Behavior of α- and β-Cyclodextrin-Encapsulated Allyl Isothiocyanate as Slow-Release Additives in Polylactide-*co*-Polycaprolactone Films

David Plackett,[1] Afshin Ghanbari-Siahkali,[2] Lajos Szente[3]

[1]*The Danish Polymer Centre, Risø National Laboratory, Frederiksborgvej 399, DK-4000 Roskilde, Denmark*
[2]*NKT Flexibles I/S, Priorparken 510, 2605 Brøndby, Denmark*
[3]*Cyclolab, Cyclodextrin Research and Development Laboratory, H-1525 Budapest, Hungary*

Received 30 August 2006; accepted 8 December 2006
DOI 10.1002/app.26344
Published online 16 May 2007 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: The natural antibacterial agent allyl isothiocyanate (AITC) encapsulated in either α- or β-cyclodextrin (CD) has previously been evaluated as a slow-release additive in polylactide-*co*-polycaprolactone (PLA–PCL) films designed for use in cheese packaging. In the research described in this article, thermogravimetric analysis (TGA) and thermogravimetric analysis in tandem with mass spectrometry (TGA–MS) were used to explore the thermal properties of CD-encapsulated AITC complexes as well as those of PLA–PCL films containing these complexes. To our knowledge, this is the first reported application of the TGA–MS technique to explore the thermal stability of CD-entrapped AITC and the first study to report differences in thermal stability of AITC in α-and β-CD cavities in the solid state. Observed differences in the thermal degradation profile of films containing the CD complexes can be explained if AITC binds more strongly to β-CD than to α-CD. This hypothesis has been reinforced by gas chromatography (GC) and high performance liquid chromatography (HPLC) studies, the results of which suggest that a new covalently bound AITC–CD complex may be formed when incorporating the β-CD complex of AITC in PLA–PCL films but not when incorporating the α-CD complex of AITC. This finding means that the α-CD complex of AITC would be preferred in situations where adequate long-term controlled release of AITC from polymer films is required, as for example in the case of active packaging applications. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 105: 2850–2857, 2007

Key words: cyclodextrins; allyl isothiocyanate; slow-release additives; biopolymer packaging; polylactide-*co*-polycaprolactone

INTRODUCTION

Allyl isothiocyanate (AITC), a major component of mustard essential oil, has attracted interest as a naturally derived preservative in active packaging of various foodstuffs.[1–3] AITC is a major flavor component in Wasabi (*Wasabia japonica* Matsum) and black mustard (*Brassia nigra* L.) and has been reported to have antibacterial activity.[4]

In research, within the European Union Fifth Framework BIOPACK project,[5] the concept of using either α- or β-cyclodextrins or CDs to encapsulate AITC was studied as a way of obtaining biopolymer packaging that might be suitable for long shelf-life storage of European cheeses. To prepare films for evaluation as cheese packaging, CD–AITC complexes were added at 5% w/w during extrusion of L-polylactide-*co*-polycaprolactone (PLA–PCL). The extruded films were tested for oxygen and water permeability and mechanical properties and, in addition, their performance in controlling molds that might colonize packaged cheeses by slow release of incorporated AITC was assessed. The mold control tests were undertaken using the complexes alone or, alternatively, using films containing these complexes in indirect contact with cheese, reflecting the normal situation in a packaged cheese product. In tests with the complexes alone before film incorporation, the β-CD–AITC complex was more effective in controlling relevant mold fungi than the α-CD–AITC complex. However, in tests with films involving indirect exposure to the fungi, the best performance was obtained with the film containing 5% w/w α-CD–AITC and a stronger antimicrobial effect was obtained in this case than with films containing either 5 or 10% w/w β-CD–AITC.

The research described in this article involved experiments designed to obtain a better understanding of the observed difference in antimicrobial effect between α-CD–AITC and β-CD–AITC when incorporated in PLA–PCL films. For example, it is possible that there might be greater loss of the AITC active ingredient from β-CD–AITC than from α-CD–AITC as a result of thermal degradation during film extrusion. There have been only a limited number of previous studies on either the release rates of AITC from CDs

Correspondence to: D. Plackett (david.plackett@risoe.dk)).
Contract grant sponsor: European Commission; contract grant number: QLK5-CT-2000–00,799.

© 2007 Wiley Periodicals, Inc.
WILEY InterScience® DISCOVER SOMETHING GREAT

or on the stability of AITC in the presence of CDs. Furuta et al.[6] examined the release rate of AITC as well as other flavor compounds at various temperatures from β-CD as encapsulation material and found that the rate was directly related to the surrounding water vapor concentration. Shiga et al.[7] discovered that the rate of release of AITC from α-, β-, or γ-CDs increased markedly with increasing relative humidity, independent of the type of CD. Release time-courses were well correlated with a modified version of Avrami's equation. There have been a number of studies on thermal decomposition of AITC in the presence of CDs[8–11]; however, these studies have dealt with AITC stability in solution rather than in the solid state.

