POST-PRINT VERSION

Pubblicato da ELSEVIER
https://www.sciencedirect.com/science/article/pii/S0308814615303514?via%3Dihub

https://doi.org/10.1016/j.foodchem.2015.12.073

Food Chemistry, Volume 199, 15 May 2016, Pages 684-693

α-Cyclodextrin encapsulation of supercritical CO2 extracted oleoresins from different plant matrices: A stability study

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Abstract

Here we describe the encapsulation in α-cyclodextrins (α-CDs) of wheat bran, pumpkin and tomato oleoresins, extracted by supercritical carbon dioxide, to obtain freeze-dried powders useful as ready-to-mix ingredients for novel functional food formulation. The stability of tocochromanols, carotenoids and fatty acids in the oleoresin/α-CD complexes, compared to the corresponding free oleoresins, was also monitored over time in different combinations of storage conditions. Regardless of light, storage at 25 °C of free oleoresins determined a rapid decrease in carotenoids, tocochromanols and PUFAs. α-CD encapsulation improved the stability of most bioactive compounds. Storage at 4 °C synergized with encapsulation in preventing degradation of bioactives. Unlike all other antioxidants, lycopene in tomato oleoresin/α-CD complex resulted to be more susceptible to oxidation than in free oleoresin, likely due to its selective sequestration from the interaction with other lipophilic molecules of the oleoresin.

Keywords

Carotenoids Cucurbita moschata (Duch.) Fatty acids Supercritical fluid extraction Tocochromanols Triticum durum (Desf.) Solanum lycopersicum (L.) Differential

scanning calorimetry Fourier transform infrared spectroscopy – attenuated total reflectance

1. Introduction

Supercritical CO2 (SC-CO2) technology has been widely used to extract food-grade oleoresins from a range of edible plant materials, including tomato berries (Lenucci et al., 2009, Lenucci et al., 2015), pumpkin (Durante et al., 2014, Shi et al., 2013) and watermelon peponides (Katherine, Edgar, Jerry, Luke, & Julie, 2008), sweet potato tubers (Spanos, Chen, & Schwartz, 1983), as well as wheat bran (Durante, Lenucci, Rescio, Mita, & Caretto, 2012). The obtained oleoresins are a rich source of bioactive molecules (mainly polyunsaturated fatty acids, tocochromanols and carotenoids) and are potentially useful as high-quality additives in the preparation of innovative cosmeceuticals, nutraceuticals and pharmaceuticals.

Tocochromanols (also known as Vitamin E) and carotenoids are fat-soluble micronutrients thought to exert beneficial effects on human health and well-being, including protection against cancer, cardiovascular diseases and age-related degenerative pathologies of many organs (Reboul et al., 2006). Tocochromanols comprise two homologous series: tocopherols, with a saturated side chain, and tocotrienols with an unsaturated side chain, further distinguished as α-, β-, δ- and γ-forms based on the chromanol head group methylation (Colombo, 2010). Carotenoids are divided in two major groups: carotenes and xanthophylls. Carotenes, such as lycopene, α-carotene and β-carotene, are hydrocarbons that are either linear or cyclized at one or both ends of the molecule. Xanthophylls, such as lutein and zeaxanthin, are the oxygenated derivatives of carotenes (Rodriguez-Amaya, 2001).

Fatty acids can be classified in saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA); some are essential for humans and must be acquired by the diet (Ristic & Ristic, 2003).

Vitamin E and carotenoids are very reactive to light, oxygen, and free radical species, whereas fatty acids are susceptible to autoxidation, enzymatic or photosensitized oxidations; this greatly limits their stability and reduce their shelf-life and health benefits (Gul et al., 2015, Hincha, 2008).

Encapsulation into micro or nano particles is the most promising technique to increase the stability of such bioactives and allows their preservation over time. Cyclodextrins (CDs) are receiving a lot of interest for their biocompatibility, non-toxicity, biodegradability and ability to form inclusion complexes with several compounds (Hedges, 1998). Complexes can be formed either in solution or in the crystalline state and water is typically the solvent of choice (Martina & Cravotto, 2015).

CDs are cyclic oligosaccharides deriving from the enzymatic conversion of starch. They consist of six (α-CDs), seven (β-CDs) and eight (γ-CDs) α-(1,4)-glucopyranose residues linked together in a toroid shaped ring forming an hydrophilic outer surface and a relatively hydrophobic central cavity (Brewster & Loftsson, 2007). CDs have different ability to form inclusion complexes with specific guest compounds depending on the relative size of CDs to the size of the guest molecule or for the presence of certain key functional groups within the guest molecule, and on the

thermodynamic interactions between cyclodextrin, guest and solvent (Del Valle, 2004, Szejtli, 1998).

The most common applications of CDs are to improve the solubility of lipophilic guest molecule; to stabilize the guest against the adverse effects of physic-chemical agents (such as oxygen, pH, light and heat); to enhance its shelf-life and reduce the concentrations of the agent required to achieve biological effect (Fang & Bhandari, 2010).

While the side rim depth is the same (~7.9 Å) for α-, β- and γ-CDs, the diameter of the internal cavity varies between 4.7–5.3 Å of α-CDs and 7.5–8.5 Å of γ-CDs (Li et al., 2007). Water solubility is also strongly affected by the number of glycosyl residues: at 25 °C α-CDs are about eight times more and 1.6 time less soluble than of β- and γ-CDs, respectively (Li et al., 2007). α-CDs also show high resistance to enzymatic hydrolysis by human salivary and pancreatic amylases, so they have special applications in food industry, especially as a natural, soluble dietary fiber, compared to β- and γ-CDs (Kondo, Nakatani, & Hiromi, 1990).

The regulatory status of CDs in food differs among countries. In USA, α, β- and γ-CDs are included in the Food and Drug Administration GRAS (Generally Recognised As Safe) list, in Japan CDs are recognized as natural products and their commercialization for food usage is only restricted by purity considerations (Li, Chen, Gu, Chen, & Wu, 2014). α-CDs are approved in Europe as soluble dietary fiber and novel food ingredient (OJ L 146/12, 2008). The Joint FAO/WHO Expert Committee on Food Additives recommends a maximum level of 5 mg/kg per day of β-CDs in foods, while for α- and γ-CDs the acceptable daily intake has not been defined because of their innocuous profiles (Astray, Gonzalez-Barreiro, Mejuto, Rial-Otero, & Simal-Gándara, 2009). This information has led to an increase in application of α-CDs in the food industries. In the latter case α-CDs are mainly used to increase the soluble dietary fiber content of food.

CDs have been applied to encapsulate a variety of bioactive components, including antimicrobials, fish oil, essential oil, flavors and antioxidants. In particular, associations of CDs with vitamin E, lutein, β-carotene and lycopene have already been published (Basu and Del Vecchio, 2001, Mele et al., 1998, Mele et al., 2002).

The purpose of this study is to evaluate the stability of bioactive compounds of tomato (TO), pumpkin (PO) and wheat bran (WBO) oleoresins, obtained by SC-CO2, encapsulated by α-CDs and stored for up to 180 days under different storage conditions. The limited stability of these oleoresins over time and/or during food processing is, in fact, the main drawback for their usage in the formulation of functional products. This is the first report on the encapsulation into α-CDs of these SC-CO2 extracted oleoresins.

2. Materials and methods

2.1. Chemicals

Tocopherols (α-, β- and γ-forms), myristic, palmitic, stearic, arachidic, palmitoleic, oleic, linoleic and linolenic acids used as standards, as well as all HPLC grade solvents were purchased from Sigma–Aldrich (Milan, Italy). Tocotrienol (α-, β- and γ-forms) and carotenoid (lutein, α-carotene, β-carotene, zeaxanthin and all-trans-

lycopene) standards were purchased from Cayman chemicals (Ann Arbor, MI, USA) and CaroteNature (Lupsingen, Switzerland), respectively. CAVAMAX® w6 food (α-CDs) were kindly provided by IMCD Italy SpA (Milano, Italy). High purity carbon dioxide (99.995%) for supercritical fluid extraction was purchased from Mocavero Ossigeno (Lecce, Italy).

2.2. Plant material and preparation of SC-CO2 suitable matrices

Open field grown red-ripe tomato (Solanum lycopersicum L.) berries of the high lycopene HLY 18 cultivar were processed in a dehydrated matrix suitable for SC-CO2 extraction as described by Lenucci et al. (2010). Tomatoes were blanched in water at 70 °C for 5 min, crushed and sieved by a Reber 9004 N tomato squeezer (Reber, Luzzara, Italy) to obtain a tomato purée free from skins, seeds and vascular tissues. The purée was centrifuged at 27000g for 10 min to remove water soluble substances. The pellet was dehydrated to constant weight by a Christ ALPHA 2–4 LSC freeze-dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) at a vacuum pressure of 0.0014 mbar. Simultaneously, the tomato seeds were recovered and vacuum-dried at 60 °C by a Salvis Lab IC40 vacuum-drying oven (Bio Instruments S.r.l., Firenze, Italy). Freeze-dried tomato pellet and dehydrated seeds were ground in a laboratory ultra-centrifugal mill (ZM200, Retsch GmbH, Haan, Germany) through 35 mesh (500 μm) and 18 mesh (1.0 mm) sieves, respectively and blended in a ratio of 1:1 by weight to obtain the tomato matrix used for SC-CO2 tomato oleoresin extraction.

The dehydrated pumpkin (Cucurbita moschata Duch.) matrix was prepared as previously reported (Durante, Lenucci, D'Amico, Piro, & Mita, 2014) from ripe open field grown peponides of the cultivar Long of Naples. Briefly, the peeled pumpkin flesh was chopped into small pieces and dehydrated by a Salvis Lab IC40 vacuum-drying oven (Bio Instruments S.r.l., Firenze, Italy) for two hours at 80 °C and to constant weight at 60 °C. Simultaneously, the seeds were recovered and vacuum-dried at 60 °C. Dried pumpkin flesh and seeds were ground in a laboratory ultra-centrifugal mill (ZM200, Retsch GmbH, Haan, Germany) through 70 mesh (210 μm) or 35 mesh (500 μm) sieves, respectively and blended in a ratio of 1:1 by weight.

Durum wheat (Triticum durum, Mill.) bran was provided by the Tomasello SpA. milling industry (Casteldaccia, PA, Italy) and processed into a matrix accordingly to Durante, Lenucci, Laddomada, Mita, & Caretto (2012). Briefly, wheat bran was oven dehydrated at 60 °C to a residual moisture content of 3%. The dehydrated material (with an average granulometry of ~600 μm) was directly used for SC-CO2 oil extraction.

All matrices were vacuum-packaged in 90 μm food grade Polyethylene/Polyamide (20/70) plastic bags (O2 permeability = 80 cm3/m2 24 h bar, at 23 °C) and stored in a freezer at −20 °C until SC-CO2 extraction.

2.3. SC-CO2 extraction of oleoresins

Aliquots (25 g each) of tomato, pumpkin and wheat bran matrices were packed into a 25 mL stainless-steel extraction vessel (ø = 1 cm2; h = 25 cm) and dynamically extracted by a Spe-ed SFE system (Applied Separations, Allentown, PA, USA) for 3 h, by flowing CO2 at a rate of ∼0.5 kg/h. The other operative parameters were: pressure = 503 bar, temperature = 86 °C for tomato oleoresin extraction, and pressure = 350 bar, temperature = 60 °C for both pumpkin and wheat oleoresin extraction.

