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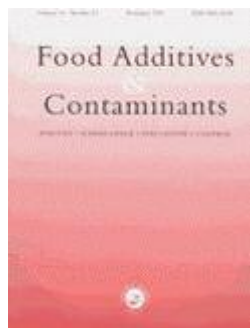
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Release of alpha-tocopherol from antioxidative low density polyethylene film into fatty food simulant: Influence of complexation in beta-cyclodextrin

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4 **1 Release of alpha-tocopherol from antioxidative low density**
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7 **2 polyethylene film into fatty food simulant: Influence of complexation**
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10 **3 with beta-cyclodextrin**
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4 **24 Abstract**
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9 26 The release of alpha-tocopherol from two formulations (with and without complexation
10 with beta-cyclodextrin) of low density polyethylene (LDPE) film was examined.
11 27 Specific migration studies were performed at $7.0 \pm 0.5^\circ\text{C}$ using plastic bags filled with
12 28 95% ethanol as fatty food simulant. The amount of complexed and free (non-
13 29 complexed) alpha-tocopherol migrating into the food simulant was followed by a high
14 30 performance liquid chromatography (HPLC). It was concluded that complexation with
15 31 beta-cyclodextrin had a significant effect on the release rate of the antioxidant. Using a
16 32 mathematical model for the description of the migration, a decrease in diffusion
17 33 coefficient (D) of one order of magnitude was calculated in the case of complexed
18 34 alpha-tocopherol compared to the free form. Total migration of alpha-tocopherol from
19 35 both films was observed, meaning that the partition coefficient of tocopherol was not
20 36 influenced by the incorporation with cyclodextrin. Thus, complexation might be the key
21 37 to a long lasting antioxidative effect of such kind of active packaging.
22 38
23 39

24 40 **Keywords:** active packaging, alpha-tocopherol, antioxidants, beta-cyclodextrin,
25 41 controlled release, diffusion coefficient;
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43 Introduction

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45 To prevent or retard any deterioration in quality of packaged foods, active packaging
46 including the concept of the controlled release of active components to foodstuffs has
47 shown the greatest potential to improve storage stability (Miltz et al. 1995, Vermeiren et
48 al. 1999, Appendini & Hotchkiss 2002, LaCoste et al. 2005). The motivation for
49 developing controlled release packaging is to transfer the active agent from the
50 polymeric carrier to the surface of the food in order to maintain a predetermined
51 concentration and thus to prolong the shelf-life without adding excess additives directly
52 to food products. This transfer of the active agent is a result of the diffusion in the
53 polymer matrix and the partition process between the polymer and the food surface. The
54 diffusion is described by the diffusion coefficient (D), while the partition is
55 characterised by the partition coefficient (K) (Garde et al. 2001, Helmroth et al. 2002,
56 Hernandez-Muñoz et al. 2002).

57

58 Controlled release of drug delivery has been used for some time (Colombo et al. 1996,
59 Kuijpers et al. 1998, Bezemer et al. 2000), and procedures for achieving release under
60 various conditions are well-established. However, considerable research on testing the
61 concept of controlled release of active compounds from food packaging did not appear
62 until the last decade (Floros et al. 2000). The promulgation of EC regulation 1935/2004
63 on materials and articles intended to come into contact with food may facilitate the
64 development of controlled release packaging concepts (EC 2004). This regulation states
65 that, unlike traditional packaging materials and articles, active packaging concepts are
66 not inert by their design. They are designed to deliberately incorporate 'active'