In our research, detailed investigations were undertaken in which the thermal degradation profiles of AITC, α- and β-CDs, α- and β-CD complexes with AITC and PLA–PCL films containing these complexes were determined using thermogravimetric analysis (TGA) and thermogravimetric analysis–mass spectrometry (TGA–MS). A thorough search of the literature has indicated that the TGA–MS analytical technique has not previously been used to study the thermal stability of CD-entrapped AITC. Gas chromatography (GC) and high performance liquid chromatography (HPLC) were also used to characterize the AITC complexes with α- and β-CDs. This article summarizes the results from these experiments and draws conclusions about the implications for the use of CD–AITC complexes as preservative additives in active packaging films.

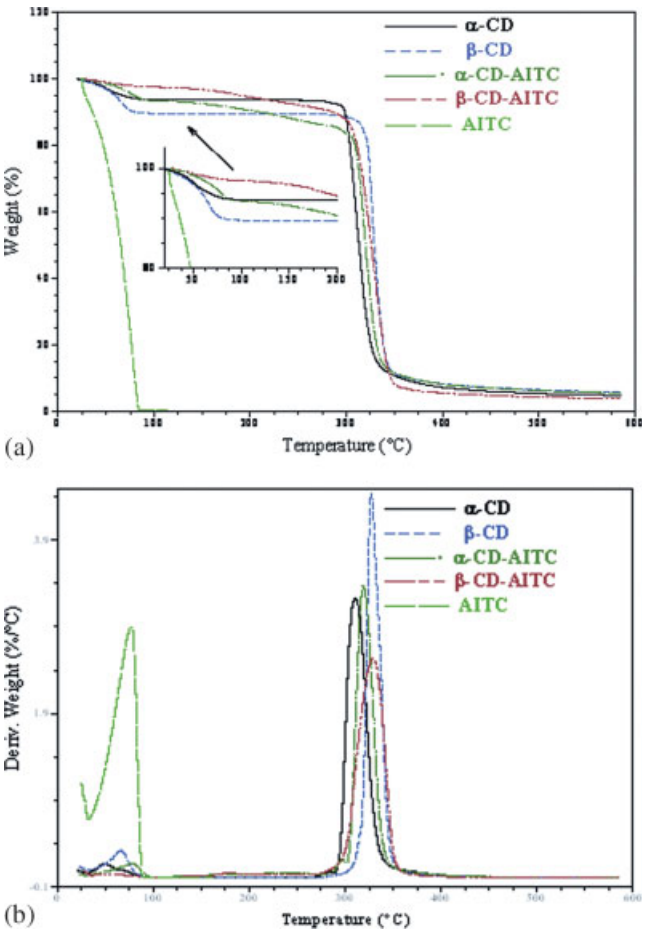

Figure 1 (a) TGA thermograms of AITC, CDs, and CD–AITC complexes and (b) DTGA thermograms of AITC, CDs, and CD–AITC complexes.

EXPERIMENTAL

Materials

A PLA–PCL copolymer was synthesized by a ring-opening polymerization procedure at Hycail b.V. (Noordhorn, The Netherlands). Stannous-2-ethylhexanoate was used as a catalyst for this synthesis in a specially configured laboratory extruder. The resulting product was a four-armed L-PLA–PCL copolymer containing 3–4% caprolactone monomer units. Films were prepared from the PLA–PCL copolymer granulate using a conventional flat die attachment on a Haake Rheomex CTW100 twin-screw extruder and were typically 150–200 μm in thickness and 130 mm in width. Cyclolab (Budapest, Hungary) provided samples of α- and β-CDs and also prepared AITC-encapsulated α- and β-CDs for antimicrobial evaluation and other tests.[5] The α- and β-complexes were found by UV analysis to contain 8.7 and 9.2% AITC, respectively. AITC (98% purity) was obtained from Fluka. PLA–PCL films containing each of the AITC–CD complexes were manufactured by dry blending of the additives at 5% w/w with PLA–PCL granulate before extrusion. Samples of AITC or the CD–AITC complexes were stored in a refrigerator at 4–6°C when not in use and the polymer films were stored under argon in a refrigerator to inhibit any polymer degradation that might otherwise be caused by hydrolysis during storage.

Characterization

The polymer films as well as AITC, the CDs and the AITC–CD complexes were examined by TGA and TGA–MS using a TA Instruments Q 500 TGA instrument in tandem with a bench-top MKS Mini-Lab mass spectrometer having detection limits from 1 to 200 atomic mass units (AMU) and a speed of up to 150 data points per second. The different gases and gas mixtures were analyzed by a newly developed precision-built quadrupole-type analyzer incorporating a closed ion source, a triple mass filter and a dual Faraday and secondary electron multiplier detector system. The analyzer configuration was selected to optimize detection level, performance, and long-term stability.

