattar et al., 1976; Gray, 1978; Hoojjat, 1988). This method is based on the assumption that a red color develops because of complexes formed between oxidation products of unsaturated fatty acids and TBA. Sinhuber et al. (1958) reported that the reactive compound in

the colorimetric reaction is malonaldehyde.

The peroxide value (PV) method has been widely used to measure lipid oxidation in edible oils (Sattar and deMan, 1976; Sattar et al., 1976; Luby et al., 1986; Kiritsakis et al., 1984; Moser et al., 1965; Boki et al., 1989; Warner et al., 1989). In this method the ability of peroxides to liberate iodine from potassium iodine or to oxidize the ferrous ion to the ferric ion is measured. Gray (1978) reported that the PV method has limitations because peroxides undergo transition to other secondary compounds. This method has been revised and updated since its initial development (Gray, 1978).

The Kreis test was one of the first tests used commercially to evaluate oxidation of fats (Sattar et al., 1976; Gray, 1978). In this test, development of a red color caused by reaction of phloroglucinol and oxidized products is measured. Mehlenbacher (1962) reported that erroneous results can be obtained as a similar color can develop from reactions not related to rancidity.

The oxirane test is another method also used to measure oxidation. Nawar (1976) reported that it is based on the addition of hydrogen halides to the oxirane group. The epoxide content is determined by titrating the sample with hydrogen bromide in acetic acid, in the presence of crystal

violet, to a bluish green end point (Sattar et al., 1976).

The measurement of carbonyl compounds is another chemical method used to evaluate lipid oxidation. The development of hydrazones, produced by the reaction of 2,4-dinitrophenylhydrazine with the oxidation products of aldehydes and ketones are measured (Henick et al., 1964). Several methods have been used, but Gray (1978) reported that in the one most commonly employed, the formation of carbonyl compounds occurs in the presence of a trichloroacetic acid catalyst. Gray (1978) reported Lea (1962) criticisms which stated that hydroperoxides can decompose under conditions of this experiment.

Physical methods are also used to determine lipid oxidation, these include uv spectrophotometry, fluorescence, infrared spectroscopy, polarography, chromatographic methods and refractometry (Gray 1978). The uv spectrophotometric method is also referred to as the conjugated diene method. This procedure is based on the principle that oxidation of polyunsaturated fatty acids is accompanied by an increase in their uv absorption. Absorbancy in the region 230 - 375 nm measures conjugated unsaturated fatty acids, while absorbancy at 234 nm and 268 nm is usually measured to monitor oxidation in diene unsaturated and triene unsaturated fatty acids respectively.

Gas chromatography has been the most widely used chromatographic system to measure lipid oxidation (Gray, 1978). This method of analysis is based on the measurement of specific compounds which are known to be typically formed during autoxidation. This method has been used by Jarvi et al. (1971) to measure rancidity in soybean oil. Samples of soybean, sunflower and low erucic acid rapeseed oils were evaluated for flavor and oxidative stability by Warner et al. 1989, using the gas chromatography method.

Infrared spectroscopy is used to follow change in functional groups as lipid oxidizes. Profiling the infrared spectra at varying times during oxidation can show a reduction in the number of bands and the consequential development of others. The refractive index method assumes that the refractive index of reactants increases with increased peroxide formation.

TYPES AND MECHANISM OF ANTIOXIDANT ACTIVITIES.

The reaction of oxygen with unsaturated lipids and polymers to cause oxidation, is basically the same. The mechanism is initiated by the removal of hydrogen from a hydrocarbon molecule, which can occur in the presence of metals, light, heat, or hydroperoxides. The resulting free radicals (R.) react with oxygen to form peroxy radicals (ROO.). In the propagation process, ROO. reacts with more RH groups to form hydroperoxides (ROOH), which are the primary

products of autoxidation.

Antioxidants (AH) can break this chain reaction by reacting with ROO. to form stable radicals which are either unreactive or form nonradical products (Frankel, 1984; and Hawkins, 1985).

$$ROO. + AH \rightarrow ROOH + A.$$

$$A. + ROO. \longrightarrow \left.\begin{matrix} \\ \\ \end{matrix}\right\} \text{Non radical products}$$

$$A. + A. \longrightarrow$$

Soybean oil is the major edible oil produced in the world (Buck, 1981). An essential characteristic of this oil is its relatively high percentage of unsaturated fatty acids. Oleic, linoleic, and linolenic acid, account for approximately 85% of its fatty acid content (Warner et al., 1984; Sherwin, 1976,; Erickson, 1983). Its iodine value (IV), (which indicates its total chemical unsaturation), is generally between 125-135 IV. Soybean oil also contains the highest level of linolenic acid esters found in any of the major edible vegetable oils (Buck, 1981). Chemically reactive sites are found in the double bonds of oleic, linoleic and linolenic acids present in the oil. These properties make soybean oil very susceptible to oxidative rancidity (Buck, 1981).

Susceptibility to oxidation is greatly reduced by the presence of various antioxidants, synergists and quenchers.

The antioxidants are those naturally found and others artificially added. Some of the natural antioxidants include tocopherols, lecithin, gum guaiac, nordihydroguaiaretic acid (NDGA), citric acid, tannins, ascorbic acid and catechol. Widely used commercial antioxidants are butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertiary butyl hydroquinone (TBHQ), trihydroxybutyrophenone (THBP), and propyl gallate (PG).

Sherwin (1976) reported that certain organic acids react synergistically with primary antioxidants in vegetable oils. Citric, phosphoric, thiodipropionic, ascorbic, and tartaric acids are used in many countries for this purpose. Lecithin has been cleared for use as an antioxidant or a synergist in vegetable oils, in various countries (Sherwin, 1976).

Warner et al. (1987) reported that β-carotene acts as a natural quencher by inhibiting singlet oxidation of unsaturated fatty acids during exposure to light in the presence of sensitizers such as chlorophyll. β-carotene occurs naturally in soybean oil where it inhibits singlet oxygen photooxidation, just as tocopherols are naturally occurring inhibitors of radical chain autoxidation (Vianni, 1980). Singlet oxygen quenchers may function by reacting chemically with singlet oxygen or by acting as screening agents to reduce the ability of incident radiation to transfer

energy necessary to form singlet oxygen. In the absence of quenchers, peroxides formed during oxidation undergo scission to radical species that accelerate the rate and ultimate level of peroxide formation (Kiritsakis et al., 1985).

β-carotene can be destroyed by exposure to incident light. The loss of β-carotene can leave an oil susceptible to photooxidation if exposed to such light. Sattar et al. (1976) studied the effect of fluorescent light on butter, butter fat, corn, rapeseed and soybean oils. The rate of oxidation increased considerable with corresponding decrease in vitamin A and β-carotene. This was probably due to the photobleaching of the carotene, which was probably a strong inhibitor of lipid oxidation in the early stages of the process (Sattar et al., 1976). Warner et al. (1987) showed that β-carotene in the presence of δ-tocopherol, behaved synergistically to prevent oxidation in olive oil. This lead them to suggest that tocopherols protect β-carotene from free radical autoxidation.

Lehmann and Slover (1976) studied the relative autoxidative and photolytic stabilities of tocopherols, and observed that α-tocopherol was highly effective in protecting γ and δ-tocopherols in methyl myristate and methyl linoleate during photolysis studies with UV light at 254 nm. However, there was only slight protection after 24 hours of exposure.

Terao et al. (1980) observed that during irradiation, β-carotene was rapidly lost from soybean oil. They also discovered that δ-tocopherols prevented the loss of β-carotene under these conditions. However, in the presence of chlorophyll, α-tocopherol and the other tocopherols were rapidly destroyed during photooxidation. This helped to speed the loss of β-carotene and thus hasten the oxidation of the oil. This deduction was also made by Carlsson et al. (1976) when they suggested that tocopherols and other phenolic compounds may act as antioxidants under suitable circumstances, and that tocopherols may also quench singlet oxygen. They reported that photooxidation (via singlet oxygen) is not directly controlled by the antioxidants commonly used to inhibit autoxidation. Warner and Frankel (1987) discovered that tocopherols protect β-carotene from free radical autoxidation. Hildebrand et al. (1984) showed that tocopherols acted synergistically with phospholipids to increase the stability of soy oil. Koskas et al. (1984) showed that α-tocopherol can act as a prooxidant at specific concentration levels. At a concentration of 3.8%, α-tocopherol showed prooxidant characteristics, while at 0.38%, it had antioxidant properties.

EFFECT OF LIGHT ON OXIDATION

Clements et al. (1973) studied the photooxidation of refined soybean oil. They investigated whether singlet oxygen-induced oxidation could be observed in refined soybean

oil. Oxidation was followed by measuring the conjugated diene absorption at 234nm. The extent of oxidation was found to be linearly dependent on the time of exposure to the light.

Formation of conjugated dienes occurs in oxidized soy oil because of the presence of fatty acids with more than one double bond. In linolenic acid, the methylene group at position 11 is activated by the two adjacent double bonds. The loss of hydrogen from this position produces a pentadienyl radical intermediate that can react with molecular oxygen to produce equal mixtures of conjugated 9- and 13-diene hydroperoxides, such as:

$$\overset{13\ \ 12\ \ 11\ \ 10\ \ 9}{-C = C - C - C = C-} \longrightarrow -C = C - C. - C = C- \qquad (1)$$

$$-C = C - C. - C = C- \longrightarrow -C = C - C= C - C. + -C. - C = C - C = C- \qquad (2)$$

$$-C = C - C= C - C. + -C. - C = C - C = C- \xrightarrow{O_2} -C = C - C = C - \underset{\underset{H}{O}}{\overset{\overset{O}{\|}}{C}}- \qquad (3)$$

$$+ -\overset{13}{C}(=O)(-O-H) - C = C - C = C-$$

(Nawar, 1985).

Clements et al. (1973) tried to determine the major sensitizers in soybean oil by initiating photooxidation, while protecting the oil with a yellow filter. Light with wavelengths less than 500nm was cut off by using this filter.

They also found that photooxidation induced by the addition of chlorophyll was reduced, but not eliminated by the filter, which indicated that absorbancy of chlorophyll both below and above 500 nm lead to photosensitization (Clements et al., 1973).

Du and Armstrong (1970) from the work of several other authors, stated that the mechanism of light-induced oxidation in fatty materials was not clear, but that oxidation could be attributed to ultraviolet light, while the effect of visible light was considered of little or no consequence. They observed that the spectral energy output of the commonly used fluorescent light tubes was primarily in the visible region (350-700 nm), and little ultraviolet light was emitted from these lamps. They also found that the unsaturated fatty acids in milk exhibit light absorption only in the far ultraviolet range (about 180 nm). Even after conjugation (during the first stages of autoxidation) and the resulting shifts in locations of the double bonds during resonance, the absorption region still was about 320 nm.

Sattar et al. (1976) reported that Chahine and deMan (1971) found that the most rapid oxidation of corn oil occurred during exposure to fluorescent light. Sattar et al. (1976) also reported that Morgan (1935) found blue light and uv light accelerated the development of rancidity, and that Coe and LeClerc (1935) observed that exposure of butter in the

wavelength range of 302 to 546.1 nm resulted in rancidity in all cases, except with green light at 546.1 nm.

Sattar et al. (1977) studied the effect of light-induced decomposition of vitamin A in chloroform , β-carotene in hexane and both products in milk fat. The loss of β-carotene and vitamin A was reduced markedly in light-exposed foods by blocking wavelengths below 465 nm. In alcohol, β-carotene has two maxima (452 and 481 nm) in the visible-absorption spectra. In petroleum ether, it has strong absorbance at 273, 453, and 481 nm (Isler et al., 1984). Thus β-carotene receives little protection by blocking UV radiation since it absorbs energy mainly in the visible region of the spectrum. Tocopherols on the other hand, should be protected by blocking UV radiation, since the UV absorption spectrum for α-tocopherol in ethanol shows maxima at 292 and 256nm (Isler and Kienzie, 1984). Therefore, loss of α-tocopherol (and subsequently the other tocopherols) can be a precursor to the loss of β-carotene, which in turn can result in lipid oxidation in oils and other food products exposed to UV radiation. Emmons et al. (1986) reported that wavelengths of 220 to 550 nm from Warm-White fluorescent light bulbs, caused a strong oxidative flavor in butter, whereas, wavelengths above 550 nm did not.

Since light has such a profound impact on the keeping quality of edible oils and other lipid containing foods, packaging can play an important roll in its shelf-life.

Sattar et al. (1976) reported that relatively simple and inexpensive changes in packaging materials result in the retention of flavor quality and light-sensitive vitamins.

EFFECT OF PACKAGING ON LIGHT SENSITIVE FOODS.

Leo (1983) stated that the container can influence the absorption of light, oxygen, heat and moisture by the product. The light source can be either artificial, incandescent or fluorescent, or natural sunlight. Of special interest was light in the ultra-violet region, with wavelengths up to 390 nm and the visible region of violet and blue having wavelengths of 390-490 nm. Oxygen was the most critical factor affecting oxidative quality, because of its role in the formation of hydroperoxides, the components normally associated with rancid oil. Oxygen can be in the headspace of a container, permeate its walls or be entrapped in the oil, or other lipid containing foods.

Nkpa et al. (1990) studied the effect of various packaging materials on the storage stability of crude palm oil. They showed that lacquered metal cans offered the greatest protection against the deleterious effects of sunlight. Colored bottles gave fairly good protection, while transparent plastic and clear glass bottles resulted in the poorest protection of the oils during storage in direct sunlight. Similar results were obtained by Warner and Mounts (1984) after evaluation of the flavor and oxidative stability

of soybean oils in amber and clear glass bottles, and in plastic bottles exposed to light.

Luby et al. (1986) determined the effect of packaging on the oxidative stability of cholesterol in butter exposed to fluorescent and daylight, and found that aluminum foil offered better protection than margarine wrap, opaque parchment, wet strength dry wax paper and polyethylene film. Similar results were obtained by Emmons et al. (1986) who determined the light transmission characteristics of wrapping materials in relationship to oxidation of butter by fluorescent light.

Bradley (1980) found that milk in glass, polycarbonate, high density polyethylene, blow-molded polyethylene, plastic bags and paperboard containers, developed a characteristic off-flavor during exposure to fluorescent light or sunlight. Kiritsakis et al. (1984) showed that olive oil packaged in transparent glass and polyethylene plastic bottles had little protection from light. Glass provided more protection than the plastic. They suggested that the higher oxidation rate in the plastic might have been due to the intrusion of oxygen as a consequence of the permeability of the plastic.

UV LIGHT ABSORBERS

In 1985, Fanelli et al. investigated the effectiveness of visible and UV light screens, compounded in polyethylene, to protect vitamins in milk from photodegradation by

fluorescent light. Six light screens (3 pigments and 3 UV absorbers), were selected for the study, all of which had FDA approval for contact with food. The three pigments created red, yellow and blue colored resin and were Quinacridone red, FD&C yellow #5 and Ultramarine blue, respectively. The three UV absorbers were Cyasorb 531™, Tinuvin 326™ and Tinuvin 662™. At the conclusion of the study, they reported that incorporation of Tinuvin 326 and Cyasorb 531 into the resin significantly reduced vitamin A loss. Tinuvin 622 did not have the same protective value, while the FD&C yellow #5, at a concentration of 0.3%, protected vitamin A for at least 24 hours. None of the UV absorbers protected riboflavin and ascorbic acid. After investigating the absorption spectra of the species involved, they showed that vitamin A had a single strong absorption band with a maximum at 325 nm. Tinuvin 326, Cyasorb 531 and FD&C yellow #5 protected vitamin A because these chemicals absorbed energy in the UV region of the spectrum. Consequently, riboflavin with its strong absorption band at approximately 450 nm was not similarly protected, even though it has several bands in the UV and visible range. Ascorbic acid absorbs in the UV range at wavelengths below 300 nm, but was not protected by the UV absorbers. They suggested that the decomposition of ascorbic acid is dependent on the presence of riboflavin, and concluded that riboflavin acts as a photosensitizer with respect to the decomposition of ascorbic acid. Shipe et al. (1983) obtained similar results when samples of pasteurized homogenized low-fat (1%) milk,

fortified with vitamin A, was exposed to light in half gallon polypropylene containers impregnated with either Tinuvin 326, Cromophtal yellow 3G or yellow 2 RLTS. Tinuvin 326 was able to reduce vitamin A loss by about 80%.