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5 67 components intended to be released into the food or to absorb substances from the food,
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7 68 therefore they may change the composition or the organoleptic properties of the food.
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9 69 These changes, however, always should comply with the Community provisions
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11 70 applicable to food, such as the provisions of Directive 89/107/EEC on food additives. In
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13 71 particular, substances such as food additives deliberately incorporated into certain active
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15 72 food contact materials and articles for release into packaged foods or the environment
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17 73 surrounding such foods, should be authorised under the relevant Community provisions.
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19 74 Besides the main requirements of use established in EC regulation 1935/2004, further
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21 75 regulations should be addressed in specific measures, to include positive lists of
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23 76 authorised substances and/or materials and articles, which should be adopted as soon as
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25 77 possible.
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33 79 Antioxidant packaging is a promising type of controlled release concepts, in which
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35 80 antioxidants are incorporated into or coated onto food packaging materials to reduce
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37 81 oxidation in the packed food (Vermeiren et al. 1999, LaCoste et al. 2005). For example,
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39 82 incorporation of synthetic antioxidant compounds, such as butylated hydroxytoluene
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41 83 (BHT) and butylated hydroxyanisole (BHA) in high-density polyethylene has been
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43 84 shown to protect cereals from oxidation (Miltz et al. 1987, Wessling et al. 2000a). In
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45 85 recent years, however, there has been a growing interest in the use of natural
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47 86 antioxidants such as tocopherols in food packaging applications, because of an
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49 87 emerging concern regarding long-term safety and negative consumer perception of
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51 88 synthetic antioxidants (Yu et al. 2002, Maisuthisakul et al. 2006). For example, BHA
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53 89 and BHT have been suspected of being responsible for liver damage and carcinogenesis
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55 90 (Onyeneho and Hettiarachchy 1992, Yu et al. 2002). Tocopherols are non-toxic
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4 91 compounds with a positive public perception, broad regulatory approvals, and
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6 92 environmentally friendly appeal to the consumers (Wessling et al. 2000b, LaCoste et al.
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9 93 2005). Besides being an effective antioxidant for reducing oxidation in foods,
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11 94 tocopherols are also excellent stabilizers for polymer processing (Al-Malaika et al.
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13 95 1994, Billingham and Garcia-Trabajo 1995, Al-Malaika et al. 1999, 2001a, 2001b).
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15 96 Therefore, tocopherols can serve dual functions when added to packaging: as a
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17 97 stabilizer for polymer processing and as an antioxidant in controlled release to reduce
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19 98 oxidation (LaCoste et al. 2005).
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26 100 The release of alpha-tocopherol from packaging material depends on several factors.
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28 101 Wessling et al. (1999) revealed that the retention of alpha-tocopherol is influenced by
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30 102 the type of the polymer as well as the fat, alcohol and organic acid content of the food
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32 103 product (Wessling et al 2000c). Heirlings et al. (2004), however, found that the polarity
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34 104 of the polymer matrix had only a slight effect on the migration rate of alpha-tocopherol.
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36 105 In this latter study the antioxidant was also adsorbed onto silica materials in order to
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38 106 give a protection during extrusion and to ensure a controlled release. By this way
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40 107 antioxidant release could be elongated for about three-four days at 7 °C. For some food
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42 108 applications, however, an even slower antioxidant release would be desired.
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49 110 Recently there has been an increasing interest in the use of cyclodextrins as a tool for
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51 111 controlled release of active compounds due to their outstanding ability to form
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53 112 molecular complexes with hydrophobic guest molecules. Cyclodextrins (CD) are
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55 113 obtained by degradation of starch. They are cyclic oligosaccharides consisting of six
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57 114 (alpha-CD), seven (beta-CD) or eight (gamma-CD) glucopyranose units, which are
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4 115 bound together by alpha (1-4)-linkages forming a torus-shaped ring structure. Due to
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7 116 their polar hydrophilic outer shell and relatively hydrophobic cavity, they are able to
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10 117 build up host-guest complexes by inclusion of suitable hydrophobic molecules (e.g.
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12 118 alpha-tocopherol). The formation of these complexes leads to significant changes of the
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14 119 solubility and reactivity of the guest molecules, but without any chemical modification
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16 120 (Szejtli 1996).
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21 122 In this study the influence of complexation by beta-cyclodextrin on the migration of
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23 123 alpha-tocopherol from LDPE was investigated. The migration of alpha-tocopherol both
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25 124 complexed in beta-cyclodextrin and non-complexed from Low Density Polyethylene
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27 125 (LDPE) polymer into a fatty food simulant was followed at 7.0 ± 0.5 °C. A migration
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29 126 model suggested by Hamdani et al. (1997) and Piringer (2000) was fitted to the
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31 127 migration profiles and diffusion coefficients were calculated. Besides protecting the
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33 128 antioxidant against high temperature during extrusion, beta-cyclodextrin is also
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35 129 expected to slow down the release of alpha-tocopherol from LDPE thus ensuring a
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37 130 longer lasting antioxidative effect.
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5 131 **Materials and methods**
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9 133 *Complexation process in beta-cyclodextrin*
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12 135 Pharma-grade beta-CD (Wacker Chemie, Germany, Lot No. 70T179, 227 g, 0.17 mol)

13 136 was suspended in 700 mL distilled water by stirring at ambient temperature. Technical

14 137 grade alpha-tocopherol [Irganox[®] E201=3,4-Dihydro-2,5,7,8-tetramethyl-2-(4,8,12-

15 138 trimethyltridecyl)-2H-1-benzopyran-6-ol] (Ciba Specialty Chemicals, Basel,

16 139 Switzerland) (43.06 g, 0.1 mol) dissolved in 50 mL 96% ethanol (analytical reagent

17 140 grade, Merck, Darmstadt, Germany) was added to the aqueous suspension by

18 141 continuous stirring. The stirring was continued for 0.5 h at 60 C and for 8 h at ambient

19 142 temperature, then the suspension was left to stay in the refrigerator for approximately 16

20 143 hours. The next day it was filtered and the filtrate was dried for 2 days at room

21 144 temperature in vacuum exsiccator beside phosphorus pentoxide till constant weight. The