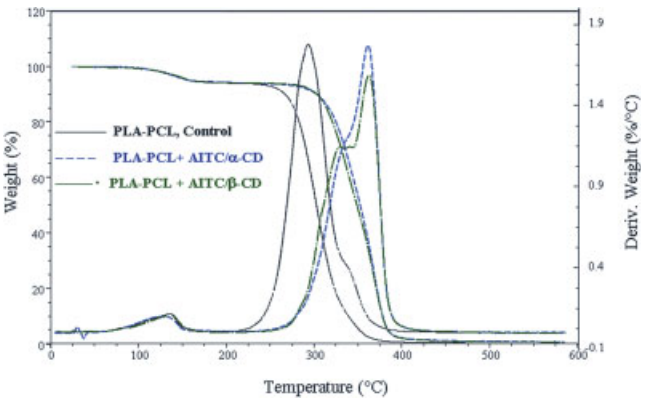

Figure 2 TGA and DTGA thermograms of PLA–PCL film and PLA–PCL film containing CD–AITC complexes.

TGA experiments were performed under nitrogen at a heating rate of 10°C/min. TGA–MS studies were carried out under a helium flow rate of 90 mL min^{-1} and heating rate of 10°C min^{-1}. Use of TGA–MS allowed the thermal degradation of the various materials to be monitored in weight terms as a function of temperature and correlated chemically with ions detected on-line via a silica-lined capillary interface heated to 150°C in the mass spectrometer.

GC for determination of AITC in CD complexes or in films involved use of a Shimadzu GC 17A Ver. 3 gas chromatograph equipped with advanced flow controller, flame ionisation detector, AOC-5000 auto injector, and a split/splitless injector port unit. Nitrogen was used as carrier and make-up gas. The column was fused silica (30 m × 0.32 mm i.d.) coated with 0.25 μm-thick SPB™-5 (Supelco). The temperature of the injector and detector was 220°C and the oven temperature program was 70°C for 1 min, increasing to 90°C at 1°C/min, and increasing again to 250°C at 50°C/min, with this final temperature held for 3 min. An AITC working standard for examination of linearity was prepared by diluting 1 g of AITC in 100 mL of DMF and injecting 1 μL of this solution into the gas chromatograph. Calibration was carried out by injecting 1 μL of either the undiluted working standard solution or this solution diluted by 2×, 2.5×, 5×, and 10×. An α-CD stock solution was prepared by diluting 625 mg of α-CD in 5 mL of DMF. Different amounts of the α-CD stock solution (20, 50, 90, 110, 150, 200, and 400 μL) were diluted to 5 mL with the undiluted AITC working standard solution and 1 μL of these solutions injected into the gas chromatograph to check the effect of matrix. AITC validation samples were analyzed by dissolving 50 mg of either α-CD–AITC or β-CD–AITC complexes in DMF and injecting 1 μL of these solutions into the gas chromatograph. Polymer samples containing CD–AITC complexes were dissolved in DMF before injection.

An HP 1050 HPLC was used for the analysis of AITC complexes. The HPLC was equipped with both evaporative light scattering and UV detectors and the column was a Nucleosil NH_2 120–7 μm (100 × 4.0 mm^2) operated at 30°C. The mobile phase was acetonitrile/water (74 : 26) with a flow rate of 1.0 mL/min. Samples were dissolved in acetonitrile/water (1 : 1) and injected directly into the HPLC. Polymer films containing the

TABLE I
TGA Data

Sample ID	Weight loss (%) at various temperatures (°C)			
	[a]RT–100	100 – 265	265 – 600	Residue
AITC	99.38	–	–	0.62
α-CD	6.27	0.21	88.75	4.77
β-CD	10.35	0.16	83.62	5.87
α-CD–AITC	6.52	6.83	81.12	5.53
β-CD–AITC	2.45	6.21	87.56	3.78
PLA–PCL	0.63	8.84	90.45	0.08
PLA–PCL/ α-CD–AITC	0.54	5.97	92.89	0.60
PLA–PCL/ β-CD–AITC	0.75	5.50	93.18	0.57

[a] RT, room temperature.

