A systematic study on the stability of selected polymer antioxidants in EU official aqueous and alternative food simulants using HPLC*

P. G. Demertzis† and R. Franz‡
Fraunhofer-Institute of Food Technology and Packaging, Giggenhaeuserstr. 35, D-85354 Freising, Germany

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Whilst considerable research has been conducted on the stability of plastics additives during processing, there is, on the other hand, a lack of studies on the stability of the same additives in food simulants in the published literature. In the work presented a systematic study has been undertaken on the stability of five selected commercial plastics additives (Irganox 3114, Irganox 1035, Irganox 245, Irganox 1098 and Irgafos P-EPQ) in the three EU-official aqueous food simulants (distilled water, 3% aq. acetic acid and 15% aq. ethanol) and in an alternative fat simulant (95% aq. ethanol) under two different time/temperature exposure conditions (10 days at 40°C and 1 h at 100°C). To enable quantitative analysis of the additives for this investigation, a simple and rapid HPLC method with UV detection was developed. The results obtained showed that the four Irganox-type additives under investigation were practically stable under the applied exposure conditions in all of the employed food simulants. Only Irganox 3114 exhibited a relatively low degree of instability in the ethanolic food simulants. Concerning Irgafos P-EPQ, a mixture of at least seven constituents, a remarkable degradation in ethanol and acetic acid solutions was observed. The developed HPLC method is also considered to be applicable for stability testing of other Irganox-type additives both in aqueous and fatty food simulants.

Keywords: polymer antioxidants, plastics additives, food simulants, migration, HPLC

Introduction

Plastics materials are widely used for the purpose of food packaging. Most of them contain small, but technically necessary amounts of additives which provide functional characteristics such as stability and plasticity and facilitate the conversion of the raw polymer to the food contact article. These chemical moieties (adjuvants) include antioxidants, plasticizers, stabilizers, processing aids, etc. and depending on their molecular weights they may migrate from the packaging into the food (Shepherd 1982, Till et al. 1987). In particular, polyolefins, the largest family by volume of commercially important thermoplastic polymers, are sensitive to thermo- and photo-oxidative degradation. Consequently, a great variety of antioxidants and light stabilizers are employed to improve their useful properties and to extend the service life of polyolefins (Munteanu et al. 1987).

The risk of possible health hazards to consumers due to migration of such substances from packaging materials into foods has been generally recognized for a long time with the consequence of establishing corresponding food regulations. In the European Union (EU), historically, the different member states had developed their own regulations for the control of food contact materials. To overcome potential barriers to free trade, the European Union is currently in an ongoing process of legislation harmonization. So far, efforts have concentrated on the regulation of plastics with the result that a so-called ‘Plastics Directive’ has been published which provides legislative control by means of positive lists of substances, i.e. monomers and other starting substances as well as additives, which can be used for the manufacture of plastics. A number of these substances permitted for...
use are toxic and, hence, transfer to foods must be restricted in order to protect consumers. Restrictions have been placed either on the quantity of a substance which may be present in a finished material or article (quantity in material, QM) or on the quantity of a substance which may migrate into foods or food simulants (specific migration limit, SML). A great number of plastics additives which will appear on the positive lists of forthcoming amendments of the 'Plastics Directive' will have SML restrictions. For a number of these substances it can be expected that they are chemically not completely stable in food simulants. As a consequence, it may be more appropriate in these cases to measure the quantity in the finished material rather than in simulants, i.e. to place a QM restriction on the substance rather than an SML (Rossi 1988).

Considerable research has been conducted on the stability of plastics additives during processing but less information is available on their stability in food simulants. For instance, it was found that BHT degraded in water, 8% and 50% ethanol solution (Schwope et al. 1987a). For the antioxidant Irganox 1010, there was virtually 100% degradation in 8% and 20% aqueous ethanol (Schwope et al. 1987b). Studies conducted on organotin stabilizers also reported degradation, although limited information on the degree of breakdown and exposure conditions were reported (Schwope et al. 1986). Since there are practically no further published results in this matter available, it is evident that there remains a need to carry out systematic studies on the stability of additives in food simulants. Key questions to be addressed are the following: which additives undergo loss in which simulants under which exposure conditions and to which degree of breakdown.