Crompton (1971) reported that Ultra violet absorbers are often used in food packaging materials to protect the plastic material as well as the foodstuff packaged, from the actinic action of ultraviolet radiation. Actinic effects may cause discoloration of both the plastic material and the foodstuff, and may also occasion changes in taste and loss of vitamins in the food.

Several other UV absorbers including Tinuvin 326, have been used in a number of applications where UV screening was necessary to extend the life of certain non-food materials. Dunn et al. (1966) showed that Tinuvin P and an antioxidant referred to as "antioxidant #2246", improved the stability (against photochemical aging) of natural rubber which had been radiation-crosslinked. Colfico (1966) reported that when Tinuvin 326 was incorporated into a coating applied to photographic reproductions on paper or film, increased protection from the sun and UV radiation resulted. Heller et al. (1962) demonstrated that incorporation of benzotriazoles into cellulose acetate, nylons, polyesters, polymethyl methacrylate, polyvinyl chloride, polyethylene and non-actinic compounds (for human skin), can protect against the damaging

effects of ultraviolet radiation. Maria et al. (1976) utilized Tinuvin to improve the light stability of polyethylene. Evans et al. (1986) showed that dyed wool can be protected from phototendering and dye fading by the 2-(2'-hydroxyaryl)-2H-benzotriazole ultraviolet absorber, sulfonated Tinuvin P™. Kolawole et al. (1982) proved that a synergistic effect was obtained when a combination of Tinuvin P and 3,5-ditert.butyl-4-hydroxybenzyl thioglycollate was used to protect acrylonitrile-butadiene-styrene (ABS) from exposure to weathering. Pyong-Nae (1989) reported that ultraviolet light stabilization of polypropylene and other polymers can be achieved by hindered amine light stabilizers (HAL), phenolic antioxidants and phosphites. Also, a synergistic effect is achieved between HALS and UV absorbers in clear coatings, metallic finishes and sparingly pigmented systems.

In general, stabilization of polymers to weathering involves retardation or elimination of primary photochemical processes similar to those involved in lipid photooxidation. This is done by preventing the UV radiation in light from reaching the polymer by using a coating or a UV screen, by UV absorbers which preferentially absorb and dissipate the UV energy harmlessly, and by addition of a compound which can remove the excited state energy from the polymer before harmful reaction can occur. This last process in known as quenching.

Ultraviolet screens function by rendering the polymer opaque to both visible and UV light (Guillet, 1972). This prevents the penetration of UV radiation into the polymer, and thus reduces degradation. The most important of these is carbon black which is one of the most effective polymer stabilizers (Guillet, 1972). Dispersed throughout a polymer (in addition to acting as a UV screen), carbon black can trap radicals produced during photooxidative processes. Heskins and Guillet (1972) have suggested that carbon black may also stabilize polymers by quenching the excited molecules in the polymer through absorption of UV radiation.

The terms UV absorbers and UV stabilizers are often used interchangeably. In the Encyclopedia of Chemical Technology (1984), it states that, "ultraviolet stabilizers are colorless or nearly colorless organic substances which protect polymeric and other light-sensitive materials from degradation by sunlight and artificial sources of UV radiation." Several different classes of compounds are used commercially to retard light-induced polymer degradation, including UV absorbers, hindered amines, nickel chelates, hindered phenols and aryl esters (Dexter, 1984). The most widely used stabilizers consist of derivatives of salicylic esters, benzotriazoles and orthohyroxybenzophenones (Guillet, 1972). Of the known UV-absorbers, the o-hydroxybenzophenones and the o-hydroxyphenylbenzotriazoles are most frequently used in industrial applications. This is

due mainly to their extreme light stability in polymeric substrates (Heller et al., 1972).

Gugumus (1984) reported that concentrations of about 0.2 - 0.3% benzophenone and benzotriazole types of UV absorbers incorporated into thick sections of HDPE, led to a considerable improvement in UV stability and extended lifetime of the material. However, with the subsequent development and use of Ni-quenchers the stabilization of thin sections of HDPE was similarly improved. A further step forward in the UV stabilization of HDPE was achieved with the hindered amine light stabilizers (HALS) (Gugumus, 1984).

An ideal UV absorber should absorb radiation between 290 and 400 nm while transmitting all visible light, as absorption directly above 400 nm causes the absorber to impart a yellowing to the substrate (Dexter, 1984). They should also be light stable and not disintegrate during long-term exposure. Dexter (1984), reported that substituted 2-(2-hyroxyphenyl)benzotriazoles approach this ideal most closely since they absorb very strongly throughout most of the UV region and show a rapid decrease in absorbancy approaching 400 nm.

The UV stabilizers (preferably derivatives of o -hydroxy-benzophenone or of 2-(2'-hydroxy-5'-methylphenyl)benzotriazole (Tinuvin) transform the absorbed

light energy into thermal energy thus preventing all sorts of photochemically initiated reactions (Werner et al., 1981). The photooxidation of polymeric materials can be represented by a simplified reaction sequence as shown by Dexter, (1984).

Initiation:

$$ROOH \xrightarrow{h\nu} RO. + .OH \qquad (1)$$

$$RCR \xrightarrow{h\nu} RCR^{*} \qquad (2)$$

photoexcited chromophore

$$RCR \rightarrow RC. + .R \qquad (3)$$

$$RC. \rightarrow R. + CO \qquad (4)$$

Propagation:

$$R. + O_2 \rightarrow ROO. \qquad (5)$$

$$ROO. + RH \rightarrow ROOH \qquad (6)$$

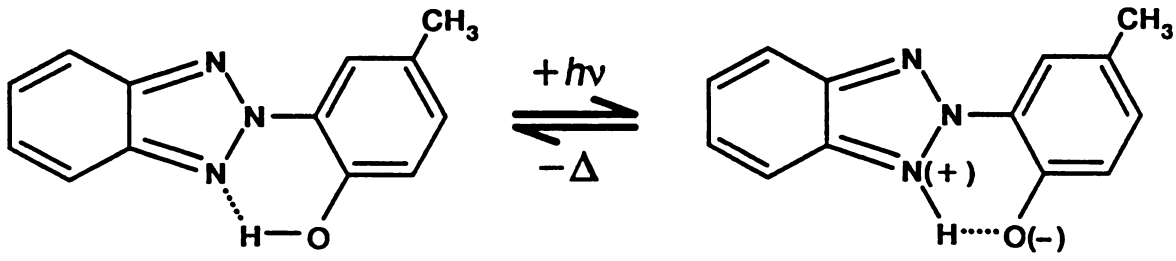
Chain branching:

$$ROOH \xrightarrow[h\nu]{\Delta \text{ or}} RO. + .OH \qquad (7)$$

$$2\ ROOH \rightarrow RO. + ROO. + H_2O \qquad (8)$$

In the presence of excess oxygen, reactions 5 and 6 can be repeated hundreds of times, which increases the concentration of hydroperoxides. These can cleave homolytically by absorption of thermal or actinic energy to yield additional radicals (equation 7) (Dexter, 1984). The ROOH in reaction 1, can come from another system undergoing oxidation. UV stabilizers can function to retard the initiation of the photooxidation process by absorption of UV energy and by quenching of photoexcited chromophores. Antioxidants can reduce oxidation by scavenging free radicals or destroying hydroperoxides to yield nonradical species. Dexter (1984) reported that many commercially available stabilizers function by more than one mechanism. Thus, 2-hydroxybenzophenones and 2-(2'-hydroxyphenyl)benzotriazoles contribute to polymer stability by absorbing UV radiation, trapping free radicals, and quenching photoexcited chromophores. Their ability to trap free radicals also gives them antioxidant properties.

The reaction of oxygen with unsaturated lipids and polymers to cause oxidation, is basically the same. The mechanism is initiated by the removal of hydrogen from a hydrocarbon molecule, which can occur in the presence of metals, light, heat, or hydroperoxides. The mechanism by which 2-(2'-hydroxyphenyl)benzotriazoles dissipate absorbed radiant energy involves tautomeric structures (1) and (2) (Werner et al., 1981).



(1) (2)

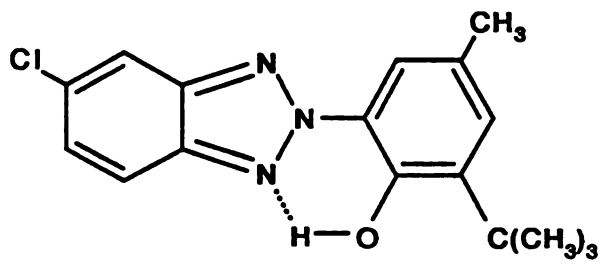
Fig 1. The mechanism of light energy absorption by a benzotriazole.

In the ground state, the phenolic structure (1) is preferred since the electron density on the oxygen atom is much greater than that on the triazole nitrogen. If UV energy is absorbed, the electron density shifts from the oxygen atom toward the triazole ring. This results in an increase in basicity of the nitrogen atom and causes the proton to jump from oxygen to the nitrogen. Tautomer (2) is unstable and rapidly loses its energy as heat, and reverts back to the ground-state structure (1). This process is efficient and accounts for the excellent light stability of 2-(2'-hydroxyphenyl)-benzotriazoles as well

as their inactivity as photosensitizers (Dexter, 1984).

Tinuvin 326

Tinuvin™ 326 is a UV absorber produced by the Ciba Geigy Co. and is chemically known as 2-(3'-tert-butyl-2'-hydroxy-5'-methylphenyl)-5-chlorobenzotriazole. It is a derivative from a benzotriazole. Tinuvin 326, a benzotriazole derivative, has a chloride molecule on the 5th position of the basal benzene ring and a tertiary butyl functional group at the #3 position of the functional benzene ring. Hence the appendices 3'-t-butyl and 5-chloro- are incorporated into the chemical name of the basic 2-(2'-hydroxyphenyl)-benzotriazole, to give (Tinuvin 326) 2-(3'-t-butyl-2'-hydroxy-5'-methylphenyl)-5-chlorobenzotriazole (Schwemmer, 1990). Capocci 1990 reported that Tinuvin 326 shows a maximum absorbancy at approximately 345 nm. A similar maximum absorbancy was found by Werner et al. 1981 for Tinuvin in a 20:20:1 ethanol/ether/pyridine solution.



Tinuvin 326

Fig 2. The structure of Tinuvin 326

The additional side chains give it various advantageous properties. Heller et al. (1962) suggested that these groups may interfere with its steric hindrance and thus rotation of the molecule in space. Dexter (1984) mentioned that such properties can increase the solubility of the UV absorber in the molten polymer, which would reduce exudation of the additive to the polymer's surface after manufacture of the material. In addition to minimizing volatility, these functional groups help to reduce reaction of the UV absorber with metal ions such as Co^{2+}, Zn^{2+}, and Cd^{2+}. Such reactions would otherwise lead to chelate formation and result in the production of colored complexes. Pure Tinuvin 326 is a yellow solid, with melting point 137-141°C and a decomposition temperature >220°C (Schwemmer, 1990).

Tinuvin 326 was approved by the FDA as a food packaging additives in April 1981 (Title 21 of the Code of Federal Regulations). The incorporation of Tinuvin 326 in containers to package oils and other food products can increase the stability of both the container and the product. This occurs due to its ability to absorb UV radiation and its antioxidant property. By reducing exposure of the oil to UV radiation, lipid oxidation can be reduced. Migration of Tinuvin 326 from the container to the oil could serve to further inhibit lipid oxidation. This technique has been successfully used in the past. Hoojjat et al. (1988) showed that the migration of BHT, an antioxidant, from high density polyethylene film into a

food product, improved the oxidative stability of the packaged oatmeal cereal.

MATERIALS AND METHODS

Test packages:

The test packages used in this study were 127 ml (4.5 oz), 0.381 mm (15 mil) thick, transparent, coextruded multilayered polypropylene cups which were provided by The Ball Plastics Division, Plastics Packaging Development Center, Evansville, IN. The material profile of the containers from outside to inside was: polypropylene 95.25 μm (3.75 mil) / regrind 80.01 μm (3.15 mil) / adhesive 9.53 μm (0.375 mil) / 44 mol. % ethylene vinyl alcohol copolymer 15.24 μm (0.6 mil) / adhesive 9.53 μm (0.375 mil) / regrind 80.01 μm (3.15 mil) / polypropylene 91.44μm (3.60 mil). One set of containers had zero percent, while the other had 0.3% Tinuvin 326 incorporated into the regrind layers, respectively. The Alusuisse™ "Easy Peel" lid stock was also provided by The Ball Plastics Division. It had a peel force of 1.36 to 1.81 kg. (3.0 to 4.0 pounds) and a break force of 2.04 to 2.9 kg. (4.5 to 6.0 pounds).

Soybean oil:

Five gallons of refined Soybean salad oil (Mikado™) were obtained from the General Food Stores Michigan State University. The label indicated that the oil contained no added antioxidants. The oil was stored at room temperature

(21°C) prior to use.

Light source:

The light source used was a Phillips, cool white, 120 volt fluorescent lamp. It had a power rating of 34 watts and a length of 120 cm. It was positioned in a light box and was used to illuminate the bleached oil samples in the containers with and without Tinuvin 326, prior to determination of the peroxide value of the bleached oil samples.

EXPERIMENTAL PROCEDURE

Extraction of Tinuvin 326 from sample containers:

Analysis for Tinuvin 326 in the containers was carried out using HPLC and UV spectrophotometric methods. The procedure involved cutting the walls of a sample container into approximately 1 cm square pieces. An average sample size of 3 g was used for each analysis. Extraction was carried out with 110 ml HPLC-grade acetonitrile (Fisher Scientific Inc.), which was mixed with the sample and the sample extracted in a Soxhlet extraction apparatus for 18 hours. On completion, the acetonitrile containing the extracted Tinuvin 326, was made up to 200 ml in a volumetric flask with HPLC grade acetonitrile. The initial sample was extracted two times more, and on each occasion 110 ml of pure HPLC grade acetonitrile was added. For each of these, the extract were made up to 100 ml in a volumetric flask. These three extracts were individually analyzed for Tinuvin 326 using UV spectrophotometric and HPLC procedures, at a wavelength of 347.3 nm. Two replicates were taken for each analytical period, and the analysis duplicated for each replicate when using the HPLC method. The result of the quantification of Tinuvin 326 in each extract was added and the total represented the level of Tinuvin 326 in the sample material.

HPLC procedure to determine Tinuvin 326 in extract:

An HPLC technique was used to measure the Tinuvin 326 in the containers. A reverse phase system was used with a Perkin-Elmer stainless steel column, 0.24 cm internal diameter and 25 cm in length. The packing material of the column was comprised of ODS-HC sil-x-1. The mobile phase was comprised of 85% acetonitrile/15% distilled water (v/v) at a flow rate of 1 ml/minute. The volume injected was 10 µl using a Hamilton microliter #701-N Syringe. Peak areas and retention times were determined using a computing integrator. The elution time of Tinuvin 326 was approximately 4.85 minutes. The high pressure liquid chromatograph (HPLC) system consisted of a Perkin-Elmer Series 3B Solvent Delivery System and a LC-100 column oven, with a Perkin-Elmer LC-75 spectrophotometric detector set at 347.3 nm. The detector was interfaced to a Spectra Physics SP4200 Computing Integrator for quantitation.

The concentration of Tinuvin 326 in the containers was determined from standard curves constructed by analyzing Tinuvin 326 solutions of known concentrations in acetonitrile. The standards were prepared by dissolving 0.01 g Tinuvin 326 powder in 25 ml acetonitrile and making up to 100 ml in a volumetric flask, to give a concentration of 100 ppm (w/v) Tinuvin 326. The Tinuvin 326 powder was supplied by Ciba-Geigy Corporation, Hawthorne, New York. Serial dilutions from this stock solution were made to give solutions of 1, 10, 60,

80 and 100 ppm (w/v), respectively. Peak areas and retention times at these concentrations were used to construct the standard curve.