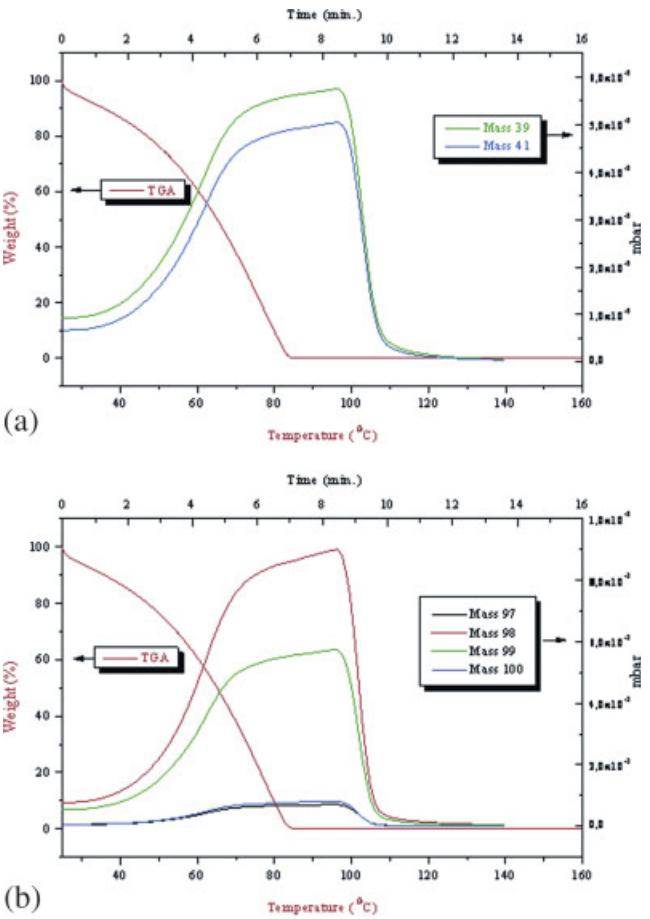

Figure 3 (a) TGA/MS of AITC showing fragment ions of mass 39 and 41 and (b) TGA/MS of AITC showing fragment ions of mass 97–100.

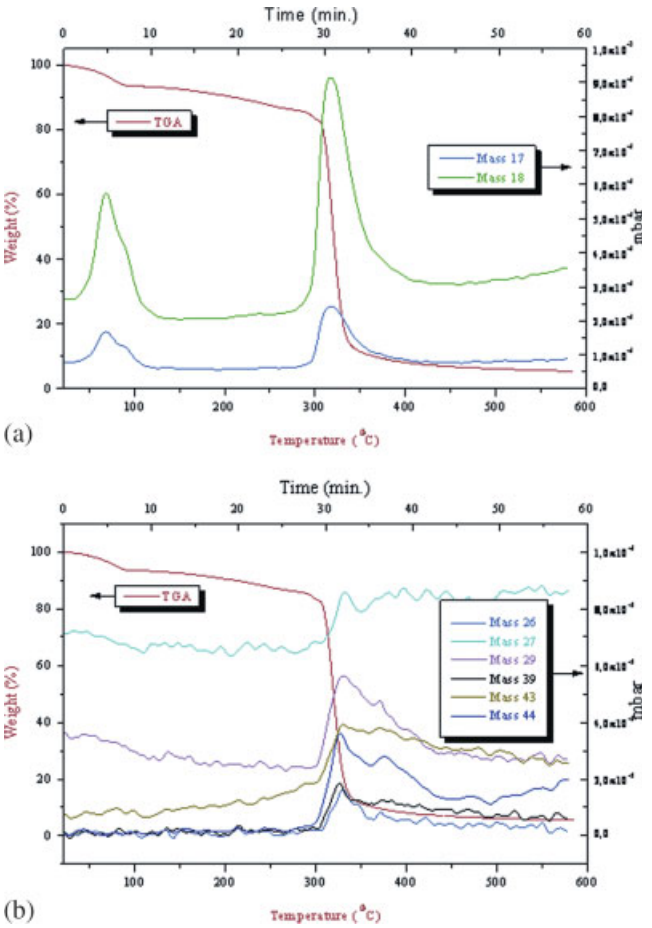

Figure 4 (a) TGA/MS of α-CD showing fragment ions of mass 17 and 18 and (b) TGA/MS of α-CD showing fragment ions in the mass range of 26–44.

CD–AITC complexes were dissolved in DMF and the solutions were then used directly for HPLC analysis.

The residual equilibrium water content of the CD–AITC complexes was determined by Karl Fischer titrimetry. The complexes were dissolved in formamide and the water content measured by dead-stop titration.

RESULTS AND DISCUSSION

The TGA and DTGA results for AITC, the CDs, and the CD complexes with AITC are shown in Figure 1(a,b), respectively. As expected, the TGA thermogram for pure AITC showed rapid weight loss because of volatilization below 100°C. Clear differences in the pattern of weight loss can be seen below 100°C, which must be associated with loss of water in the case of the CDs and might arguably be associated with loss of AITC and/or water from the corresponding AITC–CD complexes. The observed weight changes because of loss of water from the CDs [see inset in Fig. 1(a)] are in good agreement with Karl Fischer analyses, which indicated that β-CD typically contains about 12% water by weight while α-CD contains only about 7%.[12] A comparison of the onset temperatures for thermal degradation of the materials studied in Figure 1(a) is shown in the matching differential thermogravimetric analysis (DTA) plots illustrated in Figure 1(b).

The TGA results for the PLA–PCL film and the PLA–PCL film containing either 5% α-CD–AITC or 5% β-CD–AITC are shown in Figure 2. Incorporation of the CD complexes in the films enhanced the thermal stability of the polymer by about 30°C. A comparison of the thermal properties of these samples is also shown in the DTGA plots of Figure 2. The increase in the thermal degradation onset temperature appears identical for the two complexes. The weight losses of all samples studied as a function of selected temperature range and percentage residue after thermal analysis are shown in Table I.