22 145 complex obtained (214 g) contained 15.5% alpha-tocopherol as measured by the

23 146 following method. 25.0 mg complex was dissolved in 25.0 ml water-ethanol (1:1)

24 147 mixture and the absorbance was measured at 292 nm by a HP 8452 UV-VIS diode array

25 148 spectrophotometer (Hewlett-Packard Co., Palo Alto, CA, USA).
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50 150 *Heat stability measurements*

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52 152 The heat stability of free and complexed alpha-tocopherol was characterized by thermo-

53 153 gravimetric analysis (TGA) as suggested by Ferdinando et al. (2001). A MOM

54 154 Derivatograph PC (MOM, Hungary) was used to obtain differential thermo-gravimetric

55 155 (DTG) curves. Experiments were carried out on 15-20 mg of sample in aluminum-oxide
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5 156 open pan at a heating rate of 5 °C min⁻¹, under pure nitrogen at a flow rate of 40 mL
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7 157 min⁻¹. Samples were heated up to 250 °C.
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11 159 *Preparation of packaging materials*
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15 161 The experiments were carried out using Low Density Polyethylene (LDPE, Lot No. FB
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17 162 243-51) polymer provided by Tiszai Vegyi Kombinát (Tiszaújváros, Hungary). Two
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19 163 types of film were prepared: film A contained free alpha-tocopherol while film B
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21 164 comprised this antioxidant complexed into beta-cyclodextrin. The antioxidant alpha-
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23 165 tocopherol, free and complexed was mixed with the LDPE granulates at a nominal
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25 166 concentration of 2000 mg kg⁻¹ by a simple scroll extruder, and the granulates were
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27 167 transformed into films on a blown film line (Kuhne K36, Kuhne GmbH, Germany).
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29 168 Besides alpha-tocopherol (Irganox[®] E201) the films contained no further antioxidants.
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31 169 The thicknesses of film A and film B, measured by a micrometer (Mitutoyo, Mitutoyo
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33 170 Corporation, Japan), were approximately 60 µm and 50 µm, respectively.
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41 172 *Determination of the initial alpha-tocopherol concentrations*
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45 174 The exact initial alpha-tocopherol content of the polymer was determined prior to the
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47 175 migration experiment using the procedure described by Heirlings et al. (2004) with
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49 176 some slight modifications. Briefly, 1.0 g film was weighed and cut into small pieces
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51 177 (approximately 10 mm x 10 mm) then dissolved in 70 mL toluene (analytical reagent
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53 178 grade, Reanal, Budapest, Hungary). During the dissolving process the solution was
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55 179 heated up to 65 °C and stirred by a magnetic stirrer (MLW RH3, VEB MLW, Freital,
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57 180 Germany). After 15 minutes, 50 mL cold methanol (analytical reagent grade, Reanal,
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5 181 Budapest, Hungary) was added in order to precipitate the polymer. Afterwards the
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7 182 solution was filtered through a paper filter (Macherey-Nagel GmbH, 614^{1/4} Ø 150mm,
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9 183 Düren, Germany). The solvents were evaporated to dryness in a rotary evaporator
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11 184 (Heidolph VV2000, Heidolph Elektro GmbH & CO KG, Kelheim, Germany) at 50°C.
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14 185 The residue was resolved in 5 mL methanol of HPLC grade (Reanal, Budapest,
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16 186 Hungary). After filtering through a Nyfalo[®] Z269514 syringe filter (Sigma-Aldrich
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18 187 Chemie GmbH, Steinheim, Germany), the samples were analysed by a high
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21 188 performance liquid chromatography (HPLC) system described under.
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26 190 *High performance liquid chromatographic analysis*
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30 192 A Shimadzu HPLC system was used (Shimadzu Corporation, Kyoto, Japan), which
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32 193 consisted of a Shimadzu LC-10AD solvent delivery pump and a Shimadzu SPD-10A
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34 194 UV-VIS detector. Samples were injected manually through a Model 7725i injection
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36 195 valve with a 20 µL sample loop (Rheodyne, Cotati, CA, USA). A Discovery C18 (250 x
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38 196 4.6 mm, 5µm) column equipped with a Supelguard[®] guard column (20 x 4.0 mm, 5µm)
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41 197 (Supelco, Bellefonte, PA, USA) was used. Elution with a flow rate of 1.0 mL min⁻¹ was
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43 198 monitored by UV detection at 292 nm. 100% HPLC methanol was used as mobile
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46 199 phase. The injection volume was 20 µl and total analysis time was set at 13 minutes.
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49 200 Data acquisition and processing were accomplished with a workstation using Class-VP
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51 201 4.3 software (Shimadzu). Alpha-tocopherol was identified by matching of its retention
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53 202 time with the corresponding peak in the standard solution. Quantification was carried
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56 203 out by the integration of the peak areas and external calibration.
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5 205 The linearity of the determination method was measured by injecting alpha-tocopherol
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7 206 solutions of different concentrations (100, 250, 500, 750 and 1000 mg L⁻¹). Then the
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9 207 peak areas were calculated and were expressed as a function of the concentration. The
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11 208 response was linear within the examined range with a correlation coefficient of 0.9996.
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16 210 Repeatability and recovery of the HPLC analysis were evaluated by analysing five
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18 211 replicates of 50, 250, 750 and 1000 mg L⁻¹ of alpha-tocopherol standard solutions. The
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20 212 obtained relative standard deviations (RSD) were between 0.7-1.2% and the recovery
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22 213 found to be 95.8-106.0%.
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28 215 *Specific migration studies*
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30 216 Migration profiles of alpha-tocopherol from the two kinds of film were investigated
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32 217 using bags filled with food simulant. The experimental set was based on the migration
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34 218 study of Heirlings et al. (2004). As food simulant 95% ethanol (analytical reagent grade,
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36 219 Reanal, Budapest, Hungary) was used instead of the normally proposed olive oil in
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38 220 order to avoid the difficulties associated with the sample preparation of oil and the
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40 221 quantification problems arising from the originally high alpha-tocopherol content of
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42 222 olive oil.
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48 224 The migration bags with a size of 0.1 m x 0.1 m were filled entirely with 100 mL 95%
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50 225 ethanol (< 1 mL air/bag). The weight of the empty bags was approximately 1.2 g and
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52 226 1.0 g for film A and film B, respectively. After filling the bags, they were heat-sealed
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54 227 (Model N200, Cromat[®], Zagreb, Croatia) and stored at 7.0 ± 0.5 °C in a refrigerator
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56 228 with forced ventilation for 65 and 145 days in case of film A and film B, respectively.
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229 In order to provide the proper contact area and time for both sides of the bag, they were
230 turned daily.