2.4. Oleoresin encapsulation into α-CDs

Oleoresins were encapsulated in α-CDs as described by Basu and Del Vecchio (2001) with some modification. α-CDs (20 g) were dissolved in 205 g distilled water at 25 °C. Each oleoresin (∼10 g) was slowly added to the solution under continuous stirring and nitrogen sparging. Stirring was continued for 24 h under nitrogen. The samples were left to decant for 2 h to exclude oil/water phase separation. Each suspension was freeze dried to constant weight to obtain a powder (∼99% yield), thereafter called oleoresin/α-CD complex.

2.5. DSC and FTIR-ATR analyses

The DSC (differential scanning calorimetry) thermograms of α-CDs, free oleoresins and oleoresin/α-CD complexes were acquired by a calorimeter (DSC-6, Perkin Elmer, Boston, MA, USA) previously calibrated with indium metal. Each sample (2–4 mg), as described by Teixeira, Ozdemir, Hill, and Gomes (2013), was heated in a flat-bottomed-aluminum pan with a hole on the lid, at a constant scanning rate of 10 °C/min between 30 and 350 °C, under constant oxygen flow. An empty aluminum pan was used as reference. Duplicate determinations were carried out for each sample.

The FTIR-ATR (Fourier transform infrared spectroscopy – attenuated total reflectance) spectra of α-CDs, free oleoresins and oleoresin/α-CD complexes were collected between 4000 and 600 cm−1 (32 scans at a resolution of 4 cm−1), using a FT-IR spectrophotometer (670 FT-IR, Agilent, Santa Clara, CA, USA), according to Rizzi et al., 2014, Rizzi et al., 2015. Aliquots of the free oleoresins (50 μL) or oleoresin/α-CD complexes (2 mg) were deposited on the surface of the ATR device and directly subjected to spectra acquisition. Duplicate determinations were carried out for each sample.

2.6. SEM of oleoresin/α-CD complexes

The oleoresin/α-CD complexes were further critical-point-dried (K850 Critical Point Drier, Quorum Technologied LTD, Ashford, UK) using liquid CO2 and mounted on carbon adhesive stubs. Microstructure observation were carried out by SEM (scanning electron microscopy) using a ZEISS EVO HD 15 (Carl Zeiss Microscopy GmbH, Oberkochen, Germany) operated under high-vacuum at an accelerating voltage of 10 kV, at a magnification of 150×.

2.7. Evaluation of oleoresin/α-CD emulsion stability

Emulsions were prepared by suspending 10 mg of each freeze-dried oleoresin/α-CD complex in 5 mL distilled water and vigorously stirring overnight (300 rpm). The stability of the suspension against coalescence was visually assayed by monitoring the separation of the emulsion layer from the clear aqueous phase as a function of time starting from the time the suspension is left to settle.

2.8. Oleoresin/α-CD complex storage

Aliquots (0.5 g) of each oleoresin/α-CD complex were placed in 10 mL clear glass vials (23 × 46 mm), sealed with a PTFE/Silicone septa silver aluminum crimp cap (the headspace above the samples consisting of the entrapped air) and stored in different conditions of temperature (4 °C or 25 °C) and light (presence/absence). The samples exposed to light were placed 100 cm under a fluorescent light (TL-D 90 De Luxe 36W, Philips) with a radiation at sample level of 891 lux at 25 °C, while those maintained in absence of light were wrapped in aluminium foil and incubated at 4 °C or 25 °C. At predetermined time points (every ten days up to a maximum of 120 days), from each aliquot kept in the different storage conditions, oleoresin was eluted and assayed for isoprenoids (tocochromanols and carotenoids) and fatty acids as described below. Simultaneously, aliquots (0.5 g) of each SC-CO2 extracted oleoresin (free oleoresin), subjected to the same storage conditions of the oleoresin/α-CD complexes, were monitored for isoprenoid and fatty acid contents.

2.9. Elution of the oleoresins from the α-CDs

Oleoresin elution from the complex with α-CDs was carried out as described by Basu and Del Vecchio (2001). Aliquots (0.5 g) of each oleoresin/α-CDs complex (from the different storage time points) were suspended in distilled water (25 mL), heated at 60 °C and stirred, under simultaneous nitrogen sparging, for 1 h. After adding 12.5 mL hexane, the mixture was vigorously stirred (750 rpm) for 5 min. Further 37.5 mL hexane was added followed by 5 min stirring. The organic phase was separated by centrifugation (5000g, 10 min). The aqueous phase was re-extracted with 37.5 mL hexane until all pigments were removed (at least two-times). The organic phase was collected and dried under nitrogen. The obtained oleoresin was assayed for isoprenoid and fatty acids content as described below. The amount of oil present in the complexes was determined gravimetrically. Three aliquots (1.0 g) of each freshly prepared oleoresin/α-CDs complex were extensively extracted as previously described till complete discoloration of α-CDs (3–5 extractions). The organic phases were collected, evaporated and accurately weighted. The percentage of oil was calculates as the measured oil weight/oleoresin/α-CDs complex weight × 100.

2.10. HPLC analysis of isoprenoids

Triplicate aliquots (0.1 g) from free and eluted oleoresins were dissolved in 1 mL of ethyl acetate, filtered through a 0.45 μm syringe filter (Millipore Corporation, Billerica, MA, USA) and immediately analyzed by HPLC. Quali-quantitative analyses of tocopherols and carotenoids were carried out by the method of Fraser, Pinto, Holloway, and Bramley (2000), slightly modified, using an Agilent 1100 Series HPLC system equipped with a reverse-phase C30 column (5 μm, 250 × 4.6

mm) (YMC Inc., Wilmington, NC, USA). To record HPLC runs the Agilent ChemStation software was used. The mobile phases were: methanol (A), 0.2% ammonium acetate aqueous solution/methanol (20/80, v/v) (B) and tert-methyl butyl ether (C). The gradient elution was as follows: 0 min, 95% A and 5% B; 0–12 min, 80% A, 5% B and 15% C; 12–42 min, 30% A, 5% B and 65% C; 42–60 min, 30% A, 5% B and 65% C; 60–62 min, 95% A, and 5% B. The column was re-equilibrated for 10 min between runs. The flow rate was 1.0 mL/min and the column temperature was maintained at 25 °C. The injection volume was 10 μL. Absorbance was registered by diode array at wavelengths of 475 nm for carotenoids and 290 nm for tocopherols. Peaks were identified by comparing their retention times and UV–Vis spectra to those of authentic isoprenoid standards. The limit of detection was 0.4 mAU, typically in the 2 ± 10 ng range per compound.

2.11. Fatty acid analysis by GC–MS

Fatty acid derivatization was carried out according to the German Society for Fat Research (DGF – C-VI 11a) method (Lange, 2000). A methanolic solution (3 mL) of 0.5 M NaOH was added to 0.1 g of oleoresin (free or eluted). The mixture was incubated at 100 °C for 5 min in a water bath to dissolve lipids. After cooling at room temperature, 2.0 mL of boron trifluoride in methanol (12% w/v) were added and the sample incubated at 100 °C for 30 min in a water bath and then rapidly cooled in an ice bath before the addition of 1 mL of hexane for extraction. The sample was vigorously stirred for 30 s before the addition of 1 mL of a 0.6% w/v sodium chloride solution. The esterified sample was placed in a refrigerator for better phase separation. After collecting the supernatant 1.0 mL of hexane was added and sample stirred. The supernatant was collected and added to the previous fraction. The sample was concentrated to a final volume of 1.0 mL for GC–MS analysis.

The analyses were performed on an Agilent 5977E GC/MS system. Compounds were separated on DB-WAX column (60 m, 0.25 mm i.d., 0.25 mm film thickness, Agilent).The GC parameters were as follows: the temperature of the column was 50 °C after injection for 1 min, then programmed at 25 °C/min to 200 °C, at 3 °C/min to 230 °C and maintained at constant temperature of 230 °C for 23 min. Split injection was conducted with a split ratio of 5:1, the flow-rate was 1.0 mL/min, carrier gas used was 99.999% pure helium, the injector temperature was 250 °C and the column head pressure was 40 psi for 0.4 min, constant pressure at 20 psi. The MS detection conditions were as follows: transfer line temperature 250 °C, mode Scan, source and quadrupole temperature 230 °C and 150 °C respectively, scanning method of acquisition, ranging from 46 to 500, for mass/charge (m/z) was optimized. Spectrum data were collected at 0.5 s intervals. Solvent cut time was set at 2 min and 40 min retention time sufficient for separating all the fatty acids. Compounds were identified by using online NIST-library spectra and published MS data. Moreover, fatty acids standard were used to confirm MS data.

2.12. Calculation of degradation rate constants

The data were best fit by a first-order kinetic model, $\ln C = \ln C0 - k(t)$, with C: concentration (μg/g) of each analyte in free and α-CD encapsulated oleoresins at storage time t; C0: concentration (μg/g) of each analyte at initial time (before storage); t: storage time t (day), k: degradation rate constant (day−1). Degradation rate constants (k) were obtained from the slope of a plot of the natural logarithm of the average percentage retention values (C/C0 × 100) from the three free or encapsulated oleoresin stored at 4 °C vs. time. The linear regression (95% confidence limit) was used to determine the standard error of degradation rate constants.

2.13. Statistical analysis

Results are presented as the mean value ± standard deviation of three independent replicate experiments (n = 3). Statistical analysis was based on a one-way ANOVA test. Tukey's post hoc method was applied to establish significant differences between means ($p < 0.05$). All statistical comparisons were performed using SigmaStat version 11.0 software (Systat Software Inc., Chicago, IL).

3. Results and discussion

3.1. Biochemical composition of SC-CO2 extracted oleoresins from wheat bran, pumpkin and tomato matrices

Three oleoresins, differing in the relative abundance of acyclic carotenoids (lycopene), cyclic carotenes (α- and β-carotene) and xanthophylls (lutein and zeaxanthin), were extracted by SC-CO2 from dehydrated tomato, pumpkin and wheat bran matrices, respectively. The amount of carotenoids and tocochromanols (tocopherols and tocotrienols) of each oleoresin, as well as their fatty acid distribution profiles are reported in Table 1. Both pumpkin and tomato oleoresins (PO and TO, respectively) showed a high amount of total carotenoids (463.1 ± 16.5 and 446.2 ± 14.0 mg/100 g, respectively), unlike wheat brain oleoresin (WBO) in which total carotenoids were very low (2.5 ± 0.1 mg/100 g). As expected, lycopene (338.1 ± 9.7 mg/100 g), α- (233.2 ± 8.5 mg/100 g) and β-carotene (224.3 ± 7.6 mg/100 g), and lutein (1.8 ± 0.1 mg/100 g) were, respectively, the most abundant carotenoids in TO, PO and WBO accounting for more than 76%, 99% and 72% of total carotenoids, in agreement with our previous reports (Durante et al., 2012, Durante et al., 2014, Lenucci et al., 2009). All the oleoresins contained significant amounts of other carotenoids: β-carotene (71.3 ± 2.1 mg/100 g), α-carotene (27.6 ± 1.1 mg/100 g) and lutein (9.2 ± 1.1 mg/100 g) were detected in TO; lutein (5.6 ± 0.4 mg/100 g) was found in PO, and the presence of low quantities of β-carotene (0.26 ± 0.01 mg/100 g), α-carotene (0.25 ± 0.01 mg/100 g) and zeaxanthin (0.18 ± 0.02 mg/100 g) was established in WBO. Besides, TO ranked first for total tocochromanol content (1048.5 ± 16.0 mg/100 g), followed by PO (465.4 ± 21.2 mg/100 g) and WBO (221.9 ± 2.0 mg/100 g). The quali-quantitative characterization of tocochromanols evidenced a remarkable difference among oleoresins. In PO and WBO, tocopherols and tocotrienols were both detected, with the latter generally 50% more abundant, while TO was characterized by the exclusive presence of tocopherols. Among tocotrienols the β,γ-forms (which, in our system, co-migrated as a single peak) were more abundant (273.1 ± 8.6 mg/100 g and 98.1 ± 1.2 in PO and WBO respectively)

than α-tocotrienol (5.2 ± 0.5 mg/100 g and 35.9 ± 0.2 mg/100 g). Differences in the relative amounts of tocopherol forms were also detected between the oleoresins, with TO particularly rich in α-tocopherol (917.4 ± 8.5 mg/100 g), the most biologically active form of vitamin E.