The TGA–MS plots, overlaying the thermogram for AITC, are shown for AITC molecular fragments with masses of 39 and 41 in Figure 3(a) and for fragments with masses of 97–100 in Figure 3(b). Species with

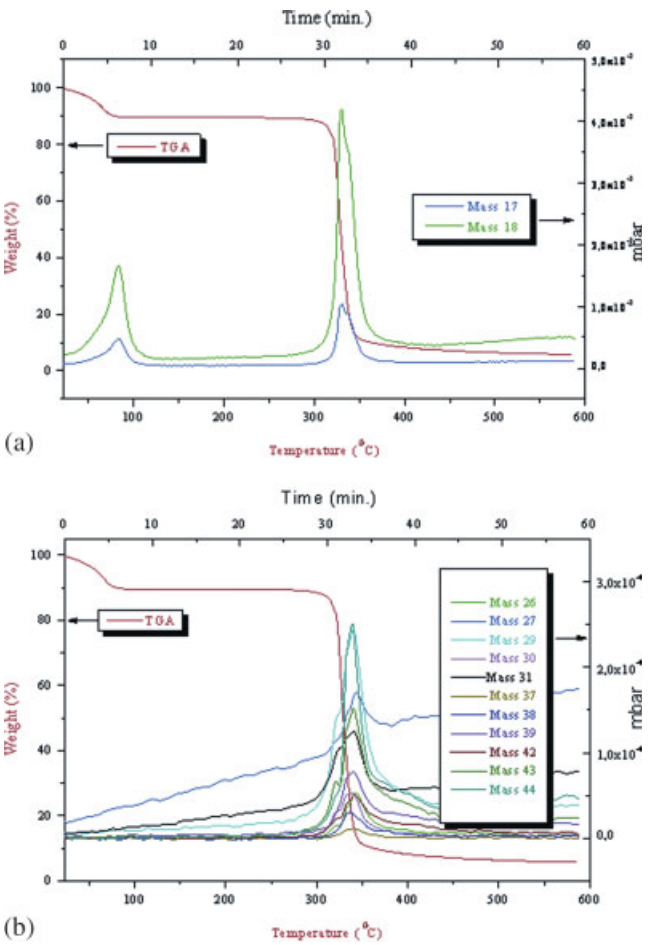

Figure 5 (a) TGA/MS of β-CD showing fragment ions of mass 17 and 18 and (b) TGA/MS of β-CD showing fragment ions in the mass range of 26–44.

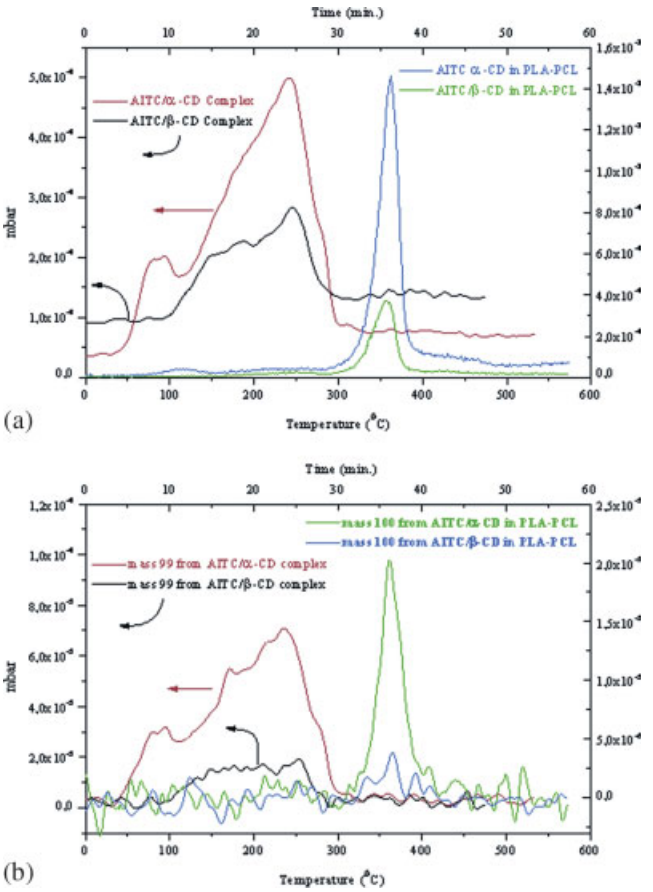

Figure 6 (a) MS traces of fragment ions of mass 41 in TGA/MS of CD–AITC complexes alone or incorporated in PLA–PCL films and (b) MS traces of fragment ions of mass 99 or 100 in TGA/MS of CD–AITC complexes alone or incorporated in PLA–PCL films.

masses of 58 and 59 were also observed and showed the same trend as shown in Figure 3(a). Fragments at mass of 39 or 41 may be assigned to allyl ($C_3H_5^+$) ions while fragments with masses of 58 or 59 are assignable to isothiocyanate ions ($N{=}C{=}S^+$). Molecular species with masses in the range 97–100 are associated with AITC parent ions.