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232 To determine the amount of alpha-tocopherol migrated in the food simulant, the whole
233 amount of food simulant (95% ethanol) was evaporated to dryness in a rotary
234 evaporator at 50 °C and the residue was resolved in 1.0-5.0 mL methanol of HPLC
235 grade, then the samples were filtered and injected into the chromatographic system. The
236 concentration of alpha-tocopherol was determined by the HPLC method described
237 above. In each time of measurements three bags of film A and B were analysed.
238 Samples were analysed once or twice a day at the beginning of the migration
239 experiment and less frequently as the migration process was nearing the equilibrium. In
240 total 72 and 90 bags of film A and film B were analysed, respectively. All prepared
241 samples were injected at least duplicate. Mean values and standard deviations were
242 calculated by Microsoft® Excel 2000 (Microsoft Corporation, USA).

243

244 *Migration modelling*

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246 A migration process was fully described by the kinetics of migrant diffusion in each
247 phase (expressed by the diffusion coefficient, D) and the chemical equilibrium
248 (expressed by the partition coefficient, K). The partition coefficient of a migrating
249 compound between the polymer and the food was defined as follows:

$$250 \quad K_{P/F} = \frac{c_{P,\infty}}{c_{F,\infty}} \quad (1)$$

251 where $c_{P,\infty}$ (mg kg⁻¹) and $c_{F,\infty}$ (mg kg⁻¹) are the equilibrium concentrations of the
252 component in the polymer and the food, respectively.

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7 254 The apparent diffusion coefficient (D) of alpha-tocopherol was determined from the
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9 255 migration versus time data, which was fitted to Fick's second law for an infinite slab in
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11 256 contact with an infinite volume of solvent (Crank 1975):

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$$\frac{M_{F,t}}{M_{F,\infty}} = 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2 \pi^2} \exp\left[-\frac{(2n+1)^2 \pi^2}{4d_p^2} Dt\right] \quad (2)$$

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17
18 258 where $M_{F,t}$ (mg) is the amount of the migrant in the food at particular time t; $M_{F,\infty}$ (mg)
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20 259 is the amount of the migrant in the food at equilibrium; d_p (cm) is the thickness of the
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22 260 polymer; D ($\text{cm}^2 \text{s}^{-1}$) is the diffusion coefficient of the migrant in the polymer and t (s) is
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24 261 the contact time.

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30 263 The diffusion coefficient (D) was determined by minimising the sum of squares in
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32 264 errors (SSE) between the estimated and measured values.

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36 37 38 266 **Results and discussion**

39 40 267 *Influence of the complexation process on heat stability*

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42 268 During extrusion, granulates and polymer additives are exposed to relatively high
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44 269 temperatures, often reaching 200 ± 30 °C depending on the type of polymers. Therefore,
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46 270 polymer additives should be stable up to this temperature range.