This paper presents a simple and rapid HPLC analytical method with UV detection to determine the stability of selected plastics additives (Irganox 3114, Irganox 1035, Irganox 245, Irganox 1098 and Irgafos P-EPQ) in the EU official aqueous food simulants, i.e. distilled water, 3% acetic acid, 15% aq. ethanol and in 95% aq. ethanol as a recognized alternative fatty food simulant. The work was carried out within a concerted action EU project (AIR3-CT94-2360) with the aim of providing stability data to the European Commission (DG III-C-1) in order to assist a decision whether legislative restrictions in the 'Plastics Directive' should be applied to the finished material or article (QM) or to migration to simulants (SML). This will then determine whether standard methods of analysis for compliance test purposes should measure the quantity of substance in the finished material or article (QM) or in simulants (SML).

Experimental

Antioxidants

The additives (antioxidants) used in this study were (i) triethylene glycol-bis-[3-(3-tert-butyl-4-hydroxy-5-methylphenyl)-propionate (=Irganox 245), (ii) thiodyethanol-bis-[3-(3,5-di-tert-butyl-4-hydroxyphenyl)-propionate] (=Irganox 1035), (iii) N,N'-hexane-1,6-diyli-bis [3-(3, 5-dii-tert-4-hydroxyphenyl)-propionamide] (=Irganox 1098), (iv) tris(3,5-di-tert-butyl-4-hydroxybenzyl)isocyanurate (=Irganox 3114) and (v) tetrakis[2,4-di-tert-butylphenyl]4,4'-biphenylylene diphosphonite (=main component of Irgafos P-EPQ). Samples of these commercially available additives were used without further purification. The chemical structures of these compounds are shown in figures 1 and 2.

Reagents

HPLC grade acetonitrile (MeCN), tetrahydrofuran (THF) and water were purchased from Fluka Chemie AG (Switzerland). Analytical grade acetic acid and ethanol for the preparation of food simulants were obtained from Merck KGaA, Darmstadt (Germany).

HPLC analysis

The chromatographic analysis was performed on a Shimadzu (Shimadzu Europa GmbH, Germany) LC-10AD liquid chromatograph equipped with a Shimadzu SPD-10A UV-VIS detector, a Shimadzu SCL-10A system controller and a Shimadzu SIL-GA auto injector equipped with an SCL-6A system controller. Elution with a flow rate of 0-6 ml/min was monitored by UV detection at 230 nm. Two columns were used: (a) Nucleosil 120-3 C18, 125 × 3 mm (Chromatographic Service GmbH, Germany) and (b) Nucleosil 5 C18AB, 125 × 3 mm (Macherey-Nagel AG, Germany). With column (b) shorter retention
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Figure 1. Chemical structures of the Irganox-type antioxidants under investigation.

Figure 2. Chemical constituents of Irgafos P-EPQ and ranges of their individual concentrations.

Sample preparation

Primary standard solutions of each of the five additives in THF at defined concentrations of approximately 0-6% for Irganox 245, 0-5% for Irganox 1035, 2% for Irganox 1098 and 1% for Irganox 3114 and Irgafos P-EPQ were prepared in duplicate by weighing the appropriate amount of each additive in a volumetric flask already partially filled with solvent, filling up the flask to the mark and mixing thoroughly. From these primary standards, spiked test samples were prepared for each additive separately in all four simulants by adding appropriate amounts of the primary standard solution into a defined volume (20 ml) of each food simulant. The concentrations of

Stability tests

The prepared spiked samples as well as unspiked samples (blank samples) were placed into 40 ml volume flasks and exposed to two different test conditions: 10 days at 40°C and 1 h at 100°C. In the case of 15% and 95% aq. ethanol, the heating for 1 h was conducted using a reflux condenser. Reflux conditions were established within 10 min by heating the samples. At the end of the 1 h reflux period, samples were cooled under running water to room temperature (cooling time = 1 min). Exposed samples
were then analysed by HPLC. Samples in distilled water, 3% acetic acid and 15% ethanol were analysed after 1:1 dilution with THF. Spiked samples and blanks were prepared and measured in triplicate and duplicate, respectively. For reference measurements, control spiked samples were also prepared as follows. Fresh primary standards of each additive were prepared in duplicate. From each primary standard, triplicate spikes at the test level were made for each of the four simulants. The resulting six control samples in each simulant were subjected to HPLC analysis without exposure to any test conditions to determine the recovery from the stability tests.