UV spectrophotometric procedure to determine Tinuvin 326 in extract:

A Perkin-Elmer Lambda 4B Double Beam UV-visible spectrophotometer was also used to measure the Tinuvin 326 content of the acetonitrile extracts. The optical density at 347.3 nm, and 1-cm path length, of each extract, was obtained after pouring approximately 3 ml into a quartz cuvette, then placing it in the cuvette holder of the spectrophotometer.

The concentration of Tinuvin 326 in the containers was determined from standard curves constructed by analyzing Tinuvin 326 solutions of known concentrations in acetonitrile. The standards were prepared by dissolving 0.01 g Tinuvin 326 powder in 25 ml acetonitrile and making up to 100 ml in a volumetric flask, to give a concentration of 100 ppm (w/v) Tinuvin 326. Serial dilutions from this stock solution were made to give solutions of 1, 5, 10, and 15 ppm (w/v), respectively. Absorbance in optical density units of these concentrations were used to construct the standard curve.

The efficiency of the extraction method was investigated by obtaining an absorbance spectrum, between

200 - 400 nm, of the container wall before the Soxhlet extraction. Another spectrum was obtain for the same container sample after the Soxhlet extraction procedure. This was done using a Perkin-Elmer (Oak Brook, Illinois) Lambda 3B UV-visible spectrophotometer which is equipped with an integrating sphere. Three samples from the 0% and three from the 0.3% Tinuvin 326 containers were cut approximately 3.0 cm x 1.5 cm and mounted directly in the sample holder of the Integrating Sphere. This instrument was used to monitor the relative change in concentration of the Tinuvin 326 in the container wall following repetitive extractions.

Loss of Tinuvin from the containers:

The rate loss of Tinuvin 326 from the test containers was determined using both HPLC, and UV spectrophotometric methods, by monitoring the change in Tinuvin content in the containers over a period of 42 days, at 21 ±1 and 35 ±1°C. respectively. The containers at 35°C were stored in a Precision Scientific, model 324 oven. The other set of test containers was stored in the laboratory, which had an average ambient temperature of 21°C ±2. The containers at both temperatures were stored in the absence of light.

Migration of Tinuvin from the containers into the oil:

The migration of Tinuvin 326 from the containers was studied over a 42 day period at 21 ±1 and 37 ±1°C.

respectively.

The samples were prepared by filling 120 ml of refined soybean oil into each of thirty 0.3% Tinuvin 326 containers. Each container was covered and heat sealed with the Alusuisse™ "Easy Peel" aluminum foil. Heat sealing was done at 182.2°C (360°F) and 275.8 kPa (40 psi) for 10 seconds using an Alloyd (Dekalb, Illinois) Blister-pack sealer.

Two of the oil filled containers were removed weekly from storage, and the concentration of Tinuvin 326 in the oil determined over a 42 day period. The container material was also analyzed for Tinuvin 326 using both UV spectrophotometric and HPLC methods over the same 42 days period.

Analysis for Tinuvin 326 in the oil was carried out using HPLC. The procedure involved removal of the container lids and stirring the oil to ensure a homogeneous mixture. From each sample, 3 ml of oil was removed with a disposable pipette and measured in a 10 ml volumetric cylinder. This was extracted with 15 ml of acetonitrile in a 50 ml separatory funnel. The mixture was shaken vigorously by hand for 20 - 30 seconds. The acetonitrile layer rose to the top after standing for several minutes. This layer was drawn off using a long-nose disposable pipette and transferred to a 100 ml test tube with a rubber cap. This extraction procedure was repeated 4 more times on the same oil sample, using 15 ml of acetonitrile

on each occasion. The extractants were combined and then centrifuged (International centrifuge, model CM, size 1, centrifuge) at 2000 rpm for 15 minutes to effect further separation of any residual oil. The acetronitrile solution above was drawn off using a long-nose disposal pipette, taking care to leave the small quantity of oil at the bottom of the test tube. The resulting solution (approximately 75 ml) was then concentrated to 5 ml using a rota-evaporator (Buchi Rota vapor, model No. 110813, rota-evaporator). The 5 ml concentrate was then analyzed for Tinuvin 326 using the HPLC procedure at a wavelength of 347.3 nm. The concentration of Tinuvin 326 in the oil was calculated from the standard (Tinuvin 326) curve.

The efficiency of the extraction method was investigated by analyzing a 40 ppm solution of Tinuvin 326 in soybean oil. Tinuvin 326 was extracted from the oil by the method described above, and the amount of Tinuvin 326 which was extracted, determined using the HPLC method. Percent recovery was obtained by comparing the amount remaining in the oil to the initial 40 ppm. The efficiency of the concentration step was investigated by concentrating a 75 ml solution of 10 ppm Tinuvin 326 to 5 ml using the Buchi rota-evaporator. The amount of Tinuvin 326 in the concentrate was determined using the HPLC method. Comparing the calculated concentration to the original 10 ppm, provided the percent recovery.

Bleaching the soybean oil:

The soybean oil was filtered through an ion exchange column of internal diameter 4.5 cm and a length of 50 cm. Made of glass, the column was prepared by first packing a 2.0 cm layer of glass wool at the bottom. On to this was placed a 2.0 cm layer of MN-Kieselgur G-HR (Macheney, Nagel and Company), then a 100 g mixture containing 50% activated carbon Darco S-51 grade (American Norit Company), 35% MN-Kieselgel G-HR and 15% Florisil adsorbent for chromatography (Fluka Chemika). Finally, two successive layers of silicic acid and anhydrous sodium sulfate, each 1.0 cm , were added to complete the packing. The column was packed under reduced pressure using a vacuum pump with a negative pressure of 248 kPa (36 psi), and pressed with a glass rod to ensure proper compaction. The respective packing materials were added to the column in small amounts in slurried form by mixing hexane with the individual materials. After passing 100 ml of hexane through the packed column, 100 g of the soybean oil were mixed with 150 ml of hexane, and poured onto the top of the column. After this mixture was sorbed by the column, 150 ml of hexane were used to elute the oil. The eluate, which contained the bleached oil and hexane, was collected in a 500 ml filtering flask,and transferred to a 500 ml round bottom flask. The hexane was evaporated using a rotary evaporator under vacuum at 30°C. The oil obtained was transferred to an amber glass bottle, flushed with nitrogen and stored at 0°C.

The column was regenerated for multiple use by eluting 150 ml each of anhydrous ethyl ether (Em Science), acetonitrile (EM Science), methanol (EM Science), acetonitrile, anhydrous ethyl ether and finally hexane (EM Science) in successive order. This was done after each batch of oil was bleached.

Bleaching of the soybean oil was necessary to remove all its natural antioxidants. This was done to insure relatively rapid oxidation of the oil during exposure to fluorescent light. The thoroughness of the bleaching procedure was determined by Kalsec Inc. Kalamazoo, Michigan. Lipid oxidation was determined on three samples of unbleached soybean oil, using a Metrohm, Model 679 Rancimat at a temperature of 110°C. For unbleached oil, an induction time of 6 hrs was found. Using the same test for three samples of bleached soybean oil, an induction time of 0.95 hr was found. A shortening of the induction time indicates a greater susceptibility to oxidation.

Irradiation System to expose the bleached oil to fluorescent light:

A total of ninety containers with 0% Tinuvin and ninety containers with 0.3% Tinuvin were used for exposure to fluorescent light. Into each of these containers, 15 ml of the bleached soybean oil were added under atmosphere pressure. These were covered and heat sealed with the Alusuisse "Easy

Peel" aluminum foil sheets, using the Alloyd Blister sealer. Heat sealing was done at 182.2°C (360°F) and 275.8 KPa (40 psi), for 10 seconds.

During illumination, samples were divided into two equal groups (90 samples each). Ninety samples, composed of forty five containers with 0% Tinuvin 326 and forty five containers with 0.3% Tinuvin 326 were placed into each of two light boxes 91 cm x 61 cm x 53 cm (36" x 24" x 21"). One box was stored at 21 and the other at 35°C for a total of 35 days. The containers were suspended near the inner roof of each box. This was accomplished by hanging them from their heat sealing lip, between rows of strings stretched between the side walls (near the roof) of each box. The fluorescent tubes were placed under the boxes a distance of approximately 64 cm (25") below the containers. The light entered a box through an adjustable slit at the bottom which controlled its intensity. Thus, the oil in the bottom of the containers was exposed to the fluorescent light. A light intensity of 538 Lux (50 foot candles) was selected following a survey of grocery aisles in several retail food stores in the East Lansing, Michigan area. A General Electric, model J-55, light meter was used to measure the light intensity. The boxes were kept in dark rooms to eliminate light from other sources. Over the 35 day period (days 1,2 4,7,14,21,25,28,32 and 35), two samples were removed of each container type, at each temperature, and the extent of lipid oxidation in the oil determined by the

peroxide value method (AOAC, 1984).

Lipid oxidation determination by peroxide value method:

Lipid oxidation of the soybean oil, as a function of storage time and temperature and level of Tinuvin 326 in the containers, was determined using the official AOAC method (1984). Peroxide value is reported as milliequivalents peroxide/kg soybean oil. Approximately 5.0 g of oil accurately weighed, were taken from the sample containers following light exposure, for each analysis . The oil was transferred to a 250 ml Erlenmeyer flask and dissolved in a 30 ml acetic acid - chloroform (3:2) mixture. To this solution was added 0.5 ml of saturated potassium iodide solution, followed by mild agitation for 1 minute. Thirty ml of distilled water were then added and the solution titrated with 0.01N sodium thiosulfate solution. One and a half ml of a 1.0% starch solution were used as the indicator, and the titration carried out until the blue color had just disappeared. The peroxide value (milliequiv. peroxide/kg sample) was obtained by the following formula:

$$PV = \frac{Vol.(ml)\ sodium\ thiosulfate\ titrated \times normality \times 1000}{weight\ of\ oil(g)}$$

RESULTS AND DISCUSSION

Standard curves and absorbance spectra profiles:

Area response units vs concentration of the standard solutions (HPLC method) are summarized in Appendix 1. The standard curve is shown in Figure 3. The absorbance of the standard solutions vs concentration (Perkin-Elmer Lambda 4B Double Beam UV-visible spectrophotometric method) is presented in Appendix 2, while the standard curve is shown in Figure 4. The slope of the regression line for the HPLC standard curve was calculated to be 3.998×10^{3}. This was used as a conversion factor in calculating the concentration of Tinuvin 326 in the container walls and in the packaged oil, from the area response units obtained using the HPLC method. The slope of the regression line for the UV spectrophotometric standard curve was 5.191×10^{-2}. The regression line is described by the following equation:

$$y=5.191\times10^{-2}(*)+2.347\times10^{-4}$$

* = concentration of Tinuvin 326 in ppm (w/v)

This was used to calculate the concentration of Tinuvin 326 in the container walls by converting optical density units from the UV spectrophotometric method into ppm Tinuvin 326 (w/v).

The absorbance spectra of the wall of the containers were obtained using a Perkin-Elmer Lambda 3B UV-visible spectrophotometer equipped with an integrating sphere. The absorbance spectra of the wall of the 0.3% Tinuvin 326 containers before and after extraction are shown in Figures 5 and 6, respectively. The absorbance spectrum of the blank (0% Tinuvin containers) is shown in Figure 7. Figure 6 and Figure 7 show almost identical absorbance spectrum. Since Figure 6 presents a spectrum of the containers with 0% Tinuvin 326, it was concluded, based on comparison with Figure 5, that the dispersed Tinuvin 326 in the container wall was removed during the Soxhlet extraction process.

The wavelength at which maximum absorbance occurred, was determined from the profile illustrated in Figure 5. From this figure, the maximum absorbance of Tinuvin 326 was determined to be at 347.3 nm. At this wavelength, the profile of the containers with 0% Tinuvin 326 shows no absorbance. The profile of the container wall (0.3% Tinuvin) after extraction also shows no absorbance at 347.3 nm. Thus, it was concluded that 347.3 nm was the wavelength of maximum absorbance for Tinuvin 326.

Similar spectra were obtained for solutions of acetonitrile containing 0.0% Tinuvin 326 and 10 ppm (w/v) Tinuvin 326. These were obtained using a Perkin-Elmer Lambda 4B Double Beam UV-visible spectrophotometer. Dexter, (1984)

reported a similar absorption spectrum for 1.0 mg/100 ml 2-(2'-hydroxy-5-methylphenyl)benzotriazole in chloroform. Yushkevichyute and Shlyapnikov, (1967) also determined the concentration of Tinuvin 326 in polyethylene using a spectrophotometric method. They found maximum absorbance at wavelengths of 276 and 296 mμ in a heptane solution.

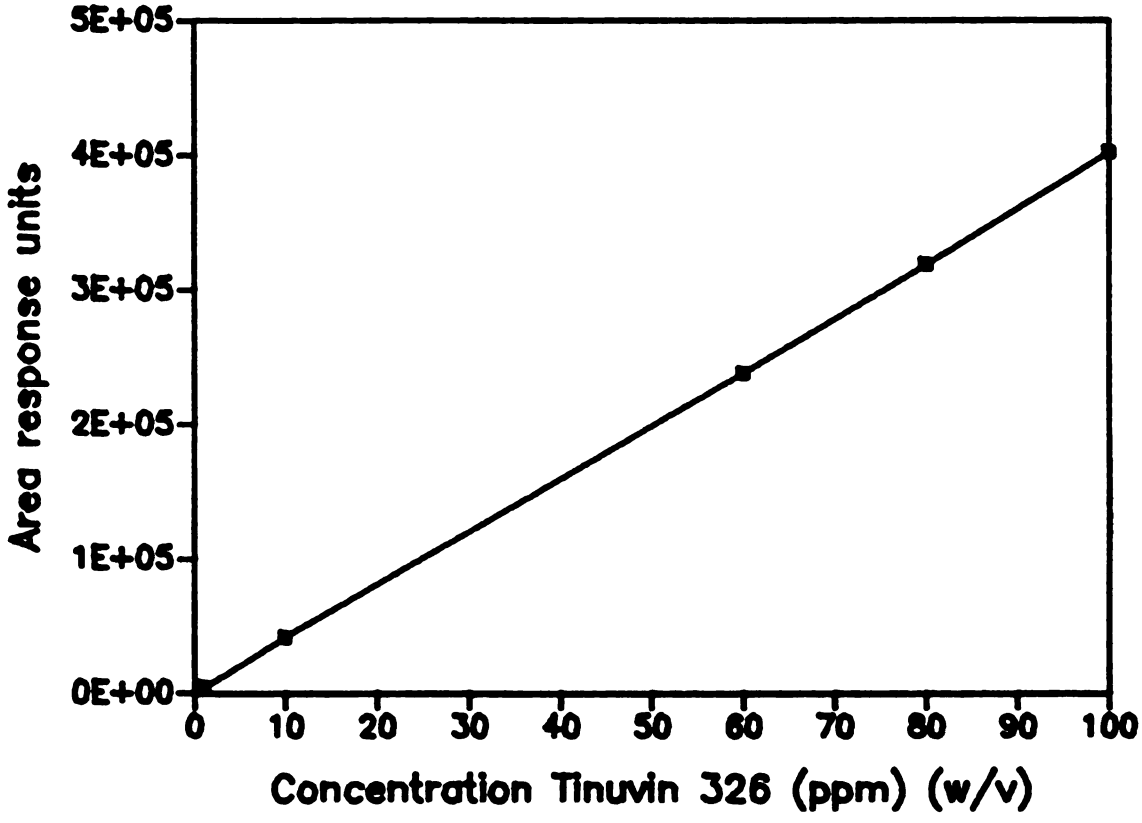


Fig. 3 Standard curve of Tinuvin 326 concentration vs area response units (HPLC method).