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52 272 Cyclodextrins are often used to increase heat stability of several compounds. In our
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54 273 experiment the effect of complexation on the heat stability of alpha-tocopherol was also
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56 274 assessed. In Figure 1 the differential thermo-gravimetric (DTG) curve of alpha-
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58 275 tocopherol/beta-cyclodextrin complex is compared to the DTG curves of the free

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4 276 components (alpha-tocopherol and beta-cyclodextrin). It can be seen that the DTG curve
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7 277 of alpha-tocopherol was flat between 50 and 190°C, which means that it is stable until
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9 278 190°C. It started decomposition at 191°C. The DTG curve of beta-cyclodextrin showed
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11 279 a single peak between 38 and 127°C (with 90°C peak temperature). This stage is related
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14 280 to the dehydration with 12.7% of water weight loss. Up to 250°C no evidence of
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16 281 decomposition were experienced. This corresponds to the information found in the
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18 282 literature, which stated that decomposition of cyclodextrins occurs only at around 300
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21 283 °C (Hedges et al. 1995).

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26 285 The solid line in Figure 1 represents the DTG curve of alpha-tocopherol/beta-
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28 286 cyclodextrin complex. Only one stage appeared on the curve between 35 and 138°C.
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30 287 This peak corresponds to the water loss process. The DTG peak minimum temperature
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33 288 was 87°C with 7.8% of water weight loss. After this peak no further weight loss was
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35 289 detected until 197°C, when the decomposition of the complex started.

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37 290 “[insert Figure 1 about here]”
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42 292 Heat stability measurements indicated that both the free alpha-tocopherol and the alpha-
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44 293 tocopherol/beta-cyclodextrin complex are stable up to 190°C. The complexation process
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46 294 did not increase the heat stability of alpha-tocopherol significantly, however, alpha-
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49 295 tocopherol is anyway quite heat stable.

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53 297 *Initial alpha-tocopherol concentrations in the films*

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55 298 The exact alpha-tocopherol content of the films was determined by HPLC-UV at the
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58 299 beginning of the migration experiment. Ten replicate analyses were performed for both
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4 300 film A (free alpha-tocopherol) and film B (complexed alpha-tocopherol). The recovery
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7 301 of the analyses, determined by subjecting solutions containing 2000 mg L⁻¹ alpha-
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10 302 tocopherol in methanol to the dissolving, filtering and evaporation procedure, was 80.6
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12 303 ± 4%. Alpha-tocopherol concentrations in film A and B found to be 1621±107 mg kg⁻¹
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14 304 and 1500± 322 mg kg⁻¹, respectively. It is assumed that a significant part of the
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16 305 originally added alpha-tocopherol (2000 mg kg⁻¹) was lost during manufacturing of the
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18 306 film, which was experienced also by other authors. For example Wessling et al. (2000b)
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20 307 found that approximately 66% of the originally added alpha-tocopherol was lost during
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22 308 LDPE film processing. Heirlings et al. (2004), however, found lower loss of alpha-
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24 309 tocopherol in LDPE films after processing but this was explained by the presence of
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26 310 other types of antioxidant in the polymer besides alpha-tocopherol. When analysing the
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28 311 initial alpha-tocopherol concentration in film B, rather high relative standard deviation
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30 312 (21.5%) was found. This was probably due to the inhomogeneous distribution of the
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32 313 complexes in the film, which was even visible. Because of the relatively polar nature of
33
34 314 the outer shell of cyclodextrin molecules, it is fairly difficult to distribute them in the
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36 315 apolar LDPE matrix. Further problems occurred due to the development of CD
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38 316 agglomerates, resulting in unfavourable optical characteristic of the film: visible white
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40 317 spots in the LDPE matrix.
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50 *Specific migration experiments*

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52 320 The migration of an additive from the packaging material into the food simulant can be
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54 321 followed by determination of its concentration either in the polymer or in the food
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56 322 simulant. This former method, however, suffers from a substantial disadvantage:
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58 323 namely, that the extraction of the analyte from the polymer matrix is often quite difficult
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4 324 and time-consuming. Therefore in the present study the migration process was followed
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7 325 by determining the amount of alpha-tocopherol migrated into the food simulant at 7.0
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9 326 ± 0.5 °C as a function of time. Progress of migration was expressed as the ratio of the
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11 327 migrated amount of alpha-tocopherol in a particular time and at equilibrium ($M_{F,t} /$
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14 328 $M_{F,\infty}$).