Table 1. Carotenoid, tocochromanolA, fatty acid composition and molecular weight of tomato (TO), pumpkin (PO) and wheat bran (WBO) SC-CO2 extracted oleoresins.

	TO	PO	WBO
Carotenoids (mg/100 g oleoresin)			
Lutein	9.2 ± 1.1[a]	5.6 ± 0.4[b]	1.8 ± 0.1[c]
α-Carotene	27.6 ± 1.1[b]	233.2 ± 8.5[a]	0.25 ± 0.01[c]
β-Carotene	71.3 ± 2.1[b]	224.3 ± 7.6[a]	0.26 ± 0.01[c]
Zeaxanthin	ND	ND	0.18 ± 0.02
All-*trans*-lycopene	338.1 ± 9.7	ND	ND
Total	446.2 ± 14.0[a]	463.1 ± 16.5[a]	2.5 ± 0.1[b]
Tocochromanols (mg/100 g oleoresin)			
α-T	917.4 ± 8.5[a]	55.3 ± 2.6[b]	56.8 ± 0.4[b]
β,γ-T	131.1 ± 7.5[a]	131.8 ± 9.5[a]	30.1 ± 0.2[b]
α-T3	ND	5.2 ± 0.5[b]	35.9 ± 0.2[a]
β,γ-T3	ND	273.1 ± 8.6[a]	98.1 ± 1.2[b]
Total	1048.5 ± 16.0[a]	465.4 ± 21.2[b]	220.9 ± 2.0[c]
Fatty acid composition (%)			
Myristic (C14:0)	0.64 ± 0.02	ND	ND
Palmitic (C16:0)	19.1 ± 1.7[a]	17.0 ± 0.9[a]	20.6 ± 1.9[a]
Stearic (C18:0)	7.9 ± 1.2[a]	8.8 ± 1.2[a]	1.2 ± 0.1[b]
Arachidic (C20:0)	0.77 ± 0.08[b]	ND	0.99 ± 0.07[a]
Palmitoleic (C16:1)	0.69 ± 0.02	ND	ND
Oleic (C18:1 $n-9$)	20.4 ± 1.1[a]	19.1 ± 3.4[a]	20.6 ± 1.9[a]
Linoleic (C18:2 $n-6$)	45.8 ± 3.2[a]	45.3 ± 3.8[a]	51.3 ± 4.2[a]
Linolenic (C18:3 $n-3$)	4.7 ± 1.3[b]	8.9 ± 2.2[a]	5.3 ± 1.2[a]
SFA	28.4 ± 3.0[a]	26.7 ± 2.1[a]	22.8 ± 2.07[a]
MFA	21.1 ± 1.1[a]	19.1 ± 3.4[a]	20.6 ± 1.9[a]
PUFA	50.4 ± 4.5[a]	54.2 ± 6.0[a]	56.6 ± 5.4[a]
PUFA/SFA	*1.8*	*2.0*	*2.5*
$n-6/n-3$	*9.7*	*5.1*	*9.7*
Approximately molecular weight (g/mol)	865.3	867.1	865.2

ND, not detected.

Data are the mean ± standard deviation of three independent replicates (n = 3). Data were submitted to one-way analysis of variance (ANOVA), Tukey's post hoc method was applied to establish significant differences among the three oleoresins ($p < 0.05$). Different letters indicate significant differences between oleoresins.

A Tocochromanols' abbreviations are as follows: α-T3, α-tocotrienol; β,γ-T3, β,γ-tocotrienol; α-T, α-tocopherol; β,γ-T, β,γ-tocopherol. Italic formatting identifies dimensionless ratios.

The fatty acid profiles of TO, PO and WBO revealed significant qualitative and quantitative differences (Table 1). In general, myristic, palmitic, stearic and arachidic (SFA), palmitoleic and oleic (MUFA), as well as linoleic and linolenic (PUFA) were detected in all oleoresins, with some minor exceptions. In TO, PO and WBO, linoleic acid was the most abundant, contributing for 45.8–51.3% to the total, followed by oleic (19.1–20.6%) and palmitic (17.9–20.6%) acids. Among all assayed oleoresins, WBO was characterized by the highest PUFA (56.6%) and MUFA (20.6%) percentages. The ratio of PUFA to SFA (PUFA/SFA) and of omega-6 to omega-3 fatty acids (n − 6/n − 3) were also calculated, being one of the most important parameters currently used to assess the nutritional quality of the lipid fraction of foods. WHO/FAO guidelines for a "balanced diet" suggest a PUFA/SFA above 0.4–0.5. PUFA/SFA ratios for TO, PO and WBO largely exceeded the suggested values setting on 1.8, 2.0 and 2.5, respectively. An n − 6/n − 3 ratio from 4 to 10 has been recommended in various countries (FAO, 1994). All SC-CO2 extracted oleoresins fell in this range confirming their high nutritional value.

An approximate estimation of the molecular weight of the three oleoresins was carried out using the on line tool "Triglyceride Molecular weight calculator" (http://www.webpages.uidaho.edu/~devs/Research/Calculators/Molecularweight_calculator.html) on the base of their fatty acid profiles (% weight). The obtained values are reported in Table 1. Since the differences between the values resulted to be very low, we used the average molecular weight 866.0 g/mol to calculate of oleoresin/CDs molar ratio for all samples.

3.2. Macro- and micro-scopic characteristics of α-CDs encapsulated oleoresins

TO, PO and WBO were encapsulated into α-CDs in a molar ratio (mol oleoresin/mol α-CDs) of approximately 0.56:1 (corresponding to 0.5:1 on a weight basis). This was the highest ratio ensuring no segregation of the oleoresin during complex formation in all samples among the three molar ratios (0.28:1, 0.56:1 and 1.12:1) tested in preliminary experiments (data not shown). The aqueous suspensions were freeze-dried, resulting into fluffy colored powdery oleoresin/α-CD complexes, with very little visible clumps (Supplementary Fig. 1). Accordingly, Basu and Del Vecchio (2001) reported that α-CDs formed powders with high carotenoid canola oil (HCCO) when mixed in equimolar or 0.5:1 (HCCO/α-CDs) molar ratio, while the use of β-CDs in a molar ratio of 1:1 determined the formation of clumpy complexes. The percentage of oil present in the complexes was 22 ± 6% with no significant differences between the different encapsulated oleoresins ($p = 0.686$; $F = 0.380$), close to the value (∼29%) reported by Basu and Del Vecchio (2001) for a molar ratio of HCCO and CDs of 0.5:1.

Clear differences in the structure of the complexes were evidenced by SEM (Fig. 1). The particles obtained by freeze-drying an aqueous solution of α-CDs showed a crystalline polygonal shape with rough surface and no apparent cracks or porosity. Their size was not uniform but generally did not exceed 100 μm (Fig. 1A). Similarly, the size of the freeze-dried WBO/α-CD complexes was small and not uniform, but the shape of the particles was irregular with coarse, clumpy surfaces (Fig. 1B). Both, the freeze-dried PO/α-CD and TO/α-CD complexes (Fig. 1C and D) showed irregular structures forming large amorphous aggregates of variable shape and size. The surface of PO/α-CD complexes appeared rather smooth but streaked with grooves, while that of TO/α-CD complexes was rough and compact. Similar structures were observed by Nunes and Mercadante (2007) for freeze-dried lycopene/β-CD complex.

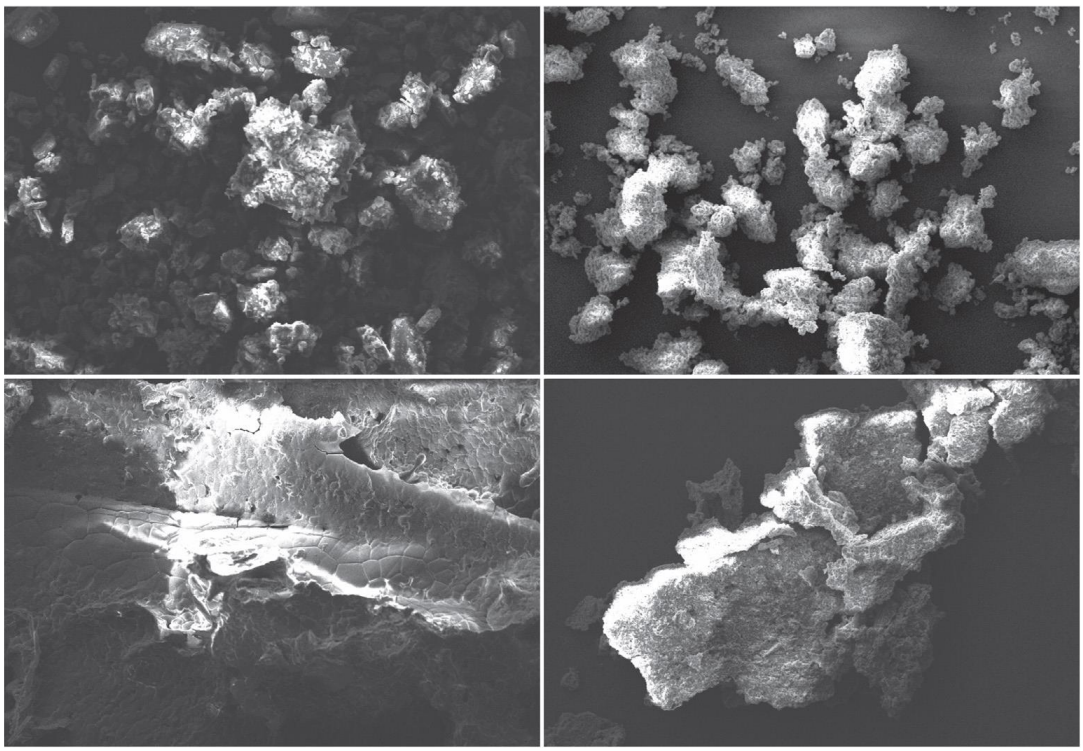


Fig. 1. Scanning electron micrographs of the pure freeze-dried α-CDs (A) and of the freeze-dried complexes WBO/α-CDs (B), PO/α-CDs (C) and TO/α-CDs (D). Accelerating voltage 10 kV, magnification 150×.

All the freeze-dried oleoresin/α-CD complexes were insoluble in distilled water, but, at a concentration of 2 mg/mL, formed a turbid emulsion stable between 6 and 12 h from preparation depending from oleoresin typology (Supplementary Fig. 2). Afterward, the suspensions tend to separate forming a microcrystalline colored sediment at the bottom, a clear region in the middle and a concentrated emulsion at the top. A similar behavior was described by Mathapa and Paunov (2013) for α- and β-CD-stabilized emulsion with several oils including n-tetradecane, tricaprylin, silicone oil, isopropyl myristate and sunflower oil. The suspended particles

obstructed 2.7 μm filters (GA/D Whatman International Ltd, England) but passed through filters of 22 μm (mirachloth, Calbiochem, San Diego, CA).