Results and discussion

Various methods have been developed for the determination of additives in plastics and/or in food simulants: liquid chromatography (HPLC or LC) (Haney and Dark 1980, Schabron and Fenska 1980, Perlestein 1983, Vargo and Olson 1985, 1986, Munteanu et al. 1987, Arpino et al. 1990, Nielson 1993, Montiero et al. 1996), gas chromatography (GC) (Perlestein and Orme 1985, Dilettato et al. 1991), thin-layer chromatography (Miles 1974) and mass spectrometry (MS) (Yoshikawa et al. 1971). HPLC is generally considered to be the most suitable method, permitting the analysis of polar and non-volatile additives which usually cannot be determined by GC. The three following different modes of liquid chromatography are the most frequently employed methods for the above-mentioned determinations: normal phase-HPLC (NP-HPLC), reverse phase-HPLC (RP-HPLC) and size exclusion chromatography (SEC). The vast majority of determinations of stabilizers was performed in the RP-HPLC mode using RP-C18 type columns and MeCN:H2O, MeCN:THF and CH3OH:H2O mixtures as the mobile phase. For many analyses the isocratic elution mode was sufficient; gradient elution was preferred for complex additive mixtures. In conclusion, RP-HPLC seems to be the most suitable technique for the analysis of antioxidants and stabilizers in plastics and in food simulants. This was taken into account in this work where one main purpose was to establish a relatively simple and quick HPLC method for the analysis of all of the five investigated additives in the four food simulants. Both RP-18 columns used (see Experimental) provided satisfying results. However, column (b) was found to be the most preferable due to yielding shorter retention times.

Figure 3 shows representative HPLC chromatograms of each of the four Irganox-type additives in 15% ethanol. Similar profiles were obtained with the other three food simulants. From the Irgafos P-EPQ chromatogram depicted in figure 4 three main peaks can be recognized. This additive is a multi-constituent product with at least seven components. The chemical structures of its constituents and commercially occurring ranges of their individual concentrations are given in figure 2. Based on bibliographic data (Ligner 1993) we assume that the first peak is attributed to low molecular weight impurities, the second one to components IV and V and the third one to components I, II, III and VI. However, a further study for clear identification of these peaks, involving possibly HPLC/MS, would be required.

The results of conducted stability tests with each of the four food simulants are presented in tables 1–3 in terms of recoveries obtained after the applied test conditions. For distilled water, 3% acetic acid and 15% ethanol, stability tests were carried out only at the highest temperature (100°C) exposure conditions, since all of the additives are practically insoluble at room temperature or even at 40°C in these simulants at the SML level. It should be noted that not only with these five additives but also with numerous other substances with an SML restriction the low solubility controls the migration and assures physically compliance with regulatory migration limits. The presented results show that the four Irganox-type additives are nearly completely recovered under the

Table 1. Recovery (%) of polymer antioxidants from all food simulants after exposure at 100°C for 1 h.

<table>
<thead>
<tr>
<th>Food simulant</th>
<th>Irganox 1098</th>
<th>Irganox 245</th>
<th>Irganox 3114</th>
<th>Irganox 1035</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>101</td>
<td>100</td>
<td>100</td>
<td>99</td>
</tr>
<tr>
<td>3% Acetic acid</td>
<td>101</td>
<td>98</td>
<td>99</td>
<td>93</td>
</tr>
<tr>
<td>15% Ethanol</td>
<td>103</td>
<td>98</td>
<td>78</td>
<td>99</td>
</tr>
<tr>
<td>95% Ethanol</td>
<td>95</td>
<td>99</td>
<td>89</td>
<td>99</td>
</tr>
</tbody>
</table>
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Figure 3. HPLC chromatographs of each of the four Irganox-type antioxidants in 15% aq. ethanol (control samples). (a) Irganox 245; (b) Irganox 1035; (c) Irganox 1098; (d) Irganox 3114.

Table 2. Recovery (%) of Irgafos P-EPQ constituents from all food simulants after exposure at 100°C for 1 h.