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19 330 The migration profiles of film A and B are compared in Figure 2. As it can be seen in
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21 331 the figure, a significant difference in the rate of migration was found between film A
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23 332 and film B. As alpha-tocopherol is fat soluble fast migration from LDPE into ethanol
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25 333 was expected. In case of film A containing alpha-tocopherol in free (non-complexed)
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27 334 form this assumption was verified. As can be seen in Figure 2, in about 400 hours
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29 335 approximately 85% of the total amount of alpha-tocopherol had migrated from film A to
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31 336 the fatty food simulant. In the first two weeks, migration showed an exponential
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33 337 increase and reached an equilibrium in the following weeks, similarly to previous
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35 338 experiments reported by Heirlings et al. (2004). Considering real food packaging
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37 339 applications, this practically means, that the high initial rate of release of antioxidant
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39 340 could inhibit the initiation step of oxidation taking place at the early stage of the
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41 341 storage. It can result in a high antioxidant concentration at the food surface and hence in
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43 342 increased diffusion rate from the surface into the bulk food due to the high
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45 343 concentration.

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51 345 Film B contained alpha-tocopherol complexed into beta-cyclodextrin with the aim to
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53 346 protect the antioxidant during film production on one hand, and to ensure slower, but
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55 347 sufficient release to the food simulant on the other hand. Migration profile of alpha-
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4 348 tocopherol released from film B into the food simulant is showed also in Figure 2. It can
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6 349 be concluded, that 85% of the total amount of alpha-tocopherol at equilibrium had
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9 350 migrated in 2324 hours from the polymer to the food simulant, while total migration
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11 351 was reached in about 3500 hours. This means that the migration rate of alpha-
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13 352 tocopherol from film B is significantly lower than that of film A.
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19 354 “[insert Figure 2 about here]”
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23 356 The initial amount of alpha-tocopherol in the bags can be calculated as follows. In the
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25 357 case of film A the initial concentration of AT was $1621 \pm 107 \text{ mg kg}^{-1}$, thus the migration
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27 358 bags weighing 1.2 g contained approximately 1.94 mg alpha-tocopherol. As the initial
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29 359 concentration in film B found to be $1500 \pm 322 \text{ mg kg}^{-1}$ and the bags weighed 1.0 g,
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31 360 therefore the initial amount of alpha-tocopherol was 1.50 mg.
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37 362 At equilibrium approximately 1.95 mg and 1.48 mg alpha-tocopherol had migrated into
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39 363 the food simulant from film A and film B, respectively. These amounts correspond to
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41 364 the total original alpha-tocopherol content of the bags, thus a total migration was
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43 365 observed for both films. It means that 95% ethanol proved to be a potent extractant and
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45 366 the partition between the polymers and the food simulant was neglectable. This finding
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47 367 is in accordance with the results of Wessling et al. (1998) and Heirlings et al. (2004) for
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49 368 free alpha-tocopherol. It can be also concluded that the partition coefficient of alpha-
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51 369 tocopherol between the polymer and food simulant was not influenced by the
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53 370 complexation.
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4 372 Reliable migration predictions require the description of the physical migration process
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7 373 by a correct mathematical equation, i.e. good agreement between predicted values and
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9 374 experimental data. The migration profiles of the two different films were fitted towards
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11 375 equation (2) to estimate the diffusion coefficient (D). It can be concluded that the model
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13 376 fitted the experimental values very well for both types of film. The sum of squares in
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15 377 errors (SSE) between the estimated and measured values was found to be as low as
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17 378 0.05. The calculated values for D were $1.53\text{E-}11 \text{ cm}^2 \text{ s}^{-1}$ and $1.68\text{E-}12 \text{ cm}^2 \text{ s}^{-1}$ for film A
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19 379 and B, respectively. There is no doubt that this difference is a consequence of the
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21 380 complexation of alpha-tocopherol in beta-cyclodextrin. It should be emphasised,
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23 381 however, that the calculated diffusion coefficient of alpha-tocopherol in film B is only
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25 382 an apparent value. In general the migration of a compound from a polymer into a food
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27 383 or simulant is of a very complex nature depending on several parameters, thus some
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29 384 simplifications are often made. Modelling this mass transfer process usually considers
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31 385 only a single phenomenon, generally the diffusion of the compound, which is then
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33 386 described by a single and constant diffusion coefficient. It is well known, however, that
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35 387 the presence of large amounts of other substances interferes with the diffusion process.
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37 388 Most of the models applied however do not take into account specific molecular
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39 389 interactions (e.g. association-dissociation processes in molecular complexes). In the
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41 390 present study it was expected that the molecular encapsulation in beta-cyclodextrin
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43 391 would also significantly influence the migration of alpha-tocopherol. The release
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45 392 kinetics of a complexed substance depends not merely on diffusion, but also on the ratio
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47 393 between its complexed and uncomplexed fractions, which is governed by the complex
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49 394 association/dissociation equilibrium characterized by the complex stability constant
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395 ($K_{1:1}$). This thermodynamic equilibrium for beta-cyclodextrin (CD) and alpha-
396 tocopherol (AT) can be described by the following equations.