3.3. Study of complex formation by DSC and FTIR-ATR

The thermal curves of α-CDs, free oleoresins and oleoresin/α-CD complexes are reported in Supplementary Fig. 3. The thermal oxidative degradation processes of α-CDs starts at temperature higher than 250 °C. All free oleoresins showed a broad exothermic peak associated to oxidation of their different constituents. Similar thermal oxidation profiles have been reported for other vegetable oils (Wang, Cao, Sun, & Wang, 2011). The onset temperatures of this peak was 153.9 °C for WBO, 195.5 °C for PO and 176.0 °C for TO, respectively. Different thermograms were obtained for oleoresin/α-CD complexes; the enveloped broad exothermic peak disappeared and was replaced by two, well defined, exothermic peaks. The onset temperature of the first peak was higher than that of the corresponding free oleoresin (166.8, 201.2 and 192.2 °C, for WBO, PO and TO/α-CD complexes, respectively); besides, a general decrease of peak intensities was observed, especially in the complexes formed with TO and PO oleoresins. These evidences indicate that α-CDs have a general protective effect on all oleoresins and offer an indirect proof of oil encapsulation as suggested by Karathanos, Mourtzinos, Yannakopoulou, and Andrikopoulos (2007) and Teixeira et al. (2013).

To further confirm oleoresins encapsulation into α-CDs, FTIR-ATR analyses were performed. The spectra of each oleoresin and α-CDs are reported in Supplementary Fig. 4. In accordance with Table 1, the typical bands of carotenoids, tocochromanols and fatty acids (Schulz & Baranska, 2007) were detected in all oleoresins, with little differences from a qualitative point of view (see caption of Supplementary Fig. 4 for peak details). FTIR-ATR assay of α-CDs (inset in Supplementary Fig. 4) gave the typical spectra with the characteristic bands at ca. 3300 cm−1 (for symmetric and anti-symmetric O–H stretching modes), 2930 cm−1 (for C–H symmetric and asymmetric stretching), at 1400–1300 cm−1 (Ikuta et al., 2014) and at 1152 cm−1 (for deformation vibrations of C–H bonds in the secondary and primary hydroxyl groups, respectively), in the regions 1076–990 cm−1 (for C–O–C stretching or C–O bonds in the hydroxyl groups of α-CDs) and 950–650 cm−1 (for vibrations of the C–H bonds and in glucopyranose cycle; more specifically, band at 865 cm−1 was ascribed to C–C–H bending and those located at 750 cm−1 and 700 cm−1 to C–C–O bending), accordingly to literature (Neacsu, Neacsu, Contineanu, Munteanu, & Tanasescu, 2013).

FTIR-ATR assay of TO/α-CD complex gave results supporting the presence of host–guest type interactions (Supplementary Fig. 5). A significant shift of the absorption band at 3318 cm−1 of α-CDs towards higher wavenumber value (3384 cm−1) was observed when the inclusion complex is considered (Supplementary Fig. 5A). This shift is likely due to a redistribution, upon complexation, of the water molecules among the different hydrogen bonds sites and to the interference with the pre-existing bonds between OH groups in the α-CD torus shaped cavity (Ikuta et al., 2014). This

was confirmed by the variations observed in the region 1400–1300 cm−1 (Supplementary Fig. 5B), at 1152 cm−1 and by the significant intensity reduction of bands at 996 cm−1 and 865 cm−1 (Supplementary Fig. 5C). Indeed, the former were ascribed to variations in the deformation vibrations of the C–H bonds in the hydroxyl groups of α-CD (Neacsu et al., 2013) and the latter to a decrease in the number of hydrogen bonds when the guest compounds replace water molecules (De Sousa et al., 2008). Interestingly, the band at 706 cm−1 shifted towards low wavenumber value (702 cm−1) after complexation (Supplementary Fig. 5D). A decrease in the frequency between the complex and its constituent molecule is attributed to the formation of hydrogen bonds and Van der Waals interactions during complexation (Hamidi, Abderrahim, & Meganem, 2010). The TO-related bands located in the regions. 3000–2800 cm−1, 1800–1700 cm−1 (indicated with asterisks in Supplementary Fig. 5A and B, respectively) and at 1160 cm−1 (marked with a black circle in Supplementary Fig. 5C) reduced appreciably their intensities, suggesting that TO compounds were blocked inside the hydrophobic cavity of α-CDs. The presence of host–guest type interactions that partially block vibrational modes could be suggested. When WBO and PO were subject to FTIR-ATR analyses, some of the so far discussed considerations could be applied (Supplementary Figs. 6 and 7). Additionally, both WBO and PO vibration bands (marked with asterisks in Supplementary Figs. 6A, B and 7A, B, respectively, and with black circles in Figs. 6C and 7C) appeared affected by oleoresin/α-CD interaction corroborating the formation of complexes also in these cases.

3.4. Effect of storage conditions on carotenoid, tocochromanol and fatty acid content of free and α-CD encapsulated oleoresins

Free and α-CD encapsulated oleoresins were stored under different conditions of light (daylight or dark) and temperature (25 °C or 4 °C). The amount of carotenoids and tocochromanols in the oleoresins, as well as their fatty acid profiles, were monitored over 120 days storage. The encapsulated oleoresins were preventively eluted from the α-CDs complex with hexane as described by Basu and Del Vecchio (2001). No significant differences were evidenced in the biochemical composition between encapsulated and free oleoresins whose profiles were qualitatively and quantitatively similar to that reported in Table 1 (data not shown).

Fig. 2 shows the results related to carotenoids' retention. Oleoresin encapsulation in α-cyclodextrins differentially improve carotenoid stability in relation to temperature, presence/absence of light, nature of the specific carotenoid, and oleoresin typology. Regardless of encapsulation, temperature seems the main factor affecting carotenoid stability. With the exception of lycopene, all carotenoids were longer preserved at 4 °C than at 25 °C. In encapsulated WBO stored at 4 °C in the dark, lutein, α-carotene, β-carotene and zeaxanthin were stable, at least, for 10 days, and their retention was approximately between 70% and 60% after 50 day storage. In free WBO subjected to the same storage conditions, carotenoid stability was significantly reduced. After

10 day storage, lutein and zeaxanthin retention was slightly higher than 80%, but it resulted below 60% after 30 days. In the short storage period (up to 20 days), the differences in retention values of carotenes between encapsulated and free WBOs were not statistically significant, but they became highly significant over time ($p < 0.001$, $F = 1717.1$ and $F = 1084.4$ for α- and β-carotene, respectively after 30 day storage, when their retention was below 10%). When free WBO was stored at 25 °C, all carotenoids were under the limit of detection after 10 day storage, regardless of the presence or absence of light. Retention was improved by oleoresin encapsulation showing a slightly higher stability in samples stored in the dark than in daylight. In PO, the retention of all identified carotenoids was highly improved by encapsulation, in all tested conditions. Best results were obtained when the encapsulated oleoresin was stored at 4 °C in the dark, where, 60–70% retention was registered for lutein, α- and β-carotene after 120 day storage, much higher than the values registered for free oleoresin (~20%). A similar trend was observed for carotenoids in TO with the exception of lycopene. Lycopene retention was, in fact, significantly lower ($p = 0.049$) in encapsulated than in free oleoresins. A number of studies pointed out the complexity of interactions between carotenoids, mainly lycopene, and α-CDs. Using a solution of highly purified lycopene (98.6%) from Guava (Psidium guajava L.) fruits in acetone, Matioli and Rodriguez-Amaya (2003) reported that lycopene formed inclusion complexes with β- and γ-CDs but not with α-CDs. The inability of α-CDs to form inclusion complexes with lycopene was also suggested by Blanch, del Castillo, Caja, Pérez-Méndez, and Sánchez-Cortés (2007) which speculated about the incompatibility between the van der Waals section of lycopene, which is approximately 5.8 Å, and the van der Waals hole of α-CDs, which is only 5.0 Å. Nevertheless, Mele et al. (2002) and Lyng, Passos, and Fontana (2005), reported that carotenoids without cyclic end groups at least on one end (such as lycopene or bixin) can form inclusion complexes with α-CDs. The same authors reported the assembly of nanoparticles of lycopene surrounded by a shell of CDs acting as amphiphilic solvating agents (Mele et al., 2002). It seems clear that the formation of stoichiometric inclusion complexes provides only one of the possible mechanisms for the formation of stable associations between carotenoids and α-CDs suggesting that simple arguments based on the steric hindrance of host and guest were oversimplified. The whole matter becomes even more complex when heterogeneous oleoresins are used. Recently, the formation of oil/water emulsion stabilized by molecularly absorbed or microcrystals of CD-oil inclusion complexes (i.e. cyclodextrinosomes) has been described (Mathapa & Paunov, 2013). In these complex systems part of the antioxidants would remain soluble in the inner core oil enabling their cooperative synergistic interaction in protecting and regenerating each other from oxidation; while others (i.e. lycopene) may be selectively included into the internal cavity of the α-CDs that form the hydrophilic shell around the oil droplet, being, at least partially, sequestered from the interaction with lipophilic stabilizing molecules and more exposed to oxygen. This is corroborated by the evidence that lycopene encapsulated in γ-CDs remained stable for 40 days in the presence or absence of light at temperature ranging from 25 to 35 °C, only when stored in the absence of oxygen (Matioli & Rodriguez-Amaya, 2003).

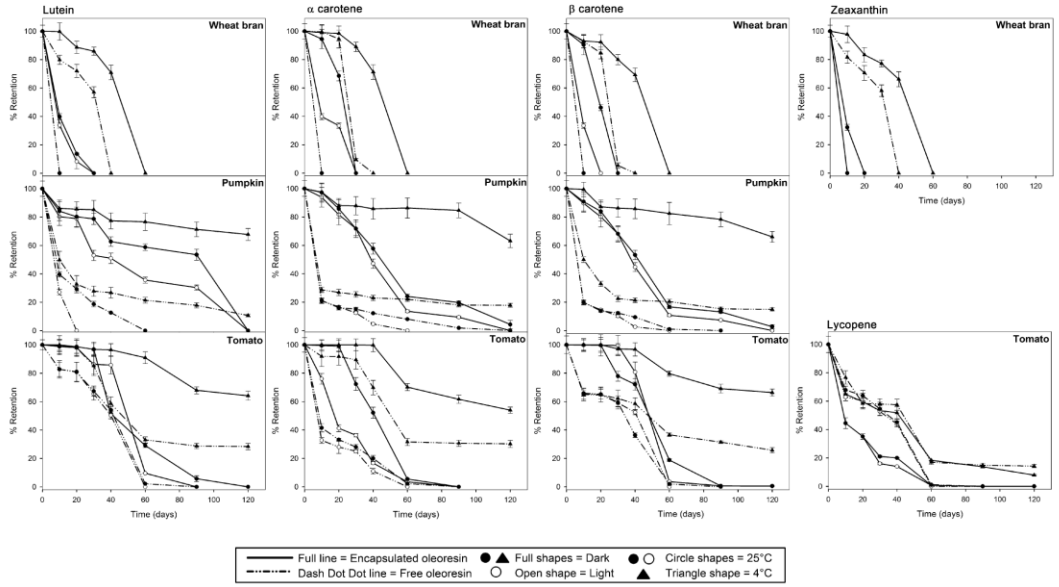


Fig. 2. Time course of the amounts of carotenoids during the storage of free and encapsulated oleoresins in different conditions. Data, expressed as percentage of retention in

Fig. 2. Time course of the amounts of carotenoids during the storage of free and encapsulated oleoresins in different conditions. Data, expressed as percentage of retention in the freshly extracted oil, are the mean ± standard deviation of three independent replicates (n = 3).