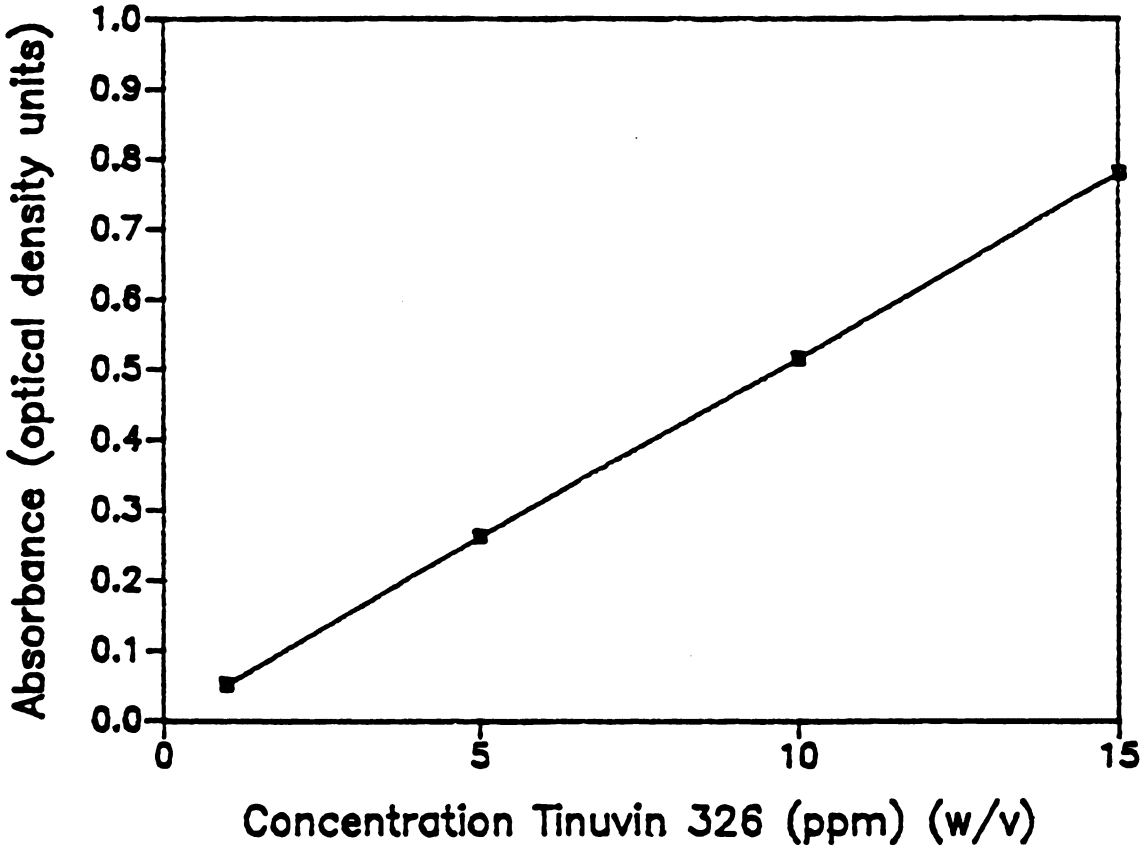


Fig. 4 Standard curve of Tinuvin 326 concentration vs absorbance (optical density units) (UV spectrophotometric method).

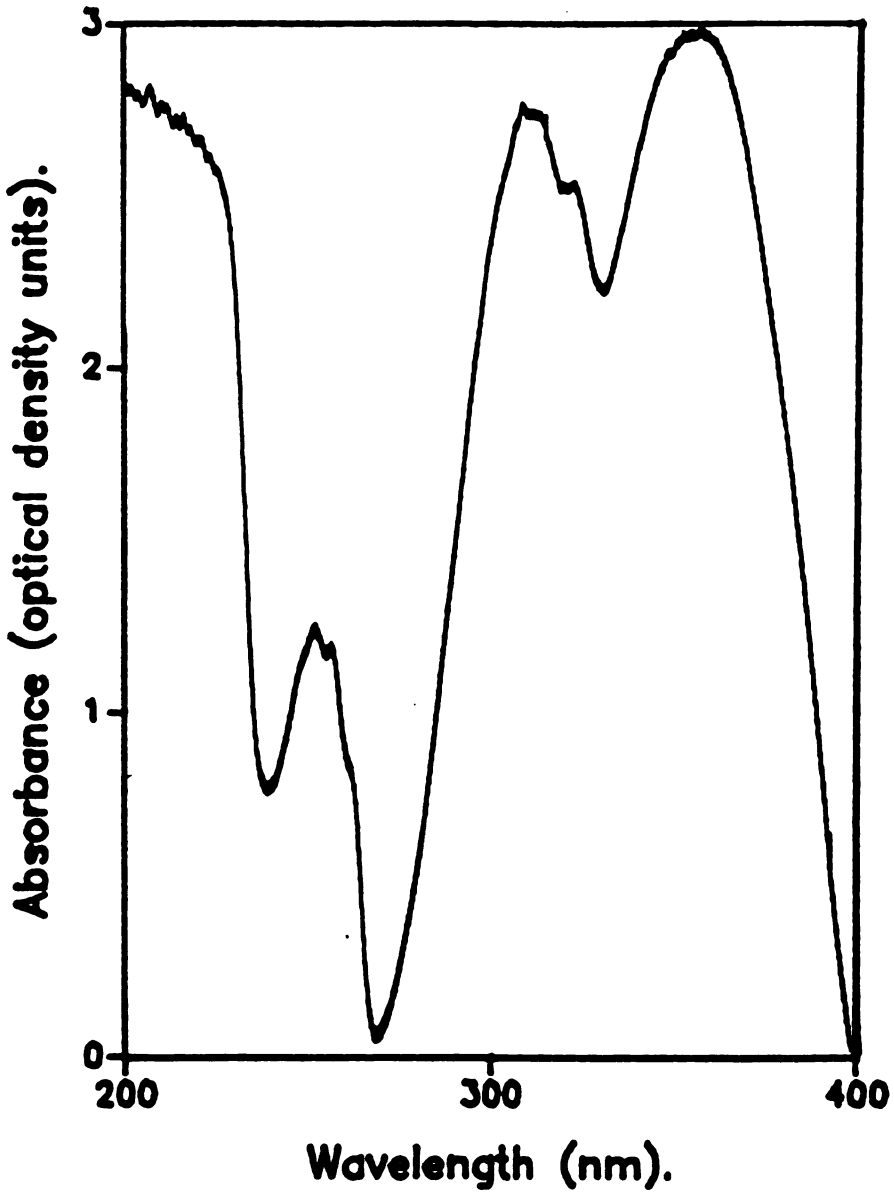


Fig. 5 Absorbance spectrum of container wall before extraction to remove Tinuvin 326.

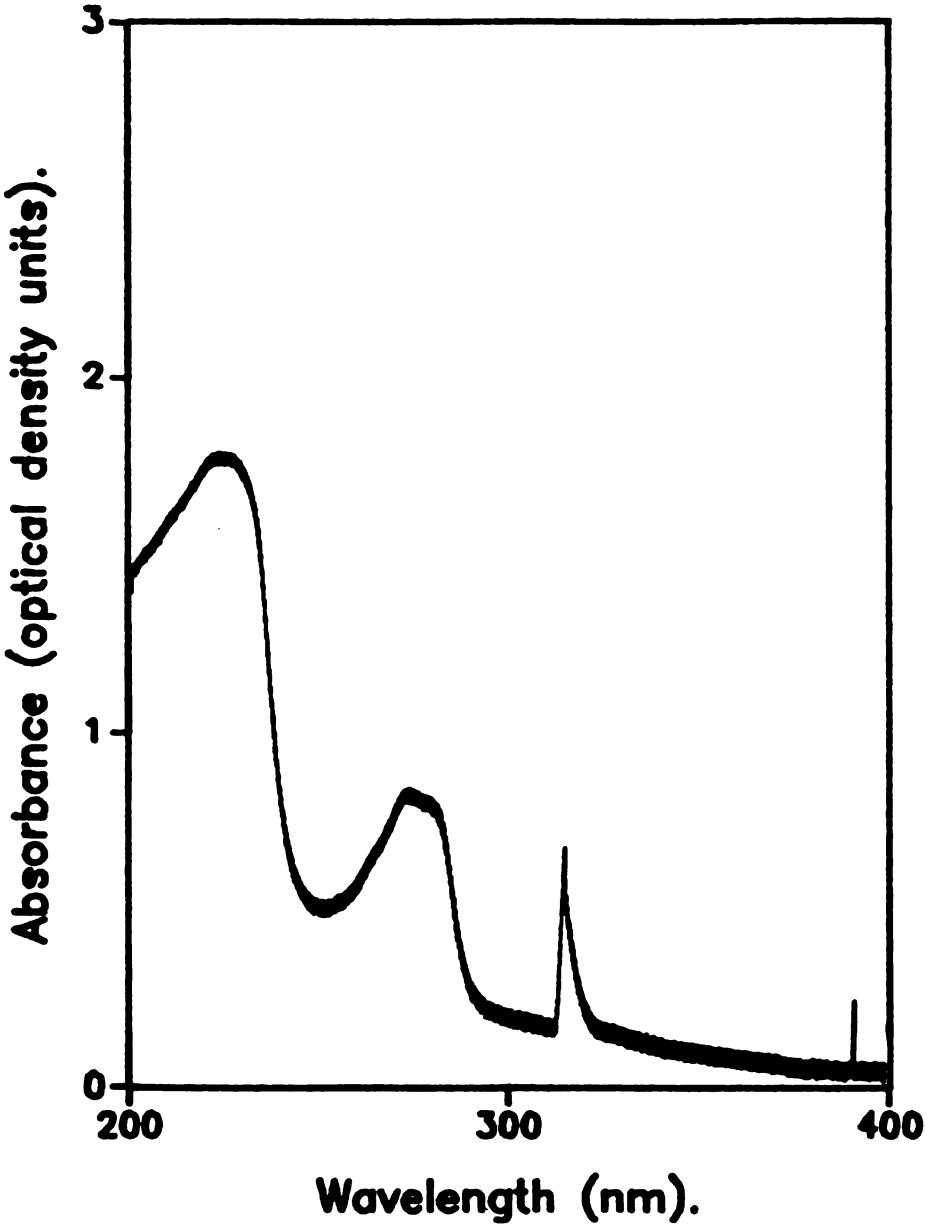


Fig. 6 Absorbance spectrum of container wall after extraction to remove Tinuvin 326.

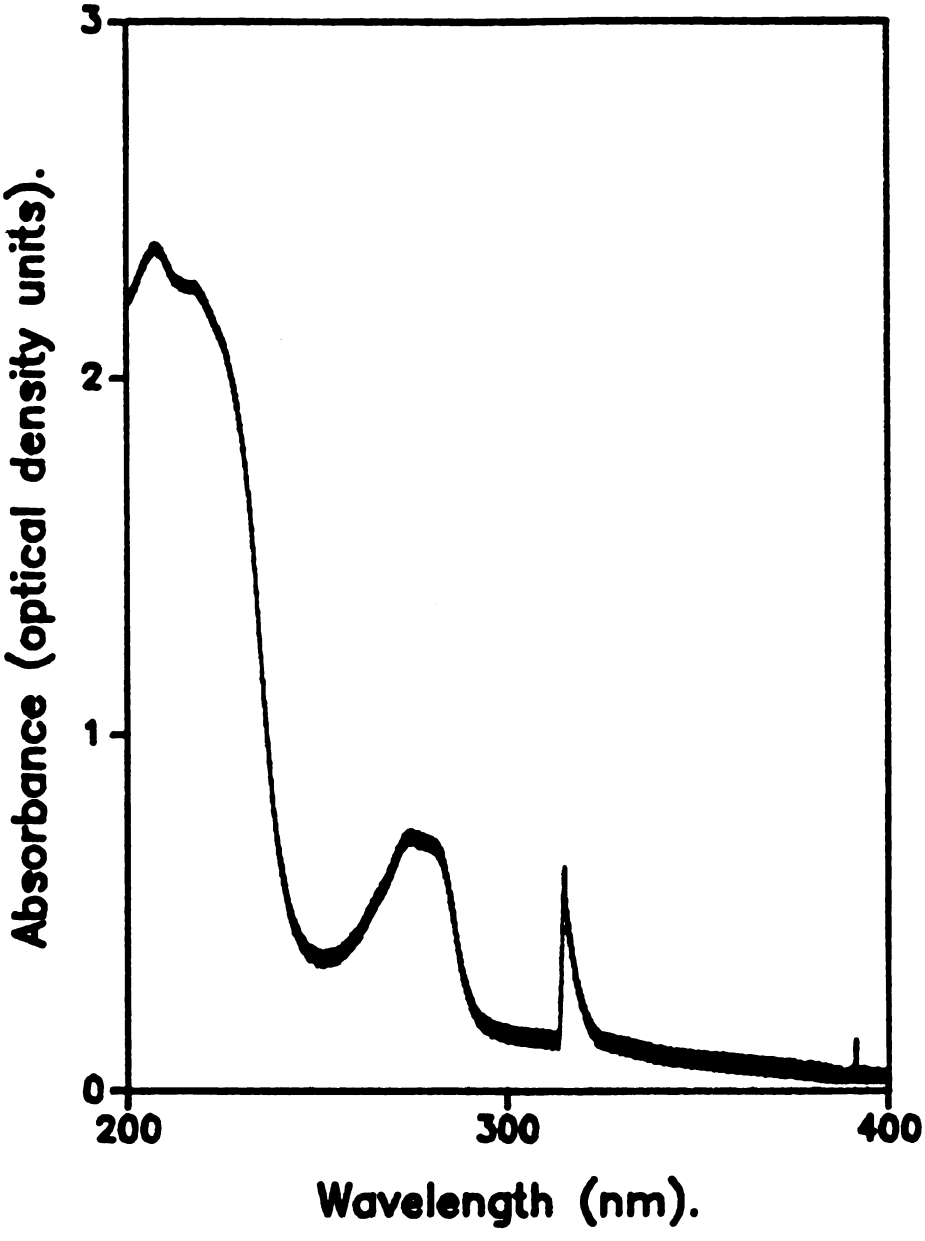


Fig. 7 Absorbance spectrum of container wall with 0.0% Tinuvin 326.

Loss of Tinuvin 326 from the container material:

In Tables 1 and 2 are summarized the percent Tinuvin 326 remaining in the container material during storage at 21°C., from zero to 42 days using the HPLC and UV spectrophotometric methods, respectively. The percent of Tinuvin 326 remaining in the containers during storage at 35°C., is shown in Tables 3 and 4. Graphs comparing results from the different storage temperatures are shown in Figures 8 and 9 (HPLC) and Figures 10 and 11 (UV spectrophotometric) respectively.

The concentration of Tinuvin 326 in the container wall (HPLC method), was calculated from the area response (au) units and the slope of the standard curve. An example of the calculation is presented in Appendix 3. Tables 5 and 6 show the data used to calculate the percentages of Tinuvin 326 at storage temperatures 21 and 35°C respectively. These are summarized in Appendix 4.

The concentration of Tinuvin 326 in the container wall was also calculated from the optical density units and the line equation of the standard curve (UV spectrophotometric method). An example of the calculation is presented in Appendix 5. The data used to calculate the percentages of Tinuvin 326, using the UV spectrophotometric method, is shown in Tables 7 and 8 for the containers at storage temperatures 21 and 35°C respectively. These Tables are included in

Appendix 6.