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$$399 \quad K_{1:1} = \frac{[CD \cdot AT]}{[CD][AT]} \quad (4)$$

400

401 where $K_{1:1}$ represents the stability constant of the beta-cyclodextrin/alpha-tocopherol
402 complex, while $[CD]$, $[AT]$ and $[CD \cdot AT]$ correspond to the solubility of beta-
403 cyclodextrin, alpha-tocopherol and their complex, respectively. As beta-cyclodextrin is
404 insoluble in 95% ethanol it can be supposed that it remains in the polymer matrix.

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406 Regarding alpha-tocopherol a partition can be expected between its complexed and
407 uncomplexed form. However, as complexation is a dynamic and usually reversible
408 process, this partition could only be interpreted for a particular moment: both
409 association and dissociation of the complexes take place at the same time. It is
410 presumable that only molecules being uncomplexed can participate in the migration
411 process. The amount of the uncomplexed alpha-tocopherol at a particular moment
412 depends on the equilibrium described above. Most of the studies of cyclodextrin
413 complex stability have been carried out in aqueous solutions and only a few workers
414 have investigated complex formation in organic solvents (Connors 1997). In general,
415 the decrease of the complex stability is assumed in function of increasing apolar nature
416 of the solvent. This can be explained by the fact, that apolar organic solvents decrease
417 the hydrophobic driving force: namely, apolar guests prefer solvents to semi-apolar

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4 418 cyclodextrin cavity. Moreover, molecules of the organic solvents can function as
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7 419 competitive guest molecules, thus displacing the primary guests.
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10 11 421 **Conclusions**

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14 422 Complexation by beta-cyclodextrin was found to be an effective tool for controlling the
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16 423 release of alpha-tocopherol from antioxidative active packaging. Complexation had a
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18 424 significant influence on the migration profile and release rate. Migration of complexed
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20 425 antioxidant was rather slow compared to that of free alpha-tocopherol. Depletion of the
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22 426 antioxidant was completed in about 1000 hours compared to about 3500 hours in case
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25 427 of free and complexed alpha-tocopherol, respectively. The slower release rate was
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27 428 proved also by the significant difference between the diffusion coefficients determined
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29 429 by using a mathematical model to describe the release rate numerically. The migration
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31 430 model fitted the experimental values very well. Rather small sum of squares in errors
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33 431 between the estimated and measured values was found. The diffusion coefficient for the
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35 432 complexed alpha-tocopherol was one order of magnitude lower than for the
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37 433 uncomplexed additive. Therefore, it can be stated that a controlled release of alpha-
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39 434 tocopherol from LDPE packaging films was achieved by inclusion complexation with
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41 435 beta-cyclodextrin.
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51
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53
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55
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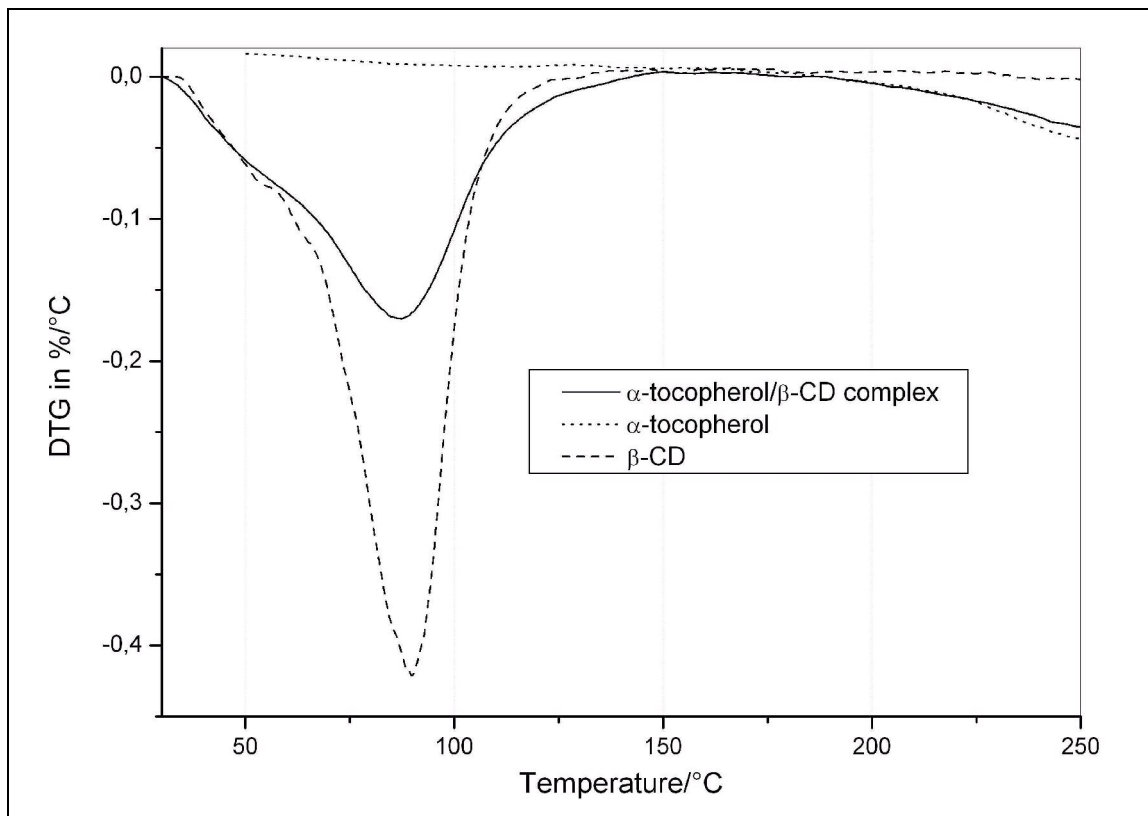