The degradation rate constants (k) for each carotenoid were calculated plotting the natural logarithm of the average percentage retention values from the three oleoresins (free and encapsulated) stored at 4 °C vs. time (d) (Table 2; Supplementary Fig. 4). Based on the correlation coefficient (r2), which was used as a parameter to determine the reaction order, it was clear that the degradation of lutein, α-carotene, β-carotene, zeaxanthin and all-trans-lycopene followed a pseudo-first-order behavior for both free and encapsulated oleoresins. In free oleoresins, carotenoid degradation rate was α-carotene $\geqslant$ lutein > zeaxanthin = lycopene > β-carotene, while, when carotenoids were encapsulated the following degradation rate was observed: lycopene $\gg$ lutein > zeaxanthin > α-carotene $\geqslant$ β-carotene. A similar order of carotenoid degradation rates was reported by Henry, Catignani, and Schwartz (1998) for free safflower seed oleoresin (lycopene > β-carotene > 9-cis-β-carotene) and by Anguelova and Warthesen (2000) in methyl linoleate (lycopene > β-carotene > α-carotene) supporting the importance of the synergism with the other constituents of the oil which may substantially change the susceptibility order based just on chemical considerations such as the number of conjugated carbon–carbon double bonds, the oxygenation degree and the configuration of the end-rings. Furthermore, with the exception of lycopene, the degradation rate constants of carotenoids were considerably lower in α-CD encapsulated oleoresins than in the free form.

Table 2. Correlation coefficient (r2) of the degradation plots (Supplementary Figs. 3 and 4) for lutein, zeaxanthin, α-carotene, β-carotene, all-trans-lycopene, α-tocotrienol (α-T3), β,γ-tocotrienols (β,γ-T3), α-tocopherol (α-T) and β,γ-tocopherols (β,γ-T), and degradation rate constant (k) of carotenoids and tocochromanols in free and α-CDs encapsulated oleoresins stored at 4 °C, in the dark.

	Free oleoresins		α-CDs encapsulated oleoresins		*n*	*p*-value
	r^2	$k \times 10^{-3}$ (day^{-1})	r^2	$k \times 10^{-3}$ (day^{-1})		
Carotenoids						
Lutein	0.9305	20.3 ± 2.6^a	0.8667	11.1 ± 1.1^b	40	<0.001
α-Carotene	0.9001	20.9 ± 2.9^a	0.7375	7.7 ± 1.0^b	41	<0.001
β-Carotene	0.9337	17.9 ± 2.9^a	0.9964	7.2 ± 0.9^b	41	<0.001
Zeaxanthin	0.9337	19.5 ± 0.7^a	0.7876	9.2 ± 1.2^b	9	<0.001
All-*trans*-lycopene	0.9549	19.3 ± 2.8^a	0.8617	22.1 ± 1.8^b	16	0.02
Tocochromanols						
α-T	0.9193	20.7 ± 2.7^a	0.8799	9.7 ± 1.3^b	34	<0.001
β,γ-T	0.9480	12.9 ± 1.1^a	0.9520	9.1 ± 0.8^b	41	<0.001
α-T3	0.7532	67.3 ± 17.7^a	0.8207	20.5 ± 5.9^b	14	<0.001
β,γ-T3	0.6775	66.4 ± 29.1^a	0.9968	15.7 ± 0.5^b	17	<0.001

k values are reported as mean ± standard error; 95% interval. Values were obtained from plots of the slopes of ln(% retention) vs. time. Data were submitted to one-way analysis of variance (ANOVA), Tukey's post hoc method was applied to establish significant differences between treatments ($p < 0.05$). Different letters indicate significant differences between free and encapsulated oleoresins k values. The sample size n and the p-values are also reported.

Fig. 3 shows the retention over time of tocopherol (T) and tocotrienol (T3) forms in free and α-CD encapsulated oleoresins stored in different conditions of light/dark and temperature (4/25 °C). Similarly to carotenoids, encapsulation in α-CDs significantly improved tocochromanols' stability, especially when stored at 4 °C, in the dark, although differences in the percentage of retention depending on the oleoresin were observed. Actually, in these storage conditions, α-T, β,γ-T and α-T3 were stable, at least, for 10 days, while β,γ-T3 retention was above 90% up to 40 days storage when WBO/α-CD complex was taken into account, while in TO/α-CD complex (that gave the best storage performance) α-T and β,γ-T retention remained stable up to 60 and 40 days, respectively.

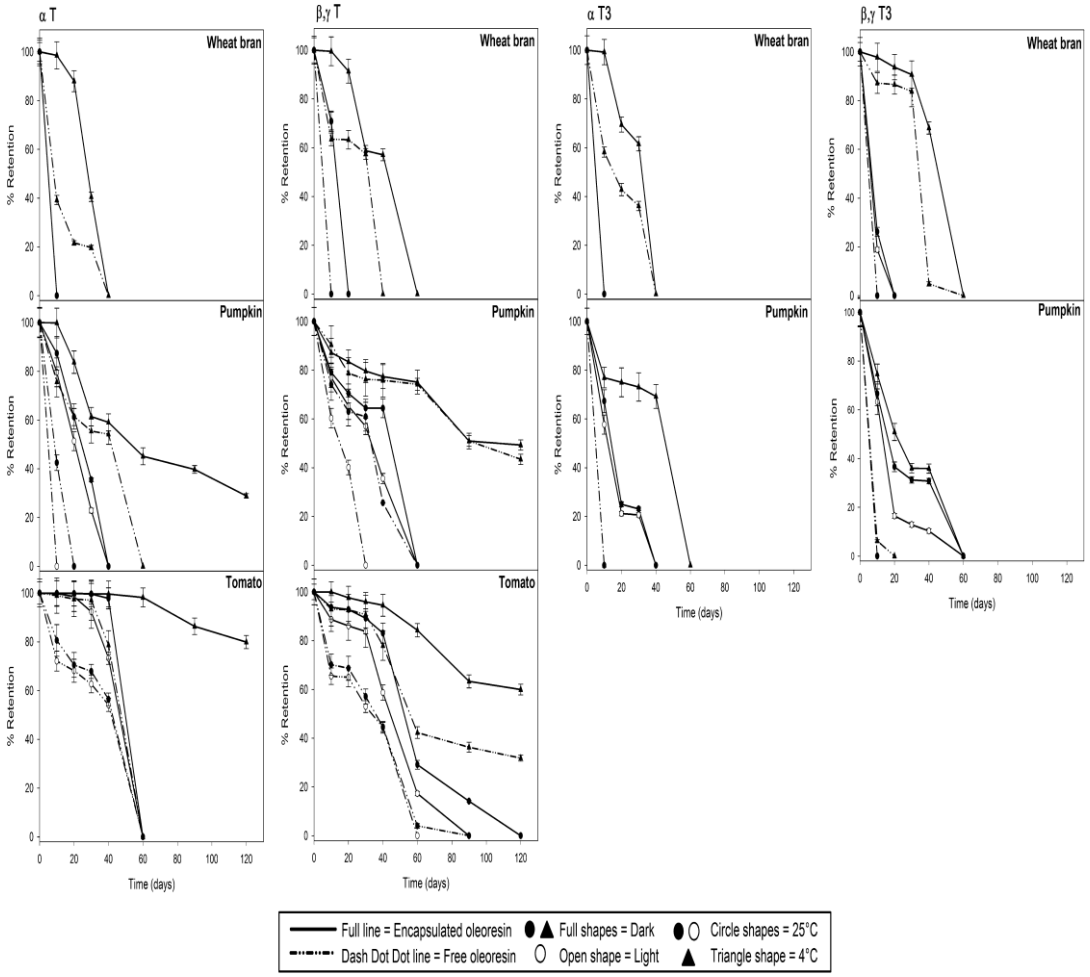


Fig. 3. Time course of the amounts of tocopherols (T) and tocotrienols (T3) during the storage of free and encapsulated oleoresins in different conditions. Data, expressed as percentage of retention in the freshly extracted oil, are the mean ± standard deviation of three independent replicates (n = 3).

Even for tocochromanols the degradation of the different forms, based on the correlation coefficient (r2), followed a pseudo-first-order behavior for both free and encapsulated oleoresins (Table 2; Supplementary Fig. 5A and B). In both free and encapsulated oleoresins, tocochromanol degradation rate was β,γ-T3 = α-T3 ≫ α-T > β,γ-T, but the numerical values were much lower in encapsulated than in free oleoresins. Tocotrienols were more sensitive and susceptible to oxidation compared to tocopherols likely in relation to the presence of three rather than two unsaturations (Sroynak, Srikalong, & Raviyan, 2013).

The changes in SFA, MUFA and PUFA percentage in free and encapsulated oleoresins at the end of the storage period are presented in Table 3A and B respectively. In all tested conditions, the results showed an increase in percentage of SFA and MUFA, and a decrease in percentage of PUFA after 60 days of storage in WBO and after 120 days in TO and PO. PUFA/SFA ratio was affected ($p < 0.05$) by storage temperature (25° C) considering that most changes occurred when storage

was done at room temperature regardless of free and encapsulated oleoresins. At 4 °C, in all encapsulated oleoresins PUFA/SFA ratios after the storage period were similar to those observed in the time 0 corresponding samples. In wheat bran, pumpkin and tomato free oleoresins PUFA/SFA ratios were significantly reduced during storage, however the values were above 0.4. The lower PUFA and higher SFA percentage during storage indicate the susceptibility of PUFA to oxidation (Cosgrove, Church, & Pryor, 1987).

Table 3. Changes in fatty acid composition in free (A) and encapsulated (B) tomato (TO), pumpkin (PO) and wheat bran (WBO) SC-CO2 extracted oleoresins stored in different conditions.

A

	TO				PO				WBO			
	Time storage (days)											
	0	120			0	120			0	60		
		25 °C, light	25 °C, dark	4 °C, dark		25 °C, light	25 °C, dark	4 °C, dark		25 °C, light	25 °C, dark	4 °C, dark
Fatty acid (%)												
C14:0	0.54^{a}	1.07^{b}	0.70^{b}	0.51^{a}	ND	ND	ND	ND	ND	ND	ND	ND
C16:0	19.11^{a}	45.69^{b}	27.02^{b}	20.54^{b}	17.93^{a}	45.13^{b}	42.08^{b}	23.04^{a}	20.63^{a}	43.28^{b}	32.48^{b}	17.08^{a}
C16:1	0.69^{a}	1.23^{b}	0.73^{a}	0.64^{a}	ND	ND	ND	ND	ND	ND	ND	ND
C18:0	7.94^{a}	14.70^{b}	11.28^{b}	7.45^{b}	8.84^{a}	25.36^{b}	17.69^{b}	8.20^{a}	1.16^{a}	2.47^{b}	1.44^{b}	1.08^{a}
C18:1	20.41^{a}	20.30^{a}	24.73^{b}	24.39^{b}	19.13^{a}	24.12^{a}	26.03^{b}	27.98^{b}	20.69^{a}	34.71^{b}	35.81^{b}	30.10^{b}
C18:2	45.80^{a}	16.01^{b}	30.43^{b}	41.75^{a}	45.21^{a}	5.39^{b}	14.20^{b}	40.00^{a}	51.26^{a}	19.54^{b}	25.32^{b}	41.09^{b}
C18:3	4.74^{a}	ND	1.17^{b}	3.94^{b}	8.89^{a}	ND	ND	0.78^{b}	5.27^{a}	ND	2.47^{b}	3.78^{b}
C20:0	0.77^{a}	ND	0.94^{a}	0.78^{a}	ND	ND	ND	ND	0.99^{a}	ND	1.48^{b}	0.87^{a}
SFA	27.98^{a}	62.46^{b}	42.94^{b}	29.28^{a}	26.77^{a}	70.56^{b}	59.77^{b}	31.24^{a}	22.08^{a}	45.55^{b}	36.40^{b}	25.03^{a}
MUFA	20.41^{a}	21.53^{a}	25.46^{a}	25.83^{a}	19.13^{a}	24.12^{a}	26.03^{b}	27.98^{b}	20.69^{a}	34.71^{b}	35.81^{b}	30.10^{b}
PUFA	50.54^{a}	16.01^{b}	31.60^{b}	45.69^{a}	54.10^{a}	5.39^{b}	14.20^{b}	40.78^{b}	56.53^{a}	19.54^{b}	27.79^{b}	44.87^{b}