The results obtained from the HPLC and UV spectrophotometric methods are essentially identical. This indicates that either method could be used to quantify Tinuvin 326 in plastics such as used in this study. The results show little or no loss of Tinuvin 326 from the containers at either temperature during storage for 42 days. Thus, there was a high level of stability of the absorber in the 0.381 mm thick polypropylene containers. UV spectrophotometric analysis was used by Yushkevichyute and Shlyapnikov (1967) to quantify levels of Tinuvin 326 (in heptane solutions) in 0.16 mm thick polyethylene. They found that an increase blooming of Tinuvin 326 occurred at the surface of the film at higher temperatures. After approximately 400 hours, the level of Tinuvin 326 stabilized at temperatures of 20, 30, and 40°C. The differences in these results and those obtained in this study may be attributed to the difference in the thickness of the plastic, the material type and the manufacturing process. In this study the Tinuvin 326 was dispersed into the regrind layers of the laminate and not exposed to the surfaces of the material. In the Yushkevichyute and Shlyapnikov (1967) study, Tinuvin 326 was incorporated into the entire mass of the plastic.

The results from the analysis for Tinuvin 326 in the containers stored at 21 and 35°C, using the HPLC and the UV

spectrophotometric methods, show averages of 68.53% recovery for the first Soxhlet extraction, 21.76% for the second and 9.72% recovery for the third Soxhlet extraction. Initially an average concentration of 0.00515% Tinuvin 326 was found in a fourth Soxhlet extraction. Since this represents approximately 1.5% of Tinuvin 326 in the containers, the results from three extractions were used to quantify the levels of Tinuvin 326 in the material.

Table 1. Relative percent of Tinuvin 326 in the multilayer containers stored at 21°C. (HPLC method).

Storage time (days)	Average % Tinuvin (absolute)
0	0.30
3	0.31
7	0.30
14	0.32
21	0.29
28	0.31
42	0.30

Table 2. Relative percent of Tinuvin 326 in the multilayer containers stored at 21°C. (UV spectrophotometric method).

Storage time (days)	Average % Tinuvin (absolute)
0	0.30
3	0.29
7	0.31
14	0.32
21	0.30
28	0.31
42	0.30

Table 3. Percent Tinuvin 326 in the multilayer containers stored at 35°C. (HPLC method).

Storage time (days)	Average % Tinuvin (absolute)
0	0.30
3	0.29
7	0.30
14	0.33
21	0.31
28	0.31
42	0.31

Table 4. Percent Tinuvin 326 in the multilayer containers stored at 35°C. (UV spectrophotometric method).

Storage time (days)	Average % Tinuvin (absolute)
0	0.31
3	0.30
7	0.29
14	0.31
21	0.31
28	0.32
42	0.32

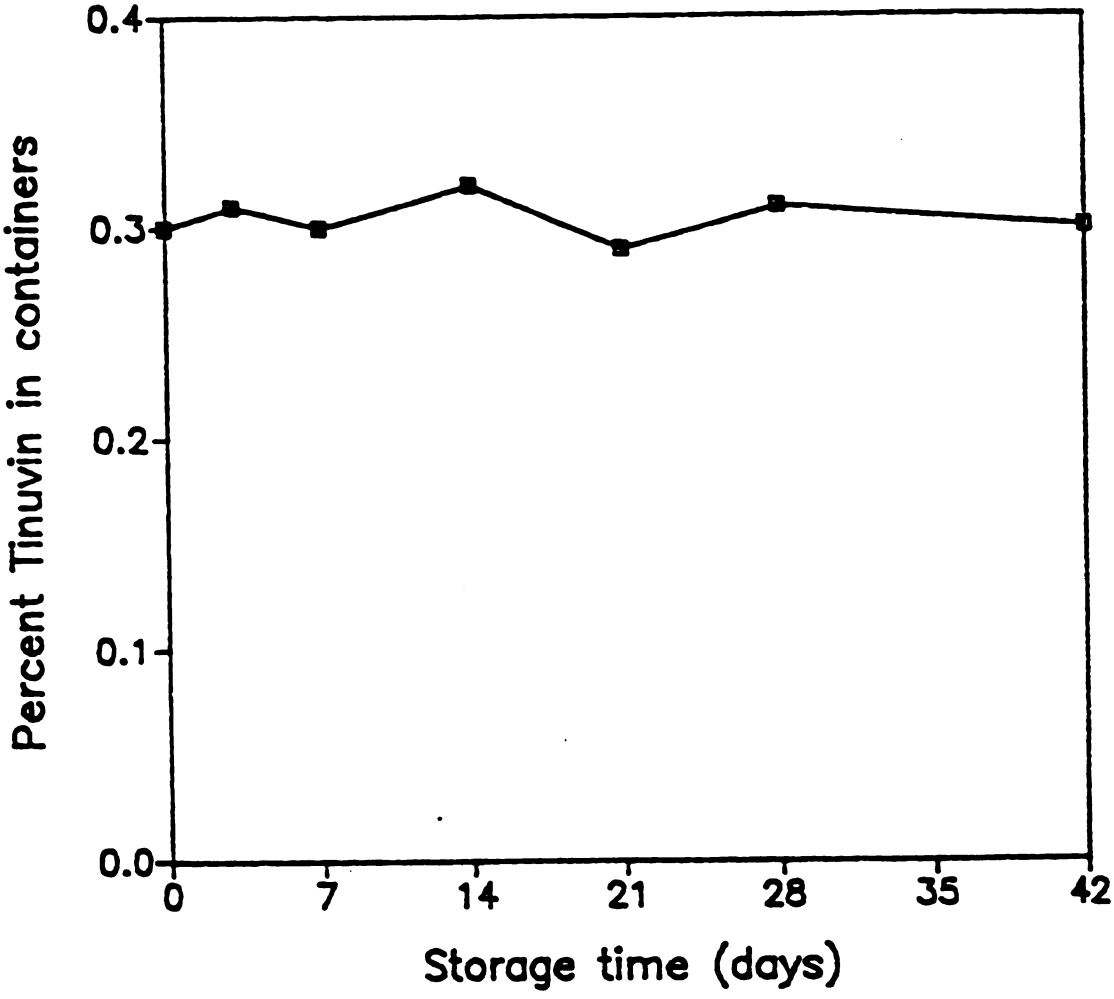


Fig. 8 Percent Tinuvin in containers stored at 21 degrees C. by HPLC method.

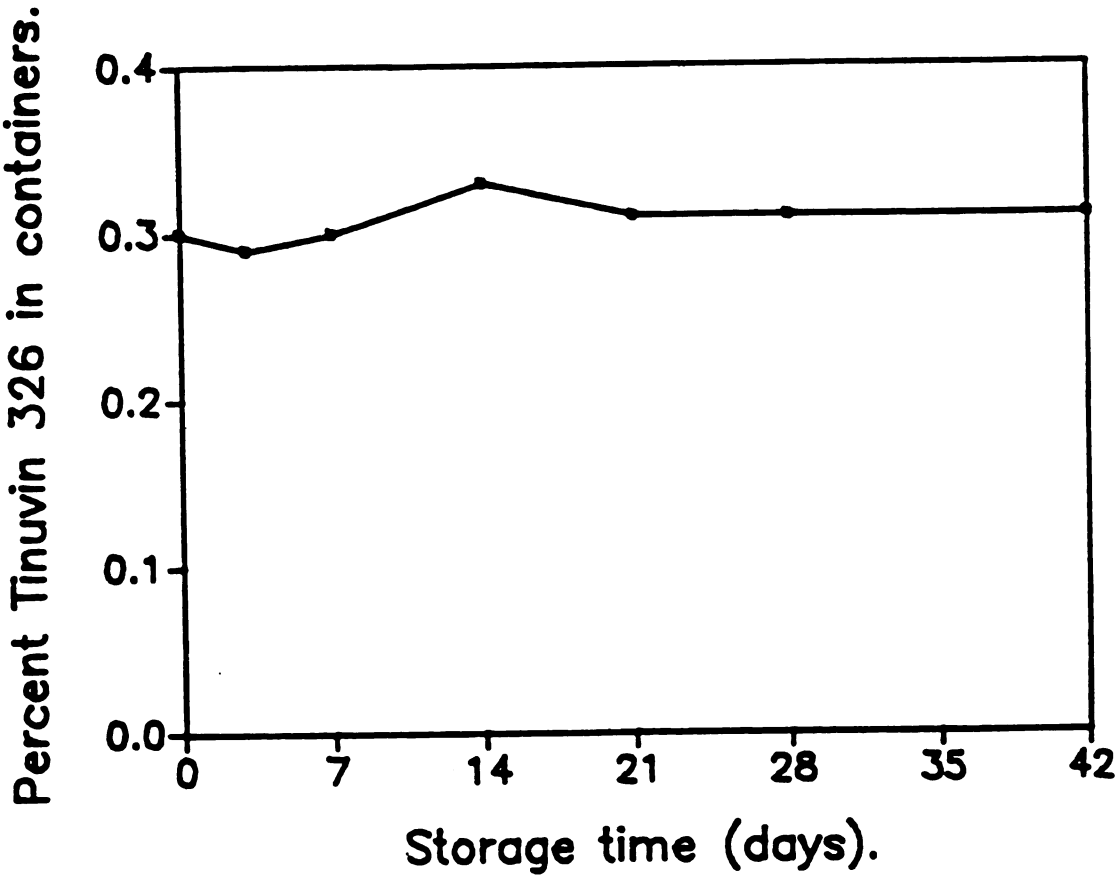


Fig 9. Percent Tinuvin 326 in multilayer container wall stored at 35 degrees C. by HPLC method.

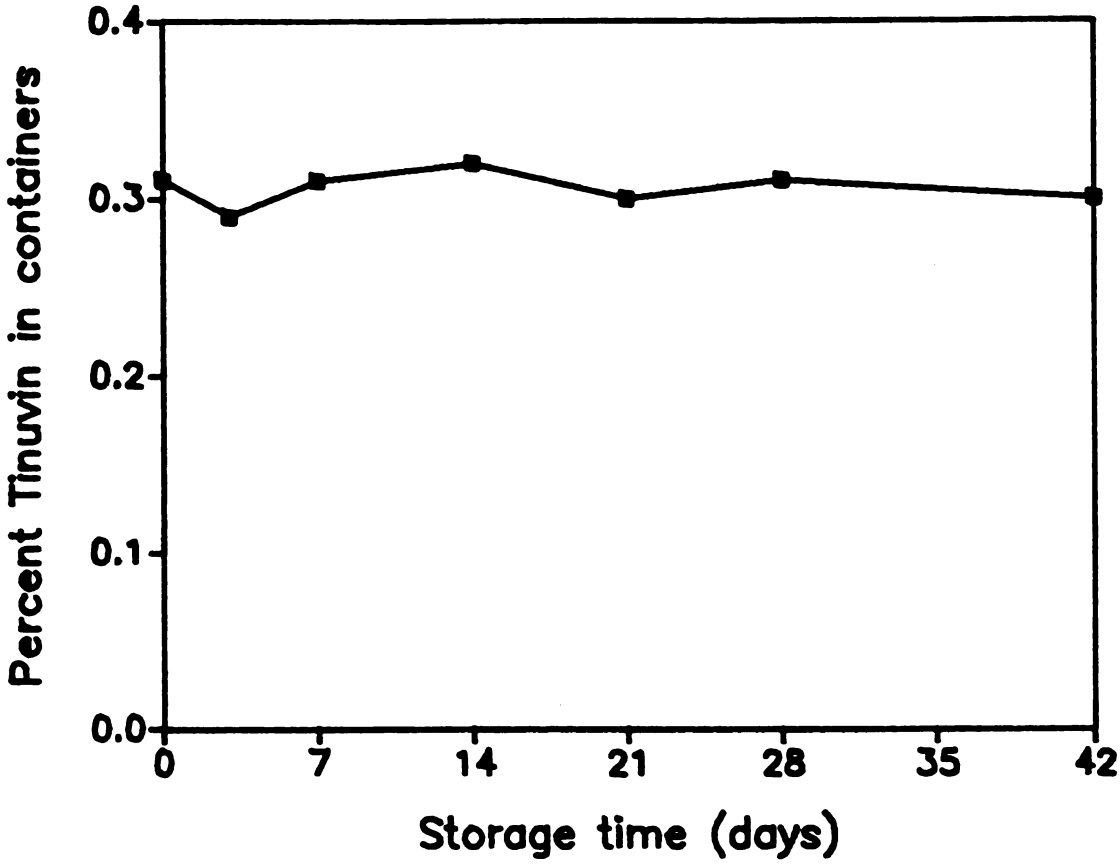


Fig. 10 Percent Tinuvin in containers stored at 21 degrees C. (UV spectrophotometric method).

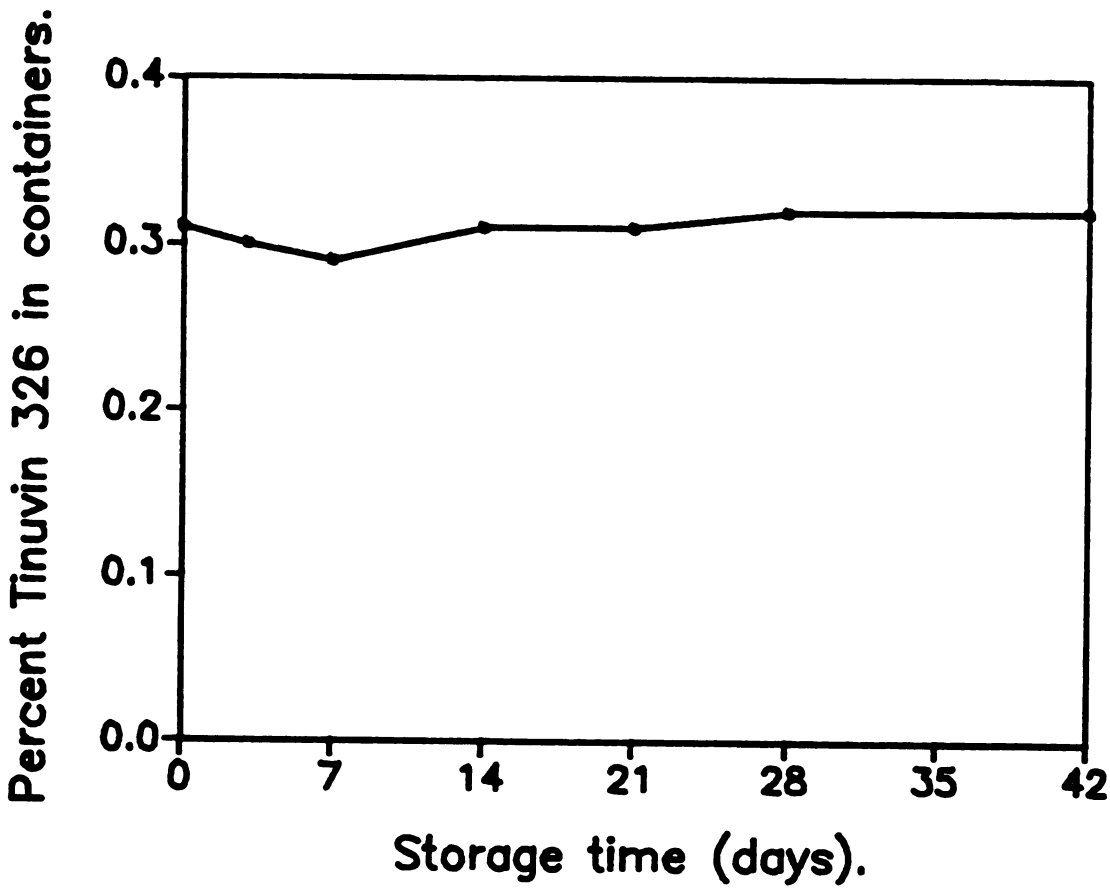


Fig 11. Percent Tinuvin 326 in containers stored at 35 degrees C. by UV spectrophotometric method.

Migration of Tinuvin 326 from the container wall into the oil:

The amount of Tinuvin 326 which migrated from the container wall into the oil at 21 and 35°C respectively, at successive storage times (HPLC method) is illustrated in Tables 9 and 10. In Figure 12, the effect of temperature and storage time on the amount of Tinuvin 326 migrating from the container wall to the oil is shown. Tables 11 and 12 display the level of Tinuvin 326 in the wall of the containers at the beginning of the study, and the level of Tinuvin 326 following storage at 21 and 35°C, respectively (HPLC method). Pictorial representation of Tables 11 and 12 are seen in Figures 13 and 14, respectively. The percent Tinuvin 326 that migrated from the containers to the oil at the two storage temperatures, is shown in Tables 13 and 14. Table 15 shows the percent recovery for extraction of Tinuvin 326 from the oil after the various storage periods. It also shows the efficiency level of the rota-evaporation concentration procedure.