Figure 1

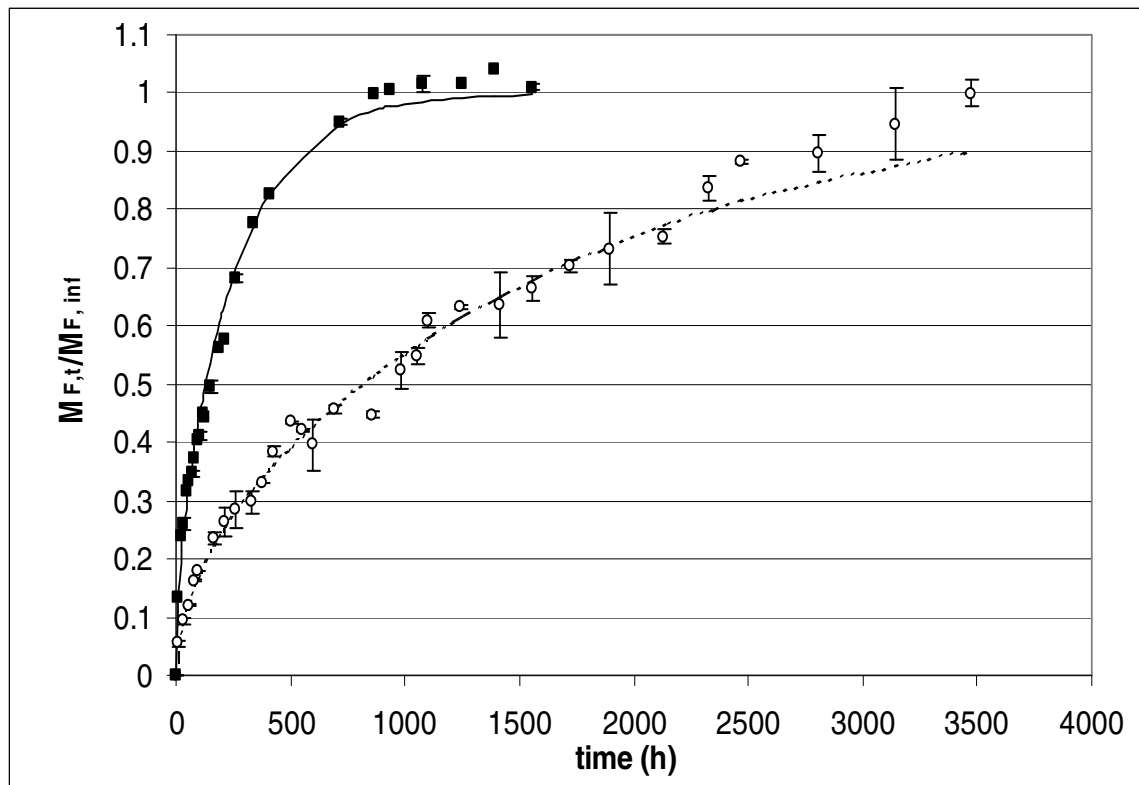


Figure 2

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3 **Figure 1** Differential Thermo-gravimetric curve (DTG) of free alpha-tocopherol
4 (dotted line), beta-cyclodextrin (dashed line) and alpha-tocopherol/beta-cyclodextrin
5 (solid line) complex
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12 **Figure 2** Migration of alpha-tocopherol from Film A (■) and Film B (○) into food
13 fatty food simulant at $7.0 \pm 0.5^\circ\text{C}$. Data expressed as the mean of three measurements
14 and error bars shows the standard deviations. Solid line (Film A) and dashed line
15 (Film B) represent the estimated migration of alpha-tocopherol based on a migration
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24 (**Film A:** LDPE 2000 mg kg⁻¹ free alpha-tocopherol; **Film B:** LDPE 2000 mg kg⁻¹
25 alpha-tocopherol complexed in beta-cyclodextrin)
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