<table>
<thead>
<tr>
<th>Food simulant</th>
<th>HPLC peak 1</th>
<th>HPLC peak 2</th>
<th>HPLC peak 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>95</td>
<td>99</td>
<td>101</td>
</tr>
<tr>
<td>3% Acetic acid</td>
<td>106</td>
<td>98</td>
<td>67</td>
</tr>
<tr>
<td>15% Ethanol</td>
<td>74</td>
<td>97</td>
<td>78</td>
</tr>
<tr>
<td>95% Ethanol</td>
<td>61</td>
<td>67</td>
<td>6</td>
</tr>
</tbody>
</table>

Figure 4. HPLC chromatogram of Irgafos P-EPQ in 15% aq. ethanol (control samples).

applied exposure conditions from all food simulants. Only Irganox 3114 showed a relatively low degree of instability in the ethanolic food simulants: after 1 h exposure at 100°C 22% instability was found in 15% ethanol and 11% instability in 95% ethanol; after 10
Table 3. Recovery (%) of all antioxidants from 95% ethanol at two different exposure conditions.

<table>
<thead>
<tr>
<th>Antioxidant</th>
<th>10 days at 40°C</th>
<th>1 h at 100°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irganox 1098</td>
<td>99</td>
<td>95</td>
</tr>
<tr>
<td>Irganox 245</td>
<td>98</td>
<td>99</td>
</tr>
<tr>
<td>Irganox 3114</td>
<td>89</td>
<td>89</td>
</tr>
<tr>
<td>Irganox 1035</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>Irgafos PEPQ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPLC peak 1</td>
<td>102</td>
<td>61</td>
</tr>
<tr>
<td>HPLC peak 2</td>
<td>92</td>
<td>67</td>
</tr>
<tr>
<td>HPLC peak 3</td>
<td>50</td>
<td>6</td>
</tr>
</tbody>
</table>

days' exposure at 40°C the same instability of 11% was obtained for 95% ethanol.

Irgafos P-EPQ was stable in water (table 2), but some of its components were sensitive to exposure to acidic or ethanolic media (tables 2 and 3). Within the mixture, peak No. 1 was not sensitive to acidic medium but fairly sensitive to the presence of ethanol: at 1 h/100°C, peak 1 exhibited a 26% decrease in 15% ethanol and a 39% decrease in 95% ethanol. However, peak 1 was not as sensitive to ethanol at the lower temperatures since the exposure of 10 days at 40°C showed no degradation for peak 1. Peak No. 2 was fairly stable in all media at 1 h/100°C, only to be sensitive to high (95%) concentration of ethanol where it degraded by 33%. Again, a longer period at the lower temperature was not as damaging. Peak No. 3 showed the most sensitivity to all media tested. Only in distilled water did peak 3 retain its full stability. The presence of 3% acetic acid led to 33% degradation, while the presence of ethanol provoked a 22% degradation when present at 15% ethanol and 94% degradation when present at 95% ethanol. Again, exposure to the higher temperature for a shorter period was more damaging (10 days at 40°C led to a 50% degradation).

The increased instability of Irgafos P-EPQ in comparison with the other Irganox-type additives could be possibly attributed to the enhanced tendency of phosphite and phosphinite compounds to undergo oxidation reactions. Studies on the influence of gamma radiation on the antioxidants present in plastic materials, such as polyethylene, polypropylene and polyvinylchloride (Allen et al. 1987a, b), showed that the degradation of the phosphite stabilizer Irgafos 168 (component V of Irgafos P-EPQ) was remarkably higher (90–100%) in comparison to that of the phenolic antioxidants Irganox 1010 and Irganox 1076 (30–50%). In another study on the effects of irradiation of the previous antioxidants in polyolefin plastics (Bourges et al. 1992), three degradation products coming from the antioxidants have been detected. However, a more detailed study would be required for elucidation of the nature of Irgafos P-EPQ instability, including identification and quantification of possible degradation products formed. Such a study was not necessary within the frame of this investigation which was only to focus on the degree of additive stability and not on the breakdown products.

Conclusions

The developed HPLC method is considered to be applicable for the stability testing of Irganox-type and other additives of similar structure in both aqueous and fatty food simulants. From the five investigated additives only Irgafos P-EPQ exhibited remarkable instability phenomena, the nature of which should be further elucidated. Concerning the food regulatory question on the placing of QM or SML restrictions, the results showed that the four Irganox-type compounds can be SML restricted, however, they are possibly limited anyway by their poor solubility in aqueous food simulants. On the other hand, the results indicated also that it would be worthwhile to consider a QM restriction for Irgafos P-EPQ. For final conclusions, stability results with olive oil food simulant should be awaited. Such a study is currently being accomplished and will be published in due course.

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