B

	TO				PO				WBO			
	Time storage (days)											
	0	120			0	120			0	60		
		25 °C, light	25 °C, dark	4 °C, dark		25 °C, light	25 °C, dark	4 °C, dark		25 °C, light	25 °C, dark	4 °C, dark
Fatty acid (%)												
C14:0	0.63^{a}	0.77^{a}	0.68^{a}	0.51^{a}	ND	ND	ND	ND	ND	ND	ND	ND
C16:0	18.40^{a}	40.39^{b}	29.23^{b}	18.36^{a}	19.20^{a}	42.32^{b}	46.74^{b}	15.34^{a}	20.36^{a}	21.75^{a}	20.88^{a}	19.11^{a}
C16:1	0.64^{a}	0.81^{a}	0.83^{b}	0.56^{a}	ND	ND	ND	ND	ND	ND	ND	ND
C18:0	7.86^{a}	12.88^{b}	10.70^{b}	8.56^{a}	8.18^{a}	21.45^{b}	16.94^{b}	9.87^{a}	1.10^{a}	1.34^{a}	1.31^{a}	1.12^{a}
C18:1	20.80^{a}	21.65^{a}	24.85^{a}	21.77^{a}	19.50^{a}	24.31^{a}	25.14^{a}	29.44^{b}	20.52^{a}	35.57^{b}	32.53^{b}	30.01^{b}
C18:2	46.50^{a}	21.08^{b}	32.02^{b}	46.14^{b}	45.10^{a}	10.92^{b}	14.18^{b}	45.60^{a}	52.13^{a}	40.12^{b}	42.11^{a}	43.31^{a}
C18:3	3.66^{a}	1.35^{b}	0.75^{b}	2.42^{a}	7.99^{a}	ND	ND	0.75^{b}	5.07^{a}	0.14^{b}	2.01^{b}	3.59^{b}
C20:0	0.62^{a}	1.07^{b}	0.94^{b}	0.68^{a}	ND	ND	ND	ND	0.82^{a}	1.08^{b}	1.16^{b}	0.86^{a}
SFA	28.41^{a}	55.11^{b}	41.55^{b}	28.11^{a}	27.38^{a}	64.76^{b}	60.68^{b}	25.21^{a}	22.20^{a}	24.17^{a}	23.35^{a}	23.09^{a}
MUFA	21.44^{a}	22.46^{a}	25.68^{a}	22.33^{a}	19.50^{a}	24.31^{a}	25.14^{a}	29.44^{b}	20.52^{a}	35.57^{b}	32.53^{b}	30.01^{b}
PUFA	50.16^{a}	22.43^{b}	32.77^{b}	49.56^{a}	53.09^{a}	10.92^{b}	14.18^{b}	46.35^{a}	57.20^{a}	40.26^{b}	44.12^{b}	46.90^{a}

Data, are the mean ± standard deviation of three independent replicates (n = 3). Standard deviation is less than 10%. Data were submitted to one-way analysis of variance (ANOVA), Tukey's post hoc method was applied to establish significant differences versus initial storage time ($p < 0.05$). ND, not detected. Different letters indicate significant differences between each treatment after 120 days storage and initial storage time.

4. Conclusions

The results reported in this work show that WBO, PO and TO extracted by SC-CO2 form complexes with α-CDs when mixed in a 0.56:1 molar ratio. DSC and FTIR-ATR analyses, in fact, demonstrated that oleoresins/α-CDs have different physical characteristics from free oleoresins. Upon storage, the retention of tocochromanols, carotenoids and fatty acids was higher in oleoresin/α-CD complexes stored at 4 °C

than in the corresponding free oleoresins, indicating that encapsulation and low temperature have a synergistic effect in preventing the degradation against oxidation of bioactives. Differences in the stability were evidenced with respect of (a) the nature of the specific bioactive and (b) the oleoresin typology. Unlike all other antioxidants which were stabilized by α-CD encapsulation, lycopene in tomato oleoresin/α-CD complex resulted more susceptible to oxidation than in free oleoresin, likely due to its selective sequestration from the interaction with other lipophilic molecules of the oleoresin. The lack of cyclic end groups at both end, makes in fact lycopene more prone to form inclusion complexes with α-CDs than cyclic carotenoids. The high complexity of the interactions between α-CDs and composite oleoresins must be taken into account in order to explain the differences in susceptibility to degradation among carotenoids.

Taken together the results suggest that PO, TO and WBO/α-CD complexes, being generally more stable than free oleoresins, can be used as ready-to-mix high-quality ingredients in the preparation of novel raw or cooked functional foods simultaneously enriched with antioxidants (i.e. carotenoids and tochocromanols), PUFA and fibers.

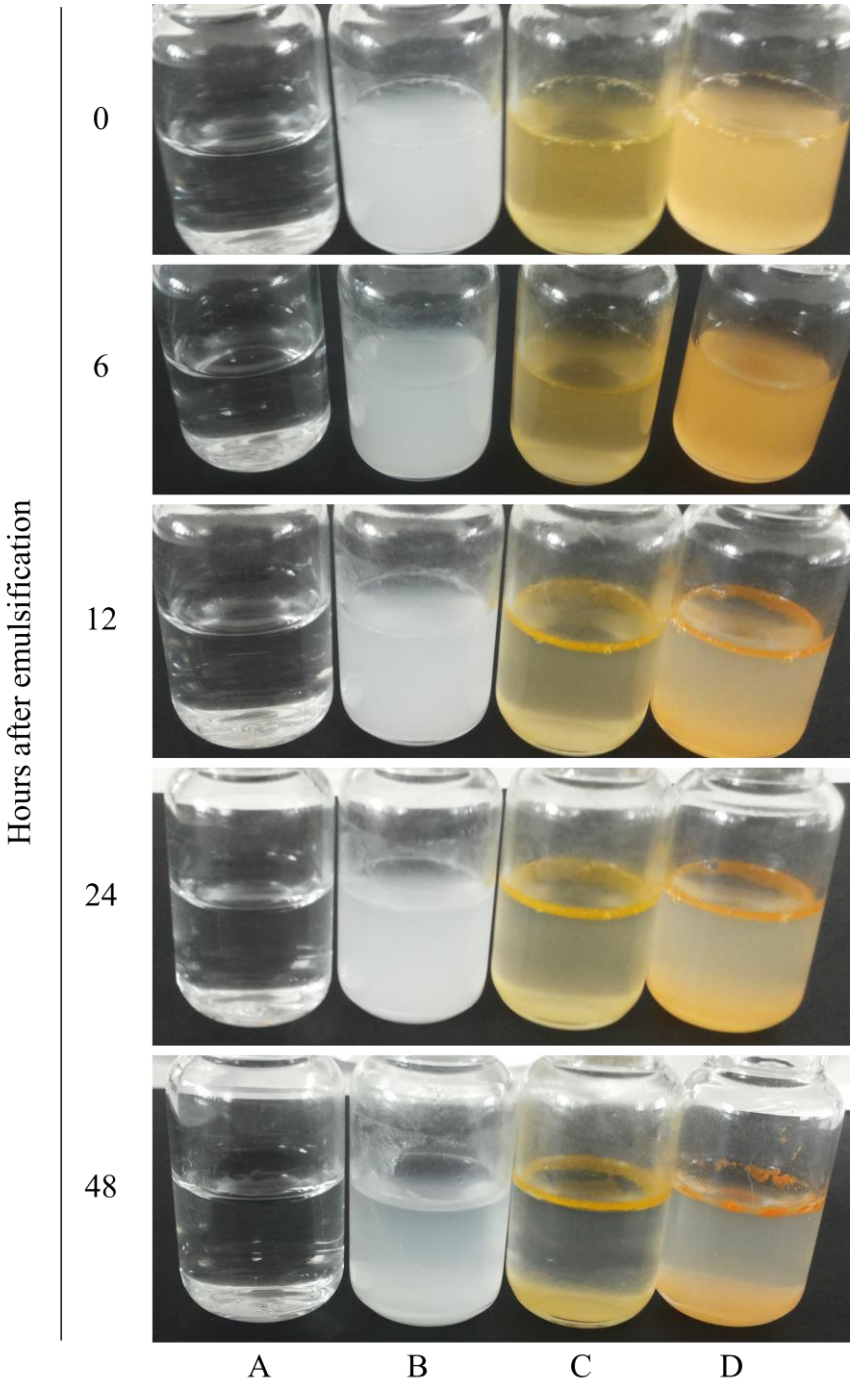
5. Conflict of interest

The authors declare no competing interests.

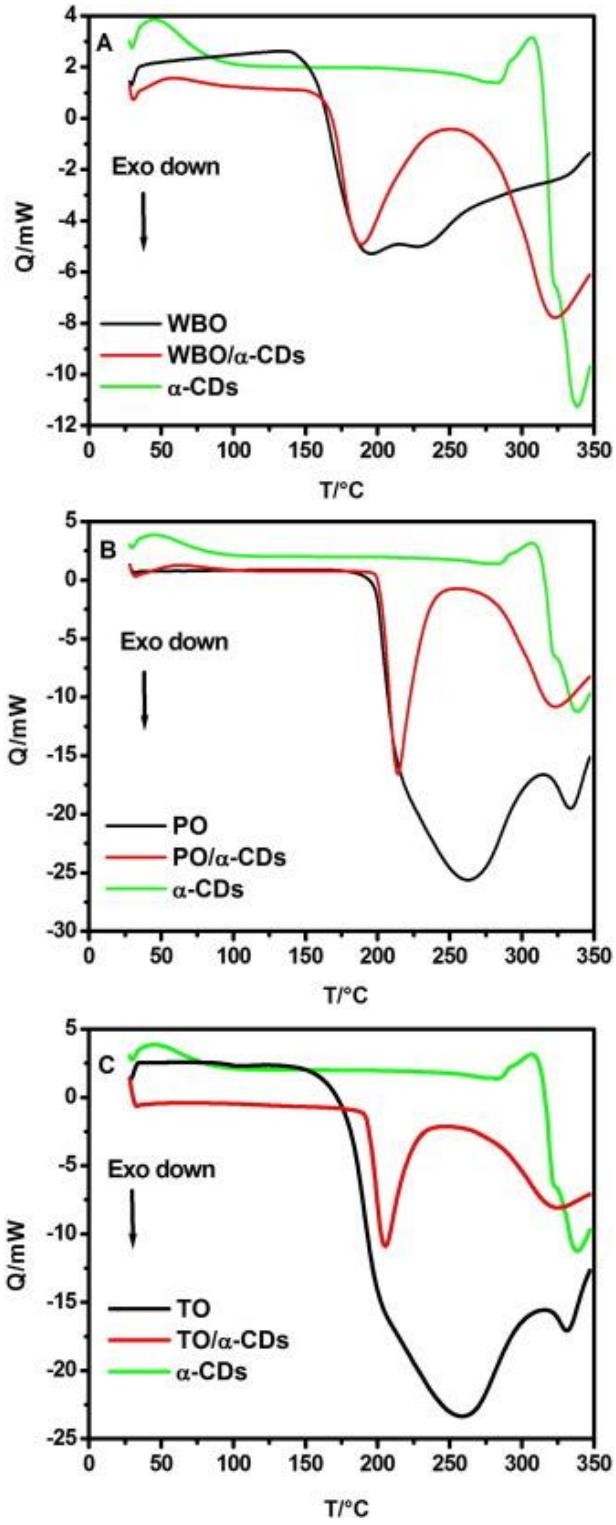
Acknowledgements

The authors are very grateful to IMCD Italia SpA Milano, Italy for kindly providing CAVAMAX® W6 (α-cyclodextrins). The research has been supported by MIUR Project “Conoscenze Integrate per la Sostenibilità e l’Innovazione del made in Italy Agroalimentare” (CISIA).