TGA–MS plots for α-CD and β-CD are presented in Figures 4(a,b) and 5(a,b), respectively. The peaks for fragments with masses of 17 and 18 from either α-CD or β-CD can be assigned to loss of water [Figs. 4(a) and 5(a)]. Loss of water occurs as the CDs decompose at temperatures over 300°C as well as at lower temperatures. This finding can be interpreted in terms of water being released as a product from thermal degradation of CDs or through release of physically trapped water as previously observed by Éhen et al.[13] As shown in Figures 4(b) and 5(b), both α-CD and β-CD also display a number of fragments in the mass range 26–44 that are released during thermal degradation at temperatures above 300°C, although there are apparent differences in the release profiles characteristic of each CD. For example, there are fragments occurring at mass units of 30, 31, 37, 38, and 42 in the TGA–MS of β-CD that are not detected in the TGA–MS of α-CD. Although not shown here, species in the mass range of 45–96 were detected in the TGA–MS results for β-CD but were not detected in the case of α-CD. These findings are in contrast to those of Éhen et al.[13] who found that the thermal fragmentation profiles of α-CD and β-CD were practically identical above 250°C and reported that the key fragments assigned to breakdown products from α-D-glucopyranose were found at mass units of 18, 22, 31, and 44.

Figure 6(a,b) present the TGA–MS traces of evolved gases for α-CD–AITC and β-CD–AITC alone and for the PLA–PCL films containing the complexes at loadings of 5% w/w. Figure 6(a) contains data for the allyl fragment of mass = 41 and Figure 6(b) shows data for AITC parent ions at mass = 99 or 100. The figures show a significantly greater release of AITC on heating PLA–PCL copolymer film containing the α-CD–AITC complex than when heating PLA–PCL film containing the β-CD–AITC complex. The figures have not been normalized on a weight basis and in fact slightly less of the film containing the α-CD–AITC (8.34 mg) was used in the experiments than the film containing the β-CD–AITC (10.34 mg), which reinforces the con-

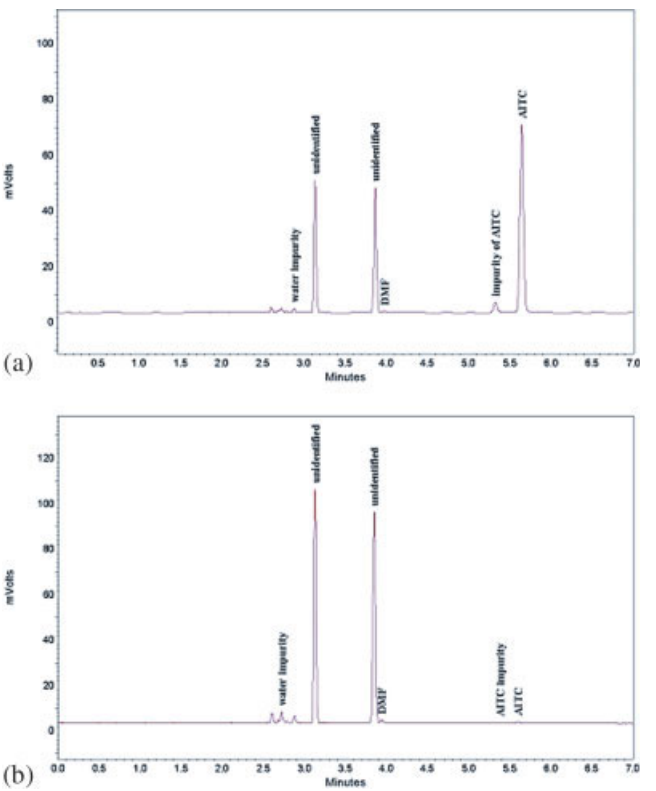

Figure 7 (a) Gas chromatogram of PLA–PCL film loaded with α-CD–AITC complex and (b) gas chromatogram of PLA–PCL film loaded with β-CD–AITC complex.