The rate of migration of Tinuvin 326 from the container wall to the oil was higher at 35°C than at 21°C. This indicates that at elevated temperatures, Tinuvin 326 is more likely to migrate than at lower temperatures. However, the level of Tinuvin 326 in the container wall remained relatively constant even at the higher temperature. These two sets of results seem to be in conflict at first glance. However, the absolute quantities of Tinuvin 326 which migrated from the containers to the oil were very low, especially at 21°C. The

maximum level of Tinuvin 326 transferred to the oil was 4.77% of the initial level of Tinuvin in the containers. This is calculated as:

$$Wt.\ Tinuvin_{326}\ transferred = \frac{(9.009\times10^{-3}g)\times4.77}{100} = 4.2973\times10^{-4}g$$

$9.009\ x10^{-3}$ = initial level of $Tinuvin_{326}$ in the container wall.

This occurred after 42 days and at a 35°C storage temperature. At 21°C, no Tinuvin 326 was detected in the oil before seven days of storage. Thus at room temperature, the migration of Tinuvin 326 from the polypropylene container to soybean oil could not be detected by the methods used during the first week of storage. Thus Tinuvin 326 exhibits stability in the plastic containers in the presence of soybean oil.

The efficiency of the extraction process to remove Tinuvin 326 from the oil was determined to be 81%. This was determined from the level of Tinuvin 326 recovered from a spiked sample of the oil (40 ppm Tinuvin 326), using the extraction procedure employed to remove migrated Tinuvin 326 from the oil. From this, a conversion factor was used to convert the experimental value to a theoretical value which represents 100% extraction. The efficiency determined for the rota-evaporator (97.78%), was assumed to be equivalent to

100%, so no factor was employed in calculation of the amount migrating. This efficiency was determined from the level of 40 ppm Tinuvin 326 recovered from a 75 ml solution which had been concentrated to 5 ml, using the same rota-evaporator. An example of the calculations used to determine the amount of Tinuvin 326 which migrated to the oil after 14 days at 21°C, is shown in Appendix 7.

Table 9. Amount of Tinuvin 326 which migrated from the container wall to the oil at 21°C, (HPLC method).

storage time (days)	Ave. conc. Tinuvin in oil (wt/vol)	Wt. Tinuvin 120 ml oil (g)
7	0.0	0.0
14	1.300×10^{-5}%	1.550×10^{-5}g
21	2.400×10^{-5}%	2.80×10^{-5}g.
28	7.200×10^{-5}%	9.300×10^{-5}g
42	5.300×10^{-5}%	6.400×10^{-5}g

Table 10. Amount of Tinuvin 326 which migrated from the container wall to the oil at 35°C, (HPLC method).

storage time (days)	Ave. conc. Tinuvin in oil (wt/vol)	Wt. Tinuvin 120 ml oil (g)
7	3.100×10^{-5}%	3.750×10^{-5}g
14	7.600×10^{-5}%	9.050×10^{-5}g
21	9.800×10^{-5}%	11.750×10^{-5}g
28	16.500×10^{-5}%	19.850×10^{-5}g
42	43.000×10^{-5}%	35.800×10^{-5}g

Table 11. Level of Tinuvin 326 in the wall of the containers in which oil was stored at 21°C (HPLC method).

Storage time (days)	% Tinuvin 326 / 100g container wall	Ave. Wt. Tinuvin 326 in the walls of the containers
0	0.30%	9.009×10^{-3} g
7	0.34%	10.271×10^{-3} g
14	0.32%	9.791×10^{-3} g
21	0.31%	9.567×10^{-3} g
28	0.31%	9.365×10^{-3} g
42	0.33%	9.968×10^{-3} g

Table 12. Level of Tinuvin 326 in the wall of the containers in which oil was stored at 35°C (HPLC method).

Storage time (days)	% Tinuvin 326 / 100g container wall	* Ave. Wt. Tinuvin 326 in the wall of the containers
0	0.30%	9.009×10^{-3} g
7	0.31%	9.520×10^{-3} g
14	0.32%	9.911×10^{-3} g
21	0.32%	9.600×10^{-3} g
28	0.32%	9.790×10^{-3} g
42	0.30%	9.113×10^{-3} g

* Average of two replicates (two injections per replicate).

Table 13. Percent Tinuvin 326 which migrated from the container wall to the oil at 21°C, (HPLC method).

storage times (days)	Relative % Tinuvin* transferred
7	0.0%
14	0.172%
21	0.311%
28	0.955%
42	0.710%

Weight of Tinuvin 326 in the wall of the container storing the oil at zero time = 9.009 $x10^{-3}$g / container.

* Relative % Tinuvin transferred from the initial weight of Tinuvin in the container wall.

Table 14. Percent Tinuvin 326 which migrated from the container wall to the oil at 35°C, (HPLC method).

storage times (days)	Relative % Tinuvin* transferred
7	0.416%
14	1.005%
21	1.304%
28	2.203%
42	4.773%

Weight of Tinuvin 326 in the wall of the container storing the oil at zero time = 9.009 $x10^{-3}$g / container.

* Relative % Tinuvin transferred from the initial weight of Tinuvin in the container wall.

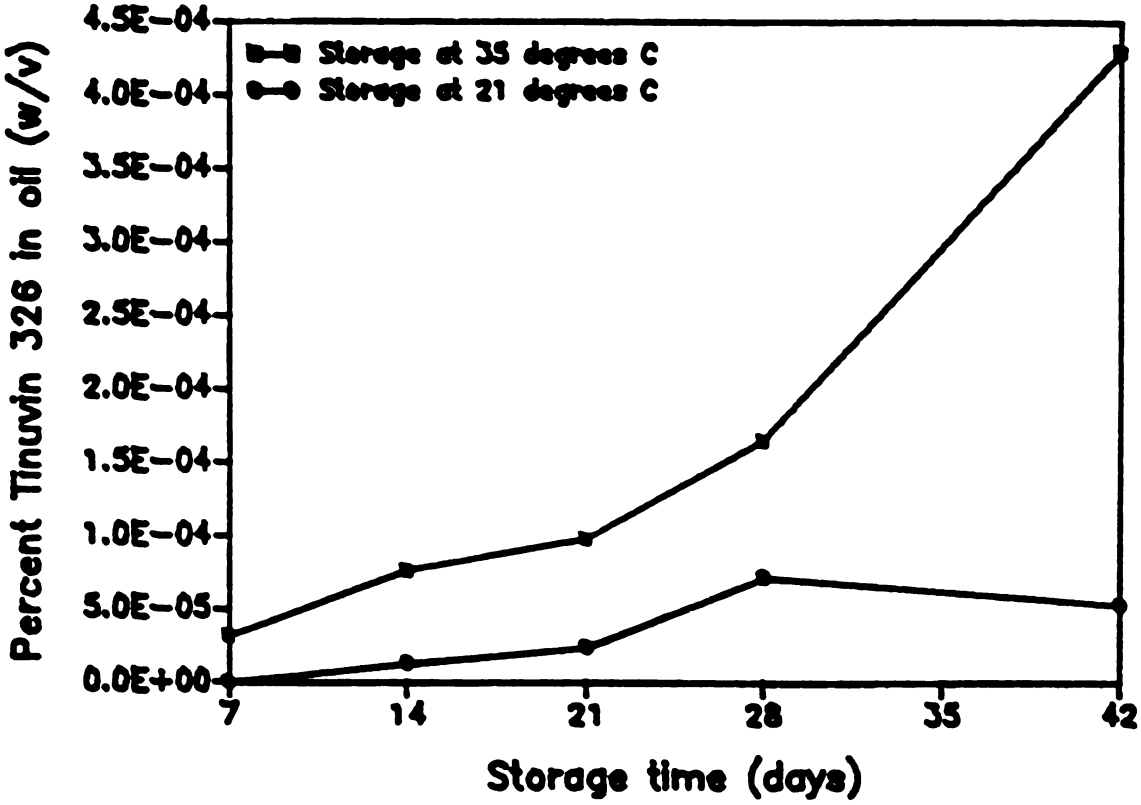


Fig. 12 Percent Tinuvin 326 which migrated from the containers to soybean oil at 21 and 35 degrees C (HPLC method).

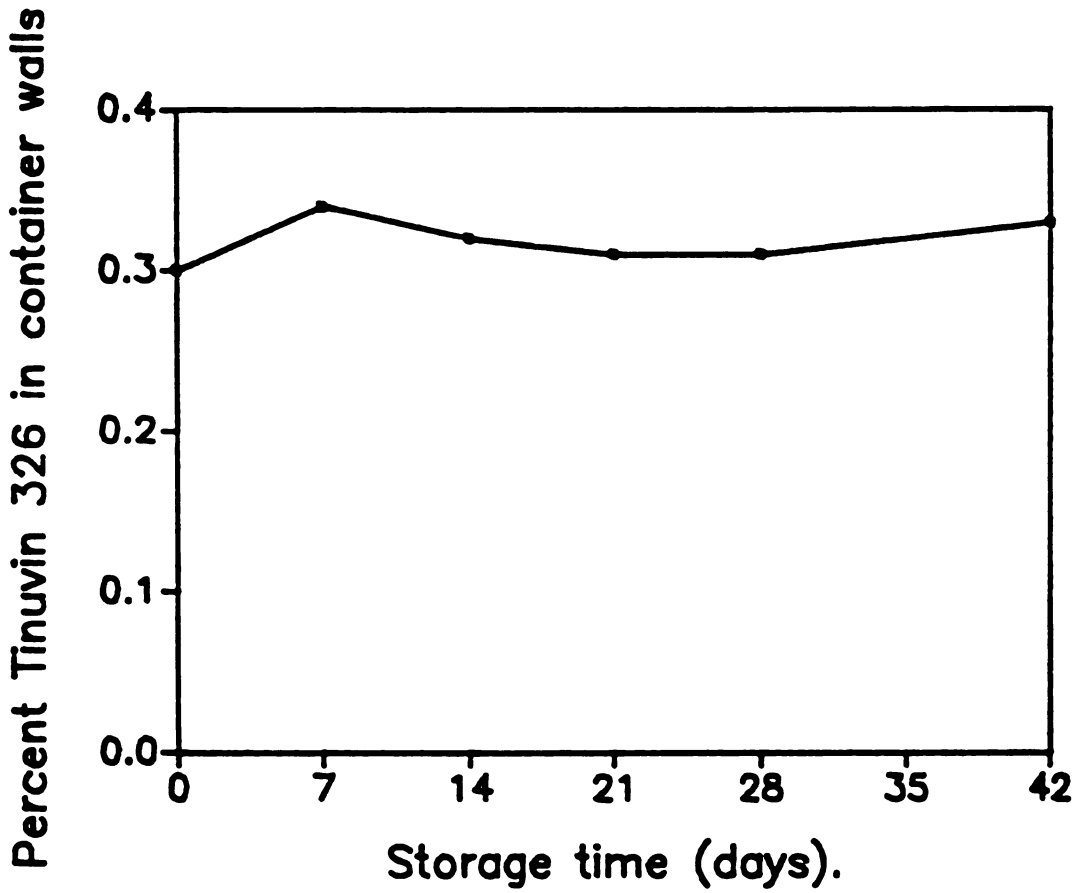


Fig. 13 Percent Tinuvin 326 in wall of containers after oil storage at 21 degrees C (HPLC method).

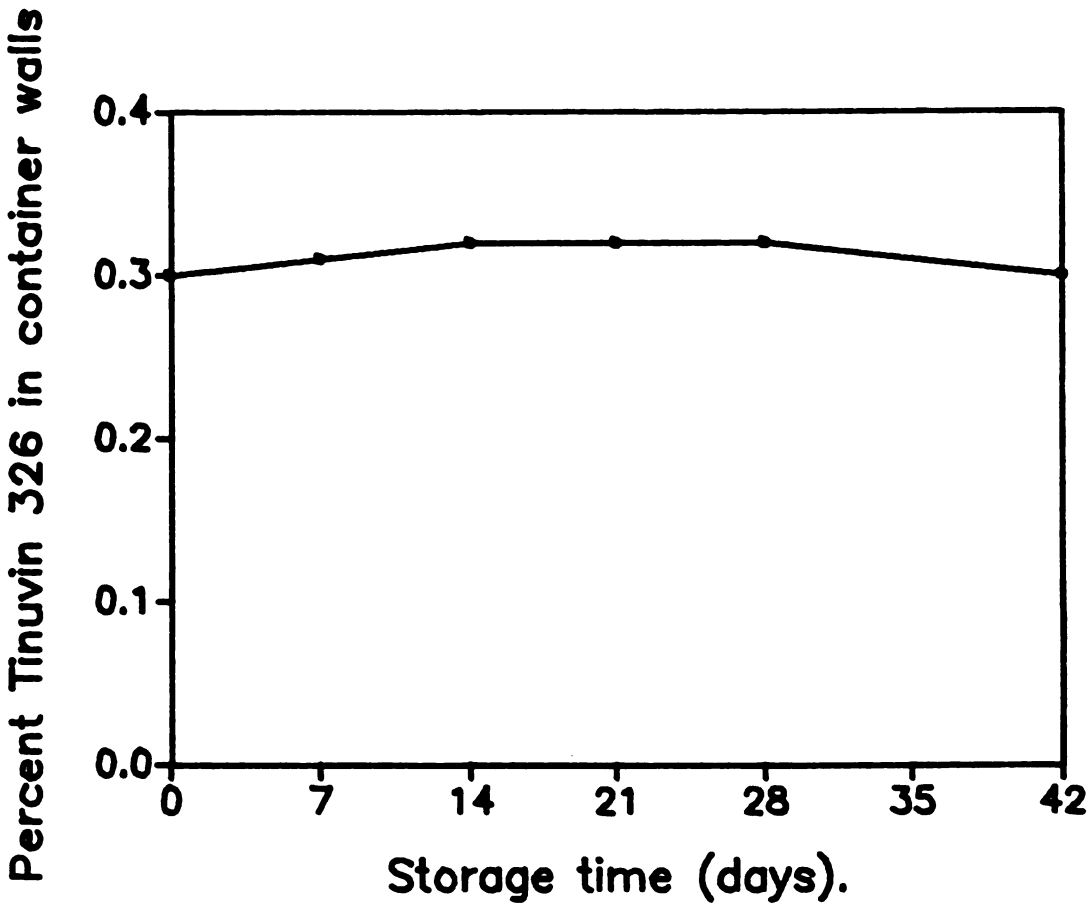


Fig. 14 Percent Tinuvin 326 in wall of containers after oil storage at 35 degrees C (HPLC method).

Table 15. Percent efficiency of the extraction process and the rota-evaporation concentration procedure.

Std. extraction trials	% efficiency (extraction process)	% efficiency (rota-evaporation)
1	79.66%	97.98%
2	81.12%	97.57%
3	82.03%	97.80%
Average	81.00%	97.78%

Lipid Oxidation Determination by Peroxide Value Method:

The peroxide value method to determine the extent of lipid oxidation in the oil samples, was chosen because of its short analytical time, simplicity and ease of reproducibility. This method of analysis has been used successfully by many researchers to determine the oxidation levels of oil samples (Vianni, 1980; Luby, 1982; Boki, 1989).