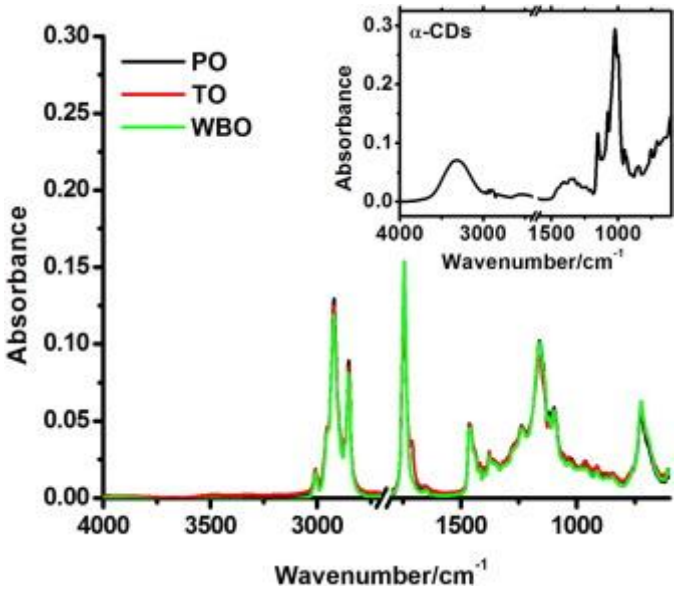
Supplementary data 1. Macroscopic aspect of the pure freeze-dried α-CDs and of the freeze-dried complexes WBO/α-CDs, PO/α-CDs and TO/α-CDs.



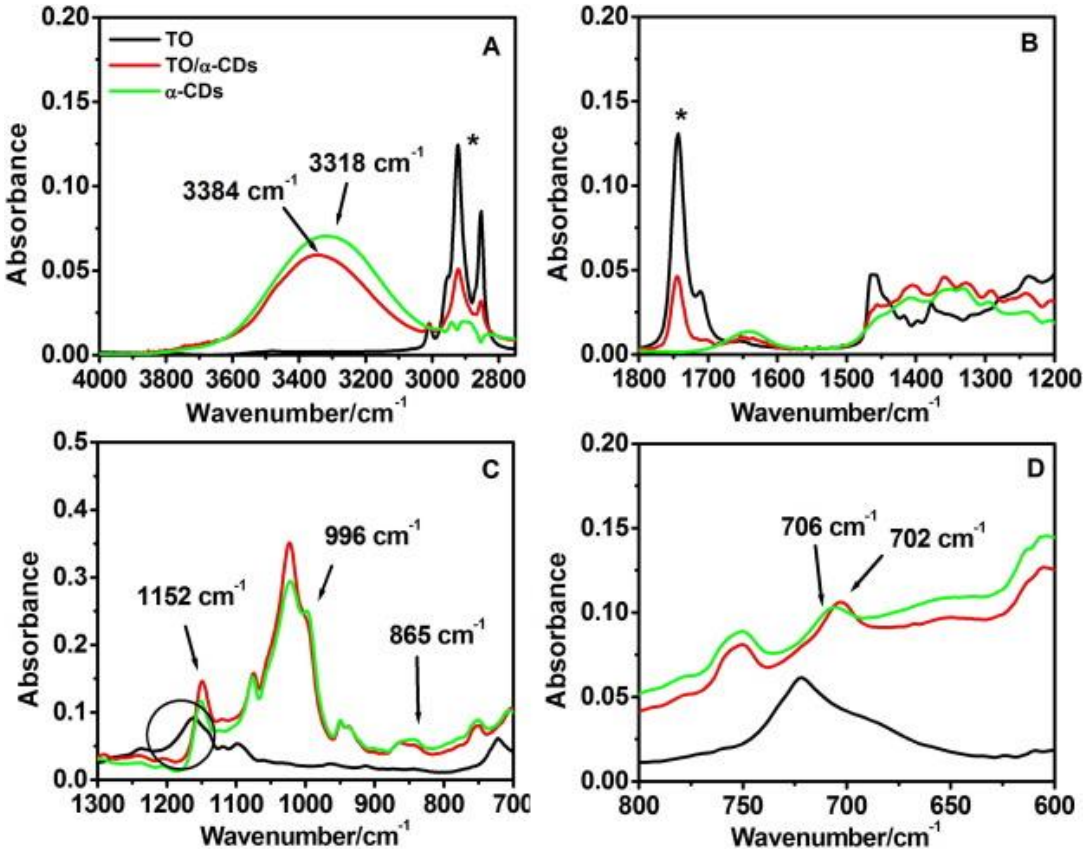
Supplementary data 2. Stability of oleoresin/α-CD emulsions over time. A = α-CDs; B = WBO/α-CDs complex; C = PO/α-CDs complex; D = TO/α-CDs complex.



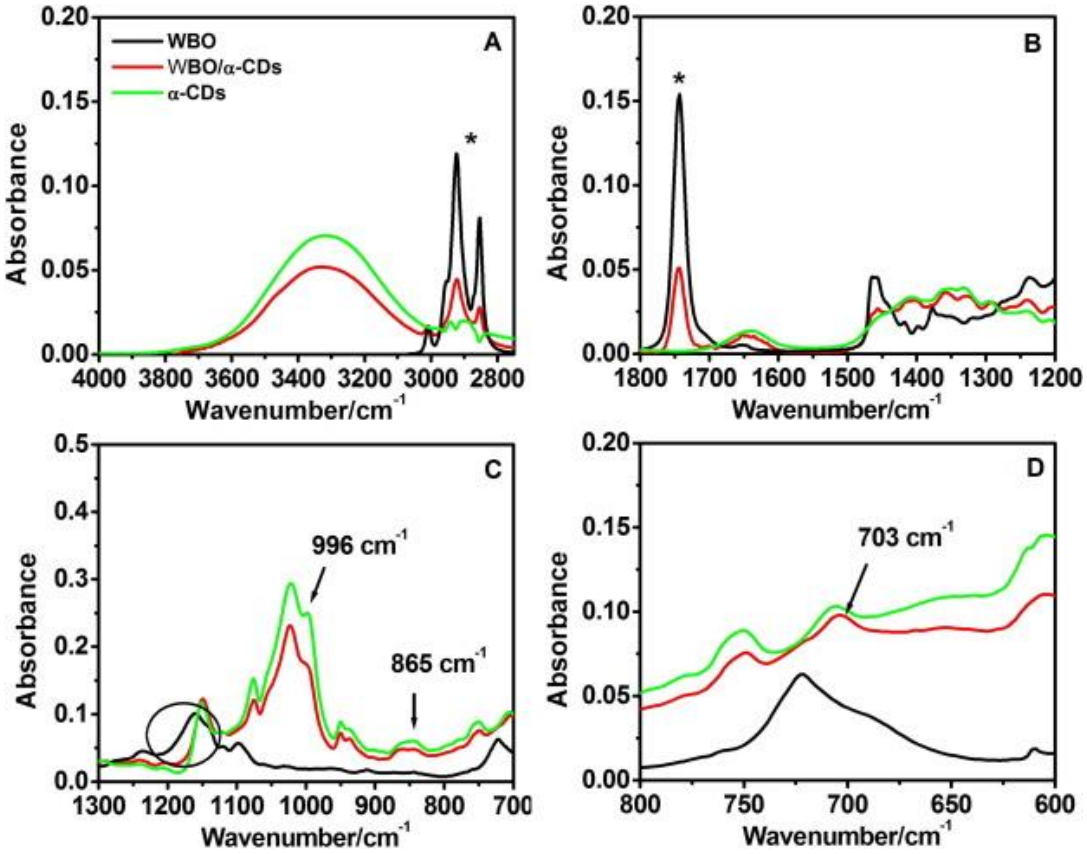
Supplementary Figure 3. DSC thermograms of α-CDs, free WBO and WBO/α-CD complex (A); α-CDs, free PO and PO/α-CD complex (B); α-CDs, free TO and TO/α-CD complex (C).



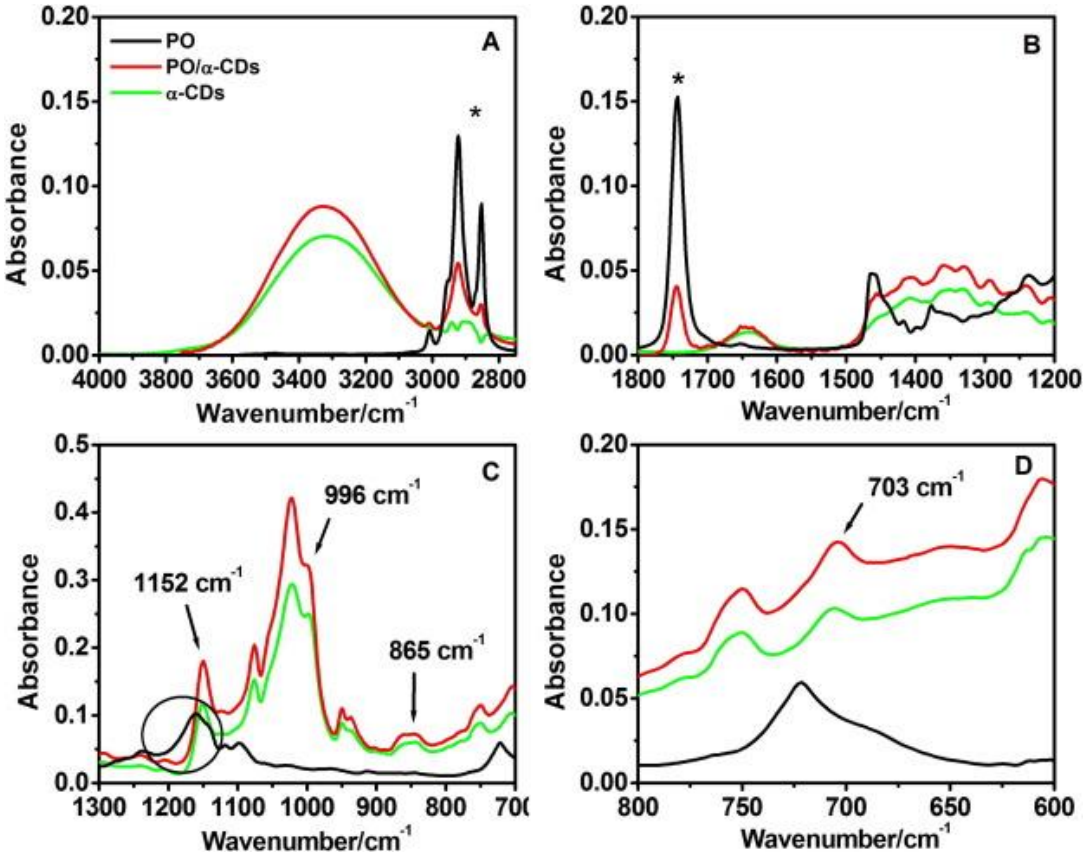
Supplementary Figure 4. Comparison between FTIR-ATR spectra (wavenumbers range 4000–600 cm−1) of free WBO, PO and TO. Inset: FTIR-ATR spectra of α-CDs. Peaks at 2922 cm−1 and 2862 cm−1 (for the asymmetric and symmetric stretching vibrations of the CH2 and CH3 moieties, respectively), at 1445 cm−1 (for CH2 scissoring), at 1360 cm−1 (for dimethyl group vibration), at 1033 cm−1 (for in plane –CH– stretching) and at 962 cm−1 (for trans conjugated alkene –CHdouble bondCH– out-of-plane deformation mode) were attributed to carotenoids. Peaks at ca. 3500 cm−1 (for OH moieties, slightly observed in the figure) and in the region 1200–1000 cm−1 (for C–O–C stretching) were attributed to tocochromanols. The contribute of lutein and zeaxanthin was also considered at these wavenumbers. Peaks at 3008 cm−1 (for –C–H stretching), in the region 3000–2800 cm−1 (for CH3 stretching), in the region 1750–1700 cm−1 (for Cdouble bondO stretching), at 1670 cm−1 (for Cdouble bondC stretching), at 1266 cm−1 (for bending of double bondC–H moiety) and at 1200–800 cm−1 (for C–C stretching) were attributed to fatty acids.