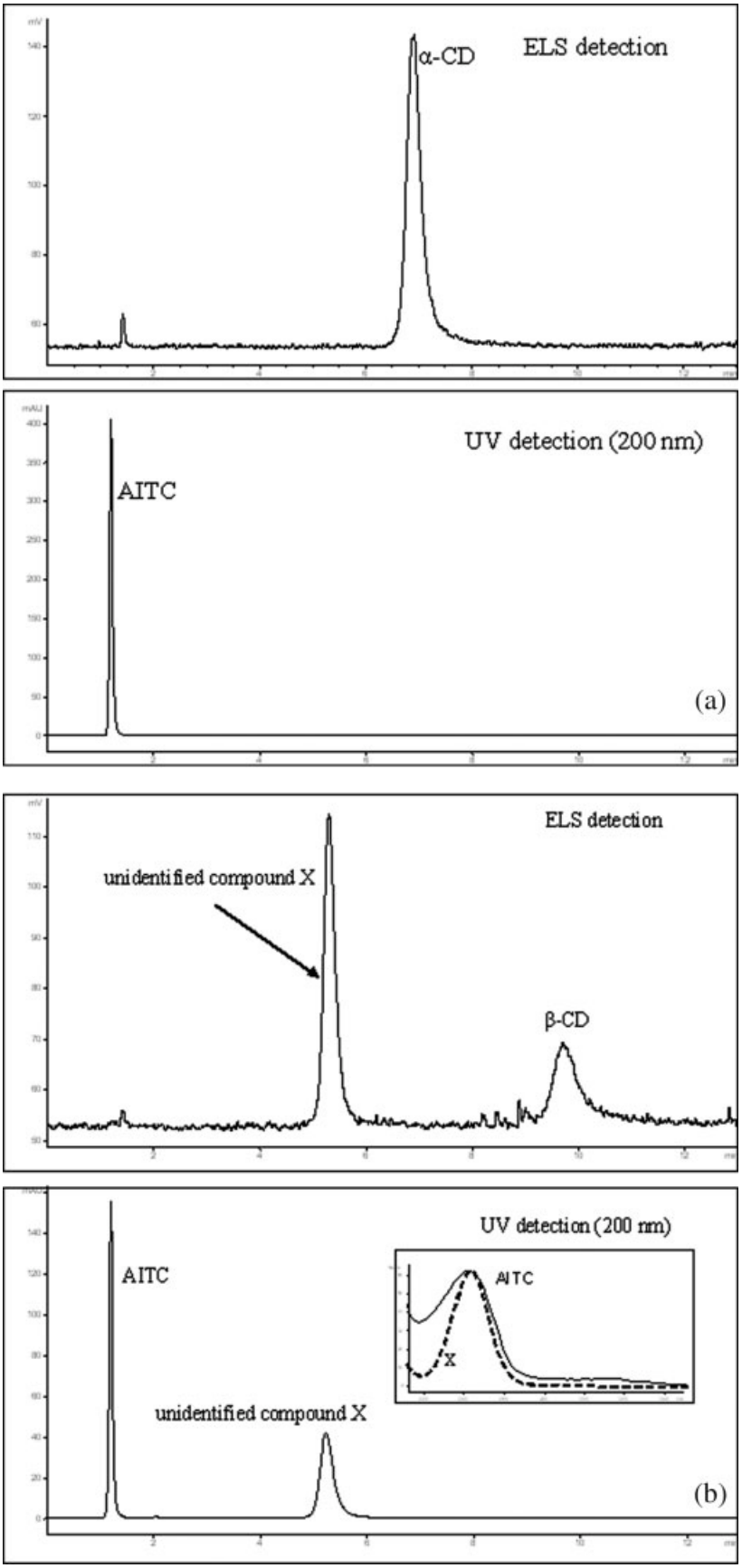

Figure 8 (a) HPLC chromatogram of α-CD–AITC complex obtained by evaporative light scattering (ELS) and UV detection and (b) HPLC chromatogram of the β-CD–AITC complex obtained by evaporative light scattering (ELS) and UV detection.

clusion that there is proportionally more AITC released from the PLA–PCL film containing the α-CD–AITC complex. The TGA–MS plots for α-CD–AITC and β-CD–AITC alone are also shown in Figure 6(a,b) and confirm that AITC release appears greater in the case of the α-form when the pure complexes are heated before film incorporation, although the difference is not as significant as when the film samples are examined. It should be noted that the peaks for fragments of mass 41 and 99 or 100 referred to in Figure 6(a,b) and associated with AITC are not found to any significant extent in the TGA–MS traces for the unmodified PLA–PCL film or the pure CDs.

The results presented in Figure 6(a,b) can be explained if AITC is bound in different ways within the α-CD–AITC and β-CD–AITC complexes, which might lead to differences in both AITC release at low temperatures and release of AITC during thermal degradation at higher temperature. Supporting evidence for this suggestion is provided by data in Table I and illustrated in Figure 1(a,b), showing that addition of AITC to β-CD results in much less weight loss observed during heating to 100°C than is the case when AITC is added to α-CD. The results in Figure 6(a,b) confirm that AITC is released to a more significant extent from α-CD–AITC than from β-CD–AITC below 100°C as well as at temperatures in the 100–265°C range.

Our findings provide a possible explanation for the antimicrobial test results previously obtained when the CD–AITC complexes or PLA–PCL films containing either of the two CD–AITC complexes were exposed to fungi known to colonize cheese products.[5] Although the β-CD–AITC complex appeared more effective than the α-CD–AITC complex in an indirect fungal exposure test, the reverse was the case when PLA–PCL film samples containing the complexes were tested, even though films were prepared with the same targeted loading of each complex. A higher content of AITC present in PLA–PCL films incorporating α-CD–AITC than in similar films containing β-CD–AITC or more strongly bound AITC in films containing β-CD–AITC, as suggested by Figure 6(a,b), would mean greater availability of AITC to volatilize in a package headspace and therefore greater antimicrobial activity when evaluating PLA–PCL films loaded with the α-CD–AITC complex.