The peroxide values of the oil packaged in containers with and without Tinuvin 326, and stored at 21°C for a duration of 35 days, are shown in Table 16. Two replicates were used for each data point. All observations were duplicated and the values reported are averages. The peroxide values for the oil in containers with and without Tinuvin 326 and stored at 21°C, increased similarly though relatively slowly during the first fourteen days of storage (Fig. 15). After fourteen days, however, the peroxide value for the oil in the containers without Tinuvin 326 increased at a faster rate than for the oil in the containers with Tinuvin 326. Analysis of variance, using a complete randomized design (Appendix 8), shows that there is a significant difference between the peroxide values of the oil stored in the containers with Tinuvin 326, and those stored in containers without Tinuvin 326. This shows that Tinuvin 326 protected the packaged oil from the effects of the fluorescent light at 21°C. During the 0 - 14 day period, the initiation step of the lipid oxidation process probably occurred. During this

period, lipid oxidation is relatively slow, but increases dramatically during the next phase (propagation stage). During the propagation stage, it is easier to observe (Fig 15) the effect of the UV absorber as it protects the oil from the influence of the fluorescent light.

Table 16. Peroxide value (milliequivalents/kg oil) of the soybean oil in the multilayered container with 0% and 0.3% Tinuvin 326, stored at 21°C

Storage time (days)	Peroxide value of oil in 0% Tin. containers	Peroxide value of oil in 0.3% Tin. containers
0	0.7985	0.7985
1	1.9462	1.7972
2	2.7208	2.4966
4	4.2665	3.8647
7	5.5849	4.5615
14	8.9848	8.3997
21	16.3081	10.3914
25	18.3367	12.8114
28	20.2853	13.0719
32	22.9514	16.4586
35	25.9298	17.1901

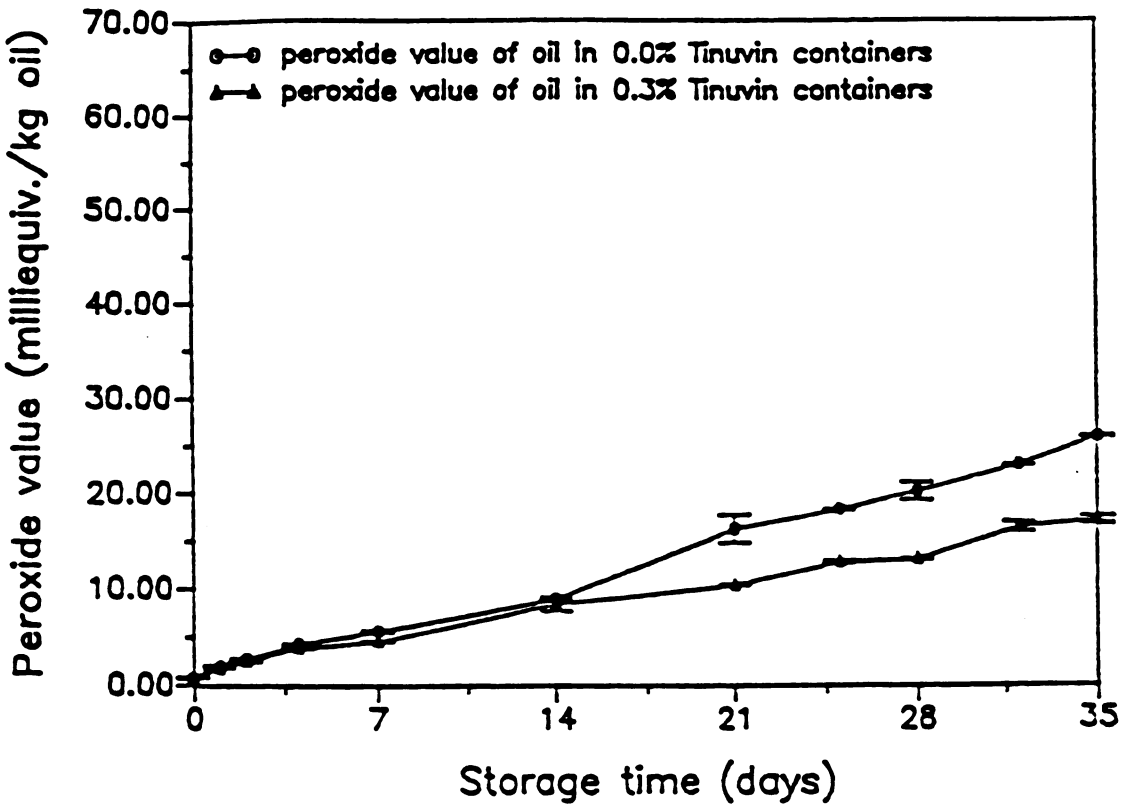


Fig. 15 Peroxide value of soybean oil in the containers 0% and 0.3% Tinuvin 326 stored at 21°C.

The peroxide values of the oil packaged in containers with and without Tinuvin 326, and stored at 35°C for a duration of 35 days, are shown in Table 17 and presented graphically in Figure 16. The results are similar to the values obtained at 21°C, except, at 35°C, the initiation step seems to be between 0 - 7 days. Tinuvin 326 protected the oil from the effect of the fluorescent light during storage at 35°C. Similar results were obtained by Shipe et al. (1983) and Fanelli et al. (1985) who showed that when Tinuvin 326 was impregnated into plastic containers, vitamin A in milk was protected from photooxidation. This protection results because of the UV absorbent characteristic of Tinuvin 326. Guillet (1972) stated that UV absorbers can preferentially absorb light and harmlessly dissipate its energy. This energy lies within the ultra violet region of the electro-magnetic spectrum. Since Tinuvin 326 absorbs light energy between 190 - 390 nm (the ultra violet range of the spectrum), the removal of this energy results in the reduction of photooxidation in the oil exposed to the fluorescent light.

Table 17. Peroxide value (milliequivalents/kg oil) of the soybean oil in the multilayered container with 0% and 0.3% Tinuvin 326, stored at 35°C

Storage time (days)	Peroxide value of oil in 0% Tin. containers	Peroxide value of oil in 0.3% Tin. containers
0	0.7985	0.7985
1	2.7437	2.4940
2	3.9432	3.9652
4	6.9170	5.9054
7	9.0223	8.4222
14	16.7877	13.8682
21	29.7837	21.9952
25	38.7162	25.7162
28	40.1460	30.4720
32	61.4133	39.2034
35	64.6554	44.1507

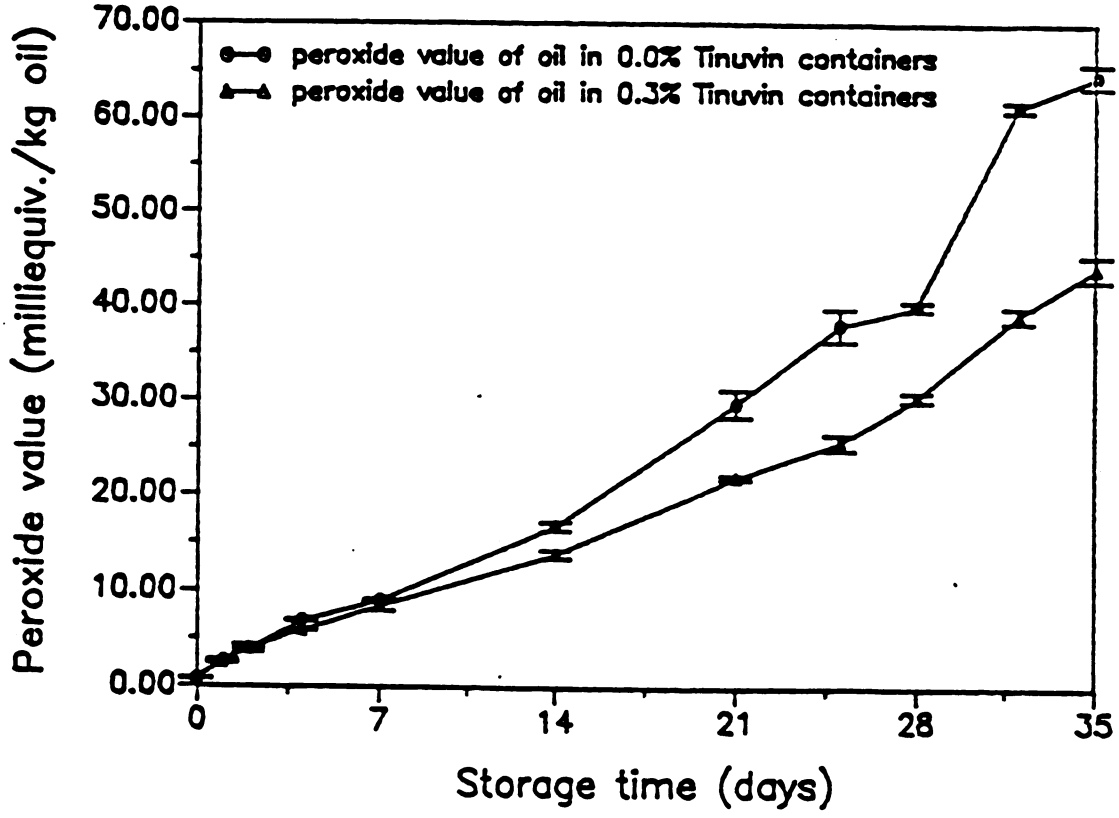


Fig. 16 Peroxide value of soybean oil in the containers with 0% and 0.3% Tinuvin 326 stored at 35°C.

CONCLUSIONS

The loss of Tinuvin 326 from the container wall of the multilayered polypropylene based cups was measured as a function of time (42 days) and temperature (21 and 35°C). Loss of Tinuvin 326 from the container wall was negligible. The results also showed that both the HPLC and the UV spectrophotometric methods could be used to determine the concentration of Tinuvin 326 in the plastic container system. There was close agreement between the results from these two methods. There was also an acceptable level of precision between the results from each method.

Migration of Tinuvin 326 from the containers to the packaged oil was greater at the higher temperature (35°C). The levels migrating to the oil were too low to noticeably affect the stability characteristics of the UV absorber in the plastic containers. The highest level of migration occurred after 42 days and at 35°C. This was determined to be 4.773% of 9.009×10^{-3}g Tinuvin 326, and is equivalent to 4.30×10^{-4}g Tinuvin 326.

Tinuvin 326 was found to have the ability to reduce the rate of lipid oxidation in soybean oil. Since it absorbs UV radiation and does not itself disintegrate between 190 - 390

nm, Tinuvin 326 functions as a UV absorber. This study also showed that cool white fluorescent light has energy levels which can contribute to lipid oxidation in oil. The oil in the cups which contained dispersed Tinuvin 326 suffered less lipid oxidation than those in cups containing no Tinuvin 326. This difference was significant at both storage temperatures.

"APPENDICES"

Appendix 1.

Table 18. Data used for the standard curve for Tinuvin 326 at several concentration levels (using HPLC).

Concentration Tinuvin 326 ppm (w/v)	Average area Response units
1	4237
10	41844
60	238410
80	319017
100	402316

Appendix 2.

Table 19. Data used for the standard curve for Tinuvin 326 at several concentration levels (using UV spectrophotometry).

Concentration of Tinuvin 326 in ppm (w/v)	Absorbance (optical density units)
1	0.051
5	0.263
10	0.516
15	0.780

Appendix 3.

From the standard curve by HPLC method,

slope = 3.998×10^{3} au/ppm

converting au/ppm to g/au units:

1 ppm = 1×10^{-6} g/ml

10 ul injected = 0.01 ml

therefore slope = (3.998×10^{3} au/1×10^{-6} g/ml)

÷ (0.01ml)

= 3.998×10^{11} au/g

= 2.501×10^{-12} g/au

area response units (inject 1) = 136765

area response units (inject 2) = 138170

average area response units = 137467.5

sample weight = 3.0394 g

137467.5 au = 3.438×10^{-7} g Tinuvin/10μl

in 200 ml solution = 6.876×10^{-3} g Tinuvin

since 200 ml solution has 3.0394g plastic,

% Tinuvin 326 = (6.876×10^{-3} g) x100

÷ 3.0394g

= 0.226% Tinuvin 326

Appendix 4.

Table 5.
HPLC analysis of containers with Tinuvin 326 stored at 21 degrees C.

Time days	Extract No.	Rep.	Sample Wt.	inj. No.	Area response	% Tinuvin	Average %
0	1	1	3.0394	1	136765		
0	1	1	3.0394	2	138170	0.2263	
0	2	1	3.0394	1	71470		
0	2	1	3.0394	2	69976	0.0582	
0	3	1	3.0394	1	23459		
0	3	1	3.0394	2	22518	0.0189	0.303
0	1	2	3.0159	1	122959		
0	1	2	3.0159	2	122530	0.2036	
0	2	2	3.0159	1	75152		
0	2	2	3.0159	2	77449	0.0633	
0	3	2	3.0159	1	29894		
0	3	2	3.0159	2	29945	0.0248	0.292
			Overall average %		=	0.298	
3	1	1	3.0079	1	162763		
3	1	1	3.0079	2	160375	0.2687	
3	2	1	3.0079	1	23117		
3	2	1	3.0079	2	22038	0.0375	
3	3	1	3.0079	1	9126		
3	3	1	3.0079	2	7561	0.0069	0.313
3	1	2	3.0392	1	125725		
3	1	2	3.0392	2	131637	0.2118	
3	2	2	3.0392	1	47425		
3	2	2	3.0392	2	45560	0.0765	
3	3	2	3.0392	1	29672		
3	3	2	3.0392	2	29771	0.0245	0.313
			Overall average %		=	0.313	
7	1	1	3.0115	1	173451		
7	1	1	3.0115	2	158044	0.2753	
7	2	1	3.0115	1	27810		
7	2	1	3.0115	2	27825	0.0231	
7	3	1	3.0115	1	14456		
7	3	1	3.0115	2	12893	0.0114	0.310
7	1	2	3.0684	1	141819		
7	1	2	3.0684	2	140642	0.2303	
7	2	2	3.0684	1	34878		
7	2	2	3.0684	2	35769	0.0288	
7	3	2	3.0684	1	35129		
7	3	2	3.0684	2	34996	0.0286	0.288
			Overall average %		=	0.299	

"Table 5 (cont'd)."

14	1	1	3.07	1	129746		
14	1	1	3.07	2	128912	0.2107	
14	2	1	3.07	1	97623		
14	2	1	3.07	2	98108	0.0797	
14	3	1	3.07	1	33303		
14	3	1	3.07	2	33667	0.0273	0.318
14	1	2	3.051	1	150929		
14	1	2	3.051	2	149863	0.2466	
14	2	2	3.051	1	76121		
14	2	2	3.051	2	78097	0.0632	
14	3	2	3.051	1	25109		
14	3	2	3.051	2	25161	0.0206	0.330
			Overall average %	=	0.324		
21	1	1	3.0225	1	102553		
21	1	1	3.0225	2	104772	0.1716	
21	2	1	3.0225	1	101230		
21	2	1	3.0225	2	102068	0.0841	
21	3	1	3.0225	1	50882		
21	3	1	3.0225	2	50727	0.0420	0.298
21	1	2	3.0667	1	108491		
21	1	2	3.0667	2	100599	0.1705	
21	2	2	3.0667	1	99601		
21	2	2	3.0667	2	102222	0.0823	
21	3	2	3.0667	1	47055		
21	3	2	3.0667	2	45970	0.0379	0.291
			Overall average %	=	0.294		
28	1	1	3.0342	1	137266		
28	1	1	3.0342	2	137973	0.2269	
28	2	1	3.0342	1	74518		
28	2	1	3.0342	2	76928	0.0624	
28	3	1	3.0342	1	31882		
28	3	1	3.0342	2	29446	0.0253	0.315
28	1	2	3.0124	1	128654		
28	1	2	3.0124	2	126095	0.2115	
28	2	2	3.0124	1	77901		
28	2	2	3.0124	2	73477	0.0628	
28	3	2	3.0124	1	31791		
28	3	2	3.0124	2	31315	0.0262	0.301
			Overall average %	=	0.308		
42	1	1	3.042	1	114032		
42	1	1	3.042	2	110820	0.1849	
42	2	1	3.042	1	93766		
42	2	1	3.042	2	95446	0.0778	
42	3	1	3.042	1	40824		
42	3	1	3.042	2	40753	0.0335	0.296

"Table 5 (cont'd)."