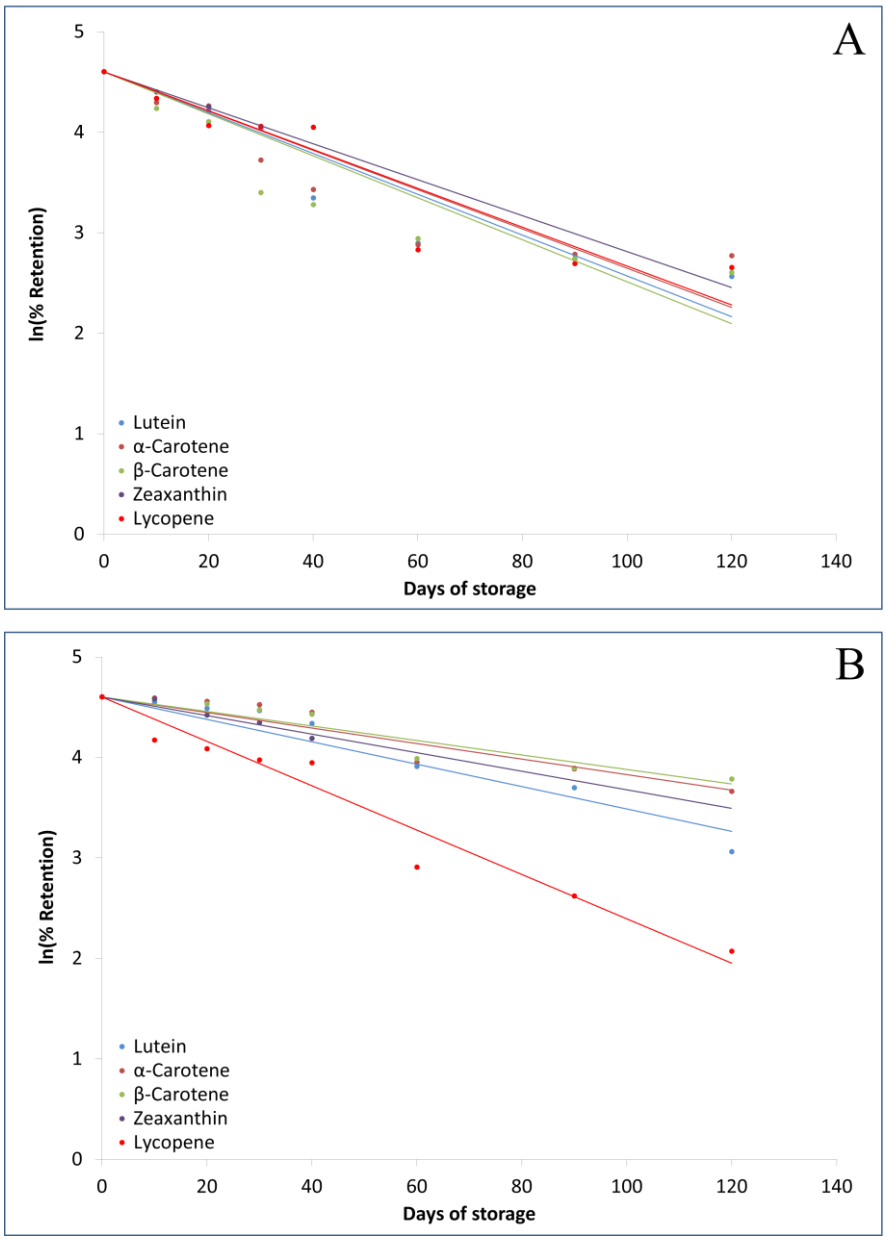
Supplementary Figure 5. Comparison between detailed views of the FTIR-ATR spectra obtained for α-CDs, free TO and TO/α-CD complex. Wavenumbers range 4000–2750 cm−1 (A), 1800–1200 cm−1 (B) 1300–700 cm−1 (C), 800–600 cm–1 (D).



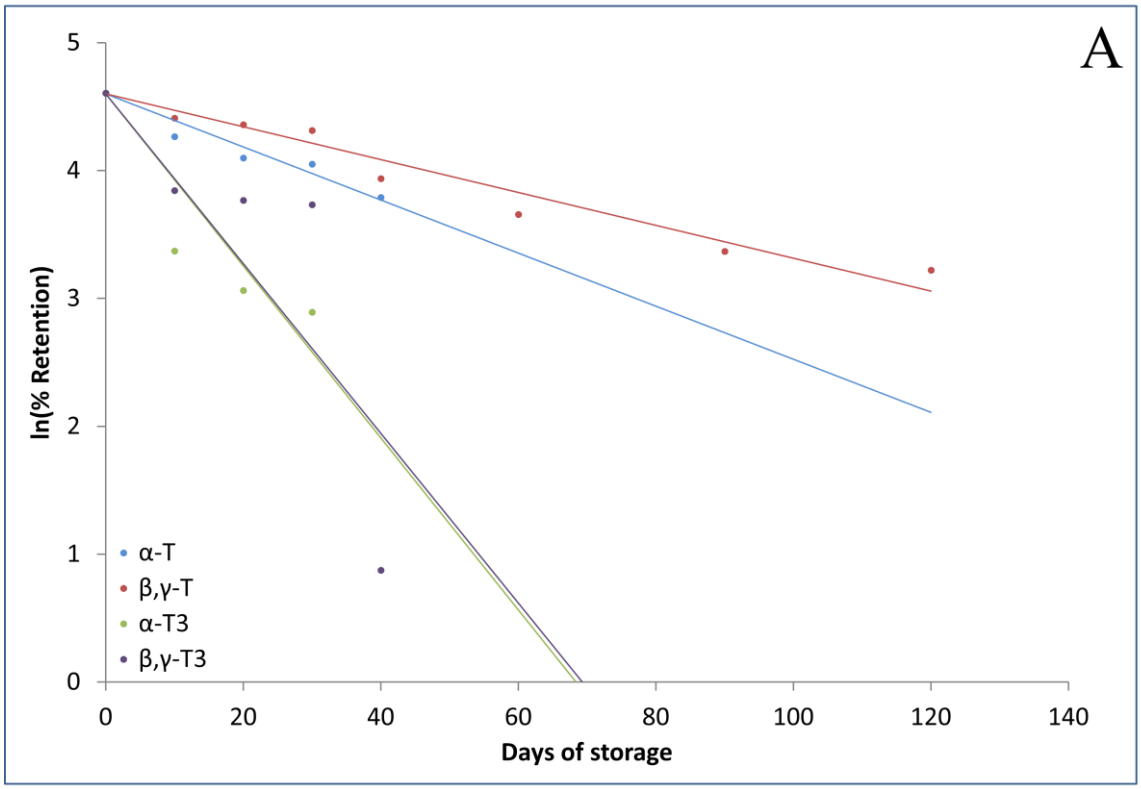
Supplementary Figure 6. Comparison between detailed views of the FTIR-ATR spectra obtained for α-CDs, free WBO and WBO/α-CD complex. Wavenumbers range 4000–2750 cm−1 (A), 1800–1200 cm−1 (B), 1300–700 cm−1 (C), 800–600 cm−1 (D).

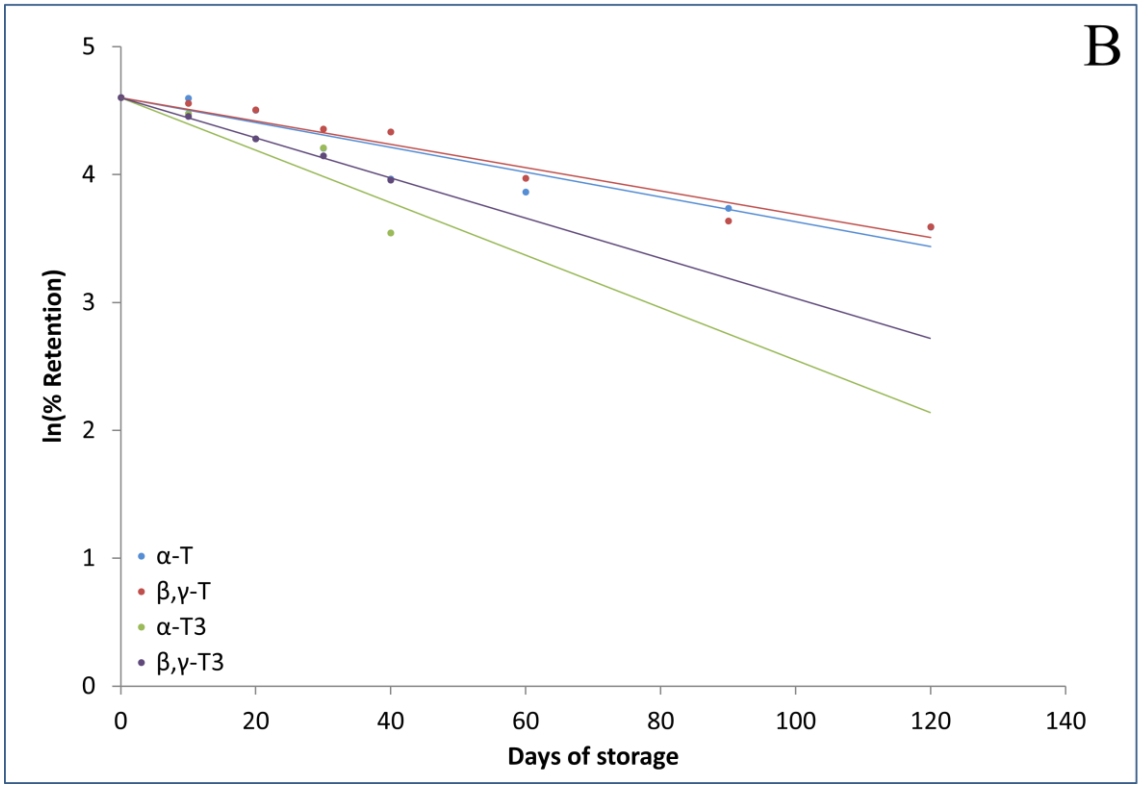


Supplementary Figure 7. Comparison between detailed views of the FTIR-ATR spectra obtained for α-CDs, free PO and PO/α-CD complex. Wavenumbers range 4000–2750 cm−1 (A), 1800–1200 cm−1 (B), 1300–700 cm−1 (C), 800–600 cm−1 (D).



Supplementary data 8. Degradation plots of carotenoids in free and α-CDs encapsulated oleoresins stored at 4 °C, in the dark.





Supplementary data 9. Degradation plots of tocochromanols in free and α-CDs encapsulated oleoresins stored at 4 °C, in the dark.

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