The results from GC analyses are shown in Figure 7(a,b) for DMF extracts from films containing either α-CD–AITC or β-CD–AITC. These figures clearly suggest that the film containing the β-CD complex contains much less available AITC than the film containing the α-CD complex. This is despite the fact that the amount injected was three times greater in the case of the film containing the β-CD complex than in the case of the film in which the α-CD complex was incorporated.

The results from HPLC analyses are shown in Figure 8(a,b) for DMF extracts isolated from the films containing either α-CD–AITC or β-CD–AITC. Figure 8(a) shows that the α-CD–AITC in the PLA–PCL film remains chemically intact since the observed peak is identical to that of an α-CD standard. In contrast, the chromatogram in Figure 8(b) shows not only a peak for the intact parent β-CD but also a peak with a retention time of 5.3 min that may be associated with a previously unreported covalent β-CD–AITC conjugate. Further research is now required to confirm the structure and the true nature of this conjugate.

CONCLUSIONS

TGA and TGA–MS analyses were employed to examine the thermal stability and the chemistry of thermal breakdown of AITC, CDs, CD–AITC complexes, and PLA–PCL films containing CD–AITC complexes. In addition, GC and HPLC were used to examine complexes extracted from PLA–PCL films. The motivation for this research was a better understanding of the performance of CD–AITC complexes within PLA–PCL films when used in trials aimed at inhibiting mold growth on packaged cheeses. The main conclusions from the research were as follows:

1. From thermal analyses, α-CD–AITC appears to have a higher percentage of less-strongly or surface-bound AITC species than β-CD–AITC. This is especially the case when the complexes are loaded in PLA–PCL films and, to the authors' knowledge, represents the first time that a mass spectrometric analytical method has been used to differentiate between lost water and lost AITC guest upon heating CD complex samples and polymer films containing such complexes.
2. GC studies show that PLA–PCL films prepared with α-CD–AITC contain chemically intact AITC but that the films loaded with β-CD–AITC contain much less volatile AITC. HPLC experiments suggest that a possible reason for the low AITC content in films loaded with β-CD–AITC, as indicated by GC, is that a covalent conjugate is formed between AITC and β-CD. The results of further research aimed at confirming the structure of the covalently bound β-CD–AITC conjugate will be released in a future publication.
3. The different behavior of AITC in terms of complexation with either α-CD or β-CD can provide an explanation for the greater effectiveness of films containing α-CD–AITC than films containing β-CD–AITC as demonstrated in earlier antimicrobial tests involving indirect exposure to selected cheese-colonizing fungi.

The research described in this paper was carried out as a follow-up to the BIOPACK project, Proactive Biobased Cheese Packaging (QLK5-CT-2000-00799), funded by the European Commission. The research does not necessarily reflect the Commission's views and in no way anticipates their future policy in this area. We thank all the participants for their cooperation and enthusiasm.

References

1. Verstichel, S.; De Wilde, B.; Fenyvesi, E.; Szejtli, J. J Polym Environ 2004, 12, 47.
2. Nielsen, P. V.; Rios, R. Int J Food Microbiol 2000, 60, 219.
3. Kuroki, J.; Yamazaki, T.; Mita, K. Packages containing microbicidal isothiocyanate for preserving produce. Jpn. Kokai Tokkyo Koho 1995, 6 pp. JP 07017576.
4. Isshiki, K.; Tokuoka, K.; Mori, R.; Chiba, S. Biosci Biotechnol Biochem 1992, 56, 1476.
5. Plackett, D. V.; Holm, V. K.; Johansen, P.; Ndoni, S.; Nielsen, P. V.; Sipilainen-Malm, T.; Södergård, A.; Verstichel, S. Pack Tech Sci 2006, 19, 1.
6. Furuta, T.; Yoshii, H.; Hashimoto, I.; Liu, X.; Linko, Y. Y.; Linko, P. In Proceedings of the 9th International Symposium on Cyclodextrins, Kluwer, Dordrecht, 1998; p 685.
7. Shiga, H.; Furuta, T.; Yoshii, H.; Hayashida, K.; Linko, P. P. In Proceedings of the 10th International Symposium on Cyclodextrins, Wacker Biochem, 2000; p 564.
8. Chen, C.-W., Ho, C.-T. J Agric Food Chem 1998, 46, 220.
9. Ohta, Y.; Takatani, K.; Kawakishi, S. Biosci Biotechnol Biochem 1999, 63, 190.
10. Ohta, Y.; Takatani, K.; Kawakishi, S. Biosci Biotechnol Biochem 2004, 68, 433.
11. Ohta, Y.; Matsui, Y.; Osawa, T.; Kawakishi, S. Biosci Biotechnol Biochem 2004, 68, 671.
12. Szejtli, J. Cyclodextrin Technology; Kluwer: Dordrecht, 1998; p 12.
13. Éhen, Z.; Giordano, F.; Sztatisz, J.; Novák, Cs. J Therm Anal Cal 2005, 80, 419.