42	1	2	3.0876	1	119432		
42	1	2	3.0876	2	118119	0.1924	
42	2	2	3.0876	1	95298		
42	2	2	3.0876	2	94971	0.0771	
42	3	2	3.0876	1	40074		
42	3	2	3.0876	2	39376	0.0322	0.302
			Overall average %		=	0.299	

Extract No. = extraction number
Rep. = replicate
Inj. No. = injection number

Table 6.
HPLC analysis of containers with Tinuvin 326 stored at 35 degrees C.

Time days	Extract No.	Rep.	Sample Wt.	inj. No.	Area response	% Tinuvin	Average %
0	1	1	3.0394	1	136765		
0	1	1	3.0394	2	138170	0.2263	
0	2	1	3.0394	1	71470		
0	2	1	3.0394	2	69976	0.0582	
0	3	1	3.0394	1	23459		
0	3	1	3.0394	2	22518	0.0189	0.303
0	1	2	3.0159	1	122959		
0	1	2	3.0159	2	122530	0.2036	
0	2	2	3.0159	1	75152		
0	2	2	3.0159	2	77449	0.0633	
0	3	2	3.0159	1	29894		
0	3	2	3.0159	2	29945	0.0248	0.292
			Overall average %		=	0.298	
3	1	1	3.0742	1	124106		
3	1	1	3.0742	2	126353	0.2038	
3	2	1	3.0742	1	91190		
3	2	1	3.0742	2	95076	0.0758	
3	3	1	3.0742	1	31928		
3	3	1	3.0742	2	31391	0.0258	0.305
3	1	2	3.0663	1	93694		
3	1	2	3.0663	2	95895	0.1547	
3	2	2	3.0663	1	97125		
3	2	2	3.0663	2	97964	0.0796	
3	3	2	3.0663	1	40431		
3	3	2	3.0663	2	41955	0.0336	0.268
			Overall average %		=	0.287	
7	1	1	3.0624	1	129159		
7	1	1	3.0624	2	130964	0.2125	
7	2	1	3.0624	1	39988		
7	2	1	3.0624	2	40880	0.0330	
7	3	1	3.0624	1	44711		
7	3	1	3.0624	2	45380	0.0368	0.282
7	1	2	3.0768	1	138254		
7	1	2	3.0768	2	129483	0.2177	
7	2	2	3.0768	1	50579		
7	2	2	3.0768	2	50349	0.0410	
7	3	2	3.0768	1	72223		
7	3	2	3.0768	2	74329	0.0596	0.318
			Overall average %		=	0.300	

"Table 6 (cont'd)."

14	1	1	3.0476	1	143807		
14	1	1	3.0476	2	145478	0.2374	
14	2	1	3.0476	1	90797		
14	2	1	3.0476	2	91023	0.0746	
14	3	1	3.0476	1	31726		
14	3	1	3.0476	2	31593	0.0260	0.338
14	1	2	3.0687	1	125261		
14	1	2	3.0687	2	123394	0.2027	
14	2	2	3.0687	1	142057		
14	2	2	3.0687	2	102060	0.0995	
14	3	2	3.0687	1	36291		
14	3	2	3.0687	2	36329	0.0296	0.332
			Overall average %	=	0.335		
21	1	1	3.0472	1	126568		
21	1	1	3.0472	2	123748	0.2055	
21	2	1	3.0472	1	76907		
21	2	1	3.0472	2	77842	0.0635	
21	3	1	3.0472	1	42894		
21	3	1	3.0472	2	41924	0.0348	0.304
21	1	2	3.0441	1	127132		
21	1	2	3.0441	2	124630	0.2069	
21	2	2	3.0441	1	108529		
21	2	2	3.0441	2	93468	0.0830	
21	3	2	3.0441	1	35767		
21	3	2	3.0441	2	35782	0.0294	0.319
			Overall average %	=	0.312		
28	1	1	3.0687	1	119549		
28	1	1	3.0687	2	127612	0.2015	
28	2	1	3.0687	1	84950		
28	2	1	3.0687	2	82620	0.0683	
28	3	1	3.0687	1	41796		
28	3	1	3.0687	2	39763	0.0332	0.303
28	1	2	3.0213	1	148498		
28	1	2	3.0213	2	148597	0.2460	
28	2	2	3.0213	1	69535		
28	2	2	3.0213	2	71865	0.0585	
28	3	2	3.0213	1	19754		
28	3	2	3.0213	2	20480	0.0167	0.321
			Overall average %	=	0.312		
42	1	1	3.0369	1	122922		
42	1	1	3.0369	2	122118	0.2018	
42	2	1	3.0369	1	92083		
42	2	1	3.0369	2	93110	0.0763	
42	3	1	3.0369	1	36437		
42	3	1	3.0369	2	33819	0.0289	0.307

"Table 6 (cont'd)."

42	1	2	3.017	1	125035		
42	1	2	3.017	2	124191	0.2066	
42	2	2	3.017	1	90538		
42	2	2	3.017	2	90694	0.0751	
42	3	2	3.017	1	32170		
42	3	2	3.017	2	33600	0.0273	0.309
			Overall average %		=	0.308	

Extract No. = extraction number
Rep. = replicate
Inj. No. = injection number

Appendix 5.

absorbance = 0.942 optical density (OD) units

sample weight = 3.0210 g

dilution factor = 4

corrected absorbance = 3.768 optical density (OD) units

substitution in y = m* + c

$y = 5.191 \times 10^{-2}$ OD/ppm(*) + 2.347×10^{-4} OD/ppm

(*) ppm = (3.768 OD) ÷ (5.191×10^{-2} OD/ppm)

- (2.347×10^{-4} OD/ppm)

= 72.587 ppm /100 ml solution

= 72.587×10^{-6} g /ml /100 ml solution

= 72.587×10^{-4} g Tinuvin 326

In 100 g plastic, % Tinuvin 326 (sample wt. = 3.0210 g)

= (72.587×10^{-4} g) x 100 ÷ 3.0210 g

= 0.240% Tinuvin 326

Appendix 6.

Table 7.
Stability studies on containers with Tinuvin 326 at 21 degrees C. using UV spectrophotometric method.

Time days	Sample wt.	Extract No.	Rep.	Corrected absorbance	Conc. Tin. in plastic	AVE. %
0	3.0210	1	1	3.768	0.2403	
0	3.0394	2	1	0.925	0.0586	
0	3.0159	3	1	0.313	0.0200	0.319
0	3.0210	1	2	3.288	0.2097	
0	3.0394	2	2	1.008	0.0639	
0	3.0159	3	2	0.389	0.0248	0.298
			Overall average % = 0.309			
3	3.0079	1	1	4.376	0.2803	
3	3.0079	2	1	0.287	0.0184	
3	3.0079	3	1	0.1	0.0064	0.305
3	3.0392	1	2	3.464	0.2196	
3	3.0392	2	2	0.606	0.0384	
3	3.0392	3	2	0.362	0.0229	0.281
			Overall average % = 0.293			
7	3.0115	1	1	2.934	0.1877	
7	3.0115	2	1	1.108	0.0709	
7	3.0115	3	1	0.736	0.0471	0.306
7	3.0638	1	2	3.706	0.2330	
7	3.0638	2	2	0.926	0.0582	
7	3.0638	3	2	0.442	0.0278	0.319
			Overall average % = 0.312			
14	3.07	1	1	3.352	0.2103	
14	3.07	2	1	1.284	0.0806	
14	3.07	3	1	0.436	0.0274	0.318
14	3.051	1	2	3.888	0.2455	
14	3.051	2	2	0.998	0.0630	
14	3.051	3	2	0.331	0.0209	0.329
			Overall average % = 0.324			
21	3.0225	1	1	2.754	0.1755	
21	3.0225	2	1	1.384	0.0882	
21	3.0225	3	1	0.67	0.0427	0.306
21	3.0667	1	2	2.77	0.1740	
21	3.0667	2	2	1.288	0.0809	
21	3.0667	3	2	0.618	0.0388	0.294
			Overall average % = 0.300			

"Table 7 (cont'd)."

28	3.0342	1	1	3.628	0.2303	
28	3.0342	2	1	0.999	0.0634	
28	3.0342	3	1	0.376	0.0239	0.318
28	3.0124	1	2	3.44	0.2200	
28	3.0124	2	2	1.016	0.0650	
28	3.0124	3	2	0.424	0.0271	0.312
			Overall average % = 0.315			
42	3.042	1	1	3.032	0.1920	
42	3.042	2	1	1.268	0.0803	
42	3.042	3	1	0.53	0.0336	0.306
42	3.0876	1	2	3.08	0.1922	
42	3.0876	2	2	1.255	0.0783	
42	3.0876	3	2	0.53	0.0331	0.304
			Overall average % = 0.305			

Extract No. = extraction number
Rep. = replicate
Conc. Tin. = concentration Tinuvin 326
Ave. = average

Table 8.
Stability studies on containers with Tinuvin 326 at 35 degrees C. using UV spectrophotometric method.

Time days	Sample wt.	Extract No.	Rep.	Corrected absorbance	Conc. Tin. in plastic	AVE. %
0	3.0210	1	1	3.768	0.2403	
0	3.0394	2	1	0.925	0.0586	
0	3.0159	3	1	0.313	0.0200	0.319
0	3.0210	1	2	3.288	0.2097	
0	3.0394	2	2	1.008	0.0639	
0	3.0159	3	2	0.389	0.0248	0.298
			Overall average % = 0.309			
3	3.0117	1	1	2.69	0.1721	
3	3.0117	2	1	1.194	0.0764	
3	3.0117	3	1	0.585	0.0374	0.286
3	3.0742	1	2	3.416	0.2141	
3	3.0742	2	2	1.208	0.0757	
3	3.0742	3	2	0.397	0.0249	0.315
			Overall average % = 0.300			
7	3.0861	1	1	2.206	0.1377	
7	3.0861	2	1	0.918	0.0573	
7	3.0861	3	1	1.31	0.0818	0.277
7	3.0624	1	2	3.37	0.2120	
7	3.0624	2	2	1.034	0.0650	
7	3.0624	3	2	0.566	0.0356	0.313
			Overall average % = 0.295			
14	3.0476	1	1	2.582	0.1632	
14	3.0476	2	1	1.51	0.0954	
14	3.0476	3	1	0.69	0.0436	0.302
14	3.0533	1	2	3.228	0.2037	
14	3.0533	2	2	1.344	0.0848	
14	3.0533	3	2	0.462	0.0291	0.318
			Overall average % = 0.310			
21	3.0472	1	1	3.05	0.1928	
21	3.0472	2	1	1.027	0.0649	
21	3.0472	3	1	0.563	0.0356	0.293
21	3.0441	1	2	3.398	0.2150	
21	3.0441	2	2	1.256	0.0795	
21	3.0441	3	2	0.484	0.0306	0.325
			Overall average % = 0.309			

"Table 8 (cont'd)."

28	3.0687	1	1	3.272	0.2054	
28	3.0687	2	1	1.117	0.0701	
28	3.0687	3	1	0.531	0.0333	0.309
28	3.0213	1	2	3.978	0.2536	
28	3.0213	2	2	0.935	0.0596	
28	3.0213	3	2	0.287	0.0183	0.332
			Overall average % = 0.320			
42	3.0369	1	1	3.234	0.2051	
42	3.0369	2	1	1.262	0.0801	
42	3.0369	3	1	0.478	0.0303	0.316
42	3.017	1	2	3.356	0.2143	
42	3.017	2	2	1.2	0.0766	
42	3.017	3	2	0.456	0.0291	0.320
			Overall average % = 0.318			

Extract No. = extraction number
Rep. = replicate
Conc. Tin. = concentration Tinuvin 326
Ave. = average

Appendix 7.

Sample calculation for 7 days storage at 35°C.

From the standard curve by HPLC method,

slope = 3.998×10^{3} au/ppm

1 ppm = 1×10^{-6} g/ml

10 µl injected = 0.01 ml

therefore slope = (3.998×10^{3} au/1×10^{-6} g/ml) ÷ 0.0 ml

= 3.998×10^{11} au/g

= 2.501×10^{-12} g/au

5 extractions of 15 ml each = 75 ml solution (conc. to 5 ml)

Area response units = 642 au

(2.501×10^{-12} g/au) x 642au = 1.6056×10^{-9} g/10 µl injected

in 5 ml concentrated soln. = (1.6056×10^{-9} g) 5ml ÷ 0.01ml

= 8.028×10^{-7} g Tinuvin 326

since extraction efficiency = 81.00%

actual weight in the oil = 8.028×10^{-7} g x 100 ÷ 81

(in 3 ml oil) = 9.9111×10^{-7} g/5 ml conc.

in 120 ml oil or a container= 9.9111×10^{-7} g x 120ml ÷ 3 ml

= 3.964×10^{-5} g Tinuvin 326

% Tinuvin in oil/container = 3.964×10^{-5} g x 100 ÷ 120

= 3.3037×10^{-5} % (w/v)

% Tinuvin transferred from container wall to the oil

= 3.964×10^{-5} g x 100 ÷ 9.009×10^{-3} g

= 0.440% Tinuvin 326

Appendix 8.

Title: Peroxide value of oil stored at 21 degrees C.

Function: FACTOR

Experiment Model Number 1:
Two Factor Completely Randomized Design

Factorial ANOVA for the factors:
Replication (Var 1: Replicate) with values from 1 to 2
Factor A (Var 2: Tinuvin 326) with values from 1 to 2
Factor B (Var 3: Time) with values from 1 to 10

Variable 4: Peroxide value (milliequiv./kg oil)

Grand Mean = 10.918, Grand Sum = 436.717, Total Count= 40

A N A L Y S I S O F V A R I A N C E T A B L E

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
Factor A	1	131.564	131.564	374.6137	0.0000
Factor B	9	1952.747	216.972	617.8050	0.0000
AB	9	106.172	11.797	33.5905	0.0000
Error	20	7.024	0.351		
Total	39	2197.507			

Coefficient of Variation: 5.43%

From the ANOVA table:
1. the observed F value for Factor A (Tinuvin 326) = 374.61
2. the observed F value for Factor A (Tinuvin 326) = 617.81

For the effect of Tinuvin, the F-distribution tables at d.f 1 (numerator) and 9 (denominator) = 10.6 at 1% level of significance

For the effect of Time, the F-distribution tables at d.f 1 (numerator) and 9 (denominator) = 10.6 at 1% level of significance

Since the observed F value for the effects of time and Tinuvin 326 exceed even the 1% level of significance, the oil in the containers with 0.3% Tinuvin 326 was better protected from the effects of the fluorescent light than the oil in the containers 0% Tinuvin 326 stored at 21°C.

Title: Peroxide value of oil stored at 35°C

Function: FACTOR

Experiment Model Number 1:
Two Factor Completely Randomized Design

Factorial ANOVA for the factors:
Replication (Var 1: replicate) with values from 1 to 2
Factor A (Var 2: Tinuvin 326) with values from 1 to 2
Factor B (Var 3: Time) with values from 1 to 10

Variable 4: Peroxide value (milliequiv./Kg oil)

Grand Mean = 23.486, Grand Sum = 939.445, Total Count= 40

A N A L Y S I S O F V A R I A N C E T A B L E

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
Factor A	1	598.110	598.110	659.7521	0.0000
Factor B	9	13139.301	1459.922	1610.3851	0.0000
AB	9	633.541	70.393	77.6484	0.0000
Error	20	18.131	0.907		
Total	39	14389.084			

Coefficient of Variation: 4.05%

From the ANOVA table:
1. the observed F value for Factor A (Tinuvin 326) = 659.752
2. the observed F value for Factor A (Tinuvin 326) = 1610.385

For the effect of Tinuvin, the F-distribution tables at d.f 1 (numerator) and 9 (denominator) = 10.6 at 1% level of significance

For the effect of Time, the F-distribution tables at d.f 1 (numerator) and 9 (denominator) = 10.6 at 1% level of significance

Since the observed F value for both the effects of time and Tinuvin 326 exceed even the 1% level of significance, the oil in the containers with 0.3% Tinuvin 326 was better protected from the effects of the fluorescent light than the oil in the containers 0% Tinuvin 326 at 35°C.

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